Effect of Curcumin on Ethanol Induced Changes in Daily Serotonin Chronometabolomics and Gene Expression in SCN and Pineal: Locomotor Rhythms

A thesis submitted to the University of Hyderabad for the award of a Ph.D. degree in Animal Sciences

By

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DECLARATION

I, Mareddy Yallamandareddy, hereby declare that this thesis entitled ***"Effect of Curcumin on Ethanol Induced Changes in Daily Serotonin Chronometabolomics and Gene Expression in SCN and Pineal: Locomotor Rhythms"*** submitted by me under the guidance and supervision of **Dr. Anita Jagota,** is an original and independent research work. I also declare that it has not been submitted previously in part or in full to this University or any other University or Institution for the award of any degree or diploma.

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CERTIFICATE

This is to certify that this thesis entitled ***"Effect of Curcumin on Ethanol Induced Changes in Daily Serotonin Chronometabolomics and Gene Expression in SCN and Pineal: Locomotor Rhythms"*** is a record of bonafide work done by Mr. **Mareddy Yallamandareddy**, a research scholar for Ph.D. programme in Animal Sciences, School of Life Sciences, University of Hyderabad under my guidance and supervision.

The thesis has not been submitted previously in part or in full to this or any other University or Institution for the award of any degree or diploma.

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Dedicated to

My Family

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INTRODUCTION

Circadian rhythms

Living organisms on earth have biological rhythms ranging from prokaryotes to higher mammals and adapted to the daily rotation of the earth on its axis (Dunlap, 1999; Harmer *et al*., 2001). A biological rhythm that persists under constant conditions and has a period of ~1 day (24 hours) is called circadian rhythm (*circa diem*, meaning about a day). The rhythms are generated in two ways - exogenous and endogenous. Exogenous biological rhythms are driven directly by the environment or another external influence (direct effect). An example of an exogenous biological rhythm is the hopping of sparrows on a perch when a light is turned on. Endogenous biological rhythms are driven by internal biological clocks and are maintained even when environmental cues are removed. Some examples of endogenous biological rhythms are sleep-wake and daily body temperature cycles. Sometimes it is difficult to determine whether the activity of an animal is due to a direct effect or that of an endogenous biological clock, because the two types of rhythms can mask each other.

The photoperiod is the most dominant environmental *Zeitgeber* (time giver) for the phase entrainment of circadian oscillators in all organisms, including cyanobacteria, fungi, green plants and metazoans. In mammals, the circadian timing system is composed of self sustained circadian oscillators. Circadian rhythms are characterized by the ability to be synchronized to environmental time cues, free running in the absence of external cues, temperature compensated and under genetic control.

The functional components of circadian time keeping system (CTS) in mammals contain three parts (Fig. 1).

1. Central or Master oscillator (Circadian Pacemaker)
2. Afferent pathways
3. Efferent pathways

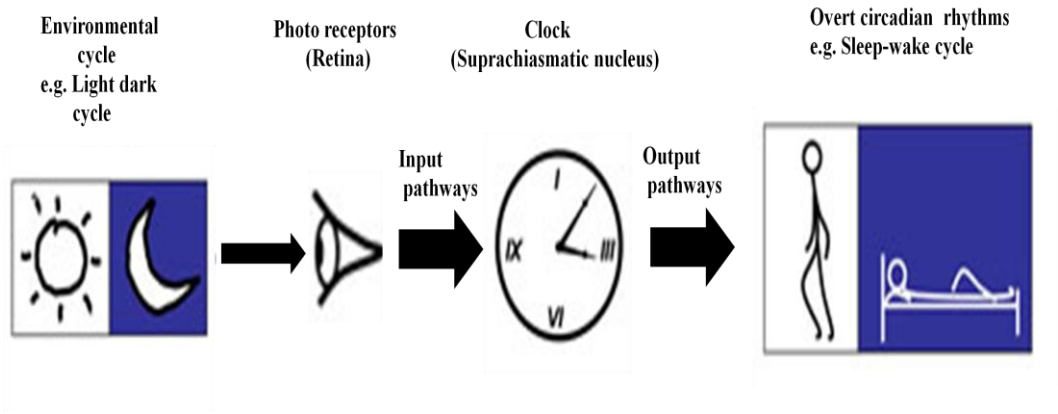


Fig. 1. Functional components of circadian time keeping system (Klein *et al.*, 1991)

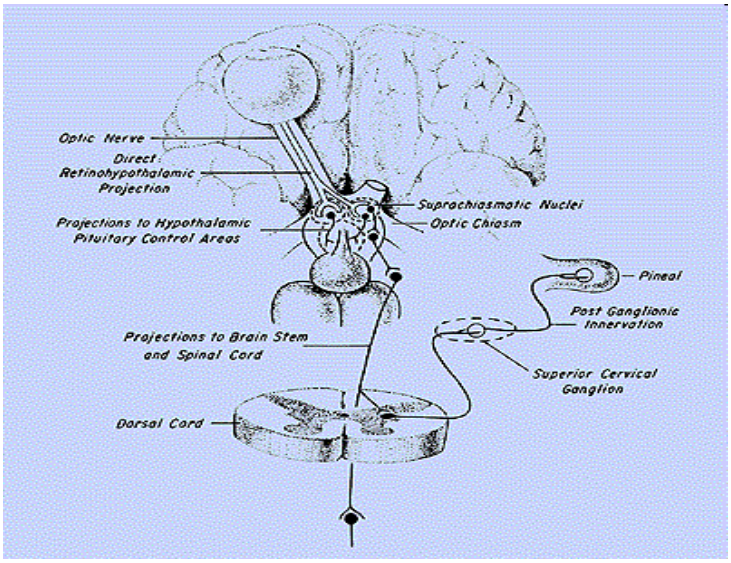
1. Master Clock: Suprachiasmatic Nucleus:

In mammals, a variety of physiological and behavioral activities show circadian rhythms (Jagota, 2006). These rhythms are primarily controlled by a small portion of the brain which is located in hypothalamus region just above the Optic chiasm (OC), lateral to third ventricle called as Suprachiasmatic nucleus (SCN) (Fig. 2A). SCN has been identified as the master circadian pacemaker (Hastings and Maywood. 2000) which resets by photic stimuli (Meijer *et al.*, 2002) as well as by nonphotic stimuli (Mrosovsky, 1992).

A. Localization and morphology:

The SCN are small paired structures in the anterior hypothalamus, just above the optic chiasm (Fig. 2B). Each nucleus contains about 10,000 neurons (Schirakawa *et al.*, 2001). The nuclei are strategically positioned for receiving visual input for light-dark entrainment through both direct and indirect retina-to-SCN pathways. SCN have heterogeneous neuronal population and divided into subdivisions, such as dorsomedial shell (DM-SCN) and ventro-lateral core (VL-SCN) (Moore, 1966) (Fig. 2C). Small, elongated and tightly packed neurons were found in DM-SCN and these cells are clustered along the walls of the blood capillaries that course through the SCN. These cells release substances into the blood which rhythmically regulates target tissues. Spherical, large and loosely packed cells were found in VL-SCN which receives photic information from optic chiasm (Jagota, 2006).

A



B **C**

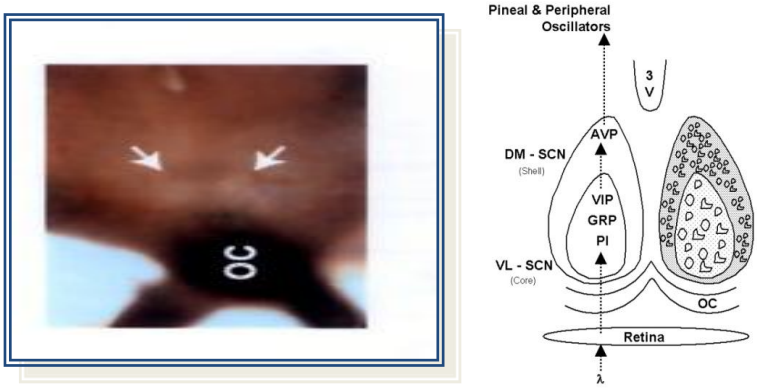


Fig. 2. **(A)** Location of SCN and Pineal in brain (Martin and Reichlin, 1987). **(B).** Structure of Suprachiasmatic nuclei**;** SCN is present in the anterior hypothalamus, immediately above the optic chiasm and lateral to third ventricle Jagota *et al.,* 2000). (**C)**. Shows the anatomical and neurochemical organizations of SCN- VL- Ventrolateral SCN and DM- dorsomedial SCN. VL region receives the photic information from retina and passes to the DM region. DM generates and relays output information to the pineal and peripheral oscillators (Jagota, 2006).

B. Neuropeptides and Neurotransmitters:

SCN have neuronal subpopulations characterized by different neuroactive substances such as acetylcholine, glutamate, neuropeptide Y (NPY), serotonin (5-hydroxy-tryptamine or 5-HT), vasoactive intestinal peptide (VIP), peptide histidine isoleucine (PHI), and arginin vasopressin (AVP) that play important role in regulation of SCN function. Small neurophysin and vasopressin-immunoreactive neurons are found in greatest density in DM-SCN (Jagota, 2006).

About one third of the SCN contain AVP neurons, which are found in dorsomedial region (Castel *et al.*, 1990). 9 to 24% of VIP neurons have been reported in SCN (Herzog *et al.*, 2004), which are predominantly present in ventral region or sometimes embedded in dorsal region of optic chiasm (Piggins & Cutler 2003). Gastrin releasing peptide (GRP), bombesin (BBS), PHI and Nerve growth factor (NGF) are also found in the VL-SCN (Card *et al.*, 1988; Van den Pol *et al.*, 1989). In addition, angiotensin II, BBS, calcitonin gene related peptide (CGRP), cholecystokinin (CCK), enkephalin (ENK), galanin, gama-aminobutyric acid (GABA), somatostatin (SS), SP, thyrotropin-releasing hormone, tyrosine hydroxylase (TH), ubiquitin, VGF (a protein induced by nerve growth factor) and Neurotensin (NT) have also been detected in adjoining areas of SCN (Watts and Swanson, 1987; Koh *et al.*, 1989, Van den Pol *et al.*, 1989).

2. Afferent Pathways:

Photic information is conveyed to the SCN by three convergent pathways: Retino-hypothalamic tract (RHT), Geniculo-hypothalamic tract (GHT), Raphe-SCN pathway (Morin, 1994). The major light input pathway to the SCN is RHT (direct), which arises from a widely distributed population of retinal ganglion cells (Moore *et al.*, 1995). The major neurotransmitter of the RHT is glutamate (Ebling, 1996). Two other peptides are also present in the RHT, substance P and pituitary adenylate cyclase-activating peptide (PACAP); modulate the entrainment process (Hamada, 1999; Chen *et al.*, 1999). Other two indirect pathways also provide retinal input to the SCN: (i) Intergeniculate leaflet (IGL) of the lateral geniculate nucleus, which receives input from the same retinal cells whose axons compose the RHT (Pickard, 1985). GHT rich in GABA, neuropeptide Y and enkephalin converges on the retinorecipient region of the SCN (Morin, 1994); (ii) Second indirect pathway from the retina to the

retinorecipient SCN is routed via the serotonergic raphe nuclei. Neuro-transmitters in both the intergeniculate leaflet and raphe pathways appear to play a role in mediating nonphotic phase shifts (behavioural arousal) (Byku *et al.*, 2000; Mistleberger *et al.*, 2000; Jagota, 2006) (Fig. 3).

3. Efferent pathways:

Efferent projections of SCN serve the purpose of conveying the information to the related centers. Efferent projections seem to have mainly AVP and VIP as neurotransmitters (Watts *et al.,* 1991). Outputs are primarily seen to the nearby hypothalamic and thalamic nuclei from the SCN, particularly to the medial preoptic nucleus, the medial part of the paraventricular nucleus of the hypothalamus, the anterior part of the paraventricular nucleus of thalamus, the medial part of the dorsomedial nucleus of hypothalamus, and principally the subparaventricular zone (Kalsbeek *et al.*, 1993; Saper *et al.*, 2005). SCN regulates a wide range of physiological outputs by two types of signals originating from the SCN. These are hormonal and neural outputs (Jagota, 2006).

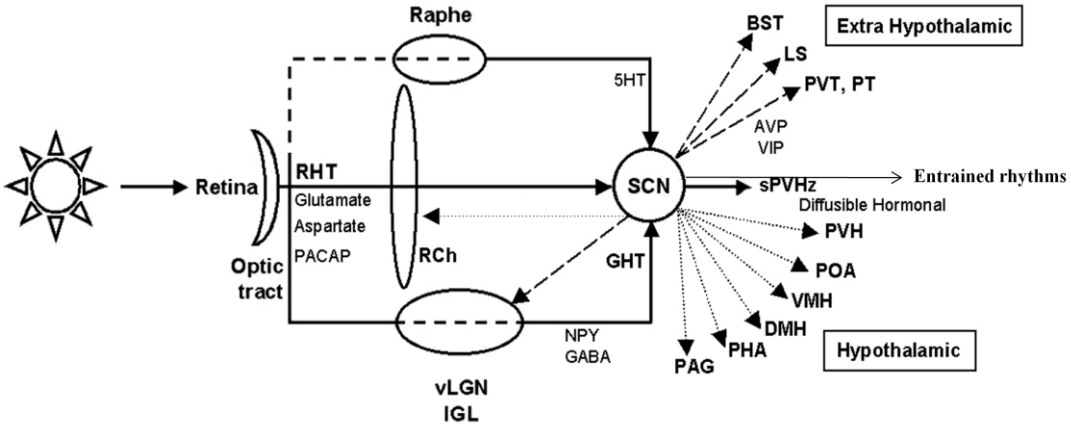


Fig. 3. Neurotransmitters involved in output and input pathways (Jagota, 2006). (i) Afferents of SCN: RHT - retinohypothalamic tract; GHT – geniculohypothalamic tract via ventral lateral geniculate nucleus (vLGN) and the intergeniculate nucleus (IGL); from median and dorsal raphe (ii) Efferents of the SCN towards various extra hypothalamic and hypothalamic targets: BST, bed nuclei of the stria terminalis; LS, lateral septal nucleus; PVT/PT, paraventricular nucleus of the thalamus, paratanial nucleus; IGL, intergeniculate leaflet; AHA anterior hypothalamic area; PVH, paraventricular nucleus of the hypothalamus; MPQ and POA, preoptic area nuclei; RCh, retrochiasmatic area; VMH, ventromedial nucleus of the hypothalamus; DMH, dorsomedial nucleus of the hypothalamus; ZI, zona incerta; PHA, posterior hypothalamic area; PAG, periaqueductal gray.

Experimental evidences proved that hormonal or diffusible factors produced by the SCN act as an important output signal for the circadian system (Ralph and Lehman, 1991; Silver and LeSauter, 1993). Transforming growth factor α (TGFα) is an important diffusible output identified in the SCN (Kramer *et al.*, 2001). TGFα is found extensively in the brain and is a member of the epidermal growth factor (EGF) family produced by both neurons and astrocytes (Junier, 2000).

In addition, the SCN also contain other neurochemicals such as Somatostatin (SS), Calbindin (Cal B), Calretinin (CALR), Galanin (Gal), Angiotensin II (ANG II), Met-Enkephalin (mENK) and Prokineticin 2 (PK2) (Reghunandanan and Reghunandanan, 2006).

SCN output pathways influence the hypothalamic region as well as extra hypothalamic regions as far as the liver, thyroid, adrenal, and salivary glands in rats. Peripheral oscillators respond to signals from SCN as well as to other inputs like periodic food availability (Leak *et al.*, 1999; Kalsbeek *et al.*, 2000; la Fleur *et al.*, 2000) (Fig. 3).

The Pineal gland: effector follower system of master clock

The mammalian pineal gland is a pyramidal shape neuroendocrine gland secreting the hormone, melatonin (Lerner *et al.*, 1959; Ganguly *et al.*, 2002; Stehle *et al.*, 2002). The gland is derived from the neural tube and located at the border between mesencephalon and diencephalon of the brain, i.e. just rostral to superior colliculi. Neuroanatomically, the pineal is described as a part of the epithalamus and thereby as a part of the diencephalon. In rodents, the gland is subdivided into two parts: (1) a superficial pineal gland located at the dorsal surface of the brain and (2) a deep pineal gland located on the brain stem. The two parts are connected through the pineal stalk (Vallarta, 1981) (Fig. 4).

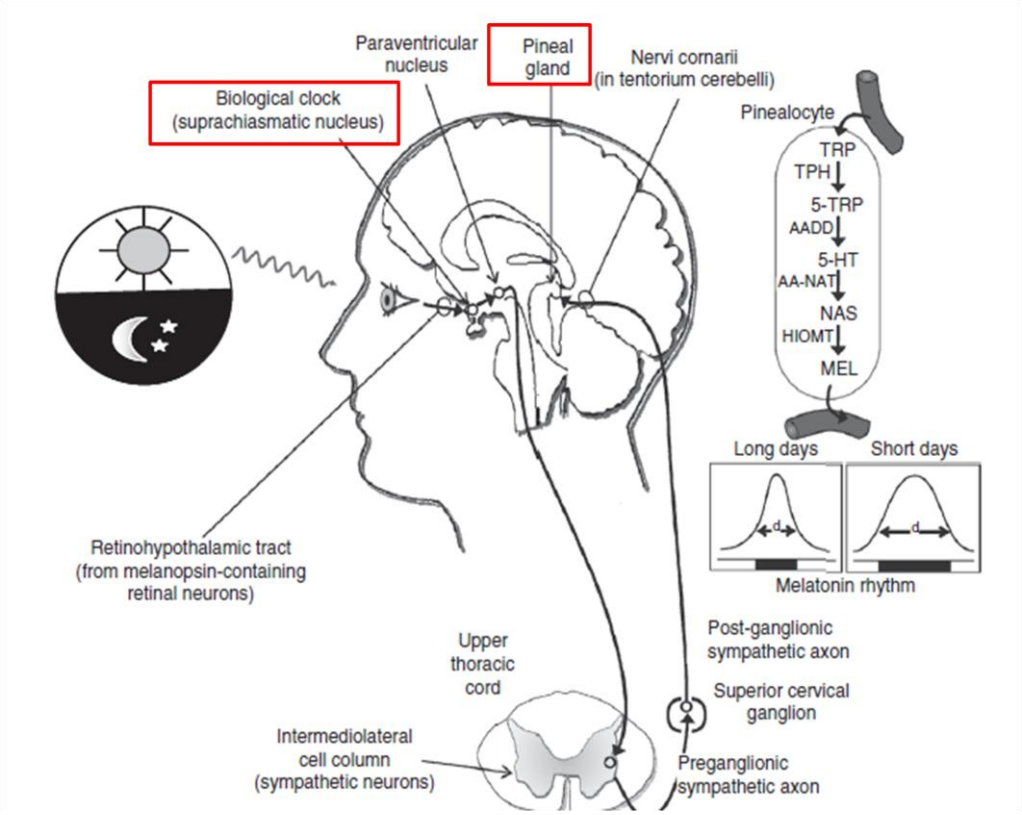


Fig. 4. Effector follower system of SCN: Pineal gland (Reiter *et al*., 2010). Melatonin synthesis (MEL) from tryptophan (TRP) in a pinealocyte. TPH, tryptophan hydroxylase; 5-TRP, 5-hydroxytryptophan; AADD, L-amino acid decarboxylase; 5-HT, serotonin, AANAT, arylalkylamine N-acetyltransferase; NAS, N-acetylserotonin; HlOMT, hydroxyindole-O-methyltransferase.

Regulation of Melatonin synthesis in Pineal:

The rhythm of melatonin production is endogenous, being generated by interacting networks of clock genes in the SCN, the major central rhythm-generating system or "clock" in mammals (Cassone and Natesan, 1997). SCN receives photic information from retina and generates output information to pineal via Intermediolateral cell column (ILCC) and SCG (Superior cervical ganglion). Ganglionic terminals release Norepinephrine and this Norepinephrine binds to adrenergic receptors. Then ATP is converted to cAMP by adenylate cyclase. This second messenger, cAMP promotes synthesis of N- acetyl transferase (NAT) regulatory enzyme via protein synthesis. NAT is key regulatory enzyme for the formation of melatonin from N-acetylserotonin. Melatonin releases into capillaries to govern various physiological functions (Nowak and Zawilska, 1998; Yonei *et al.*, 2010) (Fig. 5).

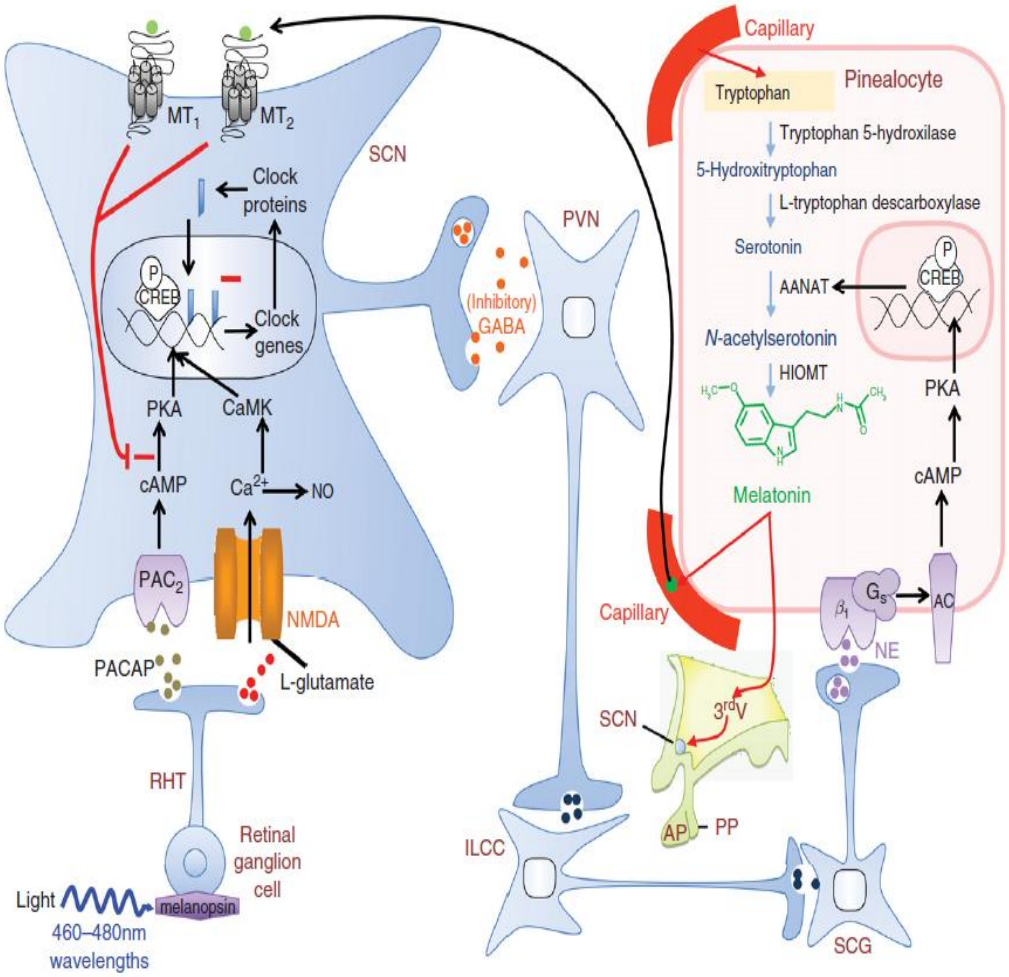


Fig. 5. Regulation of melatonin synthesis in pineal (Reiter *et al.*, 2010). Melanopsin-containing ganglion cells in retina receive blue light (~460–480 nm) and relay information to the suprachiasmatic nuclei (SCN) via the retinohypothalamic tract (RHT) by releasing glutamate and pituitary adenylate cyclase activating polypeptide (PACAP) which causes entrainment of clock gene expression in the SCN. Information from SCN neurons passes to the PVN of the hypothalamus where they release gamma-amino butyric acid (GABA). Nerve cell bodies in the PVN gives information to pinealocyte via intermediolateral cell column (ILCC) and superior cervical ganglion (SCG) by release of norepinephrine, which occurs during the night, stimulates the synthesis and release of melatonin. Melatonin is transported into the blood and cerebrospinal fluid (CSF) of the third ventricle (3rd V). Both blood and CSF melatonin acts on SCN via MT1 and MT2 receptors for resetting the circadian pacemaker and regulating circadian processes such as sleep. AANAT, arylalkylamine N-acetyltransferase; AC, adenylate cyclase; AP, anterior pituitary gland; β_1, beta-adrenergic receptor; CaMK, calmodulin kinase; Camp, cyclic adenosine monophosphate; CREB, cAMP response-element-binding protein; GS, G stimulatory protein; HIOMT, hydroxyindole-O-methyltransferase; NO, nitric oxide; PKA, protein kinase A; PP, posterior pituitary.

Melatonin

Melatonin is synthesized from tryptophan in pinealocytes and it helps regulate other hormones and maintains the body's circadian rhythm through its receptors. A melatonin receptor is a G protein-coupled receptor (GPCR) which binds melatonin. Two types G-proteins were observed (Gi (Inhibitory- Activates K

channels, inhibits adenylyl cyclase) and Go (Inhibits Ca^{2+} channels)). G proteins activate adenylate cyclase which in turn activates second messenger molecules for regulation of various physiological functions (Reppert, 1997). Three types of melatonin receptor have been identified. The MT_1 (or Mel_{1A} or MTNR1A) and MT_2 (or Mel_{1B} or MTNR1B) receptor subtypes are present in humans and other mammals (Reppert *et al.,* 1996), while an additional melatonin receptor subtype MT_3 (or Mel_{1C} or MTNR1C) has been identified in amphibians and birds (Sudgen *et al.,* 2004). In mammals, melatonin receptors are found in the brain and some peripheral organs. However, there is considerable variation in the density and location of the expression of melatonin receptors between species (Morgan *et al.,* 1994). The MT_1 subtype is present in the pars tuberalis of the pituitary gland and the suprachiasmatic nuclei of the hypothalamus. The MT_2 subtype is mainly present in the retina. The MT_3 subtype of many lower vertebrates is expressed in various brain areas (Sudgen *et al.,* 2004) (Table 1).

Table 1. Melatonin receptor subtypes

Receptor subtype	Transduction mechanism	Localization	Function
MT_1	**↓AC (Gi/o)**	pars tuberalis of the pituitary gland and the suprachiasmatic nuclei, hypothalamus, hypothalamus, cerebellum and cerebral cortex	acute inhibition of SCN firing and circadian regulation.
MT_2	**↓AC (Gi/o)**	mainly present in the retina	phase shifting effect of melatonin on circadian rhythms
MT_3	**-**	expressed in various brain areas, peripheral tissues, melanoma cells (hamster)	-------

AC, Adenylate cyclase; Gi, inhibits adenylyl cyclase; Go, Inhibits Ca^{2+} channels (Dubocovich *et al.,* 2003; Sudgen *et al.,* 2004).

Role in regulation of SCN:

The circadian release of the hormone melatonin is regulated by the SCN, which feeds back into the nucleus to modulate sleep and circadian phase through activation of the MT_1 and MT_2 melatonin receptors (Dubocovich *et al.,* 2003; Reppert *et al.,* 1996). Two G-protein coupled melatonin receptors, the MT_1 and

MT_2, inhibit neuronal activity (Liu *et al.,* 1997) and phase shift circadian firing rhythms in the SCN respectively (Hunt *et al.,* 2001; Dubocovich *et al.,* 2005; McArthur *et al.,* 1997). Recent reports have explained possible interactions between the two types of receptors as well as the role of receptors in the mammalian SCN by desensitization and internalization mechanisms (Ferguson *et al.,* 1998; Gerdin *et al.,* 2004a, b)

Therapeutic properties of melatonin:

Melatonin is a multitasking molecule used for the treatment of various diseases and disorders such as cancer, immune disorders, cardiovascular diseases, depression, seasonal affective disorder (SAD), circadian rhythm sleep disorders and sexual dysfunction.

Melatonin acts as a free radical scavenger and potent antioxidant. Melatonin as well as its metabolites (cyclic 3-hydroxymelatonin, AFMK and AMK) were highly effective in scavenging for variety of toxic radicals such as $ONOO^-$ (Peroxynitrite), $O_2^{\bullet-}$(Superoxide anion), H_2O_2 (Hydrogen peroxide), $^1O_2$ (Singlet oxygen), $NO^\bullet$ (Nitric oxide), $LOO^\bullet$ (Lipid peroxyl) and HClO (Hypochlorous acid). It was proved that melatonin and its metabolites were able to protect cells from oxidative damage due to their combined actions in detoxifying free radicals and highly efficient in reducing oxidative damage to sub cellular organelles and cells with series of melatonin's antioxidative cascade (Reiter *et al*., 2010). Oxygen (O_2) is the precursor for generating various free radicals in mitochondrial electron transport chain (ETC). Melatonin is found to be successful in reducing cellular damage and death by detoxifying the free radical generated in ETC from O_2 (Reiter *et al*., 2009) (Fig. 6). Decreased endogenous melatonin levels in ageing may contributes to free radical-mediated brain ageing (Reiter *et al*., 2000).

Melatonin has an anti-apoptotic and reduces the incidence of neurodegenerative diseases or neuronal death via apoptosis in CNS. Acute application of melatonin has been highly effective in preventing the oxidative damage and cell death in the brain and spinal cord in ischemia (Cervantes *et al*., 2008; Reiter *et al*., 2005) or trauma (Esposito *et al*., 2009; Samantaray *et al*., 2009). The chronic application of melatonin has been beneficial in neurodegenerative conditions (Pappolla *et al*., 2000; Reiter *et al*., 2004; Wang, 2009) and brain aging (Carretero *et al*., 2009; Reiter *et al.,* 1999).

Melatonin has been shown to reduce tissue damage in rats due to ischemia in both the brain (Lee *et al.*, 2007) and the heart (Dominguez-Rodriguez et al., 2006). Melatonin also inhibits the aggregation of the amyloid beta protein into neurotoxic micro aggregates which seem to underlie the neurotoxicity of this protein, causing death of neurons and formation of neurofibrillary tangles in Alzheimer's disease (Pappolla *et al.*, 1997). Studies in rats suggest that melatonin may be effective for treating Alzheimer's disease (Wang *et al.*, 2005). Some workers found that melatonin supplementation prevent the depression associated with the menopause (Bellipanni *et al.*, 2005). Several clinical studies indicate that supplementation with melatonin is effective in prevention for migraines and cluster headaches (Dodick and Capobianco, 2001; Gagnier, 2001). Melatonin has been shown to be effective in treating of seasonal affective disorder (Nathan *et al.*, 1999) and is being considered for bipolar and other disorders where circadian disturbances are involved (Lewy *et al.*, 1985; Whalley *et al.*, 1991; Bhattacharjee and Yudhijit, 2007).

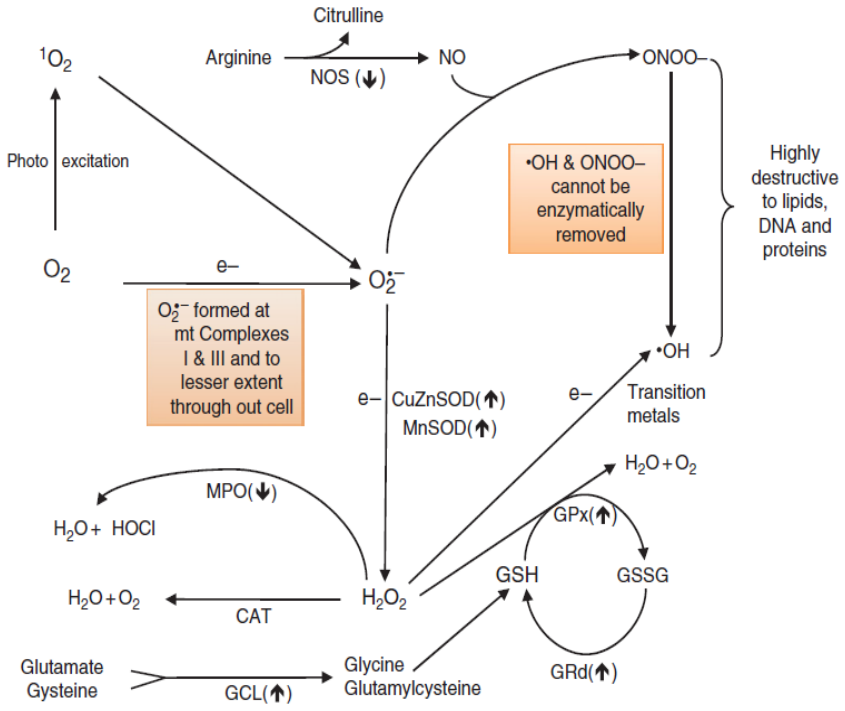


Fig. 6. Antioxidant activity of melatonin (Reiter *et al.*, 2010). Reduction of oxygen (O2) generates free radicals. •OH, hydroxyl radical; $ONOO^-$, the peroxynitrite anion; NOS, nitric oxide synthase; CAT, catalase; CuZnSOD, copper/zinc superoxide dismutase; MnSOD, magnesium superoxide dismutase; GCL, glutamylcysteine ligase; GPx, glutathione peroxidase; GRd, glutathione reductase; MPO, myeloperoxidase; $^1O_2$, singlet oxygen; $O_2^{\bullet-}$, superoxide anion radical; H_2O_2, hydrogen peroxide, HOCl, hypochlorous acid; GSH, reduced glutathione; GSSG, oxidized glutathione; e^-, electron.

Clinical trials found that melatonin was effective in treating the cancer patients (Schernhammer *et al.,* 2004; Mills *et al.,* 2005; Navara and Nelson, 2007). Melatonin acts as an oncostatic agent to inhibit tumour progression (Blask *et al*., 2005; Korkmaz *et al*., 2009; Vijayalaxmi *et al*., 2002). Melatonin presence in the gallbladder has many protective properties, such as converting cholesterol to bile, preventing oxidative stress, and increasing the mobility of gallstones from the gallbladder (Tan *et al.,* 1999; Koppisetti *et al.,* 2008). In animal models, external administration of melatonin has been shown effective in Amyotrophic lateral sclerosis (ALS) possibly due to its antioxidant effects (Weishaupt *et al.,* 2006). Many studies show that chronic melatonin supplementation in drinking water reduces body weight and abdominal fat in experimental animals, especially in the middle-aged rats (Wolden-Hanson *et al.,* 2000; Tan *et al.*, 2010). Reports found that melatonin has some effects for sexual development and seasonal timing of reproduction in higher organisms (Kinson, 1976). Exogenous melatonin has also been used in clinical trial to treat Periodic limb movement disorder (Kunz and Bes, 2001). Studies have found that the use of melatonin can help entrain the circadian clock to environmental cycles and have beneficial effects for the treatment of certain forms of insomnia (Turek and Gillette, 2004; Buscemi *et al.,* 2006; Wade *et al.,* 2007). In another study, researchers concluded that melatonin is effective in treating delayed sleep phase syndrome (Buscemi *et al.,* 2005).

Serotonin

Serotonin distribution:

Serotonin (5-hydroxytryptamine, 5-HT) is a neurotransmitter in the brain that has an enormous influence over many brain functions. Serotonin is found in three main areas of the body such as the intestinal wall, large constricted blood vessels and the central nervous system. The most widely studied effects have been observed on the central nervous system. The functions of serotonin are numerous and appear to involve control of appetite, sleep, memory and learning, temperature regulation, mood, behavior (including sexual and hallucinogenic behavior), cardiovascular function, muscle contraction, endocrine regulation, and depression (Berger *et al.,* 2009).

The activity of serotonin arises in the brain stem from clusters of neurons known as the raphe nucleus. From the brain, serotonin neurons extend to virtually all parts of the central nervous system making the branching of the serotonin network the most extensive neurochemical system in the brain (Fig. 7). The importance of this network becomes apparent when considering each serotonin neuron exerts an influence over as many as 500,000 target neurons. Due to the widespread distribution of serotonin in the nervous system, this neurotransmitter can be linked to many types of behavior (Berger *et al.*, 2009).

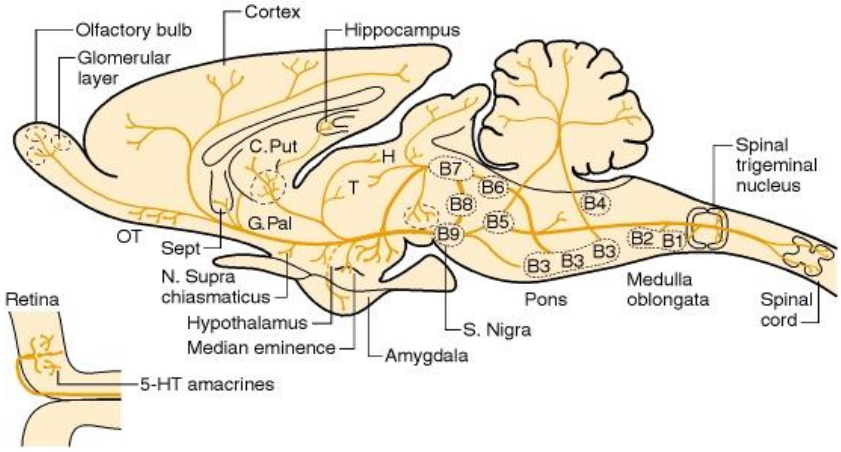


Fig. 7. Distribution of the serotonergic cell body groups in a sagittal section of the rat central nervous system and their major projections. *OT*, olfactory tuberculum; *Sept*, septum; *C. Put*, nucleus caudate-putamen; *G. Pal*, globus pallidus; *T*, thalamus; *H*, habenula; *S. Nigra*, substantia nigra (Siegel *et al*., 1999).

Serotonin Receptors:

Serotonin (5-hydroxytryptamine receptors or 5-HT) receptors are classified into 7 types: 5-HT_1, 5-HT_2, 5-HT_3, 5-HT_4, 5-HT_5, 5-HT_6 and 5-HT_7. Each type can have subtypes A, B and so on. The serotonin receptors are a group of G protein (guanine nucleotide triphosphate (GTP)-binding protein)-coupled receptors (GPCRs) except 5-HT_3, which is ligand-gated ion channels (LGICs) found in the central and peripheral nervous systems (Hoyer *et al*., 1994, Frazer and Hensler, 1999). Several types of G-proteins were found such as Gs (Stimulatory- Activates Ca^{2+} channels, activates adenylyl cyclase), Gi (Inhibitory- Activates K channels, inhibits adenylyl cyclase), Gq (Activates phospholipase C), Go (Inhibits Ca^{2+} channels), $G_{12/13}$ (Diverse ion transporter interactions). These G proteins are composed of α, β, and γ subunits. Transduction mechanism of G-protein is

multistep pathway and linked to activation or inhibition of adenylate cyclase (AC) and activation of phospholipase C (PLC). Both AC and PLC activates cAMP, second messenger molecule. This mediates various biochemical signals in order to regulate both excitatory and inhibitory neurotransmission. The serotonin receptors are activated by the neurotransmitter serotonin, which acts as their natural ligand (Nichols and Nichols, 2008) (Table 2). Like other transmitters, reuptake of serotonin released into the synaptic cleft is mainly reuptake by the presynaptic terminations by active transport with a specific carrier (Nicholas and Trevor, 1999).

The serotonin receptors modulate the release of many neurotransmitters, including glutamate, GABA, dopamine, epinephrine or norepinephrine and acetylcholine as well as many hormones including oxytocin, prolactin, vasopressin, cortisol, corticotrophin and substance P etc. The serotonin receptors influence various biological and neurological processes such as aggression, anxiety, appetite, cognition, learning, memory, mood, nausea, sleep, and thermoregulation (Fig. 8). The serotonin receptors are the target of a variety of pharmaceutical and illicit drugs including many antidepressants, antipsychotics, anorectics, antiemetics, gastroprokinetic agents, antimigraine agents, hallucinogens and entactogens (Nichols and Nichols, 2008).

Role of Serotonin receptors in Circadian rhythms:

A number of reports now implicate a role for the 5-HT_7 receptor in the regulation of circadian rhythms. Thus, 5-HT has been known to induce phase shifts in behavioural circadian rhythms (Edgar *et al.,* 1993) and neuronal activity in the SCN (Medanic and Gillette, 1992; Prosser *et al.,* 1993), the likely site of the mammalian circadian clock (Turek, 1985). The 5-HT receptor mediating this response was generally considered to be the 5-HT_{1A} receptor largely based on the ability of 8-hydroxy-*N*, *N*-dipropyl-2-aminotetralin (8-OHDPAT) to mimic the response to 5-HT (Prosser *et al.,* 1993; Cutrera *et al.,* 1996). However, 8-OHDPAT is now recognised additionally to be a 5-HT_7 receptor agonist, though at relatively high concentrations (Plassat *et al.,* 1993; Tsou *et al.,* 1994; Nelson *et al.,* 1995). 5-HT_7 receptor mRNA is expressed by cells in the SCN (Stowe and

Barnes, 1998). Treatments which either promote the levels of cAMP or mimic the effects of cAMP reproduce 5-HT's ability to induce a phase shift in neuronal activity (Prosser and Gillette, 1989; Prosser *et al.*, 1993). Furthermore, inhibitors (blockers of enzymes) and ion channels which are activated by cAMP prevent the 5-HT receptor agonist-induced response (Prosser *et al.*, 1993). In rats, mRNA for the 5-HT_{5A} receptor was seen at high levels in the SCN (Oliver *et al.*, 2000).

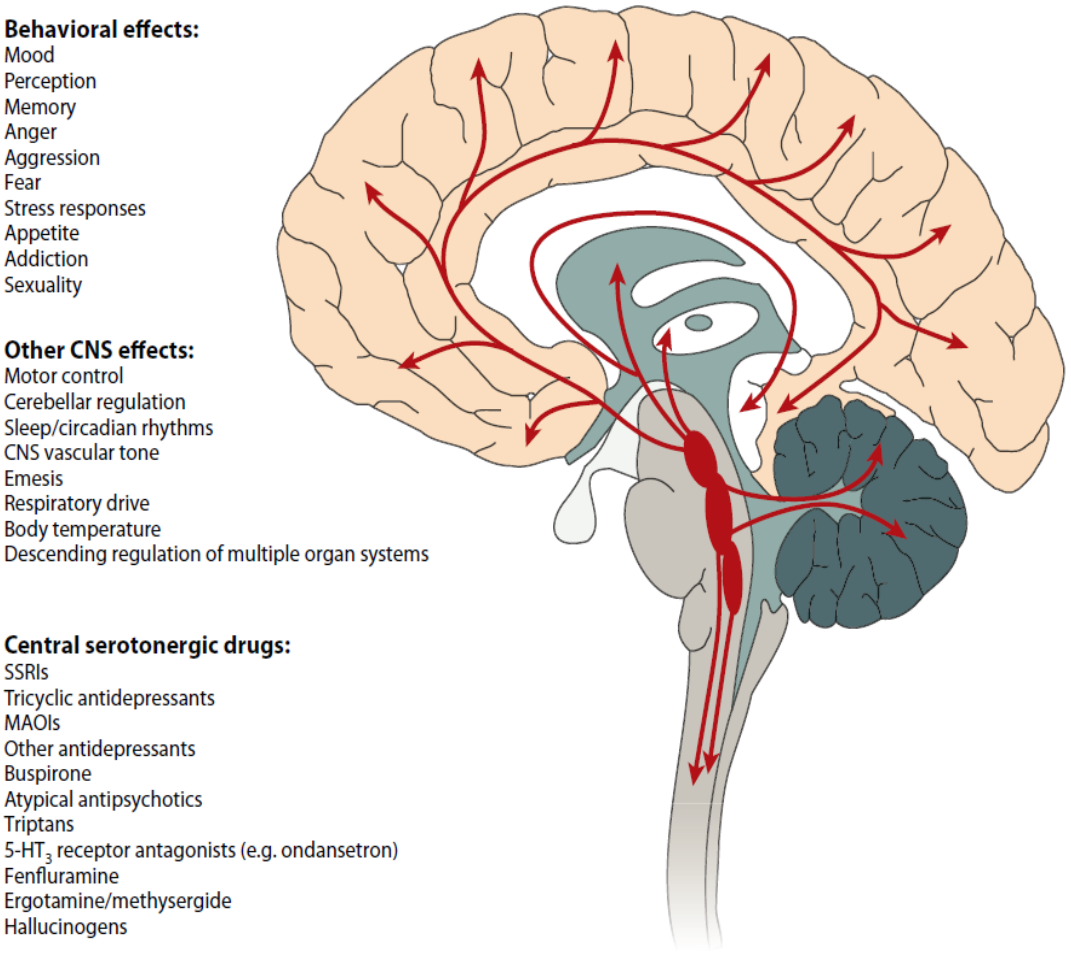


Fig. 8. Physiological effects of serotonin in central as well as peripheral regions (Berger *et al*., 2009).

Table 2. 5-HT receptor subtypes and its functions.

Receptor subtype	Transduction mechanism	Localization	Function
5-HT$_{1A}$	**↓AC (Gi/o)**	Limbic system (hippocampus, lateral septum, cortical areas), mesencephalic raphe nuclei	Hyperpolarization, modulation of Neurotransmitter release, anxiolysis, hypothermia, hyperphagia
5-HT$_{1B}$	**↓AC (Gi/o)**	Basal ganglia, striatum, amygdala, trigeminal ganglion, vascular smooth muscle	Auto receptor, locomotion, hypophagia, hypothermia, modulation of neuro-transmitter release, vasoconstriction
5-HT$_{1D}$	**↓AC (Gi/o)**	Basal ganglia, hippocampus, cortex, spinal cord, vascular smooth muscle	Auto receptor, modulation of neurotransmitter release
5-HT$_{1E}$	**↓AC (Gi/o)**	Cortex, caudate putamen, claustrum, hippocampus, amygdala	Unknown
5-HT$_{1F}$	**↓AC (Gi/o)**	Hippocampus, cortex, dorsal raphe nucleus, uterus	Speculative role in visual and cognitive function
5-HT$_{2A}$	**↑ PLC**	Forebrain, caudate nucleus, nucleus accumbens, hippocampus, olfactory tubercle, vascular smooth muscle, blood platelets	Neuronal depolarization, head twitch, hyperthermia, modulation of neuro- transmitter release smooth muscle contraction, platelet activation
5-HT$_{2B}$	**↑ PLC**	Brain, stomach fundus (rat), gut, heart, kidney, lung	Contraction of the stomach fundus, anxiety
5-HT$_{2C}$	**↑ PLC**	Choroid plexus, cortex, limbic system, basal ganglia	Hypo locomotion, hypophagia, penile erection, hyperthermia, anxiety, ↓noradrenalin and dopamine release
5-HT$_{3}$	**Ion channel (Na+, K+, Ca2+)**	Dorsal vagal complex, hippocampus, amygdala, caudate, cerebral cortex, heart, intestines	Anxiety, cognition, pain, reward /withdrawal, vomiting reflex, vasodilation, intestinal tone and secretion
5-HT$_{4}$	**↑ AC (Gs)**	Cerebral cortex, limbic areas, hippocampus, colliculus, intestines	Learning and memory, visual perception, anxiety, motor coordination, arousal, smooth muscle relaxation, modulation of neurotransmitter release
5-HT$_{5A}$	**↓ AC (Gi/o)**	Amygdala, hippocampus, caudate nucleus, cerebellum, hypothalamus, thalamus, substantia nigra, spinal cord	Modulation of exploratory behavior and locomotion
5-HT$_{6}$	**↑ AC (Gs)**	Striatum, olfactory tubercles, Nucleus accumbens, hippocampus, stomach, adrenal glands	Memory and learning, modulation of Neurotransmitter release
5-HT$_{7}$	**↑ AC (Gs)**	Thalamus, hypothalamus, hippocampus, cerebral cortex, amygdala, GI and vascular smooth muscle, heart	Circadian rhythms, smooth muscle relaxation, nociception, hypotension, modulation of REM sleep, learning and memory, LH release

Adenylate cyclase (AC); Phospholipase C (PLC); G inhibitory (Gi); G stimulatory (Gs); Go, Inhibits Ca^{2+} channels (Hoyer *et al.*, 1994; Barnes and Sharp, 1999; Nichols and Nichols, 2008).

Physiological functions of Serotonin and its receptors:

The majority of 5-HT receptors are postsynaptic but receptors such as 5-HT_{1A} and 5-HT_{1B} are mainly presynaptic and modulate serotonin release. The signalling pathways to which these receptors are coupled are known but it is hardly possible to arrange clinical effects corresponding to their stimulation. Effects of serotonin on the central nervous system are complex and numerous. Serotonin is precursor for melatonin (messenger of darkness) and involved in the regulation of circadian rhythms, sleep, mood (antidepressant action), temperature, appetite (appetite suppressant effect) by various types of receptors (Berger *et al.*, 2009). LSD or lysergide, agonist of 5-HT_2 receptors have hallucinogenic properties (Czech *et al.*, 2003; Berger *et al.*, 2009). Serotonin modulates the activity of other transmitters by various types of presynaptic and postsynaptic 5-HT receptors and plays an important role in neuroadaptation (Dayan and Huys, 2009).

Serotonin has many peripheral effects to regulate various physiological actions. Serotonin administration by intracoronary route gives a vasodilation when the coronary vessels are normal and a vasoconstriction when they are damaged and these actions are mediated by 5-HT_2 (Van Nueten *et al*., 1984). Serotonin has a positive chronotropic action by 5-HT_4 receptor stimulation and could take part in the genesis of certain rhythm disorders (Jordan, 2005). According to experimental conditions, serotonin gives either hypotension, or hypertension (Villalón and Centurión, 2007). Serotonin increases intestinal motility by stimulation of 5-HT_4 and 5-HT_3 receptors in human beings, injected by intravenous route observed in patients with carcinoid syndrome (Camilleri, 2009). 5-HT_3 stimulation elicits nausea and vomiting, and 5-HT_3 antagonists are used to avoid vomiting induced by antineoplastic treatments (George *et al*., 2009). Serotonin has an ulcerative action and induces gastric ulcerations (Jonderko and Neumann, 1982; Weiner, 1996). Serotonin has a bronchoconstrictive action; a serotonin aerosol induces dyspnea (Daum, 1992; Advenier, 1995). Serotonin induces contractions of the uterus (López, 2003). The biological diagnosis of carcinoid tumors can be checked by increased levels of serotonin in blood and excretion of abnormal amounts of 5-HIAA in urine

(Wilander *et al.*, 1989). Serotonin plays an important role in determining migraine (Silberstein, 1992). Serotonin released from platelets seems to worsen the myocardial ischemia by vaso-constriction (Fu and Longhurst, 2009).

Serotonin metabolism:

Serotonin (5-HT), an important neurotransmitter involved in the regulation of the circadian clock (Mistleberger *et al.*, 2000) synthesized from essential amino acid L-tryptophan (TRP) which then is converted to 5-hydroxytryptophan (5-HTP) by the enzyme tryptophan hydroxylase. 5-HTP is then converted to 5-HT by enzyme L- aromatic amino acid decarboxylase. Serotonin has two important pathways: (i) 5-HT is converted to N-acetyl serotonin (NAS) by N-acetylase enzyme which then is converted to melatonin (MEL) by hydroxy indole O-methyl transferase (HIOMT) enzyme. 5-HT is converted to 5-hydroxyindoleacetaldehyde by mono amine oxidase (MAO) which then is converted to 5- hydroxy indole acetic acid (5-HIAA) and 5-hydroxy tryptophol (5-HTOH) by aldehyde dehydrogenase and aldehyde reductase respectively. 5-HIAA is converted to 5-methoxy indole acetic acid (5-MIAA) and 5-HTOH is converted to 5-methoxy indole acetic acid (5- MTOH) by HIOMT enzyme; (ii) TRP is converted to N-acetyl tryptamine (NAT) by decarboxylase and acetylase enzymes respectively (Garattini and Valzelli, 1965) (Fig. 9).

The levels of serotonin related metabolites are important in regulation of various physiological, endocrine and behavioral processes. Changes in the levels of these metabolites lead to several disorders. Hyperserotonemia results in a condition known as serotonin syndrome and cardiac fibrosis, both are being toxic as well as fatal (Berger *et al.*, 2009). Elevated 5-HT is one of the most common biological findings in autism (Burgess, 2006). Low levels of 5-HT cause memory related disorders, mood disorders etc. (Berger *et al.*, 2009). 5-HIAA is the main metabolite of serotonin metabolism and excess amounts were measured in the urine to test carcinoid tumors (Mansencal *et al.*, 2010). Low levels of 5-HIAA as well as 5-HT in the cerebrospinal fluid have been associated with aggressive behavior and suicidal tendencies (Jokinen *et al.*, 2009).

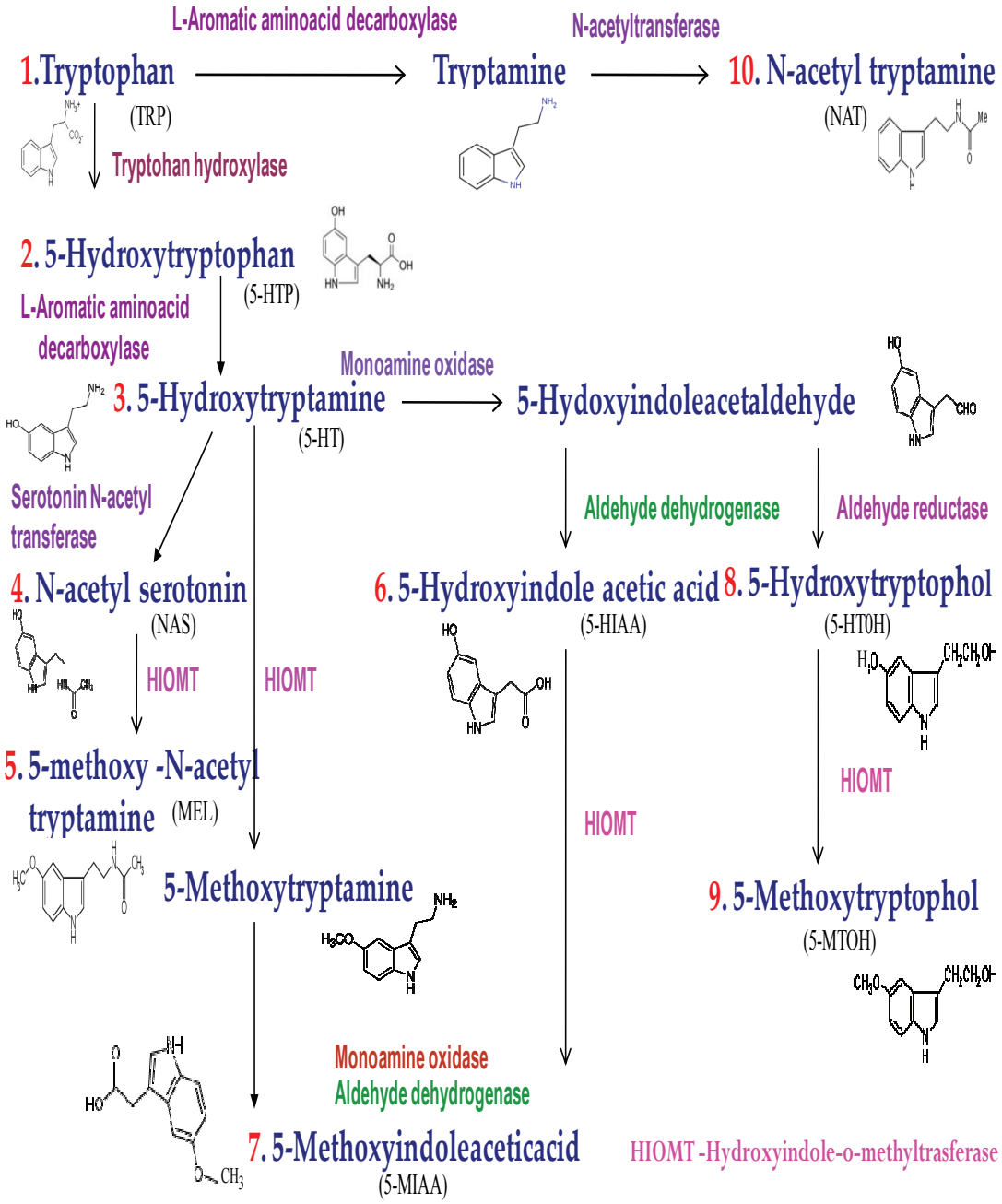


Fig. 9. Synthesis of serotonin and its related compounds (Garattini and Valzelli, 1965). TRP, tryptophan; 5-HTP, 5-hydroxytryptophan; 5-HT,5-hydroxytryptamine; NAS,N-acetylseronin; 5-MAT or MEL, 5-methoxy-N- acetyltryptamine; 5-MIAA,5-methoxyindole acetic acid; 5-HIAA, 5-hydroxy indole acetic acid; 5-HTOH, 5-hydroxytryptophol; 5-MTOH, 5-methoxytryptophol; NAT, N-acetyltryptamine.

Elevated levels of 5-HTP have been reported to cause acute serotonin syndrome (Ma *et al*., 2008) and decreased levels leads to depression (Turner and

Blackwell, 2005). Studies show that reduced levels of TRP leads to depression (Ledochowski *et al*., 1998). Low MEL levels are implicated in many pathological conditions (Karasek and Winczyk, 2006). Increased NAS levels have shown anti-depressant effects (Oxenkrug, 1999) as well as antioxidant activity (Oxenkrug, 2005). The end metabolites of serotonin metabolism such as 5-MIAA, 5-MTOH, 5-HTOH are useful as biomarkers for detection in many pathological conditions (Curtius *et al*., 1975; Das *et al*., 2008). Some studies reported that these compounds also act as potent antioxidants (Ng *et al*., 2005).

We selected 10 compounds of serotonin, its precursors and metabolites (serotonin chronometabolome) to understand serotonin metabolism in various experimental conditions planned in the present study. Since change in the metabolome is the ultimate answer of an organism to genetic alterations, disease, or environmental influences (Weckwerth, 2003). As metabolome represents the collection of all metabolites in a biological sample, which are the end products of its gene expression. The metabolic profiling can give an instantaneous snapshot of serotonin metabolism (Fig. 10).

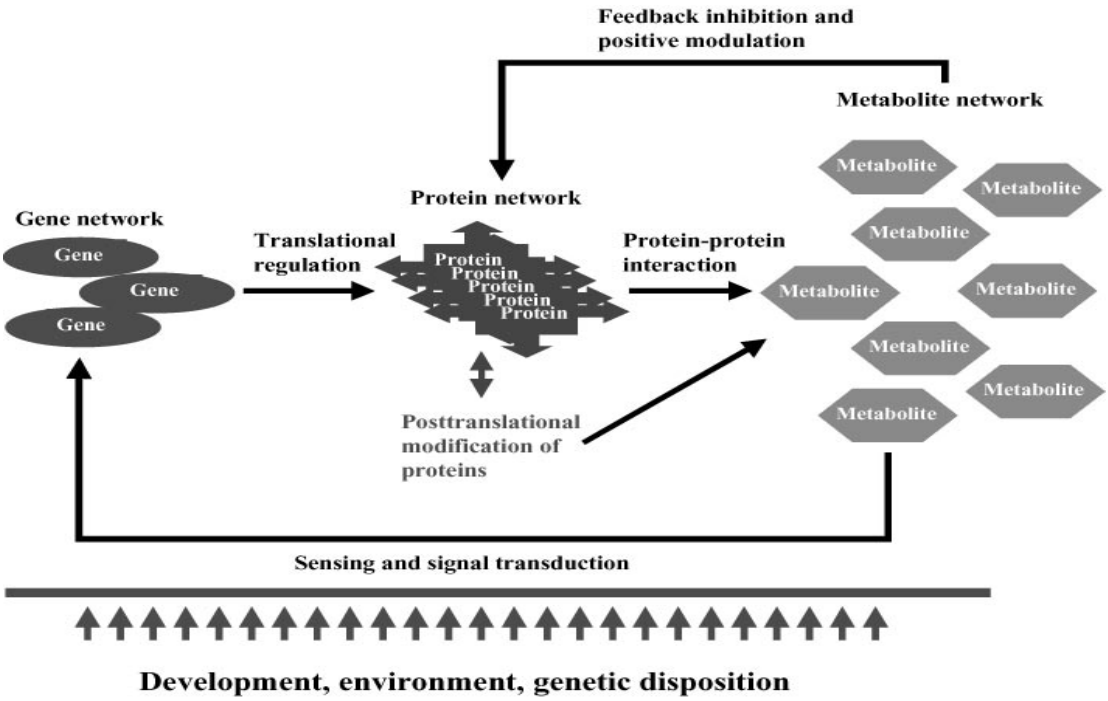


Fig. 10. Generalized pathway for metabolome analysis (Weckwerth, 2003).

Molecular Components of the Mammalian Circadian Clock:

The molecular clock of SCN is composed of multiple, single-cell circadian oscillators, which synchronized and generate coordinated circadian outputs that regulate overt rhythms by clock genes. Major clock genes such as *per* (Period), *cry* (Cryptochrome), *bmal1*(Brain and Muscle ARNT (Aryl hydrocarbon Receptor Nuclear Translocator) like protein 1), *clock* (Circadian Locomotor Output Cycles Kaput), *ror* (Retinoic acid orphan related), *rev-erbα* (Reverse-erythroblastomas *α*-virus) and *ck1ε* (Casein kinase 1 epsilon) forms transcriptional/ translational feedback loops, resulting in oscillations of expression levels of clock as well as clock-controlled genes (CCG) with ~24h cycles (Ko and Takahashi, 2006) (Fig. 11).

The transcriptions of these genes are driven by two bHLH-PAS transcription factors and activate upon binding to E-box promoter elements. RORs bind to the RRE of *bmal1* and gets activated for the formation of BMAL protein. CLOCK and BMAL proteins form the dimers. These dimers have the capacity to bind to E- box elements of genes such as *per, cry, ror, rev-erbα* and *ccg* respectively. In the cell's nucleus, the genes are transcribed to mRNAs, which is transported into the cytoplasm. These mRNAs are then translated to their protein products encoded by the circadian clock genes. In 24-hour cycle, BMAL1 and CLOCK proteins induce increased production of PER and CRY proteins. As PERs and CRYs accumulate, they inhibit their own synthesis and the protein levels decline. CK1ε protein regulates CLOCK protein levels by destabilizing PER protein. The daily light-dark cycle ultimately impinges on the control of two clock genes that reset the core clock mechanism in the SCN. Clock-controlled genes are also generated by the central clock mechanism, but their protein products transduce downstream effects. Peripheral oscillators are controlled by the SCN and provide local control of overt rhythm expression (Ko and Takahashi, 2006).

Peripheral clocks:

Studies reported that SCN, a unique circadian pacemaker orchestrates all overt rhythms in physiology and behavior. SCN is considered as a master pacemaker instead of being the only oscillator (Schibler and Sassone-Corsi, 2002). It seems that SCN and peripheral clocks are similar but not identical and SCN, act as a

master pacemaker that is capable of generating its own rhythm and maintain it independently. Peripheral oscillators need external cues such as from the SCN that acts as a reference clock to synchronize and fine-tune its rhythm (Balsalobre, 2002) (Fig. 12).

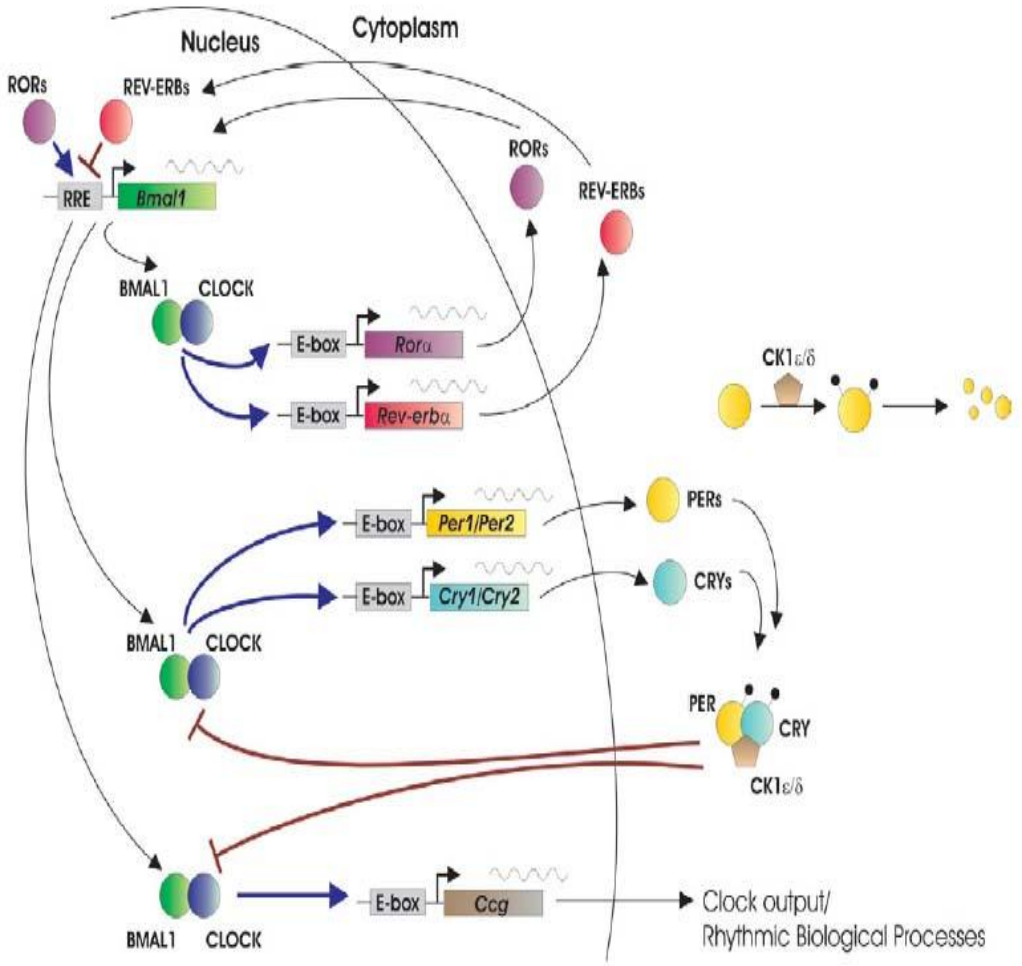


Fig. 11. Molecular mechanisms involved in regulation of clock (Ko and Takahashi, 2006).

SCN neurons in the hypothalamus controls circadian rhythmicity of peripheral tissues by CCG expression via neural and humoral signals. E-box in the promoter region of CCG can be activated by CLOCK/BMAL1 heterodimers (Balsalobre, 2002). Clock analog (MOP4) also called NPAS2 had been reported to regulate peripheral clocks and which is not found in SCN (Wang and Sehgal, 2002). MOP4 can form heterodimers with BMAL1 in vascular smooth vessel cells, which activate *mper* and *mcry* gene expression and are required for rhythmicity (Green and Menaker, 2003). Humoral factors also can entrain peripheral clocks.

These include glucocorticord (Balsalobre *et al.,* 2000), glucose (Albrecht and Eichele, 2003), serum shock (Balsalobre *et al.,* 1998), Forskolin (Yagita and Okamura, 2000), Angiotensin (Nonaka *et al.,* 2001), Noradrenaline (Akiyama *et al.,* 2003; Oishi *et al.,* 2005) etc. Behavior controlled by the SCN seems to be the most important because feeding time is the dominant Zeitgeber for peripheral clocks (Dibner *et al*., 2010).

The SCN may regulate behavioural cycles and also the time of feeding to impose its influence on slave oscillators. Feeding may change the redox state of NAD (nicotinamide adenine dinucleotide) in cells and this regulates the E-box binding ability of the CLOCK/BMAL1 and NPAS2/BMAL1 heterodimers (Rutter *et al.,* 2001). In addition, body temperature cycles can amplify the effect of chemical's entrainment of circadian rhythm and can lead to a phase-shift of circadian oscillators in peripheral tissues (Schibler and Sassone-Corsi, 2002).

The circadian clock in the suprachiasmatic nucleus is thought to drive daily rhythms of behavior by secreting factors that act locally within the hypothalamus The circadian pacemaker system of mammals has long been assumed to control various physiological functions such as locomotor activity or sleep wake cycles etc. Locomotor activity is more likely to be an index in circadian behavioral physiology. Little is known about the molecular mechanisms by which the SCN drives cycles of locomotor behavior. Researchers have been found that two neuropeptides- TGFα (transforming growth factor α) and PK2 (Prokineticin- 2) are necessary for regulating the locomotor output (Chang and Reppert, 2001; Herzog and Tosini, 2001; Kramer, 2001; Cheng, 2002; Morse and Sassone-Corsi, 2002; Albrecht and Eichele, 2003).

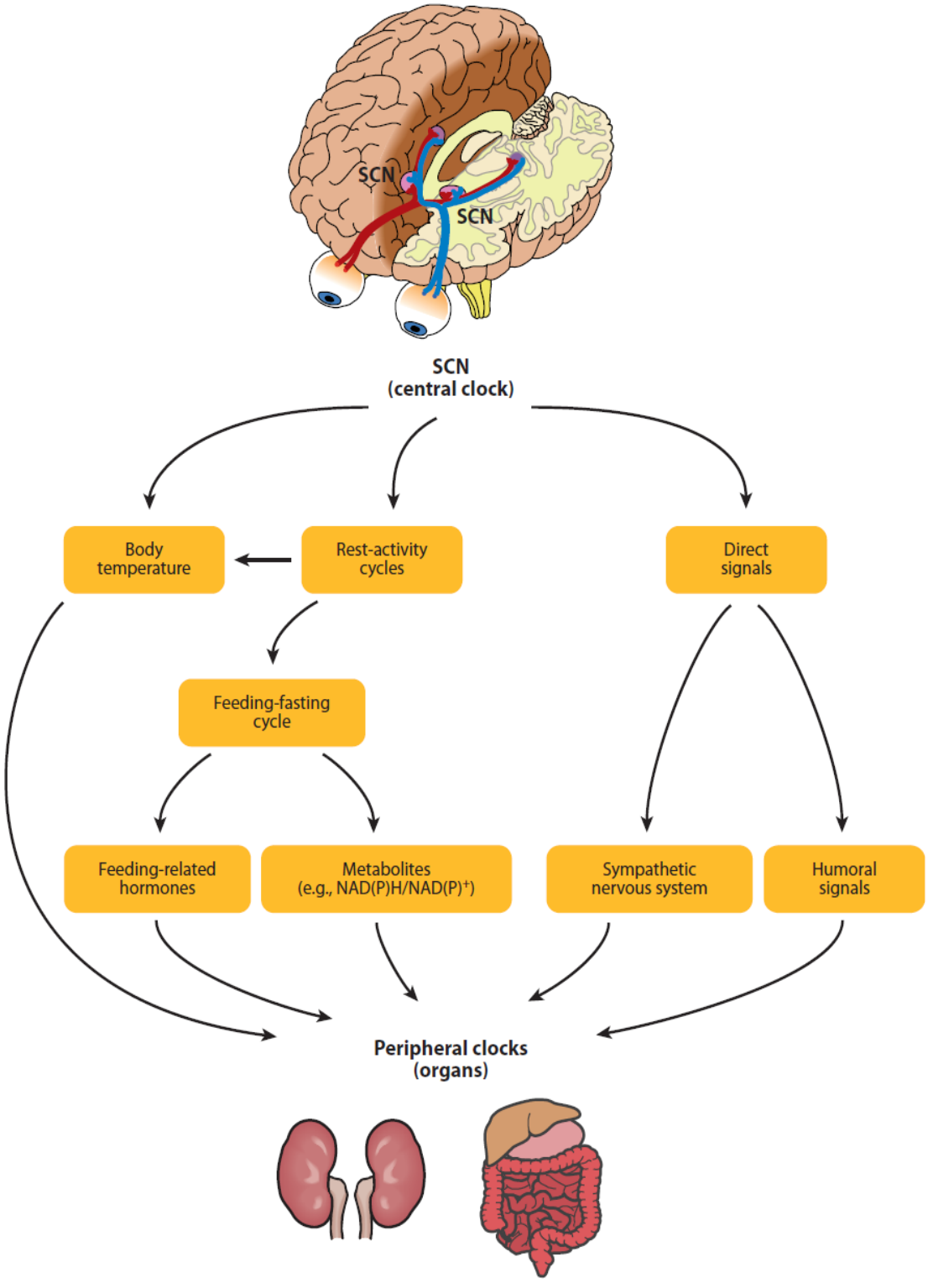


Fig. 12. Regulation Peripheral clocks by Master clock, Suprachiasmatic nucleus (Dibner *et al.*, 2010)

Circadian Rhythm Disorders

When body is out of synchronization with external cues, it leads to alteration in circadian rhythm. If it persists for a longer time, leads to circadian clock disorders. Common circadian rhythm disorders are categorized into Delayed sleep-phase syndrome (DSPS), Advanced sleep-phase syndrome (ASPS), Irregular sleep-wake rhythm or Free-running disorder, Jet-lag and Shift work disorder. Circadian rhythm disorders can be caused by many factors such as shift work, pregnancy, time zone changes, medications, changes in routine etc. (Morgenthaler *et al.,* 2007; Sack *et al.,* 2007a, b). Other Circadian Rhythm Disorders are manifested in the form of mood and sleep disorders such as Major Depression, Anxiety, Bipolar Disorders, Seasonal Affective Disorder (SAD), Insomnia, Mood Swings, Premenstrual Syndrome (PMS), Premenstrual Dysphoric Disorder (PMDD), Long or irregular menstrual cycles, Menopause Disorders, Prenatal Depression, Postpartum Depression, Attention deficit disorder or hyperactivity disorder (ADD/ADHD), Chronic Fatigue Syndrome, Fatigue, Chronic sleepiness etc. (Reid and Zee, 2009).

Treatment of Circadian Rhythm Disorders:

Circadian rhythm disorders are treated based on the type of disorder diagnosed. Therapy usually combines proper sleep hygiene techniques and external stimulus therapy such as bright light therapy or chronotherapy. Chronotherapy is a behavioral technique in which the bedtime is gradually and systematically adjusted until a desired bedtime is achieved. Bright light therapy is designed to reset a person's circadian rhythm to a desired pattern. When combined, these therapies may produce beneficial results in people with circadian rhythm disorders (Pandi-Perumal *et al*., 2008; Gooley, 2008). Exogenous melatonin with light therapy is the standard treatment for delayed sleep phase syndrome (DSPS) and non-24-hour sleep-wake syndrome (Mundey *et al.,* 2005). Melatonin can also be used as *chronobiotic* (affecting aspects of biological time structure) (Sheving *et al.,* 1979) or for treating the other circadian rhythm sleep disorders as well as jet lag or shift work. Melatonin reduces sleep onset latency to

a greater extent in people with DSPS than in people with insomnia (Buscemi *et al.*, 2004).

Aging and circadian rhythms

Aging is the progressive deterioration in behavioral, biochemical, physiological, morphological and anatomical aspects of an organism (Jagota, 2005). Alterations in circadian clock properties have been reported from our laboratory with aging (Jagota and Kalyani, 2008; Jagota and Kalyani, 2010). Oxidative stress is the important factor in aging due to free radical generation (Serra *et al.*, 2009). Melatonin deficiency may result in aging and lead to reduced antioxidant protection which may contribute to the incidence of age-related diseases (Cardinali *et al.*, 2008).

Brain aging is accompanied by a decrease in the functional capacity of neurotransmission, which is manifested by changes in storage, receptor mechanisms and loss of enzymatic activity. These alterations increase the vulnerability of the brain to the development of several physiological disturbances and neuro-psychiatric disorders closely related to age (Morgan and May, 1990). Many workers have reported a gradual deterioration of pineal functionality and morphology with age. One of the most remarkable changes is a gradual decrease in the production of the pineal hormone melatonin, which has been reported in several studies in rodents (Reiter *et al.*, 1980a, b; Pang and Tang, 1983; Tang *et al.*, 1985; Stokkan *et al.*, 1991) and the human (Iguchi *et al.*, 1982; Sack *et al.*, 1986). However, the mechanisms for the decline in pineal function with aging process and whether aging-associated body changes are secondary to pineal failure, remains to be understood. The causes of the age-associated decrease in pineal melatonin production are not clear. Aging caused decrease in mean 5-HT levels and daily 5-HT rhythmicity in SCN which could be responsible for alteration in physiological as well as behavioral functions (Jagota and Kalyani, 2010).

SCN shows alterations in peptide expression (VIP and AVP) and a reduction in the amplitude of circadian rhythms of electrical activity with aging (Watanabe *et al.*, 1995; Kawakami *et al.*, 1997). Some workers have reported that

changes at the level of the SCN affected the amplitude of daily melatonin rhythm (Humbert and Pevet, 1994), a progressive reduction in the number of pineal cells (Johnson, 1980) and a reduced activity of the enzymes involved in the formation of melatonin (Reiter *et al.,* 1981; Dax and Sugden, 1988; Stokkan *et al.*, 1991).

Serotonin is an important neurotransmitter involved in the photic as well as nonphotic regulation of circadian rhythms and is a precursor of neurohormone melatonin (Jagota and Kalyani, 2010).

Ethanol

Ethanol is mainly consumed as a beverage in the form of beer, wine, whiskey, gin, rum, brandy etc. Alcohol consumption is a critical public health issue. About 140 million people throughout the world suffer from alcohol-related disorders (WHO). Alcohol consumption has been steadily increasing in developing countries like India and developed countries (62.5 million alcohol users estimated in India).

Ethanol metabolism:

Because the body treats alcohol as a toxin or poison, elimination begins as soon as it is ingested. Approximately 2% - 10% of the alcohol is eliminated directly without being metabolized and a small amount is exhaled via the lungs while additional amounts are excreted through sweat, saliva and urine. The remaining 90% - 98% of alcohol is neutralized through metabolism (mainly oxidation) by the liver and then by excretion through the kidneys and lungs (Jones, 1998). Alcohol is metabolized in the liver, first by alcohol dehydrogenase (ADH) into acetaldehyde, which is very toxic to the body and especially to the liver, and then by acetaldehyde dehydrogenase (ALDH) into acetic acid that is finally oxidized into carbon dioxide (CO_2) and water (H_2O) (Fig. 13).The varying availability and efficiency of ADH and ALDH depends on hereditary capabilities that account the variation in people's reaction to alcohol (Bosron *et al*., 1993).

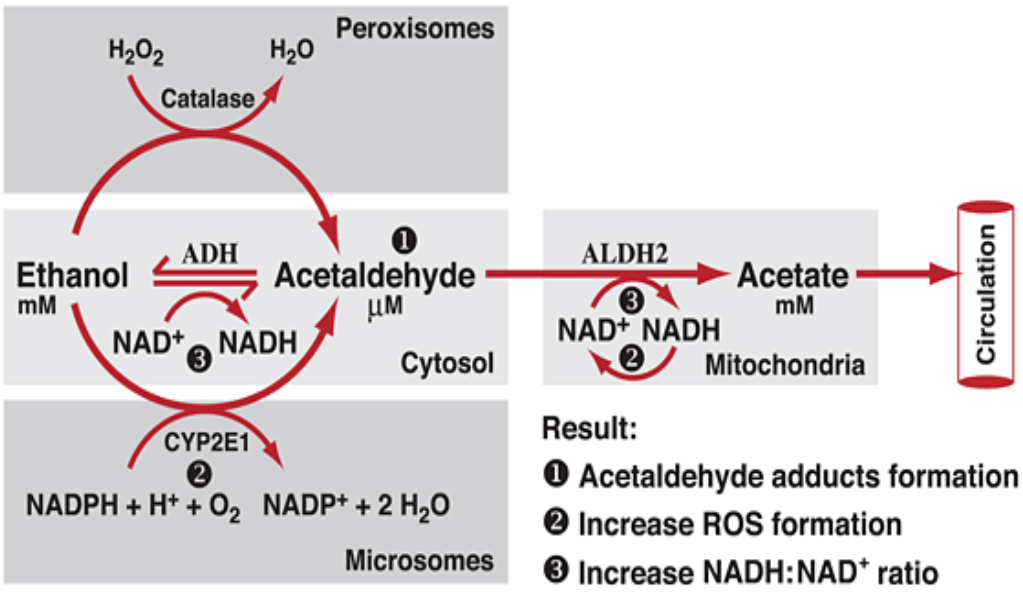


Fig. 13. Ethanol metabolism (Bosron *et al*., 1993). The enzymes alcohol dehydrogenase (ADH), cytochrome P450 2E1 (CYP2E1) and catalase contribute to oxidative metabolism of alcohol. ADH, present in cytosol converts ethanol to acetaldehyde. This reaction involves an intermediate carrier of electrons, nicotinamide adenine dinucleotide (NAD^+), which is reduced by two electrons to form NADH. ROS, reactive oxygen species.

General consequences of chronic alcohol consumption:

Alcoholism is currently listed as the third leading cause of death in our society. The majority of medical problems typically appear in the late, chronic stage of the illness. The appearances of medical complications are secondary to the primary nature of alcoholism (Fig. 14).

Physical symptoms of chronic alcoholism include hand tremor, excitability, irritability, nervousness (Aisen *et al.*, 1992; Prat *et al.*, 2009), yellow skin (jaundice) (Matull *et al.*, 2005), Parotid swelling (Maier *et al.*, 1988) and rhinophyma (Curnier and Choudhary, 2004).

Alcohol changes the motility of the intestinal tract and causes so many GI tract disturbances and diseases like dyspepsia (Keck and Higgins, 2000), Nausea & Vomiting (Dawood and Halperin, 1995), recurrent diarrhoea (Fields *et al.*, 1994),

pancreatitis (Chen *et al.*, 2006), hypoglycaemia and hyperglycaemia (Leber, 1978), GI tract bleeding (Mincis *et al.*, 1995; Zwas and Lyon, 1996), fatty liver (Cunnane, 1987), hepatitis (Ferrell, 2000), ascites (Sutton and Shields, 1995) and cirrhosis (Lefton *et al.*, 2009).

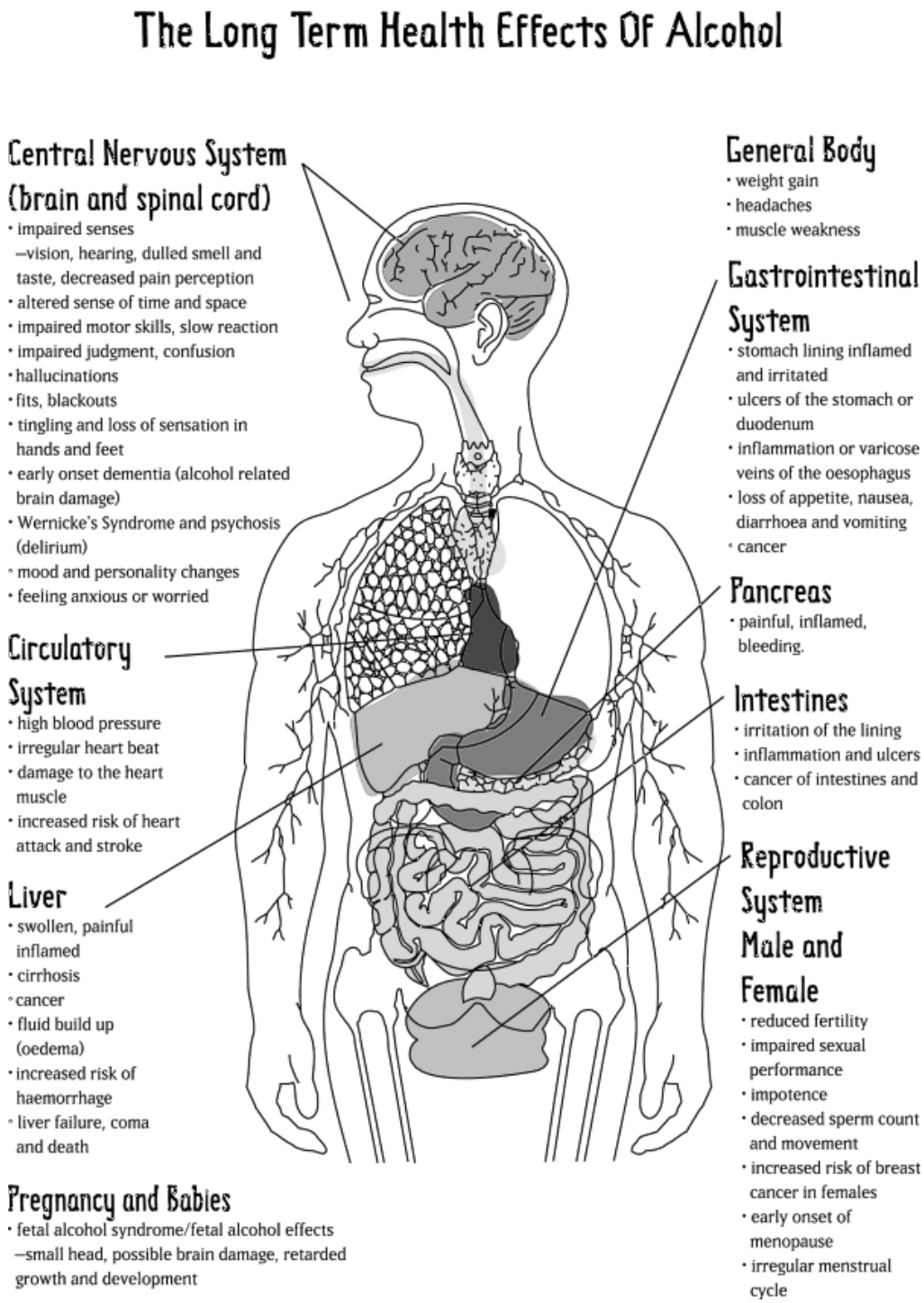


Fig. 14. Ethanol consequences in Human beings (*www.nt.gov.au)*

Generally chronic alcoholics subjected to cardiovascular diseases like cardiomyopathy (Iacovoni *et al.,* 2010), anemia (Bladé *et al.,* 2007), palpitations (Tatibouet and Tatibouet, 1982) and dilation of blood vessels (Schoppet and Maisch, 2001). Alcohol dries lung tissue and bronchi and causes them to become irritated as well as inflamed and susceptible to infection like pneumonia and chronic obstructive airways disease (COAD) (De Roux *et al.,* 2006; Olafsson and Johnson, 2004).

Alcohol is a central nervous system depressant and causes loss of memory (Finn and Hall, 2004; Lee *et al.,* 2009), seizures (Hattemer *et al.,* 2008), peripheral neuropathy (Ammendola *et al.,* 2001), ataxia (Niemann *et al.,* 2010), insomnia and hallucinations (Wolin and Mello, 1973; Brower and Perron, 2010), delirium tremens (Lutz and Batra, 2010) and Korsakoff psychosis (McKeon *et al.,* 2008).

Long-term alcohol consumption can lead to muscle atrophy, alcoholic myopathy (Fernandez-Solà *et al.,* 2007), polyuria and electrolyte imbalance (Shimizu and Yamada, 1997), nephritis (Epstein, 1997), amenorrhea (loss of menstruation) (Mello *et al.,* 1988) and impotence (McKendry *et al.,* 1983).

Complications of alcohol withdrawal:

Alcohol withdrawal (AW) is a distinctive clinical syndrome with potentially serious consequences (American Psychiatric Association 1994). Complications of AW are secondary conditions, symptoms or disorders that are caused by AW. Symptoms begin as early as 6 hours after the initial decline from peak intoxication. Initial symptoms include tremor, anxiety, insomnia, restlessness and nausea (Peyser, 1982). Particularly in mildly alcohol-dependent persons, these symptoms may comprise the entire syndrome and may subside without treatment after a few days. More serious withdrawal symptoms occur in approximately 10 percent of patients. These symptoms include a low-grade fever, rapid breathing, tremor, and profuse sweating (Kushner *et al.,* 1990). Sleep disturbances including frequent awakening, restless sleep, insomnia and night terrors are among the most common complaints in AW (Smith, 1995; Le Bon *et al.,* 1997). Depressive symptoms often are observed in patients who are intoxicated or undergoing alcohol detoxification. As many as 15 percent of alcoholics are at risk for death by suicide and recent consumption of alcohol appears to increase the danger of a

fatal outcome from self-harm (Madden, 1993). Less frequently, psychotic symptoms include delusions and hallucinations may be associated with AW (Platz *et al.,* 1995; Smith, 1995). Some researchers have hypothesized that repeated AW may predispose alcoholics to certain anxiety disorders through the process of kindling (Lepola, 1994). AW may be a contributing factor to the occurrence of alcohol-related arrhythmia (Smith, 1995). Protracted withdrawal syndrome (PWS) may develop following AW and symptoms include tremor, sleep disruption, anxiety, depressive symptoms, increased breathing rate, body temperature, blood pressure and pulse (Alling *et al.*, 1982; Schuckit *et al.,* 1991; Satel *et al.*, 1993). A majority of patients are left with an abnormal gaze, persistent ataxia and a potentially disabling memory disorder known as Korsakoff's syndrome (Charness, 1993), followed by nutritional (thiamine) deficiency known as Wernicke's syndrome (Victor *et al.,* 1989). Seizures may occur in more than 5 percent of untreated patients in acute alcohol withdrawal (Victor and Brausch, 1967; Ballenger and Post, 1978; Brown *et al.,* 1988). Another severe complication is delirium tremens, which is characterized by hallucinations, mental confusion and disorientation. The mortality rate among patients exhibiting delirium tremens is 5 to 25 percent (Wetterling *et al.,* 1994; Alvi and Gonzalez, 1995; Saitz, 1995).

Ethanol interaction with biological clock:

Interaction between alcohol and circadian rhythm has become a rapidly increasing area in Chronopharmacology and can be studied into Chronergy, Chronopharmacokinetics and Chronesthesy (Fig. 15). Chronopharmacokinetics and Chronesthesy focuses on how alcohol's effects according to the time of day at which the alcohol is administered. Chronergy is an important approach explains about simple time of day effects, to determine the influence of a drug on the individual as a whole. This can be studied into two ways: (i) Daily rhythm change the alcohol's effect on the individual. (ii) Alcohol effect's the daily rhythm. Later one is studied into acute (Period, Amplitude and Phase shift) and chronic effects (Entrainment and Disruption) (Wasielewski and Holloway, 2001). Such studies will focus on understanding the complex functional interactions between the organism's circadian rhythm and help in designing medications and behavioral interventions for treating alcohol abuse and dependence.

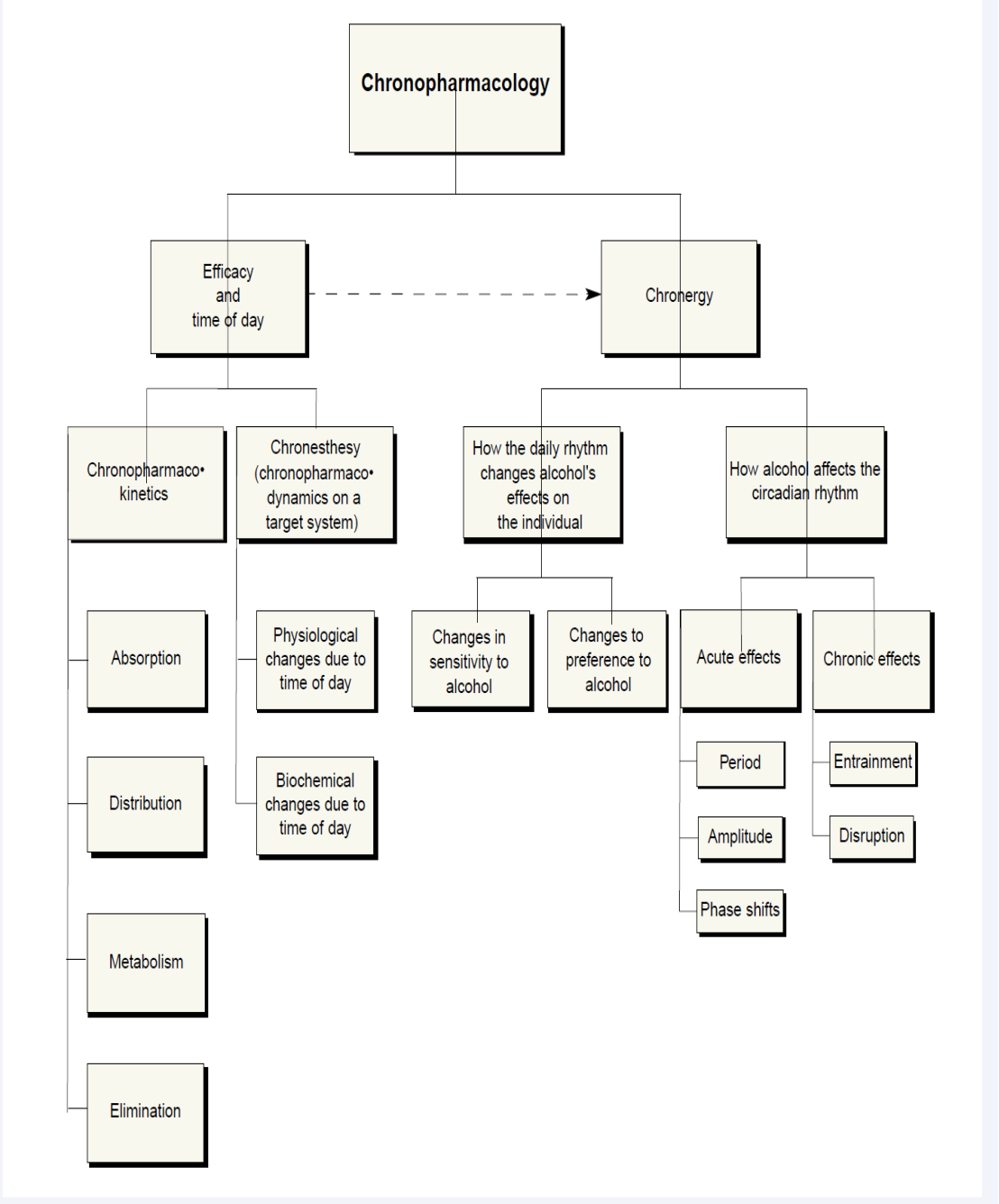


Fig. 15. The field of Chronopharmacology relates to alcohol (Wasielewski and Holloway, 2001).

Role of Serotonin in alcoholism:

Serotonin's actions have been linked to alcohol's effects on the brain and to alcohol abuse. Serotonin, along with other neurotransmitters also may contribute to alcohol's intoxicating and rewarding effects. Abnormalities in the brain's serotonin system appear to play an important role in the brain processes underlying alcohol abuse (Lovinger, 1999). Serotonin released by the signal-

emitting neuron subtly alters the function of the signal-receiving neurons in a process called neuromodulation (Nevo and Hamon, 1995). The binding of serotonin to its receptor initiates a series of biochemical events that converts the extracellular, chemical signal into an intracellular signal in the recipient cell. These changes can result either in the inhibition or the excitation of the signal-receiving neuron, depending on the cell affected. Through these mechanisms, serotonin can influence mood states, thinking patterns and behaviors (Raymond *et al.,* 2001). Serotonin's actions at the synapses normally are tightly regulated by proteins called serotonin transporters, which remove the neurotransmitter from synaptic cleft after a short period of time by transporting it back into the signal-emitting cell. Any interference with serotonin transporter function extends or diminishes the cell's exposure to serotonin, thereby disrupting the exquisite timing of nerve signals within the brain, leads to psychological problems or mental illness (Heinz *et al.,* 1998). Serotonin and its breakdown products generated after neurotransmitter has been removed from the synapse (i.e., serotonin metabolites). The concentrations of these metabolites provide an indirect measure of changes. Ethanol interacts with serotonergic synaptic transmission in the brain in several ways. Even single-episode alcohol exposure alters various aspects of serotonin's synaptic functions (LeMarquand, 1994). Alcohol interferes with the function of serotonin receptors such as 5-HT_{1A}, 5-HT_{1B}, 5-HT_2 and 5-HT_3 receptors (Overstreet *et al.,* 1994). The exact mechanisms underlying the changes in serotonin-metabolite levels are still unknown. Some of the serotonin mediated neuronal responses to alcohol may arise from interactions between serotonin and other neurotransmitters such as GABA and dopamine. Alcohol effects on serotonin may alter the activity of GABAergic neurons, which may disrupt cognition and possibly contribute to alcohol induced memory loss, impaired judgment and produce sedative effects (De Witte, 1996). Serotonin, through its interaction with the dopaminergic system may play a pivotal role in producing alcohol's rewarding effects (Bowirrat, 2005).

Pharmacotherapy of alcoholism:

Alcoholism is a serious public health problem that often results in medical, social and economic consequences throughout the world (Hunt, 1993; Rice,

1993). So the development of effective anti-alcoholism medicines is necessary. Despite great progress made in the past two decades, the development of low-toxicity and high efficiency medicines remains a challenging task for alcohol researchers. In western countries, some of the drugs like Disulfiram, Naltrexone and Acamprosate are administered to alcoholic patients to suppress withdrawal symptoms and decrease appetite for drinking. However, it is known that these chemical drugs lead to abuse, psychological dependence and adverse behavioral effects (Poulsen, 1992; Dupuy, 1995; Mann, 1996; Jones *et al.,* 2000; O'Malley *et al.,* 2000; Oncken *et al.,* 2001; Kiefer and Wiedemann, 2004). Therefore, there is need for safer medications and treatments to prevent alcoholic disorders.

Use of herbal drugs in alcohol Addiction:

Many traditional medicines and natural products obtained from medicinal plants like *Pueraria lobota, Tabernanthe iboga, Panax gingsing, Salvia miltiorrhiza and Hypericum perforatum* have been known to have preventive and therapeutic effects for alcoholism (Carai *et al.,* 2000). Recent experimental evidence suggests that novel pharmacological approaches for the treatment of alcoholism might come from natural substances. In our laboratory, we focused to check the effect of Turmeric and its active constituent, Curcumin on alcohol induced circadian rhythms.

Turmeric

Turmeric is widely consumed in India for a variety of uses such as dietary spice, medicine, textile, pharmaceutical industries and ceremonies in Hindu religion (Srimal, and Dhawan, 1973; Ammon and Wahl, 1991). Current traditional Indian medicine uses it for biliary disorders, anorexia, cough, diabetic, wounds, hepatic disorders, rheumatism and sinusitis (Jain and DeFilipps, 1991). The old Hindu texts have described it as an aromatic stimulant and carminative (Nadkarni, 1954). In Western countries, Curcumin is used as a coloring agent in cheese, spices, mustard, cereals, pickles, potato flakes, soups, icecreams, and yogurts.

Turmeric contains protein (6.3%), fat (5.1%), minerals (3.5%), carbohydrates (69.4%) and moisture (13.1%). The essential oil (5.8%) obtained by steam

distillation of rhizomes has *a*-phellandrene (1%), sabinene (0.6%), cineol (1%), borneol (0.5%), zingiberone (25%) and sesquiterpines (53%). Curcuminoid fraction (diferuloylmethane) (3–4%) is responsible for the yellow colour in turmeric and it comprises curcumin (94%), demethoxycurcumin (6%) and bis-demethoxycurcumin (0.3%). Curcumin is highly active and principle ingredient in curcuminoids (Vopel *et al.,* 1990; Ruby *et al.,* 1995) (Fig. 16).

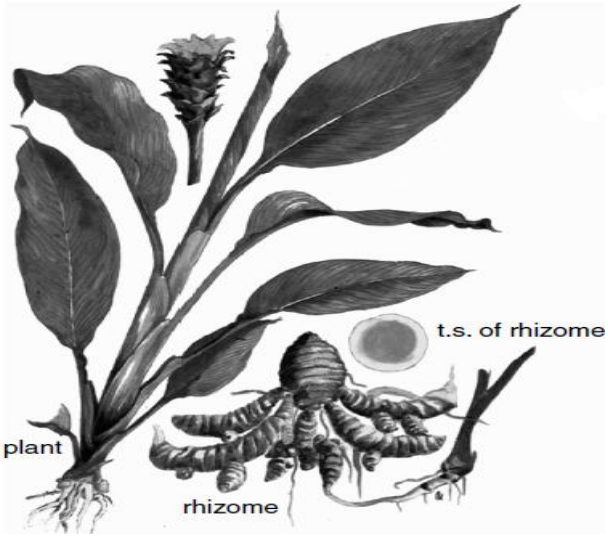


Fig. 16. *Curcuma longa* and its rhizome

Beneficial properties:

Numerous studies have found that turmeric extract of rhizome have many beneficial properties such as antioxidant (Shalini and Srinivas, 1987), antibacterial (Sankaranarayanan and Jolly, 1993), anticarcinogenic (Azuine and Bhide, 1994), anticoagulant (Kosuge *et al.,* 1984), antifungal (Naovi *et al.,* 1991), antiasthamatic (Jain *et al.,* 1979), anticonvulsant (Dhar *et al.,* 1968), antiamoebic (Dhar *et al.,* 1968), antiodema (Kosuge *et al.,* 1984), anti-inflammatory (Gupta *et al.,* 1972), anti-hepatotoxic (Kiso *et al.,* 1983), anti-ischemic effect (Arora *et al.,* 1990), antihyperlipemic (Dixit *et al.,* 1988), antimutagenic (Chang *et al.,* 1989), CNS depressant (Dhar *et al.,* 1968), antimycobacterial (Grange and Davey, 1990), antinematodal (Kiuchi *et al.,* 1989), antitumor (Itokawa *et al.,* 1985), antiulcer (Rafatullah *et al.,* 1990), treatment of gastro-intestinal disorders (Thamlikitkul *et al.,* 1989), antiviral (Cai *et al.,* 1988), anticomplement (Kinoshita *et al.,* 1986), abortifacient effect (Li, 1965), immunostimulant

(Kinoshita *et al.,* 1986), immunosuppressant (Godhwani *et al.,* 1980), hypoglycemic (Jain and Sharma, 1967), insect repellent (Dixit *et al.,* 1965), ascaricidal (Bannerjee and Nigam, 1978), insecticide (Heal *et al.,* 1950), adrenal hypertrophy effect (Liu, 1989), alkaline phosphatase inhibition (Soni *et al.,* 1992) and alkaline phosphatase stimulation (Kumazawa *et al.,* 1991) etc.

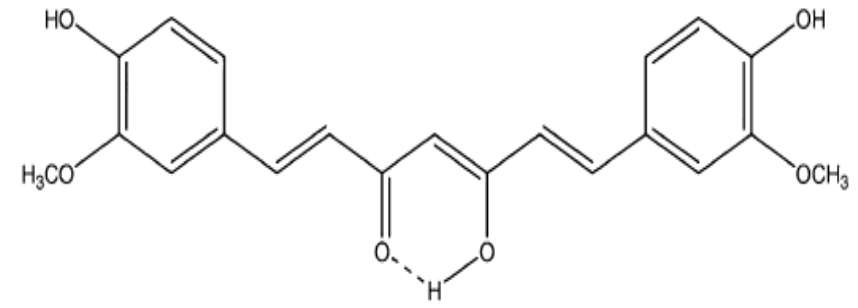
Curcumin

Curcumin is the important active ingredient responsible for the biological activity of turmeric. Curcumin, $C_2H_{20}O_6$ (m.p. 184◦C) or diferuloyl methane was first isolated in 1815. The crystalline form of Curcumin was obtained in 1910, and elucidated its structure in 1913. It is insoluble in water but soluble in ethanol and acetone (Joe *et al.,* 2004).

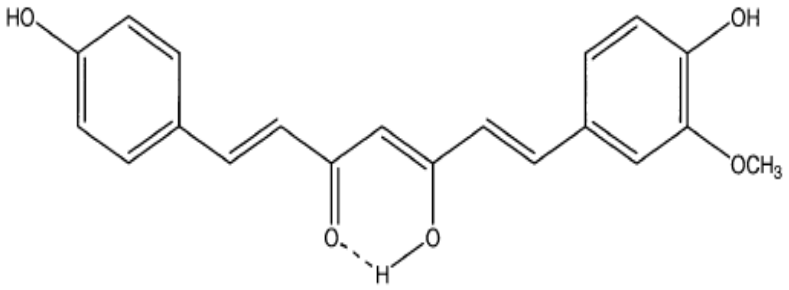
The three main curcuminoids isolated from turmeric are Curcumin, Demethoxy curcumin, and Bis-demethoxycurcumin (Fig. 17).

Mechanism of action:

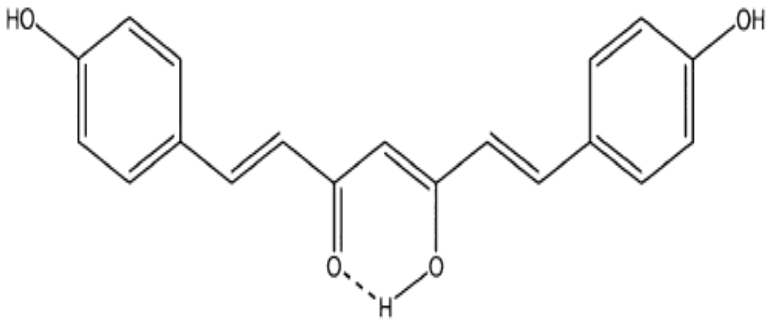
The metabolism of curcumin was first studied in mice, after intraperitoneal administration of curcumin (0.1 g/kg) in mice. Approximately 2.25 μg/ml of curcumin appeared in the plasma within the first 15 min. One hour after administration, the levels of curcumin in the intestines, spleen, liver, and kidneys were 177.04, 26.06, 26.90 and 7.51μg/g respectively. Only traces (0.41μg/g) were observed in the brain at 1 hr. The plasma was analyzed by reversed-phase HPLC to understand the nature of the metabolites of curcumin and it was found that two putative conjugates of curcumin. Treatment of the plasma with beta-glucuronidase resulted in a decrease in the concentrations of these two putative conjugates and the concomitant appearance of tetrahydrocurcumin (THC) and curcumin respectively (Pan *et al.,* 1999).



(a) Curcumin



(b) Demethoxycurcumin



(c) Bis-demethoxycurcumin

Fig. 17. Structure of Curcumin and its analogs (Joe *et al.*, 2004).

The chemical structures of these metabolites were determined by mass spectrometry analysis and suggested that curcumin was first bio-transformed to dihydrocurcumin (DHC) and THC. These compounds subsequently were converted to monoglucuronide conjugates. THC is one of the major metabolite of curcumin, stable at different pH values. THC was very stable in 0.1M phosphate buffer of various pH values. Moreover, THC was more stable than curcumin in 0.1Mphosphate buffer, pH 7.2 (37◦C). These are all suggesting that curcumin-glucuronoside, dihydrocurcumin-glucuronoside, THC-glucuronoside and THC are major metabolites of curcumin *in vivo* (Pan *et al.,* 1999). Some people studied the biotransformation of curcumin by human and rat hepatocytes and identified that hexahydrocurcumin and hexahydrocurcuminol are the major metabolites of curcumin (Ireson *et al.,* 2002).

The degradation kinetics of curcumin under various pH conditions and the stability of curcumin in physiological matrices are reported (Wang *et al.,* 1997). Trans-6- (4-hydroxy-3-methoxyphenyl)-2,4-dioxo- 5-hexenal was predicted as a major degradation product and vanillin, ferulic acid and feruloyl methane were identified as minor degradation products (Wang *et al.,* 1997). Curcumin may associate with serum albumin through hydrophobic interactions (Pulla Reddy *et al.,* 1999) and may be transported to appropriate target cells, where it elicits its pharmacological effects. Curcumin readily penetrates into the cytoplasm and is able to accumulate in membranous structures, such as plasma membrane, endoplasmic reticulum and nuclear envelope (Jaruga *et al.,* 1998a).

Beneficial properties:

Curcumin exhibits many potentially beneficial properties which appear to be sensitive in preventive and therapeutic action against a number of degenerative diseases (Fig. 18).

Curcumin has antioxidant property (Sharma, 1976) and it acts as a scavenger of oxygen free radicals (Ruby *et al.,* 1995; Subramanian *et al.,* 1994). *In vitro*, curcumin can significantly inhibit the generation of reactive oxygen species (ROS) like superoxide anions, H_2O_2 and nitrite radical generation by activated macrophages, which play an important role in inflammation (Joe and Lokesh, 1994). Curcumin also lowers the production of ROS *in vivo* (Joe and Lokesh, 1994). Its derivatives, demethoxycurcumin and bis-demethoxycurcumin also have

antioxidant effect (Unnikrishnan and Rao, 1995; Song *et al.,* 2001). Curcumin exerts powerful inhibitory effect against H_2O_2-induced damage in human keratinocytes and fibroblasts (Phan *et al.,* 2001) and in NG 108-15 cells (Mahakunakorn *et al.,* 2003). Curcumin reduces oxidized proteins in amyloid pathology in Alzheimer transgenic mice (Lim *et al.,* 2001). It also decreases lipid peroxidation in rat liver microsomes, erythrocyte membranes and brain homogenates (Pulla Reddy and Lokesh, 1994). The pro-oxidant activity appears to be mediated through generation of phenoxyl radical of curcumin by peroxidase–H_2O_2 system, which cooxidizes cellular glutathione or NADH, accompanied by O_2 uptake to form ROS (Galati *et al.,* 2002). The antioxidant mechanism of curcumin is attributed to its unique conjugated structure, which includes two methoxylated phenols and an enol form of *b*-di ketone; the structure shows typical radical-trapping ability as a chain-breaking antioxidant (Sreejayan Rao, 1994; Masuda *et al.,* 2001).

Curcumin has been shown to protect the stomach from ulcerogenic (Dasgupta *et al.,* 1969; Sinha *et al.,* 1974) and antispasmodic activity as well as antiflatulent activity in intestine (Bhavani Shankar and Sreenivasa Murthy, 1979; Srihari Rao *et al.,* 1982). Curcumin also enhances intestinal and pancreatic lipase, sucrase and maltase activity (Platel *et al.,* 1996; Platel and Srinivasan, 2000). Curcumin and its analogues have protective activity in cultured rat hepatocytes against carbon tetrachloride, D-galactosamine, peroxide and ionophore-induced toxicity (Hikino, 1985; Song *et al.,* 2001; Kang *et al.,* 2002). Curcumin also protects against diethylnitrosamine and 2-acetylaminofluorine-induced altered hepatic foci development (Shukla and Arora, 2003). Increased bile production was reported in dogs by both curcumin and essential oil of *C. Longa* (Jentzsch *et al.,* 1959; Ozaki and Liang, 1988). Curcumin protects from damage caused by myocardial infarction (Nirmala *et al.,* 1996; Sumbilla *et al.,* 2002). Curcumin has significant hypocholesteremic effect in hypercholesteremic rats (Patil and Srinivasan, 1971). Curcumin and manganese complex of curcumin offers protection against vascular dementia by exerting antioxidant activity (Vajragupta *et al.,* 2003; Thiyagarajan and Sharma, 2004).

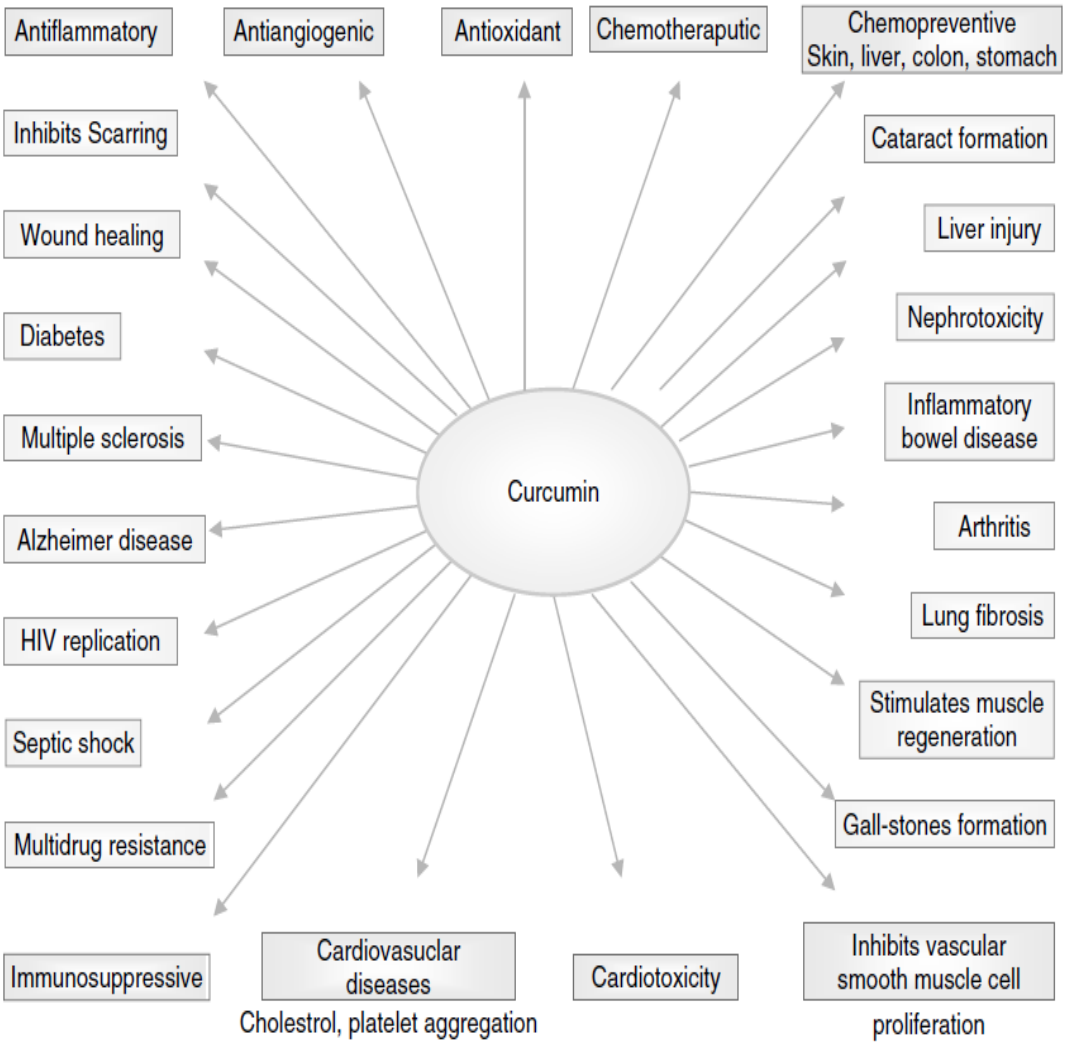


Fig. 18. Flow diagram showing effects of curcumin on various diseases (Aggarwal *et al.*, 2003).

Curcumin reduces low density lipoprotein (LDL) and very low density lipoprotein (VLDL) significantly in plasma and total cholesterol level in liver along with an increase of *a*-tocopherol level in rat plasma to offer antioxidant activity (Began *et al.*, 1999; Kamal-Eldin *et al.*, 2000). The increase in fatty acid content after ethanol-induced liver damage is significantly decreased by curcumin treatment (Akrishnan and Menon, 2001).

Curcumin is effective against carrageenin-induced oedema in rats (Ghatak. and Basu, 1972; Srihari Rao *et al.*, 1982; Brouet and Ohshima, 1995) and mice (Srimal and Dhawan, 1985). It has been reported that curcumin has anti-inflammatory effects (Yegnanarayan *et al.*, 1976) and antirheumatic activity

(Deodhar *et al.,* 1980). Curcumin stimulates stress-induced expression of stress proteins and may act in a way similar to indomethacin and salicylate has recently been reported (Kato *et al.,* 1998). Curcumin offers anti-inflammatory effect through inhibition of NF*k*B activation (Singh and Aggarwal, 1995; Bierhaus *et al.,* 1997; Surh *et al.,* 2001). Curcumin also enhances wound-healing in diabetic rats and mice (Sidhu *et al.,* 1999) and in H_2O_2-induced damage in human keratinocytes and fibroblasts (Phan *et al.,* 2001).

Curcumin acts as a potent anticarcinogenic compound by induction of apoptosis and plays an important role in its anticarcinogenic effect and antiproliferative effect (Chen *et al.,* 1996; Chen and Huang, 1998; Piwocka *et al.,* 2001; Deeb *et al.,* 2003; Duvoix *et al.,* 2003; Martin-Cordero *et al.,* 2003; Woo *et al.,* 2003; Jana *et al.,* 2004; Rashmi *et al.,* 2004). Curcumin also inhibits proliferation of rat thymocytes (Sikora *et al.,* 1997), prostaglandin synthesis in colon carcinoma (Hanif *et al.,* 1997). Curcumin exerts both pro and antimutagenic effects (Shukla *et al.,* 2002). Curcumin shows anticoagulant activity by inhibiting collagen and adrenaline-induced platelet aggregation *in vitro* as well as *in vivo* in rat thoracic aorta (Srivastava *et al.,* 1985). Curcumin also inhibits human sperm motility and has the potential for the development of a novel intravaginal contraceptive (Rithaporn *et al.,* 2003).

Curcumin shows many beneficial properties such as antidiabetic (Sajithlal *et al.,* 1998; Arun and Nalini, 2002; Suryanarayana *et al.,* 2003), antibacterial (Bhavani Shankar and Sreenivasa Murthy, 1979; Kumar *et al.,* 2001; Mahady *et al.,* 2002), antifungal (Misra and Sahu, 1977; Banerjee and Nigam, 1978; Apisariyakul *et al.,* 1995; Wuthi-Udomler *et al.,* 2000; Jayaprakasha *et al.,* 2001), antiprotozoan **(**Rasmussen et al., 2000; Gomes Dde *et al.,* 2002; Koide *et al.,* 2002), antiviral (Mazumdar *et al.,* 1995; De Clercq, 2000; Hergenhahn *et al.,* 2002; Taher *et al.,* 2003), antifibrotic (Punithavathi *et al.,* 2000) and antivenom (Ferreira *et al.,* 1992; Araujo and Leon, 2001).

Recent years have seen an increased enthusiasm in treating various diseases with natural products. Curcumin is a non-toxic, highly promising natural antioxidant compound having a wide spectrum of biological functions. It is expected that curcumin may find application as a novel drug in the near future to control various diseases.

Objectives

OBJECTIVES:

Based on this literature background the following objectives were planned.

1. Effect of Curcumin on Ethanol induced changes in daily rhythms of Serotonin Chronometabolome in SCN and Pineal.

2. To find Sensitivity of Curcumin treatment on Ethanol induced changes in daily rhythms of serotonin Chronometabolome in aging.

3. Effect of Curcumin on ethanol induced changes on daily rhythms of *per1* and *per2* expression in SCN and Pineal.

4. Effect of Curcumin on ethanol induced changes on daily locomotor activity rhythms.

Materials and Methods

MATERIALS AND METHOIDS:

Male Wistar rats of different age groups (90 day, 1yr, 2yr) were maintained at 23±1°C with LD, 12:12 (Lights on: 06:30 A.M and lights off: 6:30 P.M) two weeks prior to experiment. All rats were kept individually in polypropylene cages. Food and water were provided *ad libitum*. Dim red light was used for handling the animals in dark. Cage changing was done at random intervals. The rats were separated into six groups - (I) control; (II) ethanol treatment (ET); (III) ethanol withdrawal (EW); (IV) curcumin treated (CrT); (V) turmeric treated (TT); (VI) melatonin treated (MT).

I. Control:

Rats were maintained at standard laboratory conditions. Food and water were provided *ad libitum*.

II. Ethanol Treatment:

Ethanol (10% (v/v) in tap water) and water were given for 15 days under the two bottle free choice regimen with unlimited access for 24h/day. Food pellets were always available. Bottles were refilled everyday with a fresh solution and their positions interchanged at random intervals to avoid development of position preference. Ethanol and water intakes were recorded daily, immediately before the lights off and the values are expressed in g/kg/day (Jagota and Reddy, 2007).

III. Ethanol withdrawal:

Ethanol withdrawal was followed for 15 days after ET. Food and water were provided at *ad libitum* (Jagota and Reddy, 2007).

IV. Curcumin administration:

0.002% of curcumin in regular diet by oral free choice method for 15 days after ET (Jagota and Reddy, 2007).

V. Turmeric administration:

0.5% of turmeric in regular diet by oral free choice method for 15 days after ET (Suryanarayana *et al*., 2005).

VI. Melatonin administration:

30 µg/kg body weight of melatonin was administered in 10% ethanol in physiological saline subcutaneously at 1hr before the onset of darkness (ZT-11) for 11 days after ET (Jagota and Kalyani, 2010).

Each group contains 30 animals.

1. Sample preparation:

In each group, rats were sacrificed at various time points such as ZT-0, 6, 12, 18, 24/0 with n=6 at each time point. Brains were dissected following anaesthesia, pineal glands were separated. SCN was carefully punched out with the help of scalpel from 500µ brain slices using tissue chopper (Jagota and Reddy, 2007).

2. Antioxidant activity assay:

Add 0.3 ml of test compound (0.625 µg/ml, 6 µg/ml and 60 µg/ml) to 1.2 ml of 0.1 mM diphenyl-1-picrylhydrazyl (DPPH) (Sigma) and keep this for reaction for 20 min incubation. Color changes from deep violet to light yellow and read the absorbance at 517 nm (Dairam *et al*., 2008; Faigali and Catala., 2007).

Calculate % of inhibition or scavenging activity = A_0-A_1/A_0X 100

A_0=Absorbance of color reaction (DPPH+ Methanol)

A_1= Absorbance of test compound

Controls: Ascorbic acid and Glutathione peroxidase.
Test compounds: Melatonin, Curcumin, Turmeric, N-acetyl serotonin and 5-Hydroxytryptophan.

3. Serotonin chronometabolome assay by reverse phase high performance liquid chromatography with electrochemical detection (RP HPLC-EC):

5-HT, 5-HIAA, NAS, MEL, TRP, 5-HTP, 5-HTOH, 5-MTOH, 5-MIAA and NAT levels were assayed by using HPLC- EC method (Mefford *et al*., 1980; Grady *et al*., 1984; Jagota and Reddy, 2007).

Apparatus:

HPLC-EC (WATERS, Milford, USA) system utilized a 25 cm X 4.7mm, 5 µ pore size, C18- silica reverse phase column coupled with electrochemical detector containing carbon working electrode, ISAAC reference electrode, stainless steel auxiliary electrode maintained at 0.71V potential. Flow rate was 1 ml/min maintaining column pressure at approximately 1800 psi. All chromatographic experiments were performed at ambient temperature (25°C) in electrically shielded room.

Reagents:

Using known concentration (0.25ng-80ng) of tryptophan, 5-hydroxy tryptophan, 5-hydroxytryptamin, N-acetylserotonin, 5-hydroxyindoleacetic acid, 5-Methoxy N-acetyl 5-hydroxytryptamine, 5-Methoxytryptophol, 5-methoxyindole acetic acid, N-acetyltryptamine (Sigma) and 5-hyroxytryptophol (MP Biomed, USA) dissolved in 0.1N perchloric acid concentration, standard curves were prepared and linearity was established.

Mobile phases were made by filtering Sodium acetate-citrate (Qualigens fine chemicals) buffers containing EDTA (HI-MEDIA) and NaCl (Qualigens) (0.12g/L, to maintain proper potential in Electrochemical cell) (adjusted to pH - 4.1 with 10 N NaOH) under vacuum through 0.22 µ Supor®200 membrane filters (PALL life sciences). Methanol (Qualigens) was added to attain the appropriate concentration (10 % or 25 %) and entire mobile phase was slowly stirred and degassed. HPLC grade chemicals were used in the mobile phase preparation.

Sample preparation:

The tissue sample was homogenized with 100 µl of 0.1N Perchloric acid (Qualigens) containing sodium bisulfate (1mM) (Sd-fine Chemicals). After homogenization the tissue samples were sonicated for approximately 5 sec. The centrifugation was done at 12800g for 10 min to remove tissue debris. The supernatant was filtered through 0.22µ syringe filters (MDI membrane technologies).

HPLC analysis:

Filtered supernatant was applied to the chromatography system by using Eluant A (10% methanol; 0.1M citric acid; 0.1M sodium acetate, 50 mg/litre EDTA (pH- 4.1)) to estimate tryptophan, 5-hydroxytryptophan, 5-hydroxytryptamine, 5-hydroxytryptophol, 5-hydroxy indole acetic acid and N-acetyl serotonin and Eluant B (25 % methanol ; 0.1 M citric acid , 0.1 M sodium acetate ; 50 mg / litre EDTA; pH– 4.1)) to estimate melatonin, 5-methoxy tryptophol, 5-methoxy indole acetic acid and N-acetyl tryptamine.

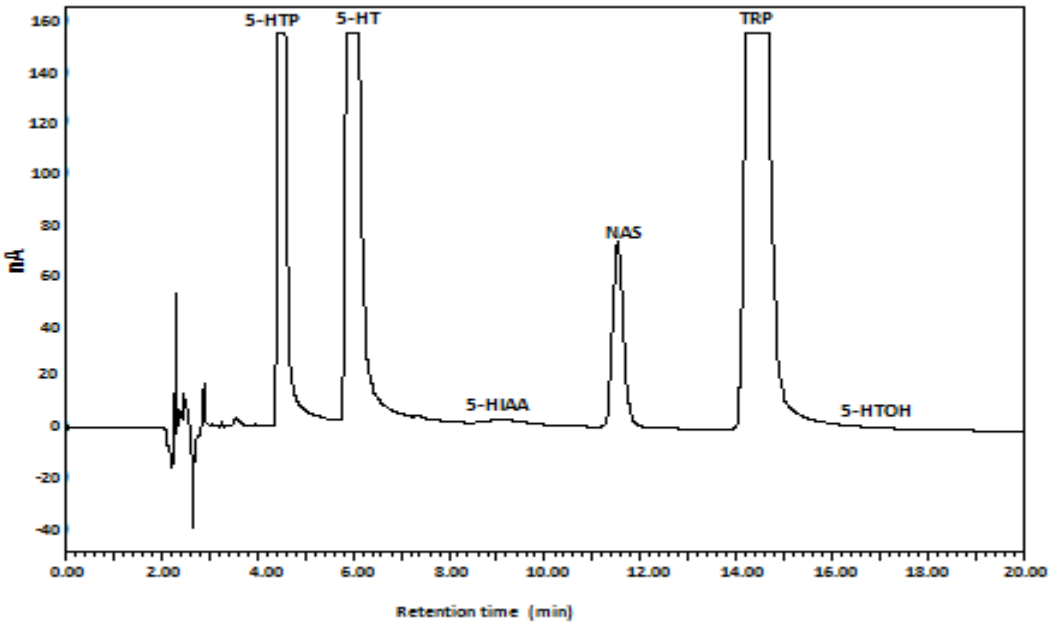
Estimation:

The amount of each compound in a sample was estimated by comparing the peak area to standard curve generated by analyzing the known amounts of standard compounds (Fig. 19; Table 3)

Protein estimation:

The protein estimation was done by using Bradford's method (Bradford, 1976). 10 µl of sample was taken for assay and diluted to 100 µl with distilled water. 1ml of Bradford reagent was added to each sample and thoroughly mixes the contents by vortex mixture. The absorbance at 595 nm was measured after 2 min and before I hr in 1 ml cuvettes against a reagent blank prepared from 0.1 ml of distilled water and 1 ml of Bradford reagent. The standard graph was prepared from 1µg to 10 µg using Bovine serum albumin (BSA). The protein concentration was estimated in sample by comparing the absorbance value with standard graph.

A



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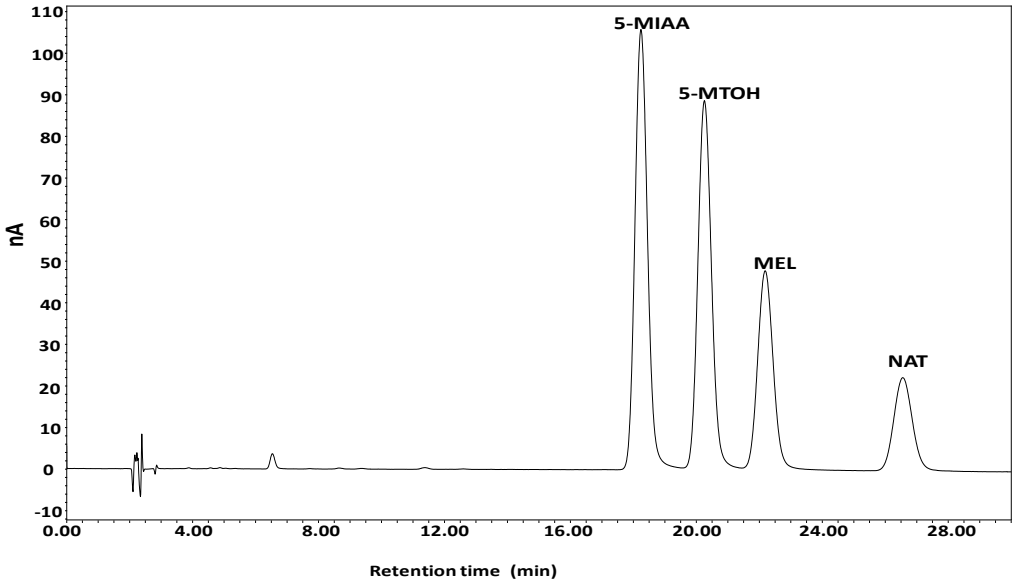

Fig. 19. Standard representative peaks of HPLC for Serotonin and its related compounds. (A) 10%methanol; 0.1N Sodium acetate; 0.1N citric acid, 50mg/litre EDTA was used for detection of compounds- 5-HTP, 5-HT, 5-HIAA, NAS, TRP and 5-HTOH. (B) 25%methanol; 0.1N Sodium acetate; 0.1N citric acid, 50mg/litre EDTA was used for detection of compounds- 5-MIAA, 5-MTOH, MEL and NAT. nA refers to nanoampere (Unit of electric current).

Table 3. Retention times (RT)s of Serotonin and its related compounds.

Compound	5-HTP	5-HT	5-HIAA	NAS	TRP	5-HTOH	5-MIAA	5-MTOH	MEL	NAT
RT	4.5	5.8	9.2	11.5	14.5	15.9	18.5	20.4	22.5	26.8

RT refers Retention time. 5-hydroxytryptophan (5-HTP); 5-hydroxytryptamine (5-HT); 5-hydroxyindole acetic acid (5-HIAA); N-acetyl serotonin (NAS); tryptophan (TRP); 5-hydroxytryptophol (5-HTOH); 5-methoxyindole acetic acid (5-MIAA); 5-methoxytryptophol (5-MTOH); melatonin (MEL); N-acetyl tryptamine (NAT).

4. Quantitation of *per1* and *per2* gene expression by Real Time - PCR:

RNA isolation protocol: (Chomczynski and Sacchi, 2006)

For 2-10 mg of SCN/ Pineal tissue, add 250 µl of TRI reagent (Sigma) and homogenize. Incubate samples for 5 min at room temperature. Add 0.1 ml of chloroform for SCN/Pineal sample. Shake samples vigorously for 15 sec. Incubate again at room temperature for 5 min. Centrifuge for 15 min at 12000g at 4˚C. Transfer upper aqueous layer to a fresh tube. Add 125 µl of isopropyl alcohol to precipitate RNA. Mix and incubate at RT for 5 min. Centrifuge for 10 min at 12000g at 4˚C. RNA will precipitate on the side/ bottom of the tube. Discard supernatant and wash RNA pellet with 250µl of 75% ethanol. Mix and Centrifuge at 12000g for 5 min at 4˚C. Repeat the wash two times. RNA pellet can be stored at -70˚C for few months in ethanol.

Redissolving RNA:

Remove supernatant and air dry the pellet for 5-10 min (don't dry completely). Dissolve the pellet in 20µl of RNase free water and incubate for 10 min at 37˚C.

RNA quantification:

The amount of extracted RNA was determined by measuring the optical density at 260 and 280 nm with Nano drop spectrophotometer and ratio should be greater than 1.8

cDNA synthesis: (Kamphuis *et al*., 2005).

Extracted RNA (1 µg) was reverse-transcribed by using iScript reverse transcriptase (Bio-rad) in a total volume of 10 µl, containing 2.0 µl 5x iScript reaction mix , 0.5 µl iScript reverse transcriptase. The reverse transcription mixture was incubated: 25 °C at 5 min, 42°C at 30 min, 85°C at 5 min, hold at 4°C to promote cDNA synthesis. The cDNA was then diluted to 1:20 in RNase-free water, and aliquots of 4 µl were used for the polymerase chain reaction (PCR).

Real time PCR:

Real-time PCR was carried out in a total volume of 10 µl containing 5 µl Power SYBR® Green (Applied biosystems), 0.5 µl Forward primer (10 pM), 0.5 µl Reverse primer (10 pM), and 4 µl sample.

Primer sequence for ***β-actin***: Forward- AGCCATGTACGTAGCCATCC
Reverse- CTCTCAGCTGTGGTGGTGAA
per1: Forward- CTGGTTCGGGATCCACGAA
Reverse- GAAGAGTCGATGCTGCCAAAG
per2: Forward- CACCCTGAAAAGAAAGTGCGA
Reverse- CAACGCCAAGGAGCTCAAGT
(Kamphuis *et al*., 2005)

PCR amplification and quantification were performed in Fast 7500 real time PCR (Applied Biosystems) as follows: denaturation for 3min at 95 °C, followed by 40

cycles of 30 s at 95 °C, 20 s at 60 °C, and 20 s at 72 °C. All amplifications were carried out in duplicate (Fig. 20).

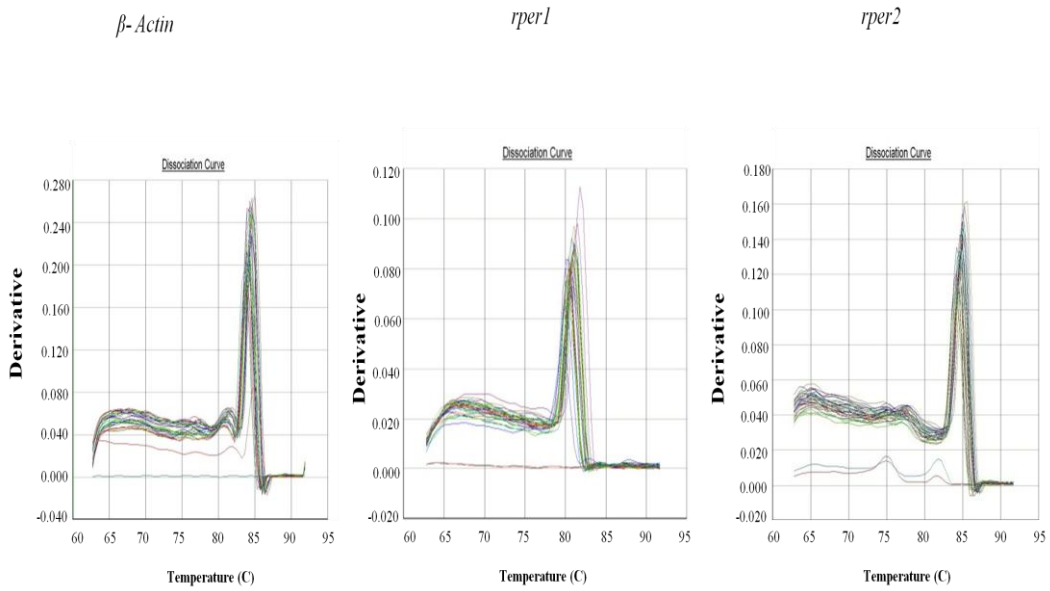

Fig. 20: Representative dissociation curves for *β-actin, rper1 and rper 2 genes.*

Quantitation:

The relative amount of RNA was calculated from the measured threshold cycles (Ct) values by Comparative ΔΔCt method (Livak and Schmittgen, 2001). The data were normalized to the amount of beta actin. Fold changes are the ratio of maximum: minimum. In general, a major problem of cDNA synthesis is the sensitivity and potential degradation of RNA during the entire sampling process. Thus, varying sample qualities need to be corrected by co-amplification of an internal standard. (house-keeping genes) such as b-actin, GAPDH etc. In an ideal setting these genes are constitutionally expressed by all cell types and should not be affected by any disease. β-actin transcript amount did not display a significant circadian variation under any of the treatment regimes (Kreuzer *et al.*,1999). Comparisons between different groups/treatments were performed using one way ANOVA (Duncan's multiple test).

5. Gross locomotor activity rhythms:

3 month male Wistar rats were used at standard laboratory conditions. The animals were housed individually in transparent polypropylene cages and locomotor activity was monitored by an infra red (I.R) motion detector (KE 28 Kit, Infra red INT Alarm).

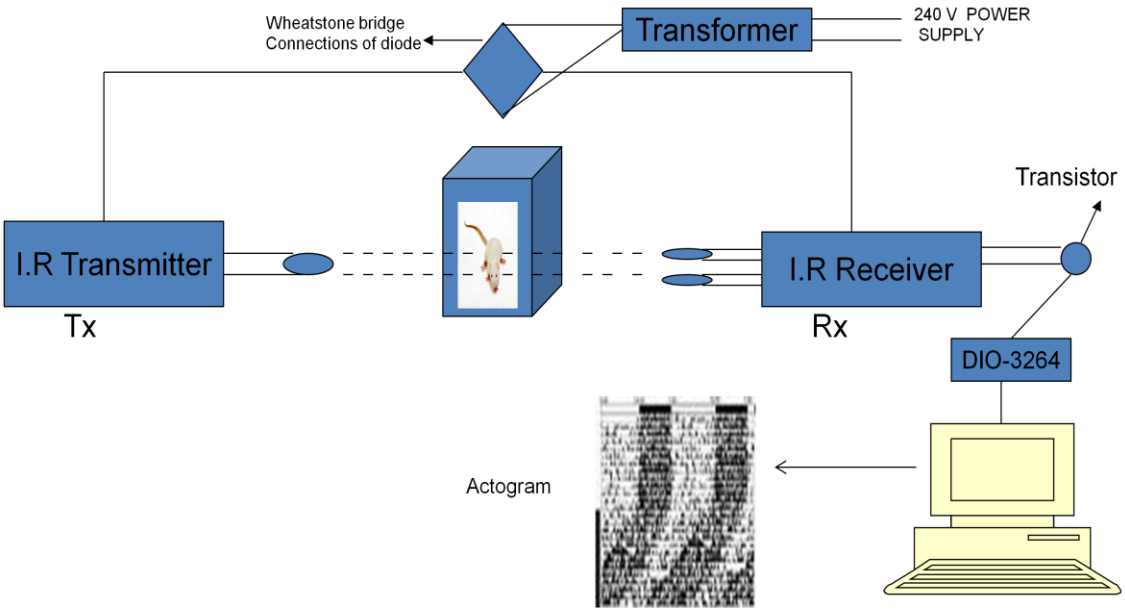

Fig. 21. Set up showing gross locomotor activity recording. Tx: Transmitter; Rx: Receiver; DIO-3264: Digital input output board fitted in computer.

The output terminals were connected to data acquisition board (DIO-3264) through transistor. When receiver (Rx) senses I.R signals from the transmitter (Tx), the output will not be generated. But when I.R between Tx and Rx is interrupted by experimental animal, the signal is generated. The signal can be

detected as double plotted actogram in computer by using Chronobiology kit (Stanford software systems, USA) (Fig. 21) (Mammen and Jagota, 2010).

6. Data analysis:

One way ANOVA was used to compare various time points within each group and comparison of different experimental groups. Max and min ratio were compared with student t- test (Jandal scientific Sigma stat). Actograms and mean activity profiles were prepared using the Chronobiology Kit and analyzed using the Kit Analyze software (Stanford Software systems, USA).

Results

OBJECTIVE 1

Effect of Curcumin on Ethanol induced changes in daily rhythms of Serotonin Chronometabolome in SCN and Pineal

1. Curcumin effect on ethanol induced changes in daily rhythms of serotonin (5-HT) chronometabolome in SCN:

5-HT:

Group I (control): 5-HT showed daily rhythmicity and levels measured at various time points such as ZT- 0, 6, 12 and 18 were 22.66 ± 2.04, 34.14 ± 1.73, 19.98 ± 0.56 and 9.95 ± 1.12 µmol/g protein respectively. The 5-HT levels were maximum at subjective mid-day (ZT-6) and minimum at subjective mid-night (ZT-18). Group II (ET): 5-HT levels at various time points such as ZT- 0, 6, 12 and 18 were 15.70 ± 5.49, 11.58 ± 2.91, 14.18 ± 4.70 and 31.46 ± 4.12 µmol/g protein respectively. 5-HT levels showed significant increase (1.5 fold) at ZT-6 ($p \leq 0.05$) though there was no significant at ZT-0, 12, 18, 24. Phase delay was observed (~12h) and both mean as well as daily pulse levels were decreased when compared with control. Group III (EW): 5-HT levels at various time points ZT- 0, 6, 12 and 18 were 22.88 ± 5.01, 45.20 ± 12.09, 39.95 ± 9.01 and 56.21 ± 19.21 µmol/g protein respectively and statistically significance was not found between time points. Phase delay (~12h) was observed. Mean was increased and daily pulse level was decreased when compared with control but rhythmicity was abolished. Group IV (CT): 5-HT levels at various time points such as ZT- 0, 6, 12, 18 and 24 were 1.45 ± 0.70, 2.52 ± 0.53, 17.11 ± 14.68, 4.64 ± 2.47 and 1.85 ± 0.43 µmol/g protein respectively. The levels were significantly increase (11.5 fold) at ZT-12 ($p \leq 0.05$). Partial restoration in phase, mean level and rhythmicity were observed ($p \leq 0.05$) but daily pulse was greatly increased ($p \leq 0.05$). Turmeric administration (Group V) showed the 5-HT levels at various time points such as ZT- 0, 6, 12, 18 and 24 were 29.34 ±7.27, 15.54 ± 6.74, 28.78 ± 5.55, 30.19 ± 7.04 and 9.68 ± 1.19 µmol/g protein respectively. Significantly difference was not observed between all time points. Restoration in mean and daily pulse was observed but rhythmicity was abolished. Group VI (MT): 5-HT levels at various time points such as ZT- 0, 6, 12, 18 and 24 were 7.14 ± 1.26, 9.56 ± 1.03, 8.35 ± 3.00, 10.86 ± 7.49 and 10.90 ± 2.38 µmol/g protein respectively. The levels showed no significant difference between all time points. Phase delay (~12h) was observed and both mean as well as daily pulse levels were decreased when compared with control and rhythm is not restored (Fig. 22, 32 and 33; Table 4).

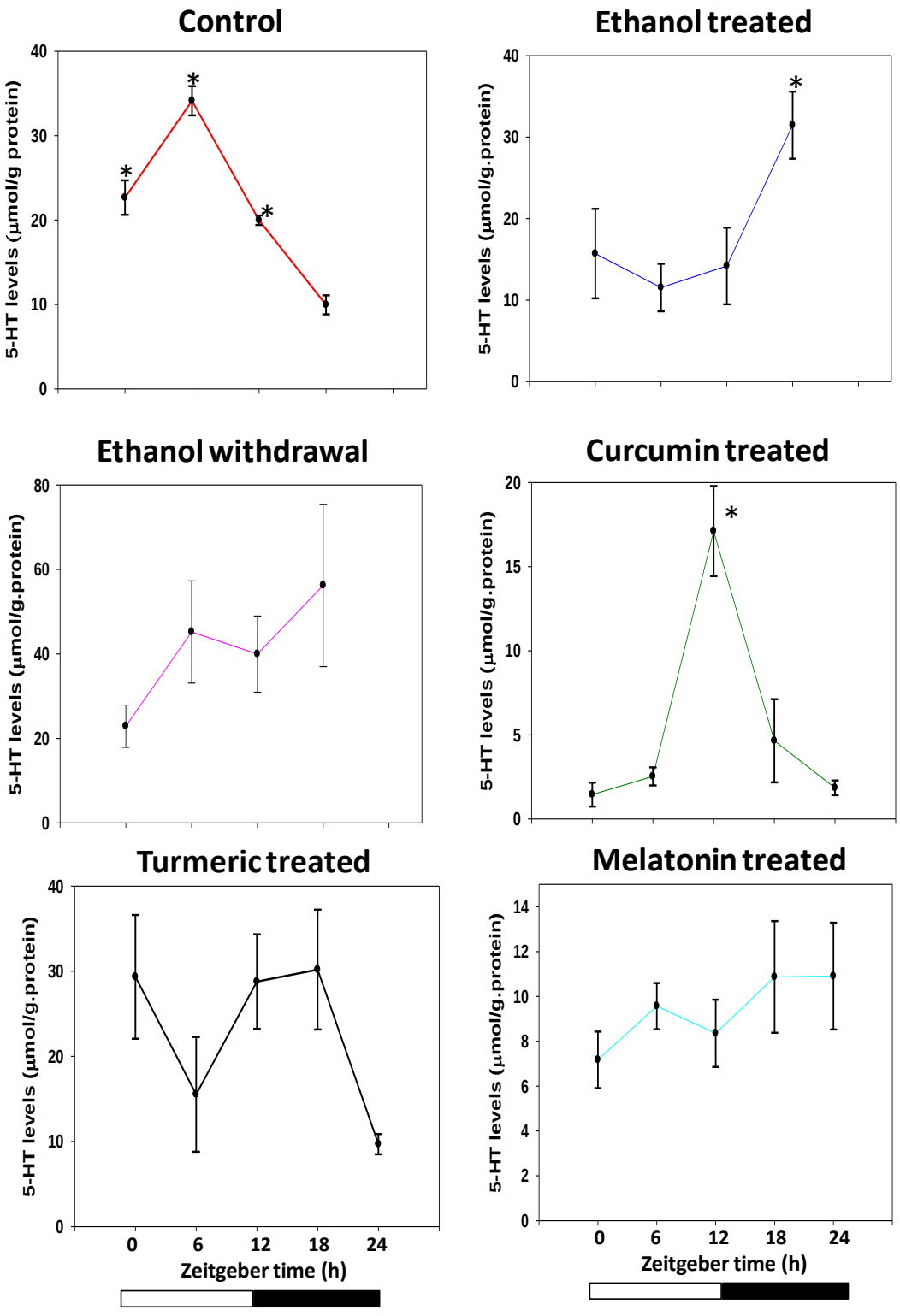

Fig. 22. Curcumin effect on ethanol induced changes in daily rhythms of 5-HT. Each value is mean ± SE, (n=6); Zeitgeber Time (ZT): ZT-0 = 6.30 A. M (Lights on); ZT-12 = 18.30 P. M (Lights off). One Way ANOVA: * Refers to comparison with lowest value of time point with other time points in each group ($p \leq 0.05$).

5-HIAA:

Group I: 5-HIAA showed daily rhythmicity and levels measured at various time points such as ZT- 0, 6, 12 and 18 were 2.83 ± 0.67, 8.76 ± 1.66, 12.05 ± 4.32 and 2.79 ± 0.46 µmol/g protein respectively. The 5-HIAA levels were maximum at ZT-12 and minimum at ZT-0. Group II: 5-HIAA levels at various time points such as ZT- 0, 6, 12 and 18 were 3.39 ± 0.74, 1.49 ± 0.35, 0.81 ± 0.26 and 1.02 ± 0.21 µmol/g protein respectively. 5-HIAA levels showed significant decrease (5 fold) at ZT-0 ($p \leq 0.05$). Phase delay (~12h) was observed and mean was decreased ($p \leq 0.05$) though no change in daily pulse when compared with control. Group III: 5-HIAA levels at various time points such as ZT- 0, 6, 12 and 18 were 2.46 ± 0.43, 2.11 ± 0.47, 1.30 ± 0.42 and 1.82 ± 0.36 µmol/g protein respectively and showed no significant difference between all time points. Phase delay (~12h) was observed and both mean ($p \leq 0.05$) as well as daily pulse levels were decreased when compared with control. Group IV: 5-HIAA levels at various time points such as ZT- 0, 6, 12, 18 and 24 were 2.73 ± 0.96, 3.58 ± 0.87, 3.88 ± 1.63, 4.44 ± 1.12 and 1.04 ± 0.23 µmol/g protein respectively. Significant difference was not observed between all time points. Phase delay (~6h) was observed and mean was decreased ($p \leq 0.05$) though no change in daily pulse when compared with control. Group V: showed 5-HIAA levels at various time points such as ZT- 0, 6, 12, 18 and 24 were 1.35 ± 0.43, 2.98 ± 1.18, 1.26 ± 0.32, 1.45 ± 0.27 and 1.87 ± 0.39 µmol/g protein respectively. Significant difference was not observed between all time points. Phase advance (~6h) was observed and mean was decreased significantly ($p \leq 0.05$) but not in daily pulse when compared with control. Group VI: 5-HIAA levels at various time points such as ZT-0, 6, 12, 18 and 24 were 1.62 ± 0.60, 4.14 ± 0.53, 0.98 ± 0.29, 0.95 ± 0.33 and 2.37 ± 0.72 µmol/g protein respectively. The levels were significantly different at ZT-6 ($p \leq 0.05$). Phase advance (~6h) was observed and mean was decreased significantly ($p \leq 0.05$) but though no change in daily pulse when compared with control (Fig. 23, 32 and 33; Table 1).

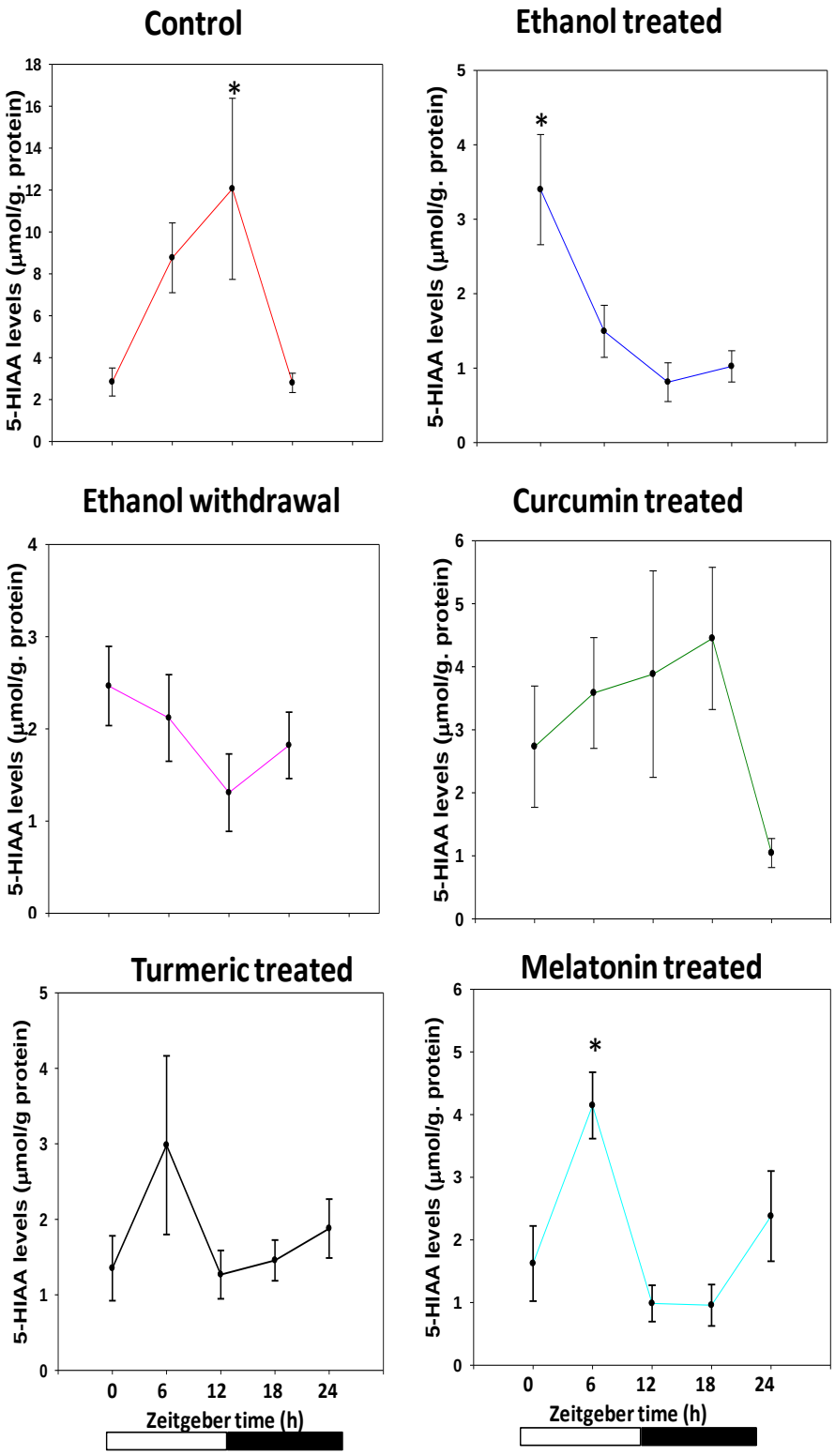

Fig. 23. Curcumin effect on ethanol induced changes in daily rhythms of 5-HIAA. Each value is mean ± SE, (n=6); Zeitgeber Time (ZT): ZT-0 = 6.30 A. M (Lights on); ZT-12 = 18.30 P. M (Lights off). One Way ANOVA: * Refers to comparison with lowest value of time point with other time points in each group ($p \leq 0.05$).

5-HTP:

Group I: 5-HTP showed daily rhythmicity and levels measured at various time points such as ZT- 0, 6, 12 and 18 were 0.82 ± 0.02, 0.66 ± 0.05, 0.93 ± 0.07 and 0.80 ± 0.07 µmol/g protein respectively. The 5-HTP levels were maximum at ZT-12 and minimum at ZT-0. Group II: 5-HTP levels at various time points such as ZT- 0, 6, 12 and 18 were 8.69 ± 2.88, 18.37 ± 6.95, 7.91 ± 1.64 and 15.14 ± 1.36 µmol/g protein respectively. 5-HTP levels showed no significant difference between time points and rhythm was abolished. Phase advance was observed (~6h) and both mean ($p \leq 0.05$) as well as daily pulses were increased when compared with control. Group III: 5-HTP levels at various time points such as ZT- 0, 6, 12 and 18 were 8.82 ± 2.36, 18.67 ± 6.7, 9.10 ± 1.20 and 15.32 ± 4.31µmol/g protein respectively, showed no significant difference between time points and rhythm was abolished. Phase advance (~6h) was observed and both mean ($p \leq 0.05$) as well as daily pulses were increased when compared with control. Group IV: 5-HTP levels at various time points such as ZT- 0, 6, 12, 18 and 24 were 1.08 ± 0.22, 1.35 ± 0.38, 1.58 ± 0.28, 3.39 ± 0.84 and 0.87 ± 0.19 µmol/g protein respectively. The levels were significantly increased by 3.8 fold at ZT-18 ($p \leq 0.05$). Partial restoration in mean, pulse and rhythm was observed but phase delay (~6h) was observed. Group V: 5-HTP levels at various time points such as ZT- 0, 6, 12, 18 and 24 were 3.90 ± 0.79, 2.16 ± 0.74, 4.00 ± 0.27, 8.33 ± 2.23 and 5.39 ± 1.11 µmol/g protein respectively. The levels were significantly increase (4 fold) at ZT-18 ($p \leq 0.05$). Phase delay (~6h) was observed and both mean as well as daily pulse was increased when compared with control. Group VI: 5-HTP levels at various time points such as ZT- 0, 6, 12, 18 and 24 were 1.95 ± 0.43, 1.02 ± 0.13, 1.95 ± 0.75, 1.63 ± 0.36 and 14.12 ± 3.64 µmol/g protein respectively. The levels were significantly increase (12 fold) at ZT-24 ($p \leq 0.05$). Phase delay (~12h) was observed and both mean as well as daily pulse ($p \leq 0.05$) was increased when compared with control but it fails to restore rhythmicity (Fig. 24, 32 and 33; Table 4).

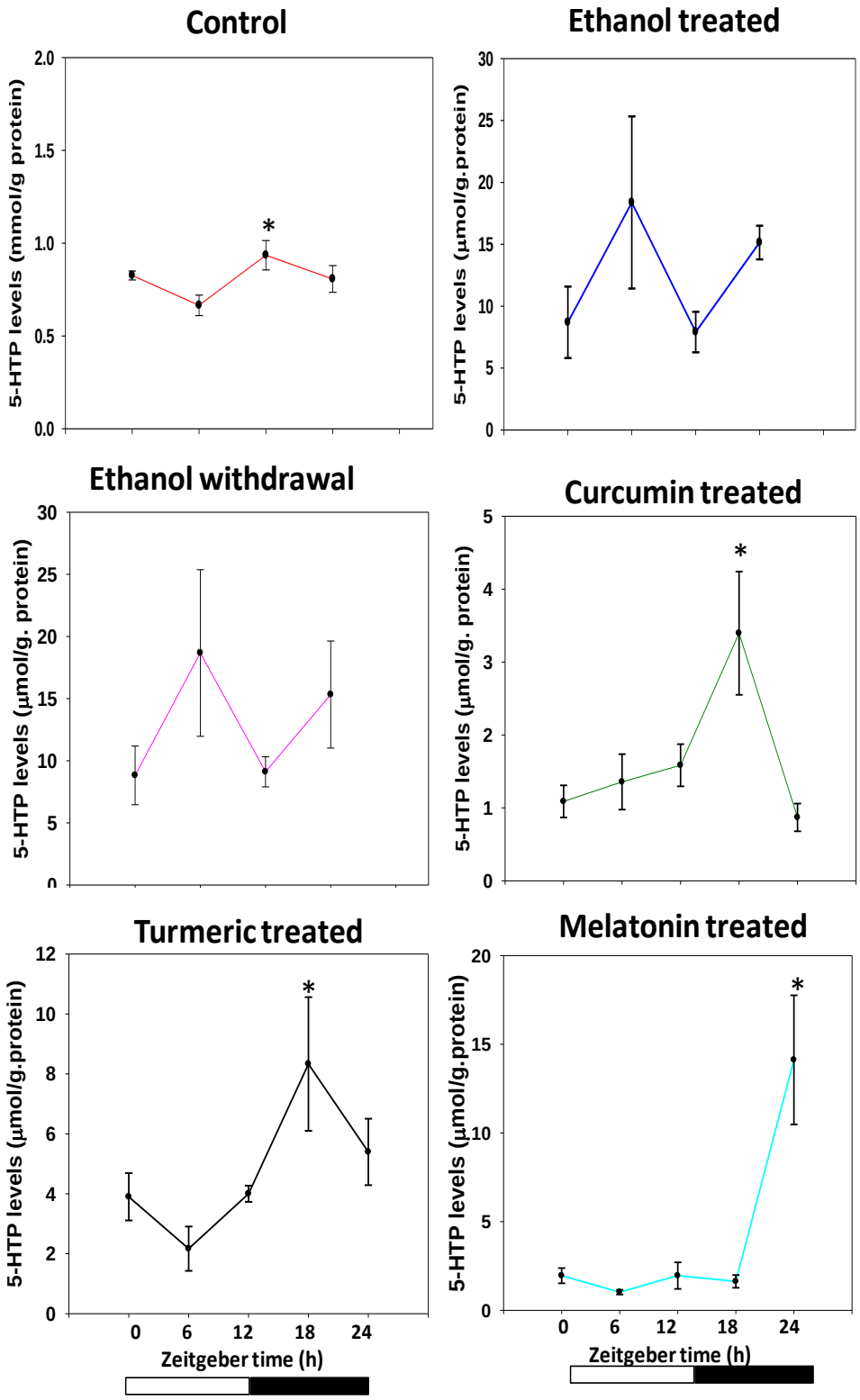

Fig. 24. Curcumin effect on ethanol induced changes in daily rhythms of 5-HTP. Each value is mean ± SE, (n=6); Zeitgeber Time (ZT): ZT-0 = 6.30 A. M (Lights on); ZT-12 = 18.30 P. M (Lights off). One Way ANOVA: * Refers to comparison with lowest value of time point with other time points in each group ($p \leq 0.05$).

5-HTOH:

Group I: 5-HTOH showed daily rhythmicity and levels were measured at various time points such as ZT- 0, 6, 12 and 18 were 0.44 ± 0.01, 0.38 ± 0.018, 0.49 ± 0.015 and 0.47 ± 0.01 µmol/g protein respectively. The 5-HTOH levels were maximum at ZT-12 and minimum at ZT-6. Group II: 5-HTOH levels at various time points such as ZT- 0, 6, 12 and 18 were 10.42 ± 2.50, 1.90 ± 0.43, 5.70 ± 2.48 and 1.44 ± 0.47 µmol/g protein respectively. 5-HTOH levels showed significant increase (7.5 fold) at ZT-0 ($p \leq 0.05$). Phase advance (~12h) was observed and both mean and daily pulse ($p \leq 0.05$) was increased when compared with control but rhythmicity was abolished. Group III: 5-HTOH levels at various time points such as ZT- 0, 6, 12 and 18 were 3.00 ± 0.83, 2.54 ± 0.67, 0.40 ± 0.12 and 0.37 ± 0.07 µmol/g protein respectively, showed significant increase (7.5 fold) at ZT-0 and (6 fold) ZT-6 ($p \leq 0.05$). Phase advance (~12h) was observed and both mean as well as daily pulse ($p \leq 0.05$) was increased when compared with control. Group IV: 5-HTOH levels at various time points such as ZT- 0, 6, 12, 18 and 24 were 0.50 ± 0.13, 1.06 ± 0.28, 0.76 ± 0.12, 0.59 ± 0.19 and 0.51 ± 0.10 µmol/g protein respectively. The levels were significantly increase (2 fold) at ZT-6 ($p \leq 0.05$). Phase advance (~6h) was observed and both mean as well as daily pulse was increased when compared with control. Rhythmicity was restored. Group V: 5-HTOH levels at various time points such as ZT- 0, 6, 12, 18 and 24 were 3.80 ± 0.84, 5.46 ± 2.00, 3.66 ± 1.71, 3.43 ± 1.68, and 3.18 ± 0.59 µmol/g protein respectively. The levels were showed no significant difference between time points and fail to restore rhythmicity. Phase advance (~6h) was observed and both mean ($p \leq 0.05$) as well as daily pulse was increased when compared with control. Group VI: 5-HTOH levels at various time points such as ZT- 0, 6, 12, 18 and 24 were 1.92 ± 0.48, 2.86 ± 0.49, 2.69 ± 0.64, 1.23 ± 0.30 and 6.14 ± 1.67 µmol/g protein respectively. The levels were significantly increase (3 fold) at ZT-24 ($p \leq 0.05$). Phase advance (~12h) was observed and both mean ($p \leq 0.05$) as well as daily pulse was increased when compared with control but rhythmicity was abolished (Fig. 25, 32 and 33; Table 4).

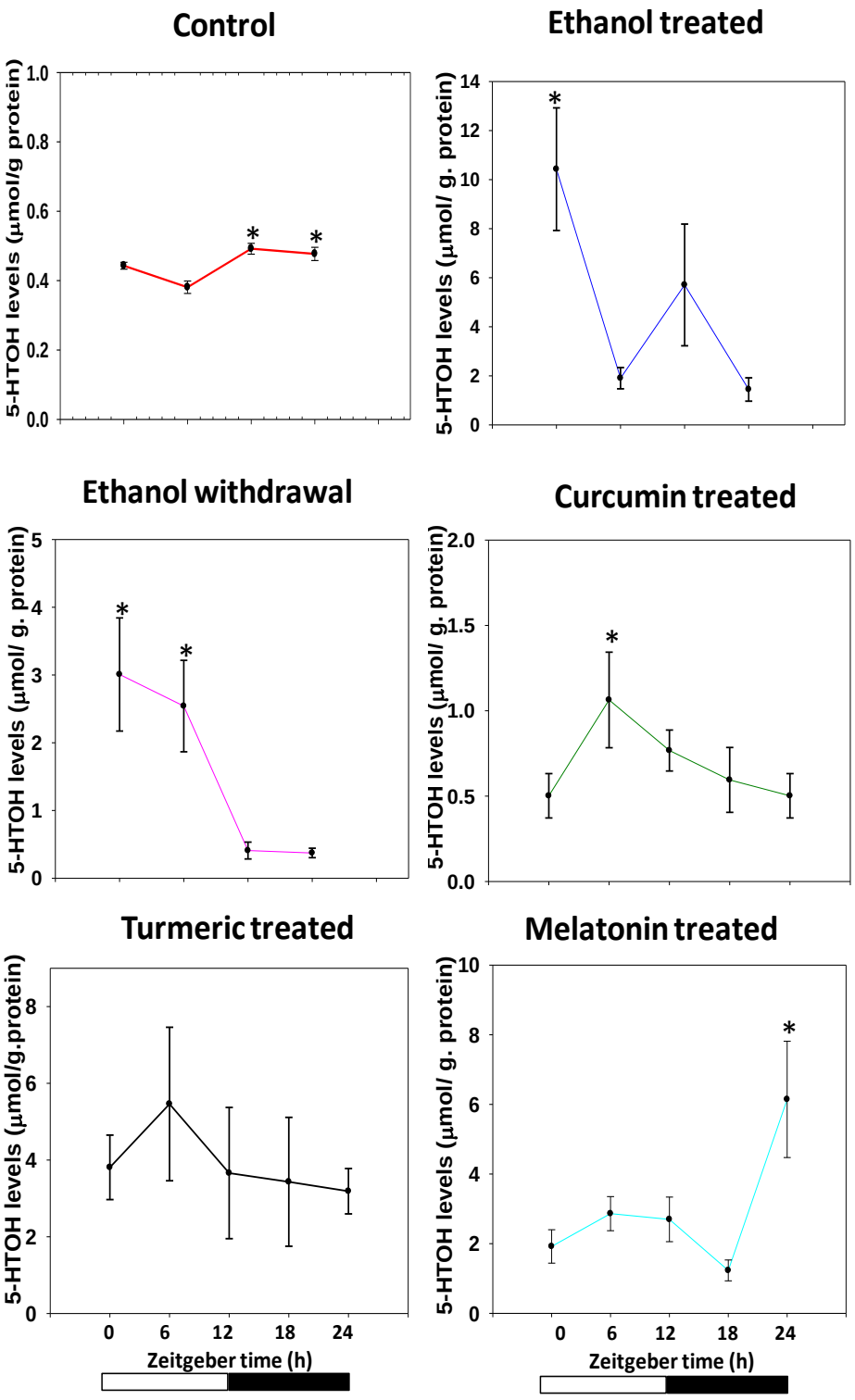

Fig. 25. Curcumin effect on ethanol induced changes in daily rhythms of 5-HTOH. Each value is mean ± SE, (n=6); Zeitgeber Time (ZT): ZT-0 = 6.30 A. M (Lights on); ZT-12 = 18.30 P. M (Lights off). One Way ANOVA: * Refers to comparison with lowest value of time point with other time points in each group ($p \leq 0.05$).

NAS:

Group I: NAS showed daily rhythmicity and levels measured at various time points such as ZT- 0, 6, 12, 18 and 24 were 0.16 ± 0.05, 0.07 ± 0.0007, 0.32 ± 0.08, 0.91 ± 0.18 and 0.21 ± 0.07 µmol/g protein respectively. The NAS levels were maximum at subjective mid-night (ZT-18) and minimum at subjective mid-day (ZT-6). Group II: NAS levels at various time points such as ZT- 0, 6, 12 and 18 were 4.64 ± 1.9, 3.61 ± 1.01, 2.01 ± 0.59 and 5.51 ± 0.71 µmol/g protein respectively. NAS levels were showed no significant difference at all time points. Both mean and daily pulse ($p \leq 0.05$) was increased when compared with control but rhythmicity was abolished. Group III: NAS levels at various time points such as ZT- 0, 6, 12, 18 and 24 were 2.74 ± 0.87, 7.41 ± 1.61, 5.30 ± 0.91 and 10.03 ± 3.18 µmol/g protein respectively and rhythmicity was abolished. Mean was increased whereas daily pulse was decreased significantly ($p \leq 0.05$). Group IV: NAS levels at various time points such as ZT- 0, 6, 12, 18 and 24 were 0.87 ± 0.30, 0.63 ± 0.38, 0.91 ± 0.32, 0.45 ± 0.23 and 0.80 ± 0.20 µmol/g protein respectively. The levels were showed no significant difference at all time points. Mean level was increased and daily pulse was decreased ($p \leq 0.05$) with phase advance (~6h) and even fails to restore rhythmicity. Group V: NAS levels at various time points such as ZT- 0, 6, 12, 18 and 24 were 2.68 ±1.55, 13.12 ± 5.99, 4.93 ± 1.69, 6.75 ± 2.08 and 3.95 ± 1.23 µmol/g protein respectively. Statistically significant difference was not observed at all time points. Mean level was increased ($p \leq 0.05$) and daily pulse was decreased with phase advance (~6h) but rhythmicity was restored. Group VI: NAS levels at various time points such as ZT- 0, 6, 12, 18 and 24 were 14.52 ± 2.83, 15.34 ± 2.34, 20.06 ± 7.31, 6.49 ±2.51 and 4.27 ± 2.36 µmol/g protein respectively. The levels were significantly increase (5 fold) at ZT-12 ($p \leq 0.05$). Mean level was increased and daily pulse was decreased significantly ($p \leq 0.05$). Rhythmicity was restored (Fig. 26, 32 and 33; Table 4).

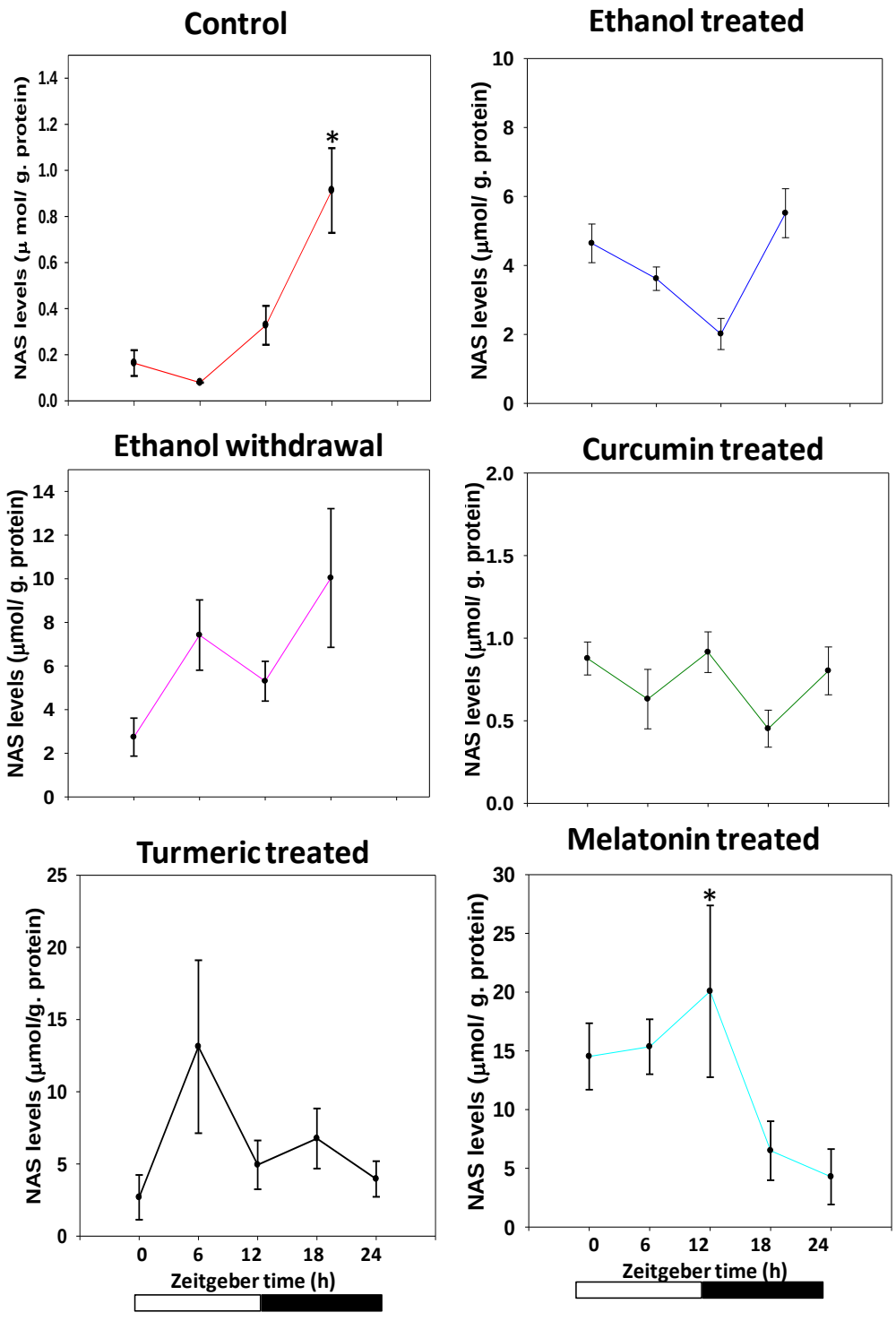

Fig. 26. Curcumin effect on ethanol induced changes in daily rhythms of NAS**.** Each value is mean ± SE, (n=6); Zeitgeber Time (ZT): ZT-0 = 6.30 A. M (Lights on); ZT-12 = 18.30 P. M (Lights off). One Way ANOVA: * Refers to comparison with lowest value of time point with other time points in each group ($p \leq 0.05$).

TRP:

Group I: TRP showed daily rhythmicity and levels measured at various time points such as ZT- 0, 6, 12 and 18 were 0.28 ± 0.08, 0.61 ± 0.08, 0.91 ± 0.05 and 0.14 ± 0.09 µmol/g protein respectively. The TRP levels were maximum at ZT-12 and minimum at ZT-18. Group II: TRP levels at various time points such as ZT- 0, 6, 12 and 18 were 0.45 ± 0.06, 0.32 ± 0.07, 0.11 ± 0.02 and 0.24 ± 0.07 µmol/g protein respectively. TRP levels were showed significant increase at ZT-0 (4 fold) and ZT-6 (3 fold) ($p \leq 0.05$). Both mean as well as daily pulse ($p \leq 0.05$) was decreased with phase advance (~12h) but rhythmicity was not affected. Group III: TRP levels at various time points such as ZT- 0, 6, 12 and 18 were 0.29 ± 0.06, 0.12 ± 0.02, 0.05 ± 0.007 and 0.11 ± 0.06 µmol/g protein respectively, were showed significant increase (60 fold) at ZT-0 ($p \leq 0.05$). Both mean and daily pulse ($p \leq 0.05$) was decreased with phase advance (~12h) but rhythmicity was not affected. Group IV: TRP levels at various time points such as ZT- 0, 6, 12, 18 and 24 were 0.13 ±0.04, 0.16 ± 0.05, 0.38 ± 0.11, 0.19 ± 0.04 and 0.28 ± 0.008 µmol/g protein respectively. The levels were showed significantly increase (3 fold) at ZT-12 ($p \leq 0.05$). Both mean and daily pulse ($p \leq 0.05$) was decreased but rhythmicity was not affected. Group V: TRP levels at various time points such as ZT- 0, 6, 12, 18 and 24 were 6.74 ± 1.36, 7.70 ± 4.77, 4.51 ± 1.54, 3.43 ± 0.95 and 13.37 ± 2.015 µmol/g protein respectively. The levels were showed no significant difference between all time points. Mean was increased and daily pulse was decreased significantly ($p \leq 0.05$) with phase delay (~12h) but rhythmicity was abolished. Group VI: TRP levels at various time points such as ZT- 0, 6, 12, 18 and 24 were 5.93 ± 2.08, 3.03 ± 1.03, 9.02 ± 2.64, 4.84 ± 2.48 and 15.59 ± 3.64 µmol/g protein respectively. The levels were significantly increase (5 fold) at ZT-24 ($p \leq 0.05$). Mean was increased and daily pulse was decreased significantly ($p \leq 0.05$) with phase advance (~12h) but rhythmicity was abolished (Fig. 27, 32 and 33; Table 4).

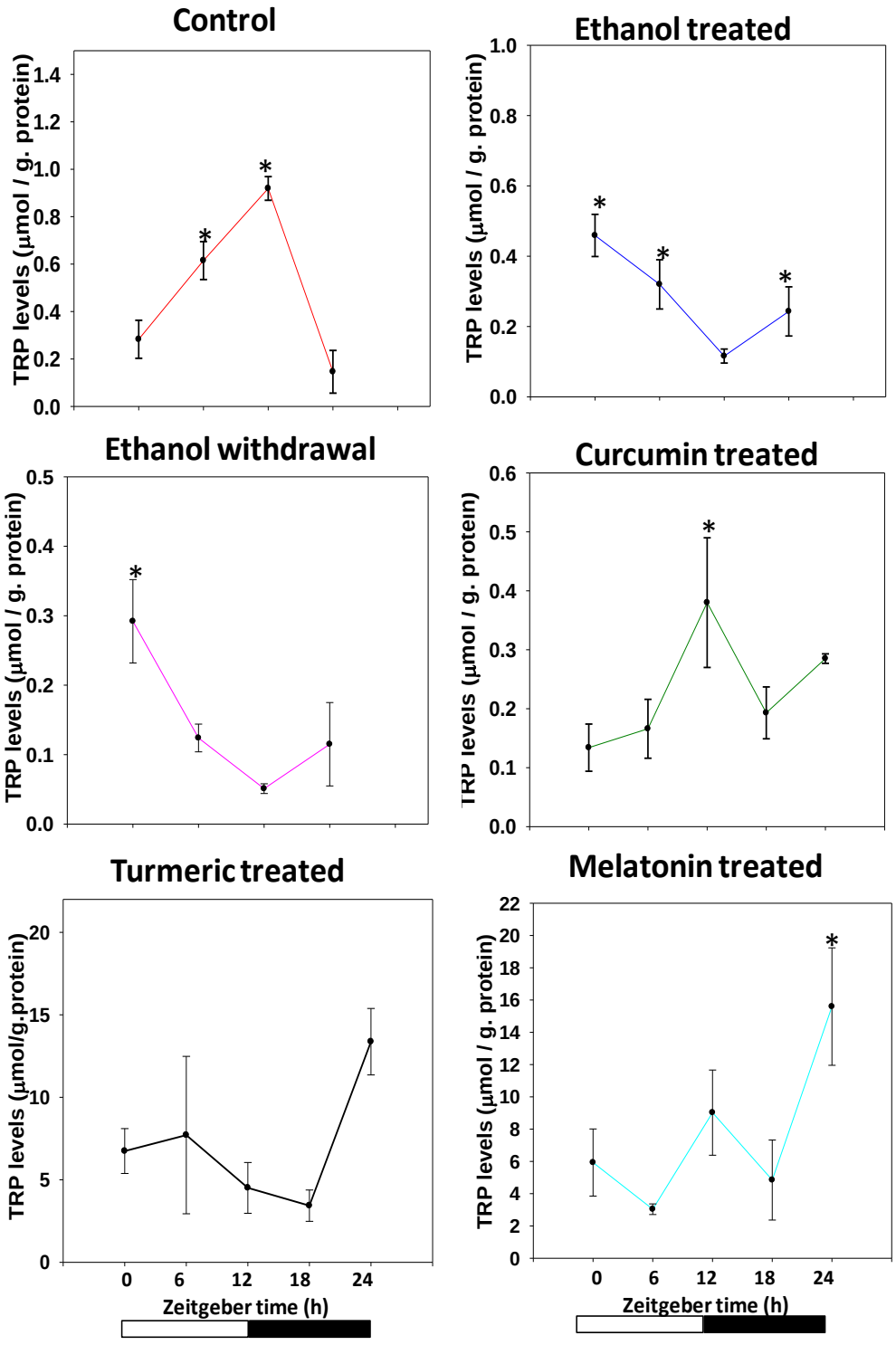

Fig. 27. Curcumin effect on ethanol induced changes in daily rhythms of TRP. Each value is mean ± SE, (n=6); Zeitgeber Time (ZT): ZT-0 = 6.30 A. M (Lights on); ZT-12 = 18.30 P. M (Lights off). One Way ANOVA: * Refers to comparison with lowest value of time point with other time points in each group ($p \leq 0.05$).

5-MTOH:

Group I: 5-MTOH showed daily rhythmicity and levels measured at various time points such as ZT- 0, 6, 12 and 18 were 20.99 ± 3.2, 22.89 ± 1.4, 15.15 ± 2.07 and 12.27 ± 0.55 µmol/g protein respectively. The 5-MTOH levels were maximum at subjective mid-day (ZT-6) and minimum at subjective mid-night (ZT-18). Group II: 5-MTOH levels at various time points such as ZT- 0, 6, 12 and 18 were 8.39 ± 2.7, 44.54 ± 13.59, 0.88 ± 0.14 and 1.44 ± 0.60 µmol/g protein respectively. 5-MTOH levels showed significant increase was observed (50 fold) at ZT-6 ($p \leq 0.05$). Mean level was decreased but daily pulse was increased significantly ($p \leq 0.05$). Group III: 5-MTOH levels at various time points such as ZT- 0, 6, 12 and 18 were 9.04 ± 2.2, 13.25 ± 2.6, 9.18 ± 2.7 and 11.25 ± 2.3 µmol/g protein respectively, showed no significant difference at all time points and rhythmicity was abolished. Both mean and daily pulse was decreased but it was not statistically significant. Group IV: 5-MTOH levels at various time points such as ZT- 0, 6, 12, 18 and 24were 6.64 ± 1.03, 10.53 ± 2.7, 22.81 ± 4.7, 26.49 ± 4.6 and 11.50 ± 3.1 µmol/g protein respectively. The levels were significantly increase at ZT-12 (3 fold) and 18 (4 fold) ($p \leq 0.05$). Mean levels were decreased and daily pulse was increased with phase delay (~12h) and rhythmicity was restored. Group V: 5-MTOH levels at various time points such as ZT- 0, 6, 12, 18 and 24 were 1.94 ± 0.35, 3.42 ± 0.85, 3.88 ± 0.62, 1.40 ± 0.26 and 3.82 ± 1.60 µmol/g protein respectively. The levels were showed no significant difference between all time points and rhythmicity was not restored. Mean level was decreased significantly and daily pulse was increased with phase delay by ~6h. Group VI: 5-MTOH levels at various time points such as ZT- 0, 6, 12, 18 and 24 were 1.75 ± 0.44, 8.74 ± 0.90, 1.05 ± 0.38, 1.17 ± 0.28 and 2.99 ± 0.55 µmol/g protein respectively. The levels were significantly increased (8 fold) at ZT-6 ($p \leq 0.05$). Mean level was decreased ($p \leq 0.05$) and daily pulse was increased with restoration of rhythm and phase (Fig. 28, 32 and 33; Table 4).

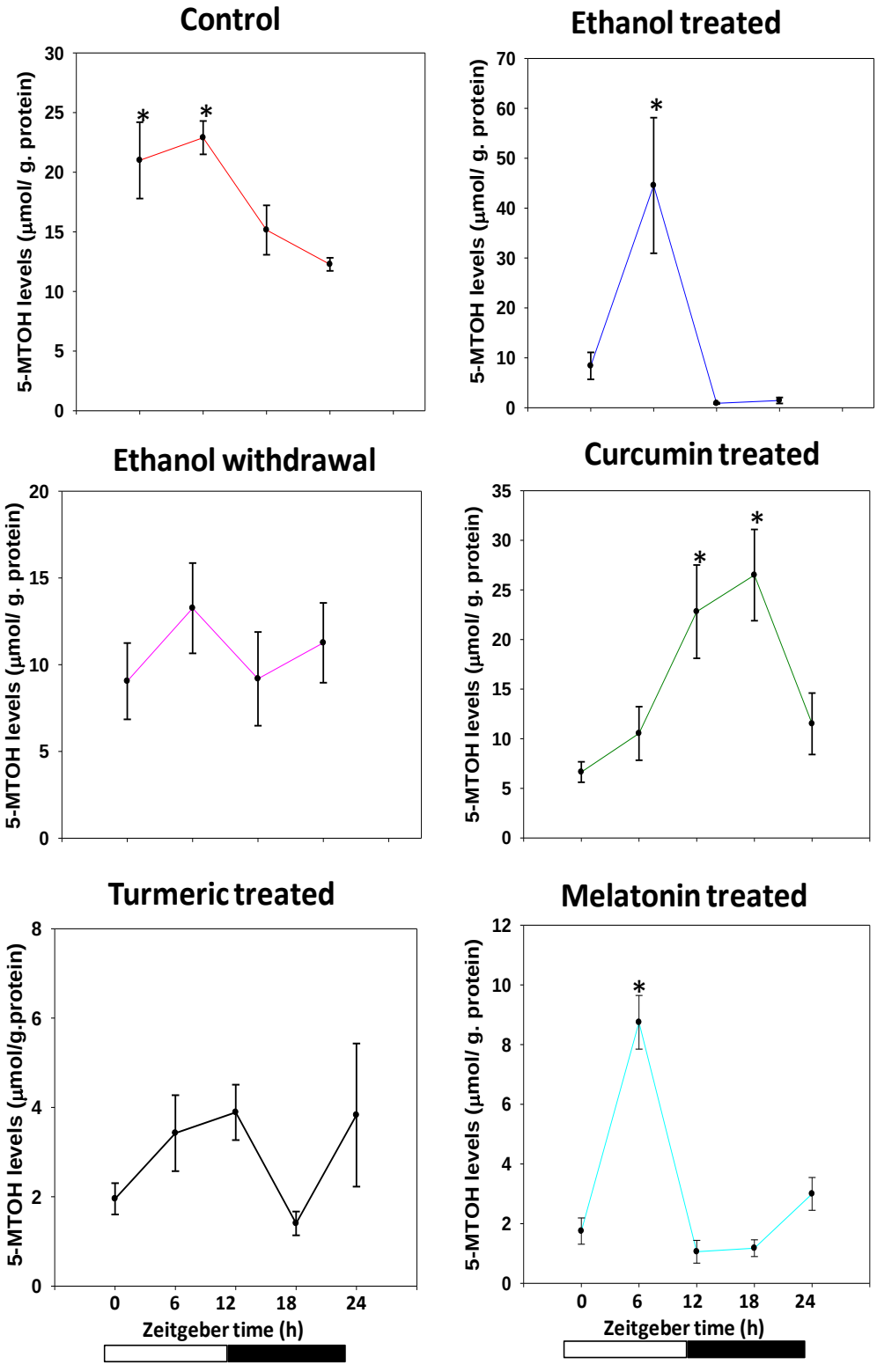

Fig. 28. Curcumin effect on ethanol induced changes in daily rhythms of 5-MTOH. Each value is mean ± SE, (n=6); Zeitgeber Time (ZT): ZT-0 = 6.30 A. M (Lights on); ZT-12 = 18.30 P. M (Lights off). One Way ANOVA: * Refers to comparison with lowest value of time point with other time points in each group ($p \leq 0.05$).

MEL:

Group I: MEL showed daily rhythmicity and levels measured at various time points such as ZT- 0, 6, 12 and 18 were 0.64 ± 0.04, 0.04 ± 0.03, 0.68 ± 0.02 and 2.93 ± 0.57 µmol/g protein respectively. The MEL levels were maximum at subjective mid-night (ZT-18) and minimum at subjective mid-day (ZT-6). Group II: MEL levels at various time points such as ZT- 0, 6, 12 and 18 were 1.06 ± 0.18, 0.52 ± 0.15, 0.24 ± 0.9 and 0.63 ± 0.05 µmol/g protein respectively. MEL levels were showed significant increase (5 fold) at ZT-0 ($p \leq 0.05$). Both mean and daily pulse was decreased ($p \leq 0.05$) with phase delay by ~6h without affecting rhythmicity. Group III: MEL levels at various time points such as ZT- 0, 6, 12 and 18 were 1.03 ± 0.22, 1.42 ± 0.25, 0.88 ± 0.25 and 1.39 ± 0.46 µmol/g protein respectively, were showed no significant difference between time points and rhythm was abolished. Both mean and daily pulse was decreased ($p \leq 0.05$) with phase advance by ~12h. Group IV: MEL levels at various time points such as ZT- 0, 6, 12, 18 and 24 were 3.28 ± 1.4, 4.70 ± 0.04, 12.08 ± 2.4, 10.51 ± 2.2 and 0.43 ± 0.09 µmol/g protein respectively. The levels were significantly increase at ZT-12 (25 fold) and 18 (23 fold) ($p \leq 0.05$). Mean level was increased whereas daily pulse was decreased significantly ($p \leq 0.05$) with phase advance by ~6h. Restoration of rhythmicity was observed. Group V: MEL levels at various time points such as ZT- 0, 6, 12, 18 and 24 were 0.28 ± 0.07, 0.28 ± 0.08, 0.35 ± 0.103, 0.36 ± 0.08 and 0.95 ± 0.10 µmol/g protein respectively. The levels were significantly increase (4 fold) at ZT-24 ($p \leq 0.05$). Mean as well as daily pulse ($p \leq 0.05$) was decreased with phase delay by ~6h. Rhythmicity was restored. Group VI: MEL levels at various time points such as ZT- 0, 6, 12, 18 and 24 were 0.25 ± 0.06, 0.21 ± 0.03, 0.34 ± 0.09, 0.32 ± 0.12, 1.00 ± 0.30 µmol/g protein respectively. The levels were significantly increased (4 fold) at ZT-24. Mean as well as daily pulse ($p \leq 0.05$) was decreased with phase delay by ~6h. Rhythmicity was restored (Fig. 29, 32 and 33; Table 4).

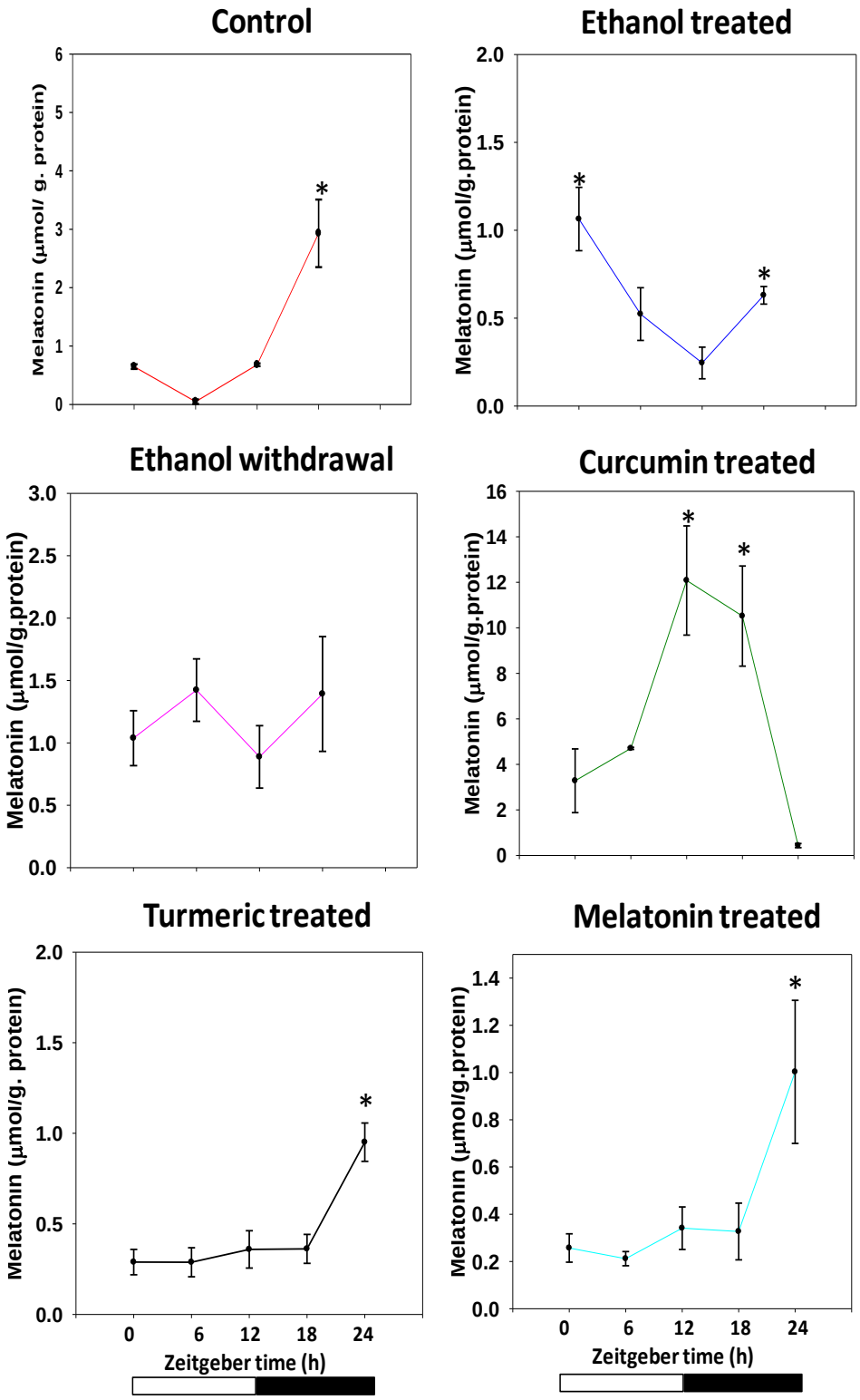

Fig. 29. Curcumin effect on ethanol induced changes in daily rhythms of MEL. Each value is mean ± SE, (n=6); Zeitgeber Time (ZT): ZT-0 = 6.30 A. M (Lights on); ZT-12 = 18.30 P. M (Lights off). One Way ANOVA: * Refers to comparison with lowest value of time point with other time points in each group ($p \leq 0.05$).

5-MIAA:

Group I: 5-MIAA showed daily rhythmicity and levels measured at various time points such as ZT- 0, 6, 12 and 18 were 0.84 ± 0.16, 2.80 ± 1.3, 1.20 ± 0.40 and 0.16 ± 0.03 µmol/g protein respectively. The 5-MIAA levels were maximum at subjective mid-day (ZT-6) and minimum at subjective mid-night (ZT-18). Group II: 5-MIAA levels at various time points such as ZT- 0, 6, 12 and 18 were 0.52 ± 0.23, 0.33 ± 0.08, 0.14 ± 0.03 and 0.29 ± 0.08 µmol/g protein respectively. 5-MIAA levels were showed no significant difference between all time points. Both mean and daily pulses ($p \leq 0.05$) were decreased without affecting the rhythmicity but phase advance was observed by ~6h. Group III: 5-MIAA levels at various time points such as ZT- 0, 6, 12 and 18 were 0.33 ± 0.11, 0.48 ± 0.06, 0.17 ± 0.03 and 0.90 ± 0.3 µmol/g protein respectively, were showed significant increase (5 fold) at ZT-18 ($p \leq 0.05$). Both mean and daily pulses ($p \leq 0.05$) were decreased with abolition in rhythmicity but phase delay was observed by ~12h. Group IV: 5-MIAA levels at various time points such as ZT- 0, 6, 12, 18 and 24 were 2.58 ± 0.46, 1.64 ± 0.63, 4.30 ± 0.85, 5.82 ± 0.81 and 0.23 ± 0.06 µmol/g protein respectively. The levels were significantly increase at ZT-0 (1.5 fold), 12 (2.5 fold) and 18 (3 fold) ($p \leq 0.05$). Both mean ($p \leq 0.05$) and daily pulse were increased with restoration in rhythmicity but phase delay was observed by ~12h. Group V: 5-MIAA levels at various time points such as ZT- 0, 6, 12, 18 and 24 were 0.25±0.03, 0.54 ± 0.18, 0.33 ± 0.07, 0.39 ± 0.11 and 0.66 ± 0.13 µmol/g protein respectively. The levels were showed no significant difference at all time points. Both mean and daily pulses ($p \leq 0.05$) were decreased with abolition in rhythmicity. Group VI: 5-MIAA levels at various time points such as ZT- 0, 6, 12, 18 and 24 were 0.29 ± 0.06, 0.23 ± 0.06, 0.37 ± 0.08, 0.21 ± 0.04 and 0.51 ± 0.13 µmol/g protein respectively. Significant difference was not observed between time points. Both mean and daily pulses ($p \leq 0.05$) were decreased with abolition in rhythmicity (Fig. 30, 32 and 33; Table 4).

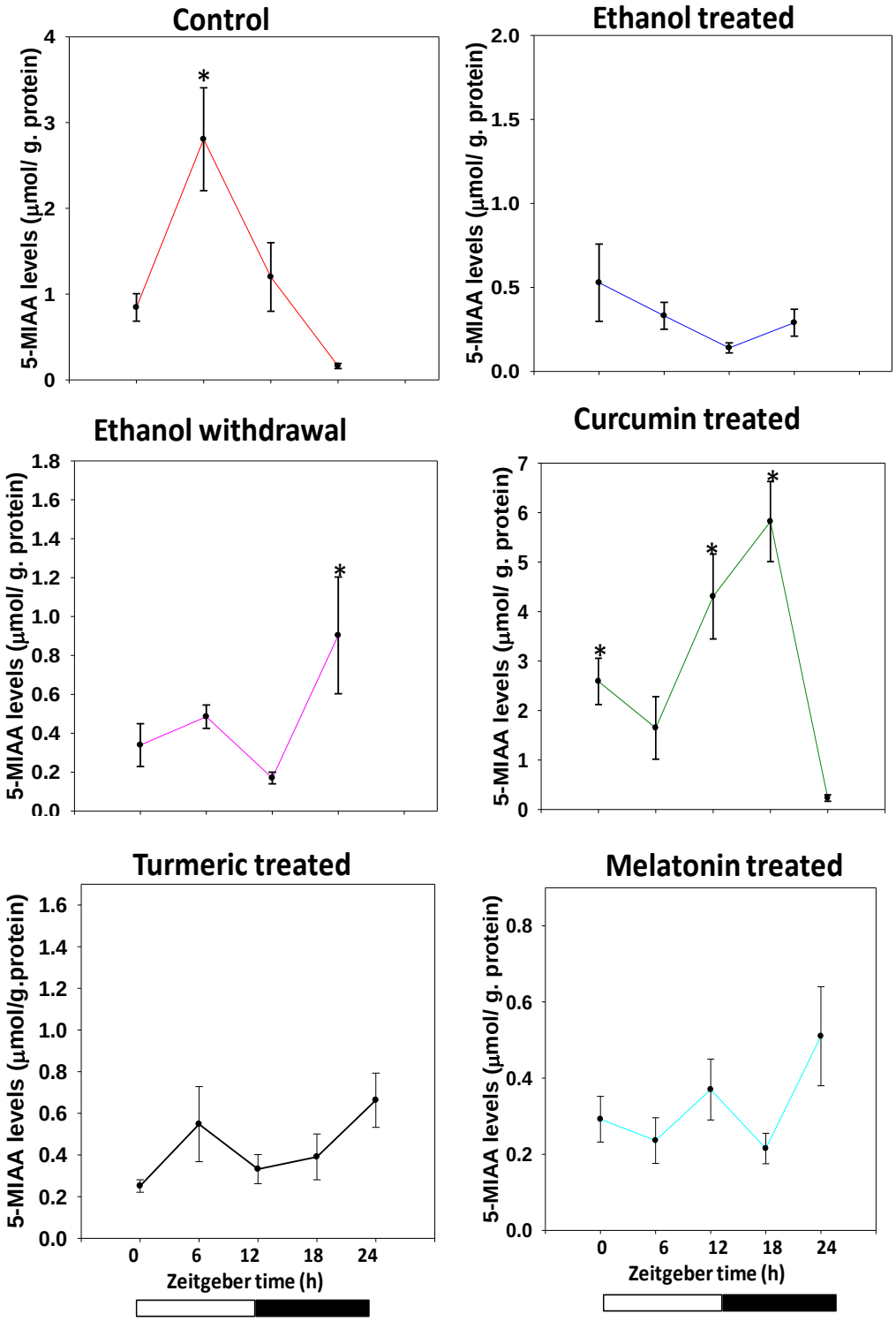

Fig. 30. Curcumin effect on ethanol induced changes in daily rhythms of 5-MIAA. Each value is mean ± SE, (n=6); Zeitgeber Time (ZT): ZT-0 = 6.30 A. M (Lights on); ZT-12 = 18.30 P. M (Lights off). One Way ANOVA: * Refers to comparison with lowest value of time point with other time points in each group ($p \leq 0.05$).

NAT:

Group I: NAT showed daily rhythmicity and levels measured at various time points such as ZT- 0, 6, 12 and 18 were 0.11 ± 0.02, 0.24 ± 0.09, 0.06 ± 0.001 and 0.02 ± 0.002 µmol/g protein respectively. The NAT levels were maximum at subjective mid-day (ZT-6) and minimum at subjective mid-night (ZT-18). Group II: NAT levels at various time points such as ZT- 0, 6, 12 and 18 were 0.055 ± 0.001, 0.05 ± 0.02, 0.03 ± 0.01 and 0.03 ± 0.01 µmol/g protein respectively. NAT levels were showed no significant difference between time points. Both mean and daily pulses were decreased significantly without affecting the rhythmicity. Group III: NAT levels at various time points such as ZT- 0, 6, 12 and 18 were 0.04 ± 0.008, 0.07 ± 0.02, 0.03 ± 0.009 and 0.11 ± 0.03 µmol/g protein respectively, showed significant increase (33 fold) at ZT-18 ($p \leq 0.05$). Both mean and daily pulse ($p \leq 0.05$) was decreased with abolition in rhythmicity. Phase delay (~12h) was also observed. Group IV: NAT levels at various time points such as ZT- 0, 6, 12, 18 and 24 were 0.13 ± 0.04, 0.16 ± 0.05, 0.38 ± 0.11, 0.19 ± 0.045 and 0.028 ± 0.008 µmol/g protein respectively. The levels were significantly increased at ZT-12 ($p \leq 0.05$). Both mean ($p \leq 0.05$) and daily pulse was increased with restoration in rhythmicity. ~6h phase delay was observed. Group V: NAT levels at various time points such as ZT- 0, 6, 12, 18 and 24 were 0.02 ± 0.01, 0.02 ± 0.01, 0.01 ± 0.003, 0.01 ± 0.002 and 0.03 ± 0.008 µmol/g protein respectively. The levels were showed no significant difference at all time points. Both mean and daily pulse was decreased significantly ($p \leq 0.05$) with restoration in rhythmicity. Group VI: NAT levels at various time points such as ZT- 0, 6, 12, 18 and 24 were 0.008 ± 0.001, 0.01 ± 0.002, 0.01 ± 0.002, 0.01 ± 0.002 and 0.03 ± 0.008 µmol/g protein respectively. The levels were significantly increased at ZT-24 ($p \leq 0.05$). Both mean and daily pulse was decreased significantly ($p \leq 0.05$) with restoration in rhythmicity (Fig. 31, 32 and 33; Table 4).

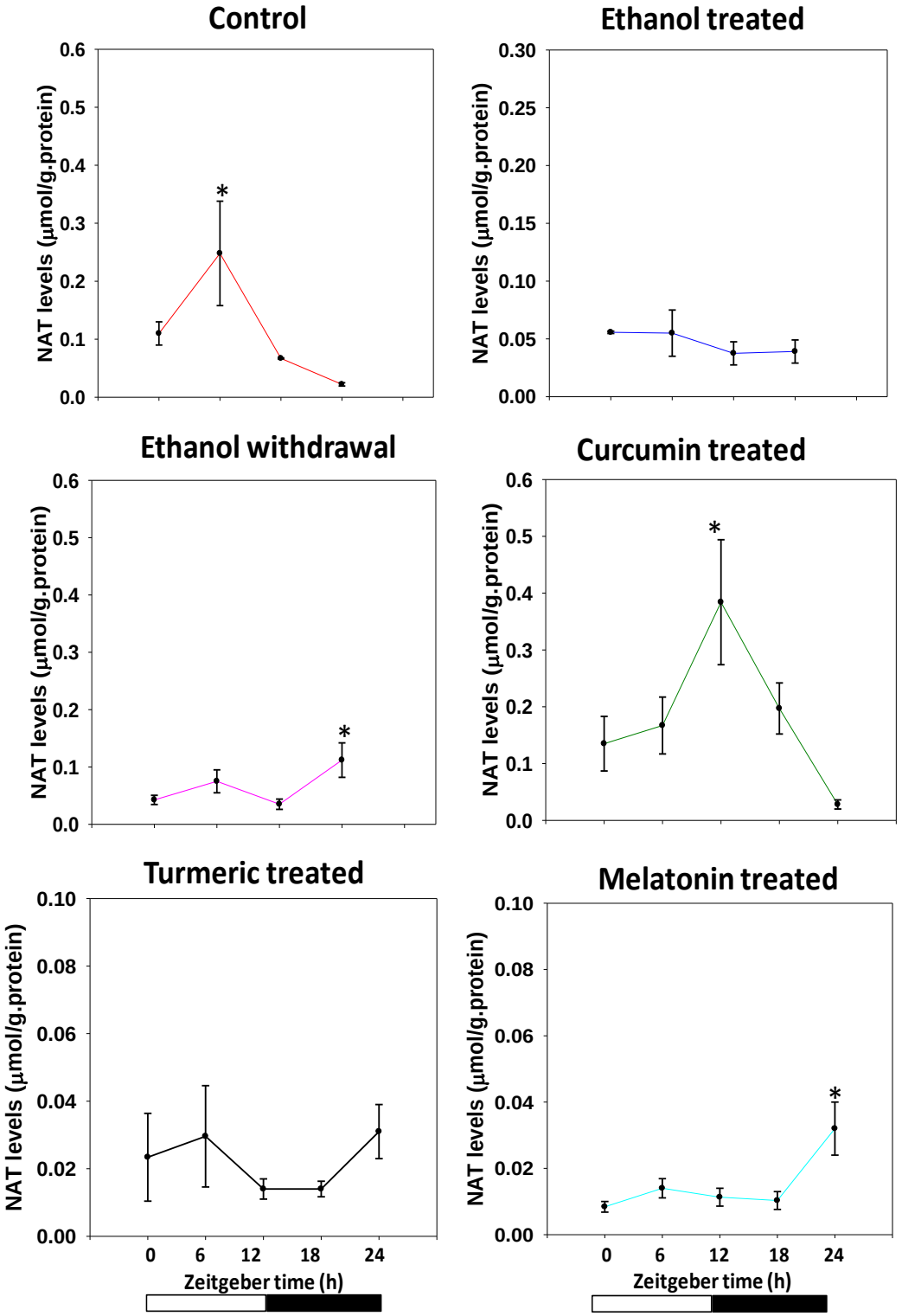

Fig. 31. Curcumin effect on ethanol induced changes in daily rhythms of NAT. Each value is mean ± SE, (n=6); Zeitgeber Time (ZT): ZT-0 = 6.30 A. M (Lights on); ZT-12 = 18.30 P. M (Lights off). One Way ANOVA: * Refers to comparison with lowest value of time point with other time points in each group ($p \leq 0.05$).

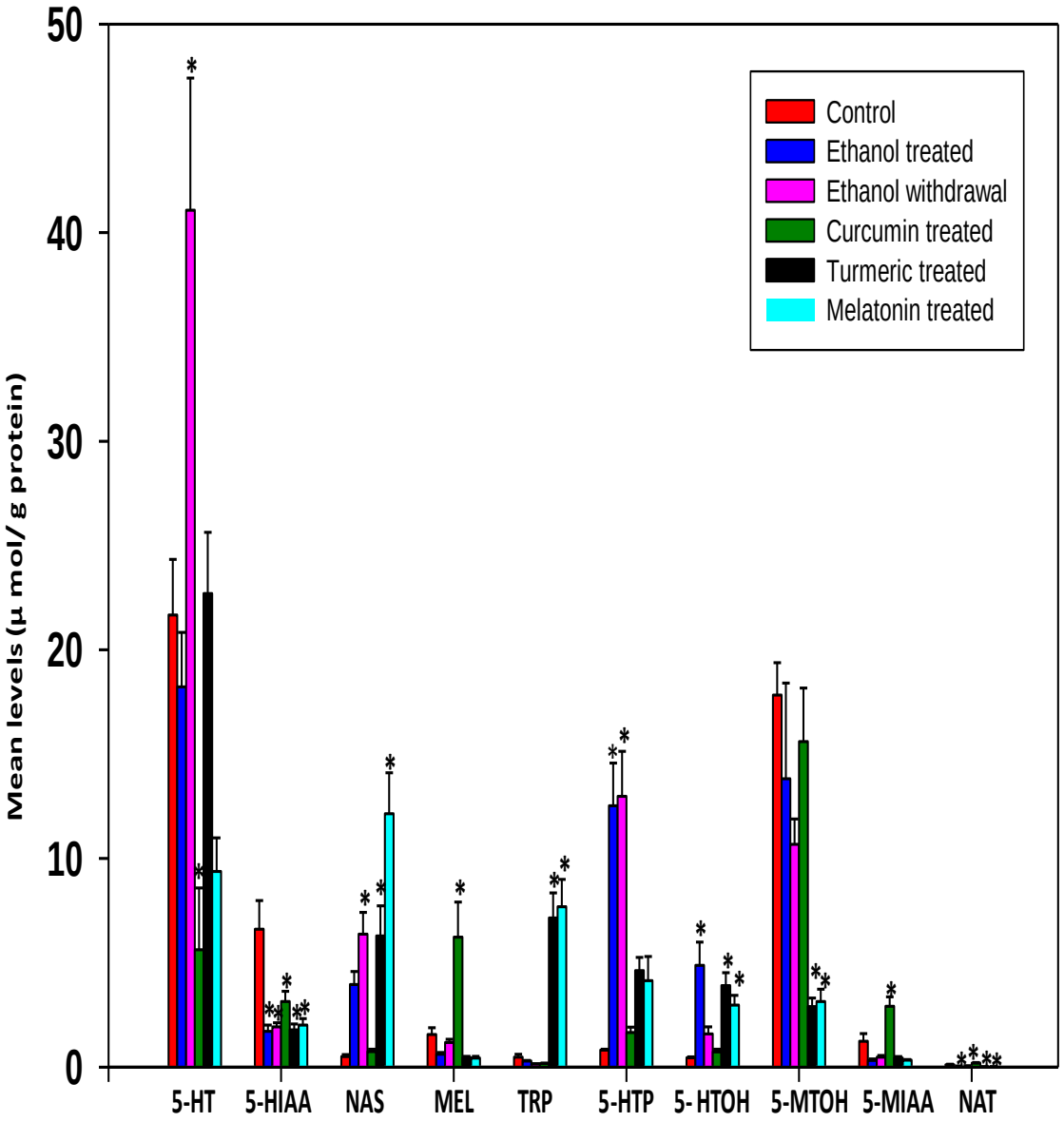

Fig. 32. Curcumin effect on ethanol induced changes in daily mean levels of serotonin chronometabolome in SCN. Each value is mean ± SE, (n=6); One Way ANOVA: * Refers to comparison with control ($p < 0.05$).

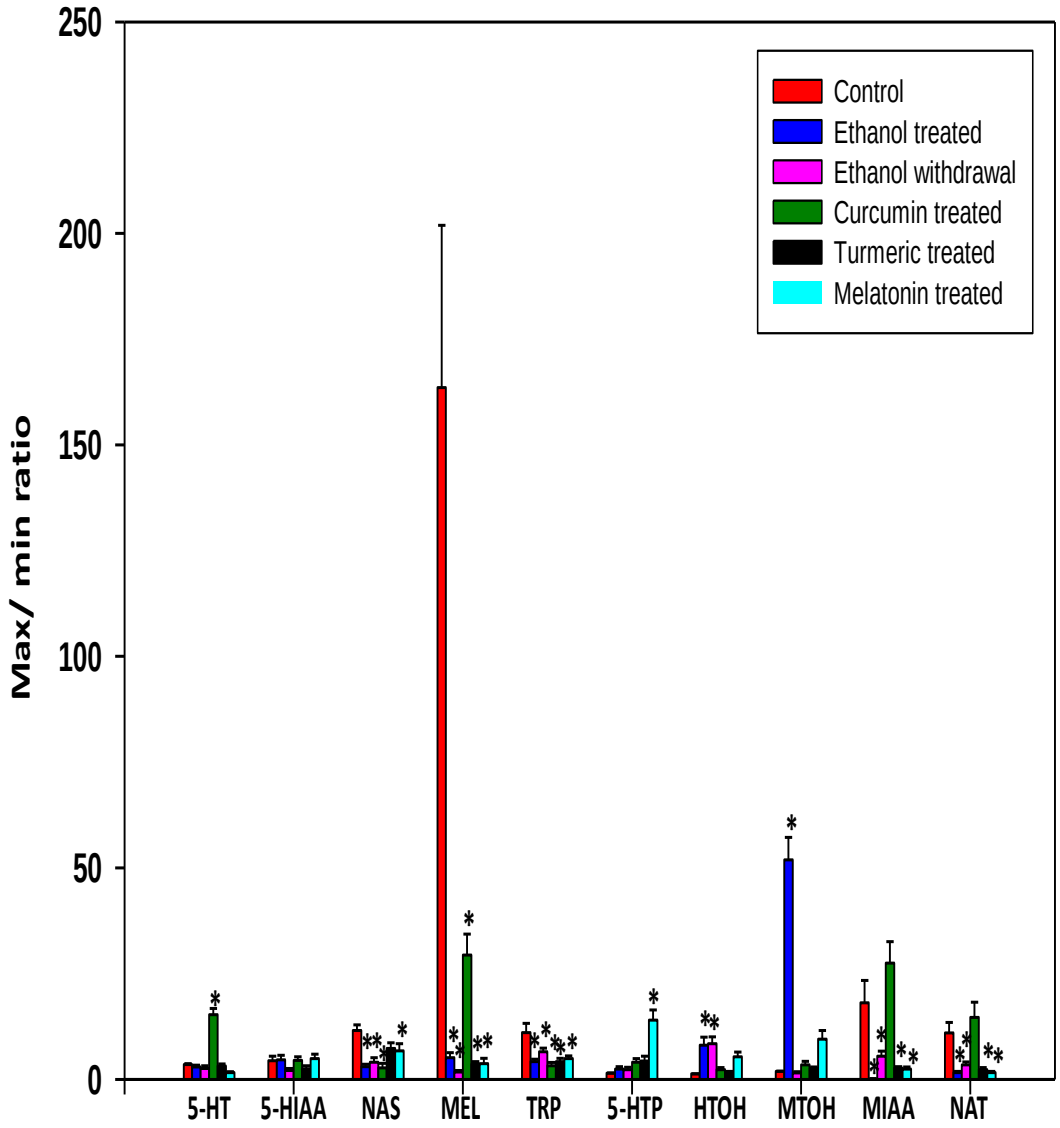

Fig. 33. Curcumin effect on ethanol induced changes in daily pulse levels of serotonin chronometabolome in SCN. Each value is mean ± SE, (n=6); One Way ANOVA: * Refers to comparison with control ($p < 0.05$).

Table 4. Curcumin effect on ethanol induced changes in daily mean and pulse levels of serotonin chronometabolome in SCN.

EXP		5-HT	5-HIAA	5-HTP	5- HTOH	NAS	TRP	5-MTOH	MEL	MIAA	NAT
C	Mean	21.6±2.67	6.6± 1.37	0.8±0.05	0.44±0.04	0.49±0.1	0.46±0.14	17.8± 1.5	1.56± 0.32	1.23± 0.3	0.11 ± 0.02
	Max:Min	3.472±0.24	4.4±1.01	1.4±.0.09	1.29±0.04	11.5±1.34	11.0±4.1*	1.8±0.08	163±78.4*	18.0±5.3	11.0±2.46
	Rhythm	Persistent	Persistent	Persistent	Persistent	Persistent	Persistent	Persistent	Persistent	Persistent	Persistent
ET	Mean	18.2± 2.62	1.72±0.2*	12±2.04*	4.86±1.1*	3.96±0.62	0.28±0.03	13.8±4.59	0.61±0.08	0.32±0.06	0.03± 0.008*
	Max:Min	2.90±0.48	4.65±1.05	2.42±0.61	8.09±1.9*	2.99±0.5*	4.22±0.5*	51±10.3*	5.10±1.24*	5.38±1.1*	1.60 ± 0.25*
	Rhythm	Persistent	Persistent	Abolished	Abolished	Abolished	Persistent	Persistent	Persistent	Persistent	Persistent
EW	Mean	41.07±6.33	1.92±0.2*	12.9±2.1*	1.58±0.35	6.37±1.0*	0.14±0.02	10.66±1.2	1.18±0.154	0.47±0.09	0.053 ±0.011
	Max:Min	2.57 ± 0.61	2.11±0.45	2.27±0.60	8.40±1.6*	4.0±1.08*	6.52±0.8*	1.55±0.28	1.75±0.34*	5.46±1.2*	3.42 ± 0.74*
	Rhythm	Abolished	Persistent	Abolished	Persistent	Abolished	Persistent	Abolished	Abolished	Abolished	Abolished
CT	Mean	5.61±2.97*	3.1±0.49*	1.65±0.25	0.73 ±0.12	0.73±0.12	0.18±0.12	15.6± 2.57	6.22±1.68*	2.91± 0.4*	0.192 ± 0.03*
	Max:Min	15.3±9.44*	4.46±0.87	4.09±0.79	2.27±0.49	2.7±1.02*	3.22± 0.8*	3.42±0.86	29.3±4.94*	27.4±5.04	14.62 ± 3.60
	Rhythm	Persistent	Persistent	Persistent	Persistent	Abolished	Persistent	Persistent	Persistent	Persistent	Persistent
TT	Mean	22.6±2.9	1.78±0.2*	4.62±0.63	3.90±0.6*	6.29±1.4*	7.15±1.2 *	2.90±0.4*	0.450±0.05	0.43±0.05	0.023±0.004*
	Max:Min	3.16±0.48	2.51±0.69	4.34±1.11	1.58±0.27	7.32±3.30	4.21±0.7*	2.61±0.29	3.57±0.62*	2.67±0.3*	2.320±0.45*
	Rhythm	Abolished	Persistent	Persistent	Persistent	Persistent	Abolished	Abolished	Abolished	Persistent	Persistent
MT	Mean	9.37±1.615	2.01±0.3*	4.13±1.16	2.97±0.4*	12.1±1.9*	7.68±1.3*	3.14±0.5*	0.42±0.084	0.32±0.04	0.015±0.002*
	Max:Min	1.56±0.255	4.91±1.05	14.0±2.3*	5.30±1.13	6.74±2.6*	4.8±0.67*	9.49±2.05	3.73±1.25*	2.45±0.4*	1.576±0.32*
	Rhythm	Abolished	Persistent	Abolished	Abolished	Persistent	Abolished	Abolished	Abolished	Abolished	Persistent

Each value is mean ± SE, (n=6); One Way ANOVA: * Refers to comparison with control (p< 0.05).

2. Curcumin effect on ethanol induced changes in daily rhythms of serotonin (5-HT) chronometabolome in Pineal:

5-HT:

Group I: 5-HT showed daily rhythmicity and levels measured at various time points such as ZT- 0, 6, 12 and 18 were 133.79 ± 15.94, 235.63 ± 3.19, 36.24 ± 4.19 and 64.02 ± 4.23 µmol/g protein respectively. The 5-HT levels were maximum at subjective mid-day (ZT-6) and minimum at subjective mid-night (ZT-18). Group II: 5-HT levels at various time points such as ZT- 0, 6, 12 and 18 were 136.60 ± 17.24, 53.14 ± 9.42, 20.41 ± 3.33 and 12.88 ± 3.69 µmol/g protein respectively. 5-HT levels showed significant increase at ZT-0 (11 fold) and ZT-6 (4 fold) ($p \leq 0.05$). Decrease in mean level ($p \leq 0.05$) and increase in daily pulse ($p \leq 0.05$) were observed without affecting the rhythmicity. Phase advance was observed by ~6h. Group III: 5-HT levels at various time points such as ZT- 0, 6, 12 and 18 were 19.20 ± 6.46, 40.69 ± 15.30, 19.68 ± 9.01 and 6.02 ± 0.91 µmol/g protein respectively, showed no significant difference at all time points. Daily pulse was increased ($p \leq 0.05$) and mean level was decreased without affecting the rhythmicity. Group IV: 5-HT levels at various time points such as ZT- 0, 6, 12, 18 and 24 were 59.35 ± 14.31, 191.40 ± 44.21, 92.08 ± 10.49, 91.25 ± 14.36 and 64.15 ± 6.21 µmol/g protein respectively. The levels were significantly increase (3.5 fold) at ZT-6 ($p \leq 0.05$). Restoration in daily pulse, phase and partial restoration in mean levels were observed. Group V: 5-HT levels at various time points such as ZT- 0, 6, 12, 18 and 24 were 38.96 ± 9.89, 9.22 ± 1.90, 42.09 ± 6.11, 45.83 ± 4.09 and 47.41 ± 12.42 µmol/g protein respectively. The levels were significantly decreased (5 fold) ZT-6 ($p \leq 0.05$). Mean level was decreased ($p \leq 0.05$) and daily pulse was increased with abolition in rhythmicity and ~6h phase delay. Group VI: 5-HT levels at various time points such as ZT- 0, 6, 12, 18 and 24 were 15.87 ± 2.22, 2.58 ± 0.72, 11.411 ± 3.44, 7.96 ± 1.75 and 20.44 ± 8.591 µmol/g protein respectively. The levels were showed no significant difference between all time points. Mean level was decreased ($p \leq 0.05$) and daily pulse was increased with abolition in rhythmicity and ~6h phase advance (Fig. 34, 44 and 45; Table 5).

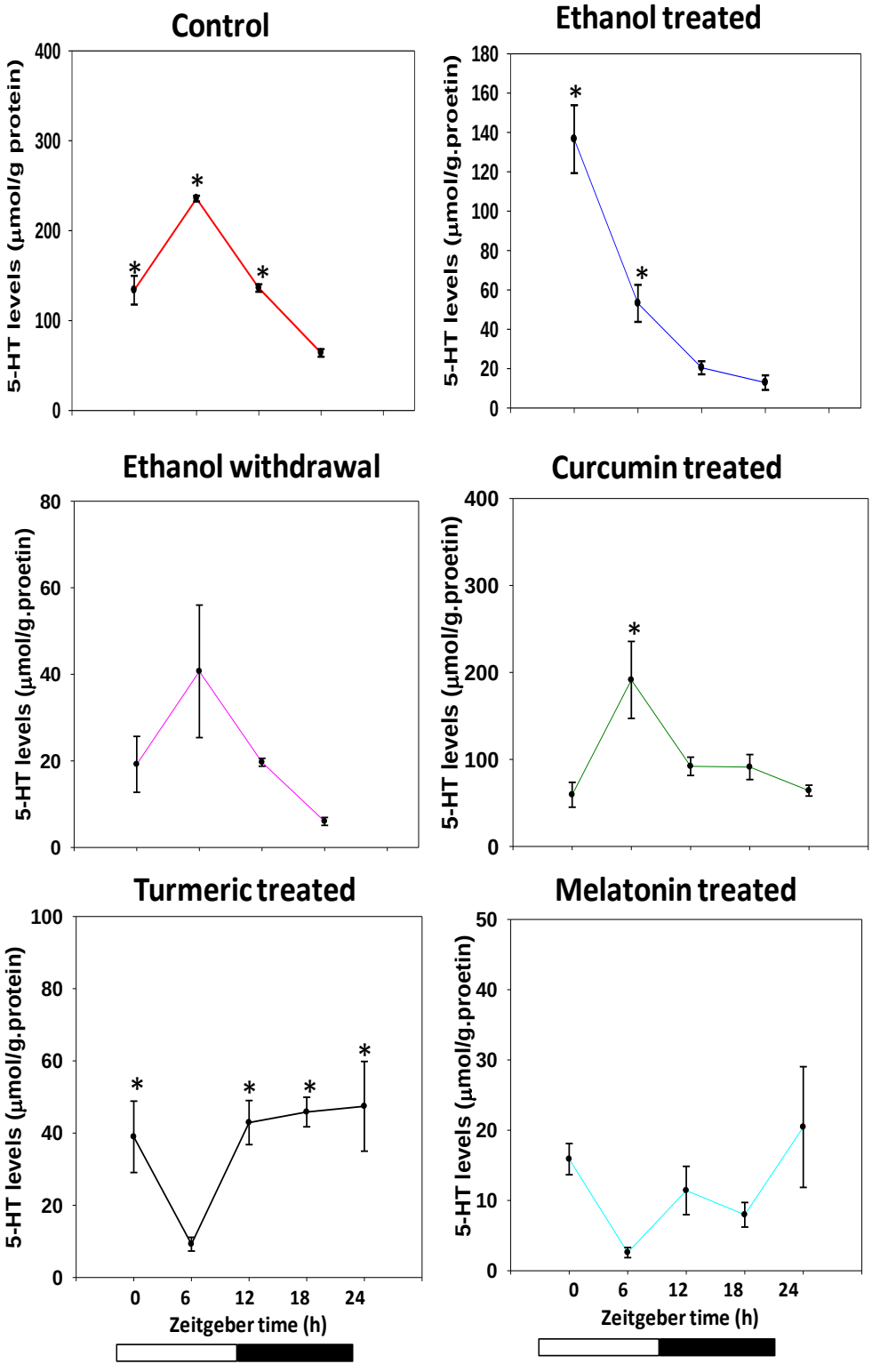

Fig. 34. Curcumin effect on ethanol induced changes in daily rhythms of 5-HT. Each value is mean ± SE, (n=6); Zeitgeber Time (ZT): ZT-0 = 6.30 A. M (Lights on); ZT-12 = 18.30 P. M (Lights off). One Way ANOVA: * Refers to comparison with lowest value of time point with other time points in each group ($p \leq 0.05$).

5-HIAA:

Group I: 5-HIAA showed daily rhythmicity and levels measured at various time points such as ZT- 0, 6, 12 and 18 were 11.98 ± 1.54, 18 ± 1.81, 10.75 ± 1.125 and 2.79 ± 0.71 µmol/g protein respectively. The 5-HIAA levels were maximum at ZT-6 and minimum at ZT-18. Group II: 5-HIAA levels at various time points such as ZT- 0, 6, 12 and 18 were 1.51 ± 0.24, 2.21 ± 0.50, 1.37 ± 0.32 and 2.15 ± 0.71 µmol/g protein respectively. 5-HIAA levels were showed no significant difference at all time points. Both mean and daily pulses were greatly decreased with abolition in rhythmicity and ~12h phase delay. Group III: 5-HIAA levels at various time points such as ZT- 0, 6, 12 and 18 were 13.69 ± 0.72, 30.95 ± 8.65, 15.06 ±2.13 and 8.56 ±1.43 µmol/g protein respectively and showed significant increase (4 fold) at ZT-6 ($p \leq 0.05$). Increase in mean level ($p \leq 0.05$) and decrease in pulse level was observed with restoration in phase as well as rhythmicity. Group IV: 5-HIAA levels at various time points such as ZT- 0, 6, 12, 18 and 24 were 10.08 ± 4.62, 11.39 ± 4.01, 4.04 ± 1.10, 2.18 ± 0.63 and 8.77 ± 0.61 µmol/g protein respectively. Significant difference was not observed at all time points ($p \leq 0.05$). Decrease in both mean and daily pulse levels were observed with restoration in phase as well as rhythm. Group V: 5-HIAA levels at various time points such as ZT- 0, 6, 12, 18 and 24 were 8.40 ± 2.65, 4.15 ± 0.68, 7.27 ± 1.91, 9.56 ± 0.83 and 10.72 ± 2.68 µmol/g protein respectively. The levels were showed no significant difference at all time points. Decrease in both mean and daily pulse levels were observed with restoration in rhythm but not in phase (advance ~6h). Group VI: 5-HIAA levels at various time points such as ZT- 0, 6, 12, 18 and 24 were 2.46 ± 0.41, 18.15 ± 5.76, 1.76 ± 0.39, 4.60 ± 0.95 and 8.83 ± 2.41 µmol/g protein respectively. The levels were significantly increase (9 fold) at ZT-6 ($p \leq 0.05$). Mean level was decreased and daily pulse level was increased with restoration in rhythm as well as phase (Fig. 35, 44 and 45; Table 5).

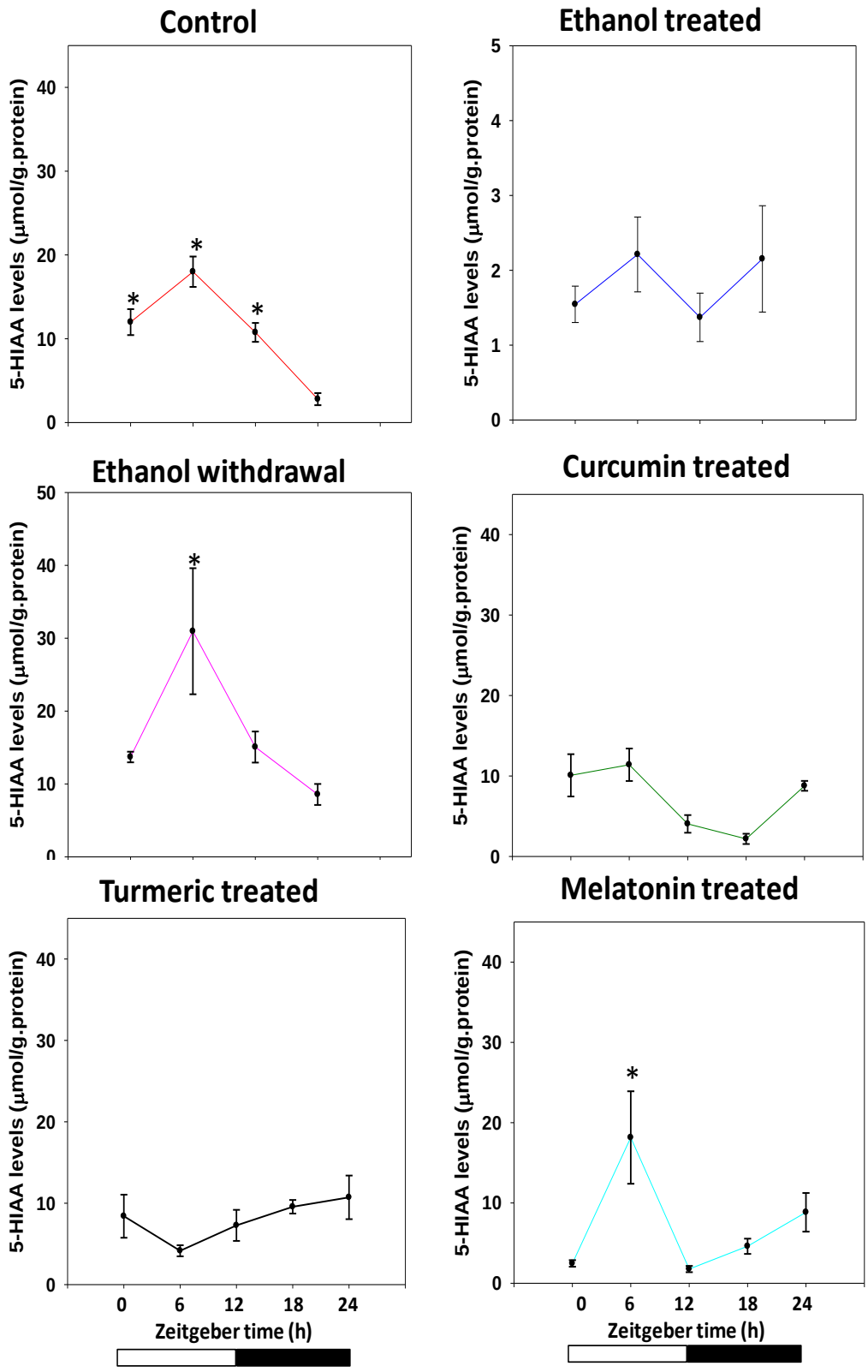

Fig. 35. Curcumin effect on ethanol induced changes in daily rhythms of 5-HIAA. Each value is mean ± SE, (n=6); Zeitgeber Time (ZT): ZT-0 = 6.30 A. M (Lights on); ZT-12 = 18.30 P. M (Lights off). One Way ANOVA: * Refers to comparison with lowest value of time point with other time points in each group ($p \leq 0.05$).

5-HTP:

Group I: 5-HTP showed daily rhythmicity and levels measured at various time points such as ZT- 0, 6, 12 and 18 were 5.86 ± 0.20, 4.54 ± 0.59, 3.96 ± 0.23 and 3.53 ± 0.92 µmol/g protein respectively. The 5-HTP levels were maximum at ZT-0 and minimum at ZT-12. Group II: 5-HTP levels at various time points such as ZT- 0, 6, 12 and 18 were 10.74 ± 6.68, 7.92 ± 2.01, 2.46 ± 0.73 and 9.11 ± 2.03 µmol/g protein respectively. 5-HTP levels were showed no significant difference all time points. Increase in mean as well as daily pulse was observed without affecting rhythmicity. Group III: 5-HTP levels at various time points such as ZT- 0, 6, 12 and 18 were 16.89 ± 3.20, 28.84 ± 11.25, 17.06 ± 1.27 and 9.15 ± 2.21 µmol/g protein respectively, showed no significant difference at all time points. Increase in mean ($p \leq 0.05$) as well daily pulse was observed with ~6h phase delay. Group IV: 5-HTP levels at various time points such as ZT- 0, 6, 12, 18 and 24 were 2.32 ± 0.35, 2.97 ± 0.98, 2.18 ± 0.35, 4.04 ± 0.86 and 1.95 ± 0.51 µmol/g protein respectively. Significant difference was not observed at all time points. Partial restoration was observed in mean as well as daily pulse. Rhythmicity was not restored and ~6h phase advance was observed. Group V: 5-HTP levels at various time points such as ZT- 0, 6, 12, 18 and 24 were 2.04 ± 0.38, 1.89 ± 0.37, 0.93 ± 0.19, 1.10 ± 0.19 and 2.61 ± 0.83 µmol/g protein respectively. The levels were showed no significant difference at all time points. Decrease in mean and increase in daily pulse was observed with restoration in rhythmicity. Group VI: 5-HTP levels at ZT- 0, 6, 12, 18 and 24 were 15.17 ± 3.09, 24.54 ± 7.96, 3.06 ± 0.92, 3.75 ± 0.98 and 33.74 ± 9.42 µmol/g protein respectively. The levels were significantly increase at ZT-6 (5 fold) and 24 (10 fold) ($p \leq 0.05$). Both mean and daily pulse was increased significantly ($p \leq 0.05$) with restoration in rhythmicity. Phase delay was observed by ~6h (Fig. 36, 44 and 45; Table 5).

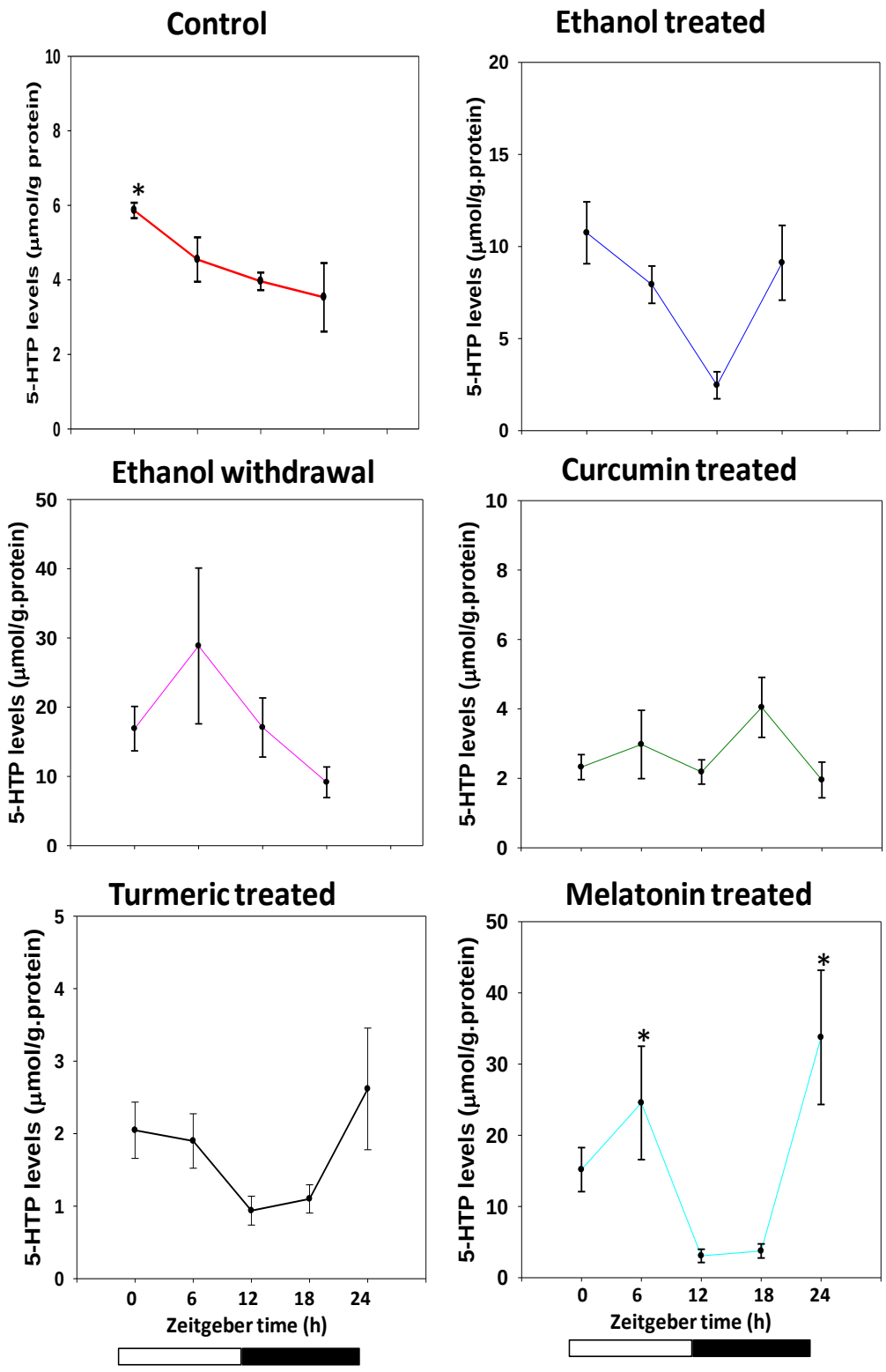

Fig. 36. Curcumin effect on ethanol induced changes in daily rhythms of 5-HTP. Each value is mean ± SE, (n=6); Zeitgeber Time (ZT): ZT-0 = 6.30 A. M (Lights on); ZT-12 = 18.30 P. M (Lights off). One Way ANOVA: * Refers to comparison with lowest value of time point with other time points in each group ($p \leq 0.05$).

5-HTOH:

Group I: 5-HTOH showed daily rhythmicity and levels measured at various time points such as ZT- 0, 6, 12 and 18 were 4.18 ± 1.21, 1.55 ±0.33, 0.99 ± 0.23 and 1.64 ± 0.63 µmol/g protein respectively. The 5-HTOH levels were maximum at ZT-0 and minimum at ZT-12. Group II: 5-HTOH at various time points such as ZT- 0, 6, 12 and 18 were 18.52 ± 6.87, 2.44 ± 0.82, 0.55 ± 0.10 and 4.67 ± 0.35 µmol/g protein respectively. 5-HTOH levels were showed significant increase (35 fold) at ZT-0 ($p \leq 0.05$). Daily pulse ($p \leq 0.05$) as well as mean was increased without affecting the rhythmicity. Group III: 5-HTOH levels at various time points such as ZT- 0, 6, 12 and 18 were 8.97 ± 3.18, 11.43 ± 3.85, 16.99 ± 4.97 and 12.17 ± 3.07 µmol/g protein respectively, showed no significant difference between all time points. Increase in mean ($p \leq 0.05$) and decrease in daily pulse level was observed without affecting the rhythmicity. Phase delay was observed by ~12h. Group IV: 5-HTOH levels at various time points such as ZT- 0, 6, 12, 18 and 24 were 4.73 ± 3.64, 11.49 ± 4.77, 1.628 ± 0.61, 1.23 ± 0.87 and 0.71 ± 0.45 µmol/g protein respectively. Significantly difference was not observed at all time points. Increase in mean as well as daily pulse was observed with ~6h phase delay. Group V: 5-HTOH levels at various time points such as ZT- 0, 6, 12, 18 and 24 were 5.43 ± 2.69, 8.35 ± 1.71, 3.44 ± 0.77, 3.86 ± 0.52 and 4.54 ± 0.657 µmol/g protein respectively. The levels were showed no significant difference at all time points. Increase in mean and decrease in daily pulses were observed with ~6h phase delay. Group VI: 5-HTOH levels at various time points such as ZT- 0, 6, 12, 18 and 24 were 20.19 ± 3.70, 18.92 ± 6.22, 49.74 ± 13.98, 15.69 ± 3.75 and 15.71 ± 4.02 µmol/g protein respectively. The levels were significantly increased (3 fold) at ZT-12 ($p \leq 0.05$). Increase in mean and decrease in daily pulse ($p \leq 0.05$) was observed with ~12h phase delay (Fig. 37, 44 and 45; Table 5).

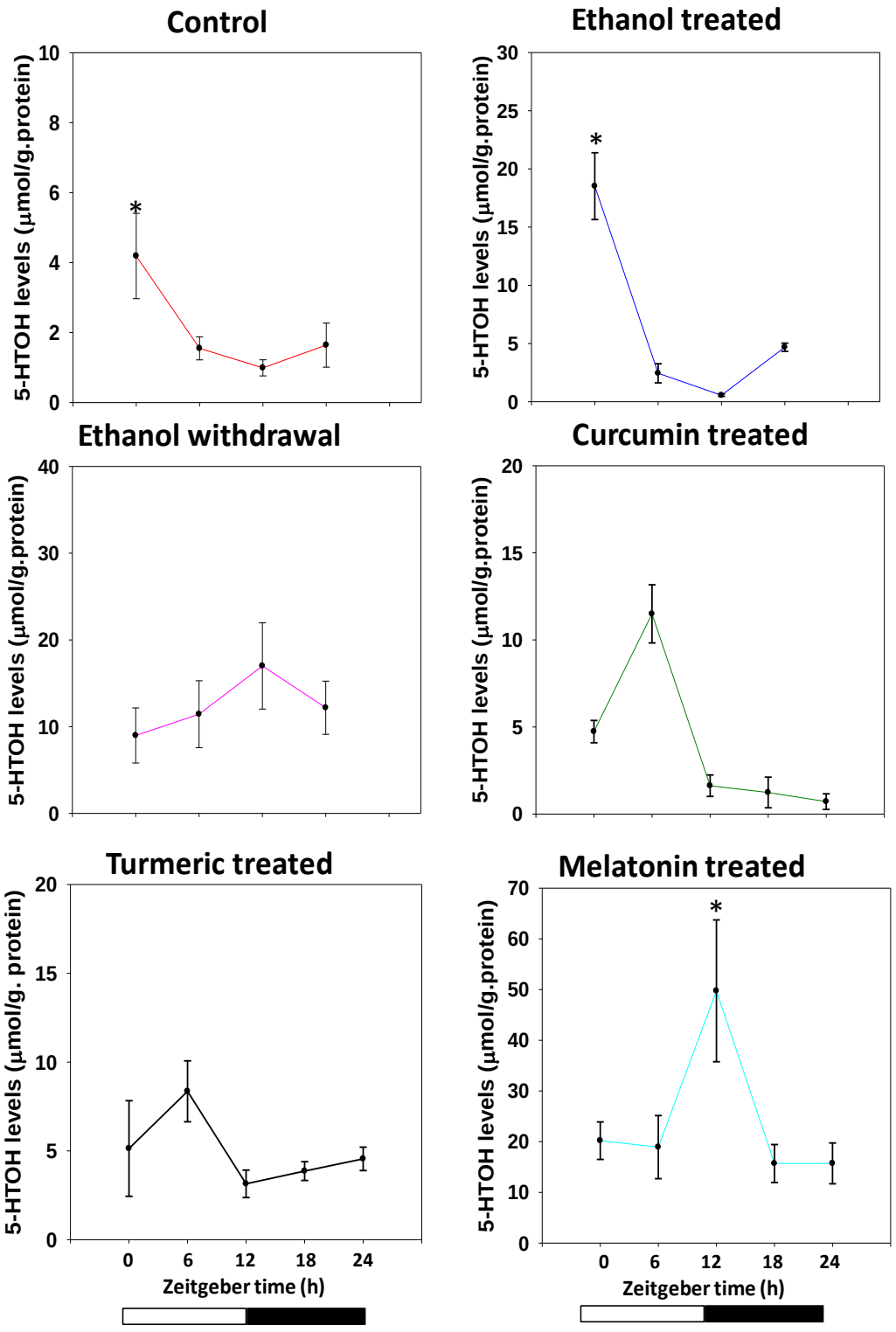

Fig. 37. Curcumin effect on ethanol induced changes in daily rhythms of 5-HTOH. Each value is mean ± SE, (n=6); Zeitgeber Time (ZT): ZT-0 = 6.30 A. M (Lights on); ZT-12 = 18.30 P. M (Lights off). One Way ANOVA: * Refers to comparison with lowest value of time point with other time points in each group ($p \leq 0.05$).

NAS:

Group I: NAS showed daily rhythmicity and levels measured at various time points such as ZT- 0, 6, 12 and 18 were 1.00 ± 0.20, 0.86 ± 0.34, 4.46 ± 0.43 and 6.89 ± 0.31 µmol/g protein respectively. The NAS levels were maximum at subjective mid-night (ZT-18) and minimum at subjective mid-day (ZT-6). Group II: NAS levels at various time points such as ZT- 0, 6, 12 and 18 were 1.05 ± 0.40, 1.53 ± 0.22, 0.87 ±0.23 and 2.68 ± 0.68 µmol/g protein respectively. NAS levels were showed significant increase (3 fold) at ZT-18 ($p \leq 0.05$). Both mean and daily pulse was decreased without affecting the rhythmicity. Group III: NAS levels at various time points such as ZT- 0, 6, 12 and 18 were 0.91 ± 0.41, 1.34 ± 0.41, 0.85 ± 0.24 and 1.88 ± 0.17 µmol/g protein respectively and levels were showed no significant difference at all time points. Both mean and daily pulse was decreased with abolition in rhythmicity. Group IV: NAS levels at various time points such as ZT- 0, 6, 12, 18 and 24 were 0.73 ± 0.43, 0.31 ± 0.18, 0.20 ± 0.004, 1.100 ± 0.58 and 0.24 ± 0.05 µmol/g protein respectively. The levels were showed no significant difference at all time points. Both mean and daily pulse was decreased with restoration in rhythmicity. Group V: NAS levels at various time points such as ZT- 0, 6, 12, 18 and 24 were 29.96 ± 10.13, 23.31 ± 3.97, 35.75 ± 7.43, 50.54 ± 12.82 and 70.46 ± 20.68 µmol/g protein respectively. The levels were showed no significant difference at all time points. Mean level was increased significantly ($p \leq 0.05$) and decrease in daily pulse was observed without restoration in rhythmicity and ~6h phase delay was also observed. Group VI: NAS levels at various time points such as ZT- 0, 6, 12, 18 and 24 were 113.17 ± 19.19, 59.91 ± 16.21, 73.89 ± 26.18, 1.96 ±0.44 and 5.38± 1.65 µmol/g protein respectively. The levels were significantly increased at ZT-0, 6, 12 ($p \leq 0.05$). Significant increase in mean as well as daily pulse was observed without restoration in rhythmicity and ~6h phase delay was observed (Fig. 38, 44 and 45; Table 5).

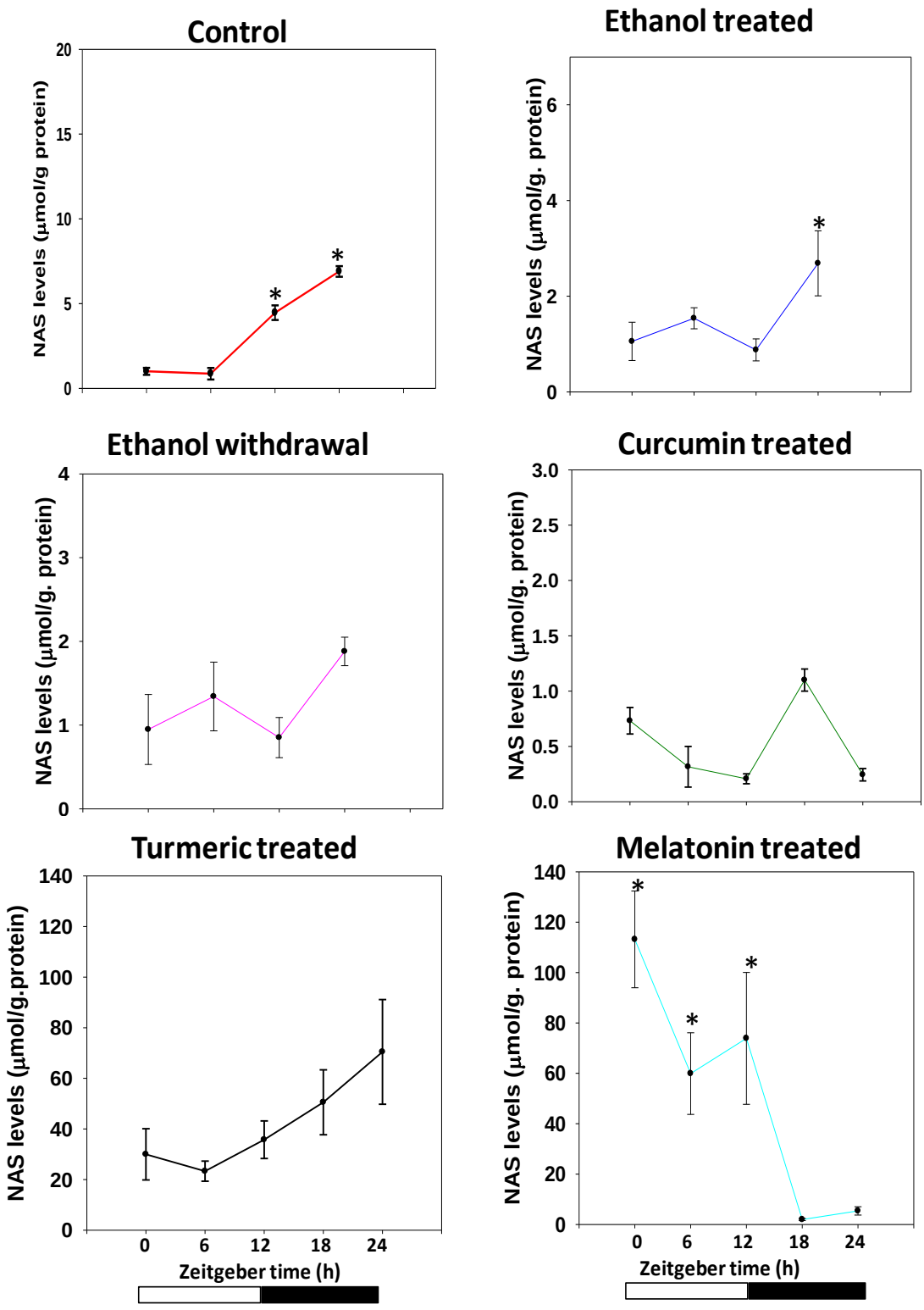

Fig. 38. Curcumin effect on ethanol induced changes in daily rhythms of NAS. Each value is mean ± SE, (n=6); Zeitgeber Time (ZT): ZT-0 = 6.30 A. M (Lights on); ZT-12 = 18.30 P. M (Lights off). One Way ANOVA: * Refers to comparison with lowest value of time point with other time points in each group ($p \leq 0.05$).

TRP:

Group I: TRP showed daily rhythmicity in control and levels measured at various time points such as ZT- 0, 6, 12 and 18 were 0.11 ± 0.006, 0.14 ± 0.03, 0.17 ± 0.04 and 0.84 ± 0.10 µmol/g protein respectively. The TRP levels were maximum at ZT-18 and minimum at ZT-0. Group II: TRP levels at various time points such as ZT-0, 6, 12 and 18 were 0.12 ± 0.05, 0.28 ± 0.14, 0.15 ± 0.06 and 0.49 ± 0.12 µmol/g protein respectively. TRP levels were showed significant increase (4 fold) at ZT-18 ($p \leq 0.05$). Both mean and daily pulse was decreased with lost in rhythmicity. Group III: TRP levels at various time points such as ZT- 0, 6, 12 and 18 were 0.16 ± 0.05, 0.22 ± 0.09, 0.33 ± 0.13 and 0.07 ± 0.01 µmol/g protein respectively, were showed no significant difference at all time points. Both mean and daily pulse was decreased with restoration in rhythmicity. Group IV: TRP levels at ZT- 0, 6, 12 and 18 and 24 were 1.24 ± 1.00, 0.18 ± 0.03, 0.11 ± 0.01, 4.17 ± 1.94 and 0.23 ± 0.06 µmol/g protein respectively. The levels were significantly increased (38 fold) at ZT-18 ($p \leq 0.05$). Increase in daily pulse and mean was observed with restoration in rhythmicity. Group V: TRP levels at various time points such as ZT- 0, 6, 12, 18 and 24 were 5.42±1.11, 2.96± 0.56, 4.01 ± 0.49, 3.93 ± 0.50 and 6.98 ± 1.74 µmol/g protein respectively. The levels were showed no significant difference at all time points. Increase in mean ($p \leq 0.05$) and decrease in daily pulse was observed without restoration in rhythmicity. Phase delay was observed by ~6h. Group VI: TRP levels at various time points such as ZT- 0, 6, 12, 18 and 24 were 2.87 ± 0.49, 0.24 ± 0.05, 4.69 ± 1.46, 2.40 ± 0.45 and 0.51 ± 0.17 µmol/g protein respectively. The levels were significantly increased at ZT-0 and 12 ($p \leq 0.05$). Significant increase in mean and daily pulse was observed ($p \leq 0.05$) without restoration in rhythmicity. Phase advance was observed by ~6h (Fig. 39, 44 and 45; Table 5).

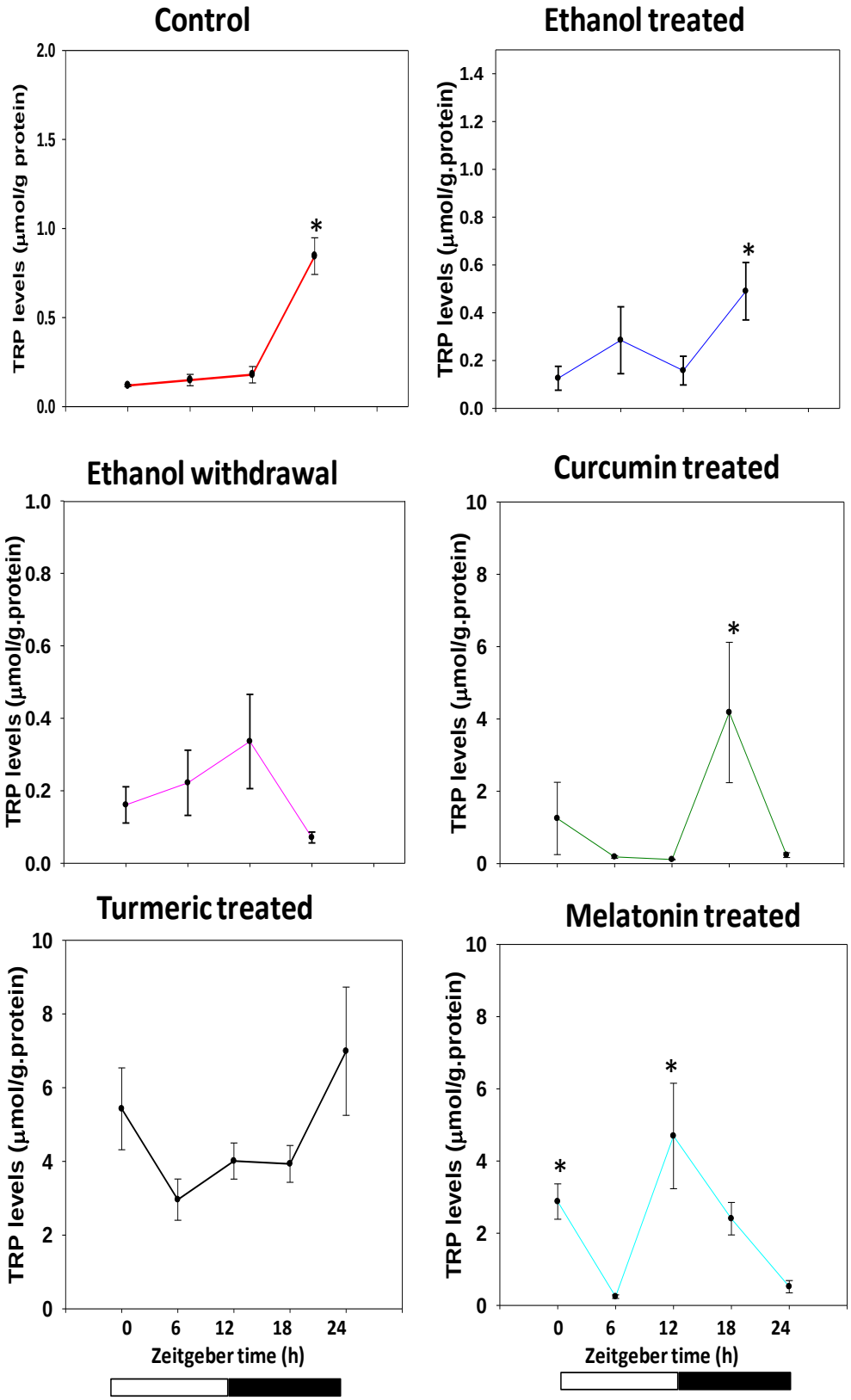

Fig. 39. Curcumin effect on ethanol induced changes in daily rhythms of TRP. Each value is mean ± SE, (n=6); Zeitgeber Time (ZT): ZT-0 = 6.30 A. M (Lights on); ZT-12 = 18.30 P. M (Lights off). One Way ANOVA: * Refers to comparison with lowest value of time point with other time points in each group ($p \leq 0.05$).

5-MTOH:

Group I: 5-MTOH showed daily rhythmicity and levels measured at various time points such as ZT- 0, 6, 12 and 18 were 25.36 ± 4.09, 29.86 ± 2.32, 13.70 ± 1.87 and 10.16 ± 0.357 µmol/g protein respectively. The 5-MTOH levels were maximum at subjective mid-day (ZT-6) and minimum at subjective mid-night (ZT-18). Group II: 5-MTOH levels at various time points such as ZT- 0, 6, 12 and 18 were 5.65 ± 1.02, 8.14 ± 0.96, 5.57 ± 0.28 and 21.66 ± 6.12 µmol/g protein respectively. 5-MTOH levels showed significant increase (4 fold) at ZT-18 ($p \leq 0.05$). Decreased in mean and increased in daily pulse was observed with abolition in rhythmicity. Phase delay was observed by ~12h. Group III: 5-MTOH levels at various time points such as ZT- 0, 6, 12 and 18 were 3.78 ± 0.35, 5.69 ± 0.61, 7.22 ± 1.24 and 4.51 ± 0.94 µmol/g protein respectively, showed significant increase (2 fold) at ZT- 12 ($p \leq 0.05$). Decrease in mean as well as daily pulse was observed with restoration in rhythmicity. Phase delay was observed by ~6h. Group IV: 5-MTOH levels at various time points such as ZT- 0, 6, 12, 18 and 24 were 29.70 ± 8.08, 129.18 ± 7.64, 15.85 ± 2.88, 20.12 ± 4.31 and 15.84 ± 5.783 µmol/g protein respectively. The levels were significantly increase at ZT-6 ($p \leq 0.05$). Significant increase in mean as well as daily pulse was observed ($p \leq 0.05$) with restoration in rhythmicity. Phase restoration was observed. Group V: 5-MTOH levels at various time points such as ZT- 0, 6, 12, 18 and 24 were 6.01 ± 2.46, 10.08 ± 2.82, 7.85 ± 1.89, 1.21 ± 0.25 and 11.39 ± 2.96 µmol/g protein respectively. The levels were significantly increased at ZT-6 and 24 ($p \leq 0.05$). Decrease in mean level and significant increase in daily pulse ($p \leq 0.05$) with restoration in rhythmicity. Phase restoration was also observed. Group VI: 5-MTOH levels at various time points such as ZT- 0, 6, 12, 18 and 24 were 40.11 ± 13.07, 116.81 ± 37.35, 16.80 ± 5.55, 12.20 ± 4.19, 10.21 ± 2.64 µmol/g protein respectively. The levels were significantly increased at ZT-6 ($p \leq 0.05$). Significant increase in mean as well as daily pulse was observed ($p \leq 0.05$) with restoration in rhythmicity. Phase restoration was also observed (Fig. 40, 44 and 45; Table 5).

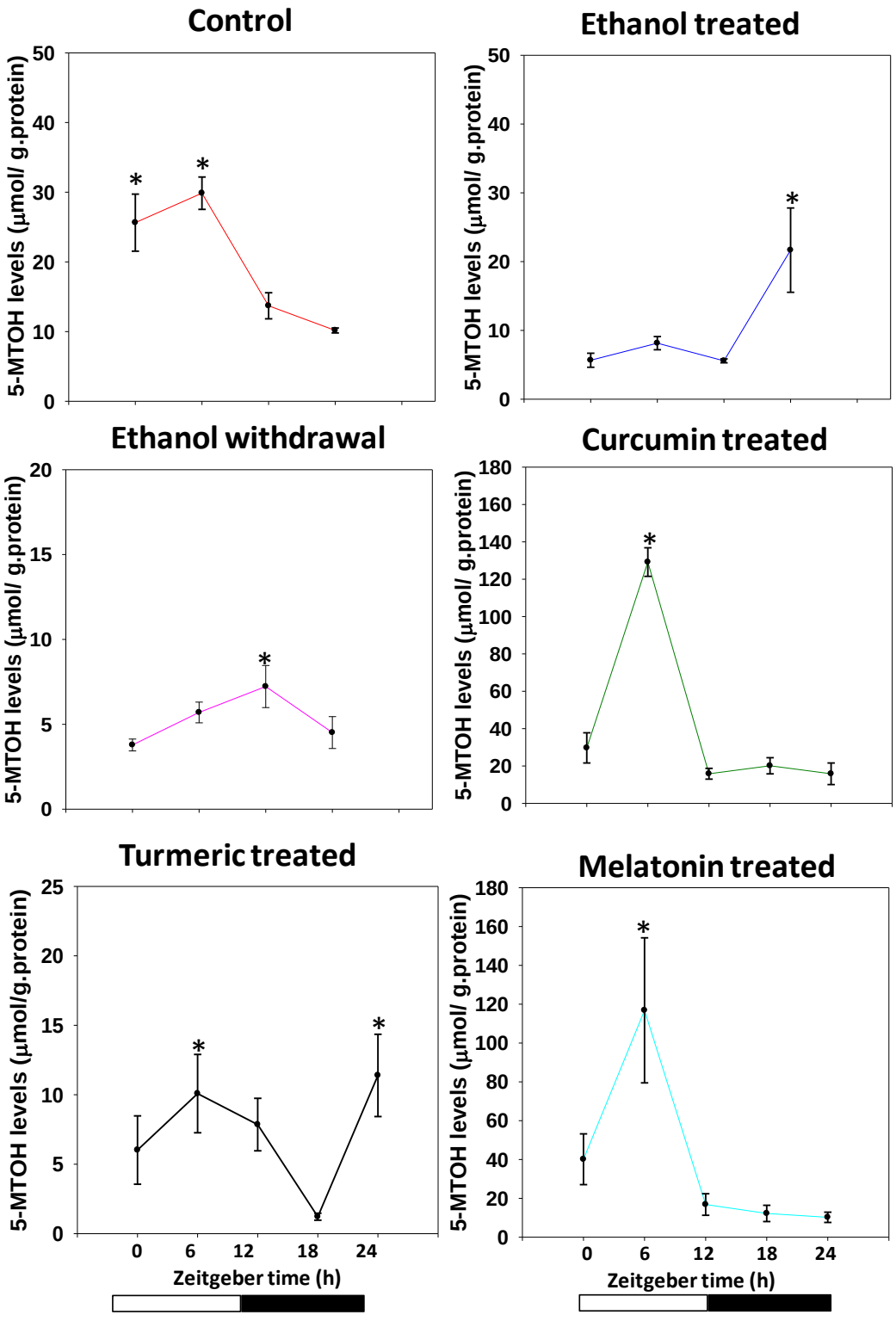

Fig. 40. Curcumin effect on ethanol induced changes in daily rhythms of 5-MTOH. Each value is mean ± SE, (n=6); Zeitgeber Time (ZT): ZT-0 = 6.30 A. M (Lights on); ZT-12 = 18.30 P. M (Lights off). One Way ANOVA: * Refers to comparison with lowest value of time point with other time points in each group ($p \leq 0.05$).

MEL:

Group I: MEL showed daily rhythmicity and levels measured at various time points such as ZT- 0, 6, 12 and 18 were 3.27 ± 0.66, 1.10 ± 0.16, 3.79 ± 0.85 and 8.53 ± 1.10 µmol/g protein respectively. The MEL levels were maximum at subjective mid-night (ZT-18) and minimum at subjective mid-day (ZT-6). Group II: MEL levels at various time points such as ZT- 0, 6, 12 and 18 were 0.20 ± 0.03, 0.38 ± 0.10, 0.30 ± 0.06 and 0.47 ±0.11 µmol/g protein respectively. MEL levels were showed no significant difference at all time points. Decrease in mean as well as daily pulse was observed with abolition in rhythm. Group III: MEL levels at various time points such as ZT- 0, 6, 12 and 18 were 5.40 ± 0.35, 3.41 ± 0.45, 5.99 ± 0.83 and 3.66 ± 0.50 µmol/g protein respectively, were showed significant increase at ZT-0, 12 and 24 ($p \leq 0.05$). Increase in mean and decrease in daily pulse was observed without restoration in rhythm. Phase advance was observed by ~6h. Group IV: MEL levels at various time points such as ZT- 0, 6, 12, 18 and 24 were 2.16 ± 0.39, 1.85 ± 0.32, 1.52 ± 0.37, 9.42 ± 5.53 and 1.32 ± 0.47 µmol/g protein respectively. The levels were showed no significant difference at all time points. Restoration in mean, daily pulse, rhythm and phase were observed. Group V: MEL levels at various time points such as ZT- 0, 6, 12, 18 and 24 were 0.78 ± 0.35, 0.39 ± 0.08, 0.35 ± 0.15, 0.24 ± 0.08 and 0.74 ± 0.43 µmol/g protein respectively. The levels were showed no significant difference at all time points. Significant decrease in mean as well as daily pulse was observed without restoration in rhythmicity. Phase delay was observed by ~6h. Group VI: MEL levels at various time points such as ZT- 0, 6, 12, 18 and 24 were 0.52 ± 0.07, 5.98 ± 2.04, 1.03 ±0.28, 0.49 ±0.182 and 1.26 ± 0.18 µmol/g protein respectively. The levels were significantly increase (12 fold) at ZT- 6 ($p \leq 0.05$). Decrease in mean and increase in pulse level was observed with restoration in rhythmicity. Phase advance was observed by ~12h (Fig. 41, 44 and 45; Table 5).

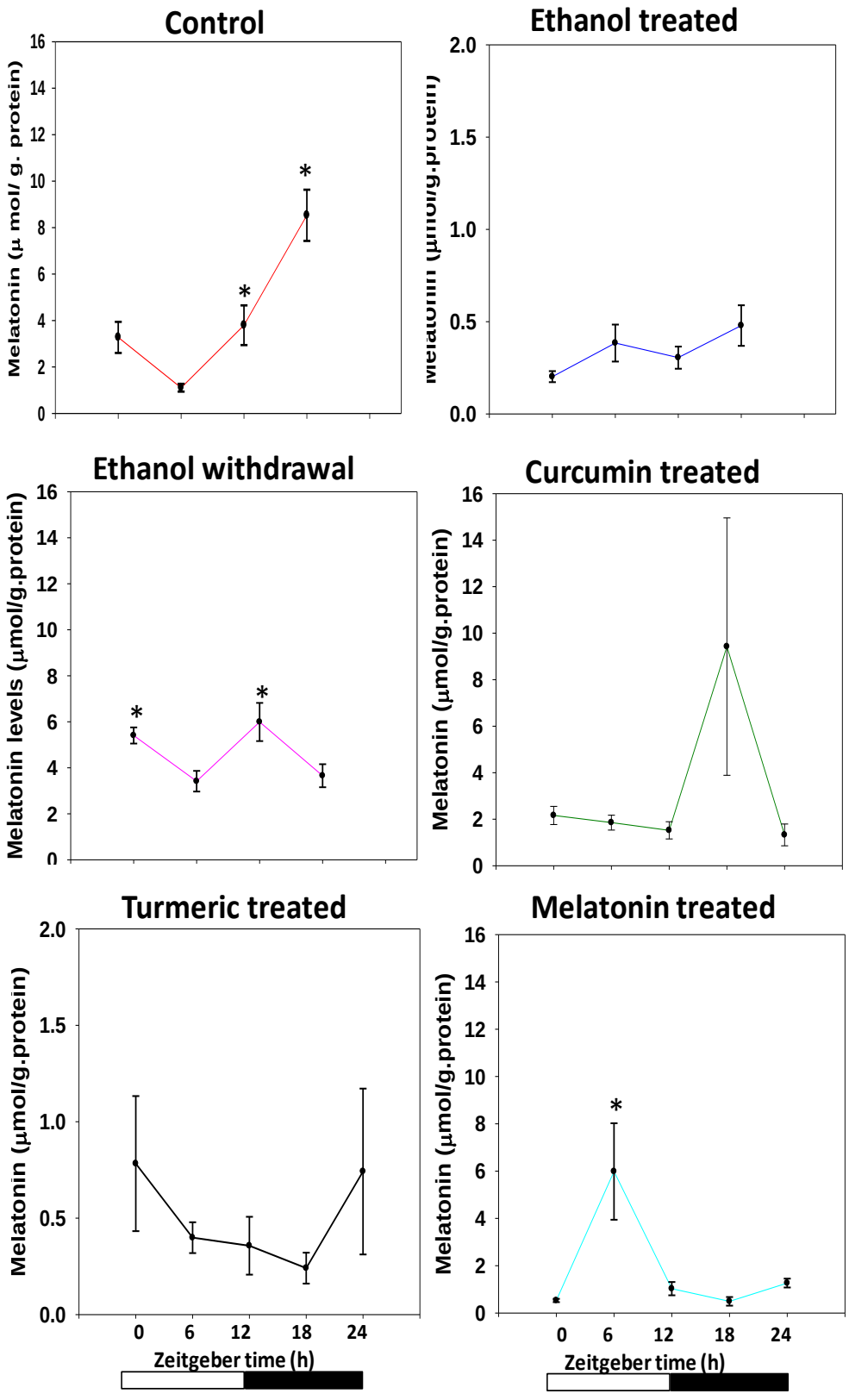

Fig. 41. Curcumin effect on ethanol induced changes in daily rhythms of MEL. Each value is mean ± SE, (n=6); Zeitgeber Time (ZT): ZT-0 = 6.30 A. M (Lights on); ZT-12 = 18.30 P. M (Lights off). One Way ANOVA: * Refers to comparison with lowest value of time point with other time points in each group ($p \leq 0.05$).

5-MIAA:

Group I: 5-MIAA showed daily rhythmicity and levels measured at various time points such as ZT- 0, 6, 12 and 18 were 2.02 ± 0.31, 3.40 ± 0.69, 1.07 ± 0.41 and 0.47 ± 0.13 µmol/g protein respectively. The 5-MIAA levels were maximum at subjective mid-day (ZT-6) and minimum at subjective mid-night (ZT-18). Group II: 5-MIAA levels at various time points such as ZT- 0, 6, 12 and 18 were 0.16 ± 0.04, 0.33 ± 0.09, 0.54 ± 0.14 and 0.75 ± 0.20 µmol/g protein respectively. 5-MIAA levels were showed significant increase (5 fold) at ZT-18. Decrease in mean ($p \leq 0.05$) and daily pulse were observed without affecting the rhythmicity. Group III: 5-MIAA levels at various time points such as ZT- 0, 6, 12 and 18 were 0.73 ± 0.14, 0.71 ± 0.21, 1.78 ± 0.42 and 0.82 ± 0.19 µmol/g protein respectively, were showed significant increase (2.5 fold) at ZT-12 ($p \leq 0.05$). Decrease in mean and daily pulse was observed without affecting the rhythmicity. Phase delay was observed by ~6h. Group IV: 5-MIAA levels at various time points such as ZT- 0, 6, 12, 18 and 24 were 1.331 ± 0.38, 1.94 ± 0.85, 0.63 ± 0.18, 3.86 ± 1.66 and 1.01 ± 0.37 µmol/g protein respectively. The levels were significantly increase (6 fold) at ZT- 18 ($p \leq 0.05$). Restoration in mean, daily pulse without restoration in rhythm was observed. Phase delay was observed by ~12h. Group V: 5-MIAA levels at various time points such as ZT- 0, 6, 12, 18 and 24 were 0.55±0.19, 0.57 ± 0.10, 0.24 ± 0.05, 0.19 ± 0.03 and 0.83 ± 0.39 µmol/g protein respectively. The levels were showed no significant difference at all time points. Decreased in mean and daily pulse was observed with restoration in rhythm. Phase advance was observed by ~6h. Group VI: 5-MIAA levels at various time points such as ZT- 0, 6, 12, 18 and 24 were 0.56 ± 0.16, 1.18 ± 0.72, 0.88 ± 0.38, 0.32 ± 0.10 and 1.30 ± 0.41 µmol/g protein respectively. Statistical significance was not observed between all time points. Decreased in mean and daily pulse was observed with restoration in rhythm (Fig. 42, 44 and 45; Table 5).

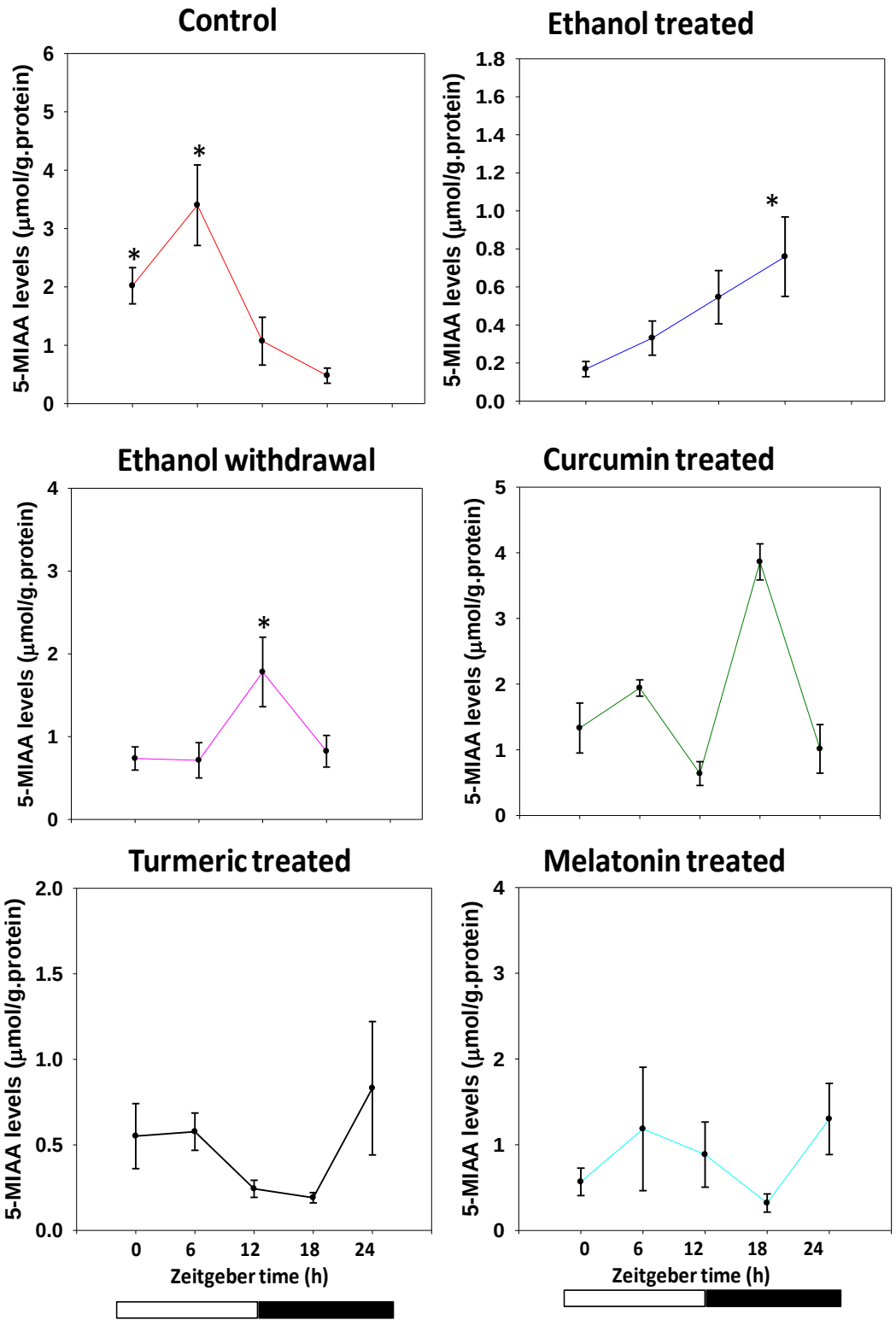

Fig. 42. Curcumin effect on ethanol induced changes in daily rhythms of 5-MIAA. Each value is mean ± SE, (n=6); Zeitgeber Time (ZT): ZT-0 = 6.30 A. M (Lights on); ZT-12 = 18.30 P. M (Lights off). One Way ANOVA: * Refers to comparison with lowest value of time point with other time points in each group ($p \leq 0.05$).

NAT:

Group I: NAT showed daily rhythmicity and levels measured at various time points such as ZT- 0, 6, 12 and 18 were 0.25 ± 0.07, 0.39 ± 0.02, 0.65 ± 0.01 and 0.22 ± 0.06 µmol/g protein respectively. The NAT levels were maximum at ZT-12 and minimum at ZT-18. Group II: NAT levels at various time points such as ZT- 0, 6, 12 and 18 were 0.22 ± 0.11, 0.43 ± 0.38, 0.53 ± 0.007 and 0.17 ± 0.05 µmol/g protein respectively. NAT levels showed significant increase (5 fold) at ZT-12 ($p \leq 0.05$) though there was no significant difference at ZT-0, 18, 24. Decrease in mean ($p \leq 0.05$) and increase in pulse level was observed without affecting the rhythm. Phase advance was observed by ~6h. Group III: NAT levels at various time points such as ZT- 0, 6, 12, 18 and 24 were 0.02 ± 0.006, 0.04 ± 0.01, 0.31 ± 0.22 and 0.03 ± 0.01 µmol/g protein respectively, showed no significant difference at all time points. Decrease in mean ($p \leq 0.05$) and increase in pulse level was observed without affecting the rhythm and phase. Group IV: NAT levels at various time points such as ZT- 0, 6, 12, 18 and 24 were 0.22 ± 0.05, 0.01 ± 0.002, 0.01 ± 0.002, 0.01 ± 0.003 and 0.008 ± 0.05 µmol/g protein respectively. The levels were significantly increased at ZT-0, 6 and 18 ($p \leq 0.05$). Partial restoration in mean as well as daily pulse was observed without restoration in rhythm. Phase delay was observed by ~6h. Group V: NAT levels at various time points such as ZT- 0, 6, 12, 18 and 24 were 0.071±0.05, 0.01±0.002, 0.011 ± 0.002, 0.013 ± 0.003 and 0.08 ±0.05 µmol/g protein respectively. The levels were showed no significant difference at time points. Significant decrease in mean levels ($p \leq 0.05$) and partial restoration in daily pulse and rhythm was observed. Phase delay was observed by ~12h. Group VI: NAT levels at various time points such as ZT- 0, 6, 12, 18 and 24 were 0.028 ± 0.006, 0.018 ± 0.01, 0.02 ± 0.010, 0.01 ± 0.006, 0.14 ± 0.04 µmol/g protein respectively. The levels were significantly increased at ZT-24 ($p \leq 0.05$). Significantly decrease in mean and increase in daily pulse was observed without restoration in rhythmicity. Phase delay was observed by ~12h (Fig. 43, 44 and 45; Table 5).

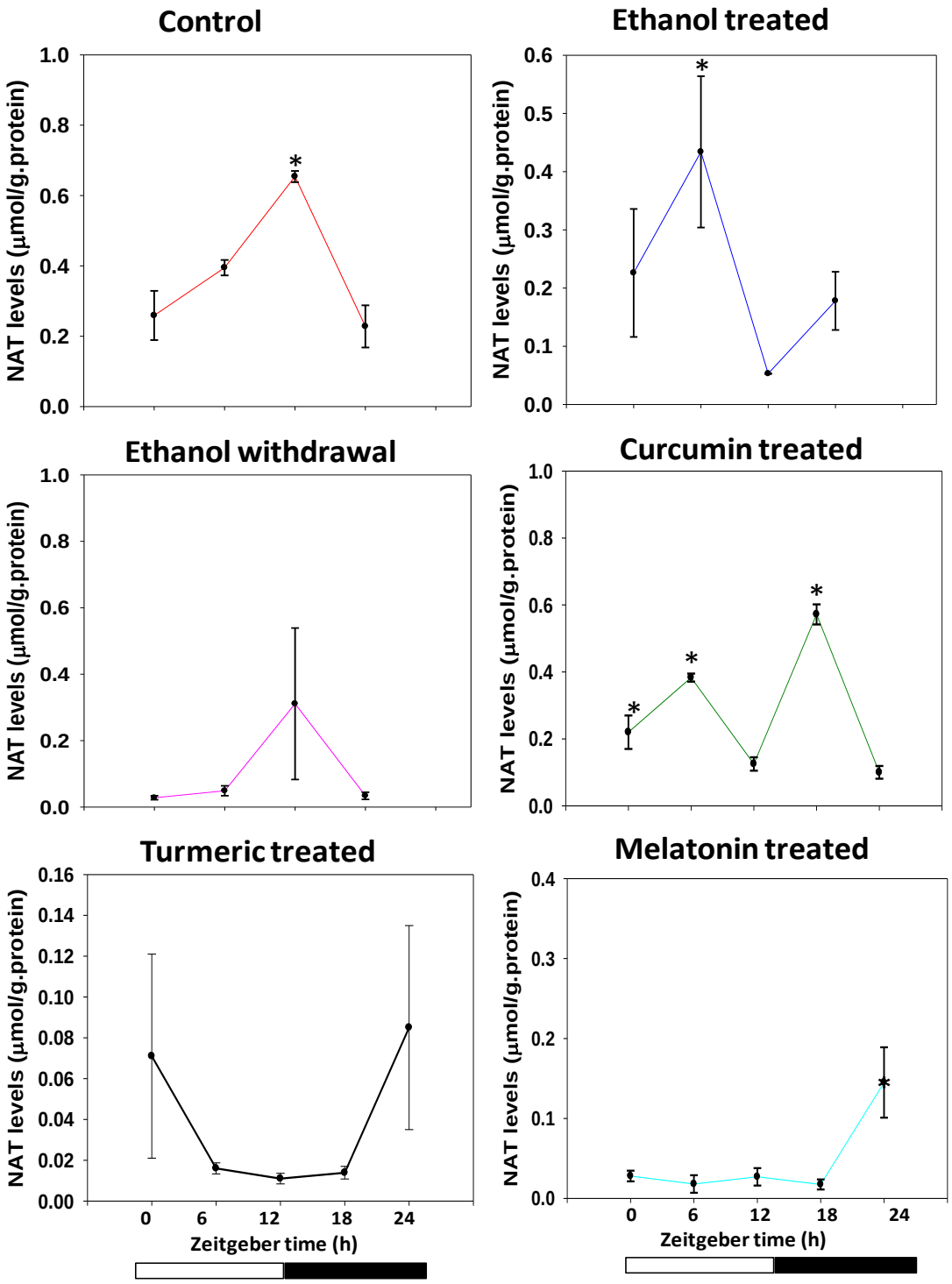

Fig. 43. Curcumin effect on ethanol induced changes in daily rhythms of NAT. Each value is mean ± SE, (n=6); Zeitgeber Time (ZT): ZT-0 = 6.30 A. M (Lights on); ZT-12 = 18.30 P. M (Lights off). One Way ANOVA: * Refers to comparison with lowest value of time point with other time points in each group ($p \leq 0.05$).

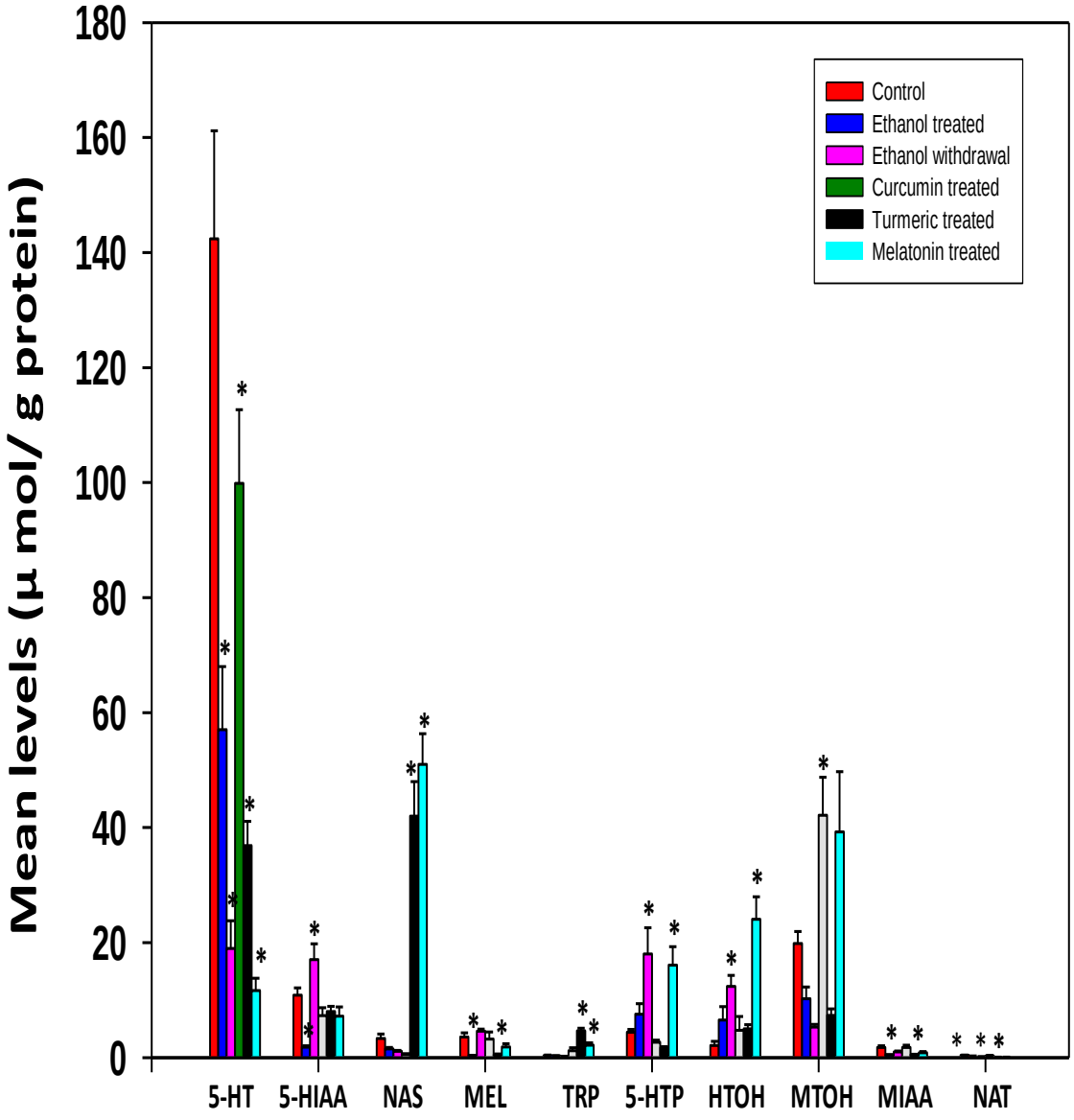

Fig. 44. Curcumin effect on ethanol induced changes in daily mean levels of serotonin chronometabolome in Pineal. Each value is mean ± SE, (n=6); One Way ANOVA: * Refers to comparison with control ($p < 0.05$).

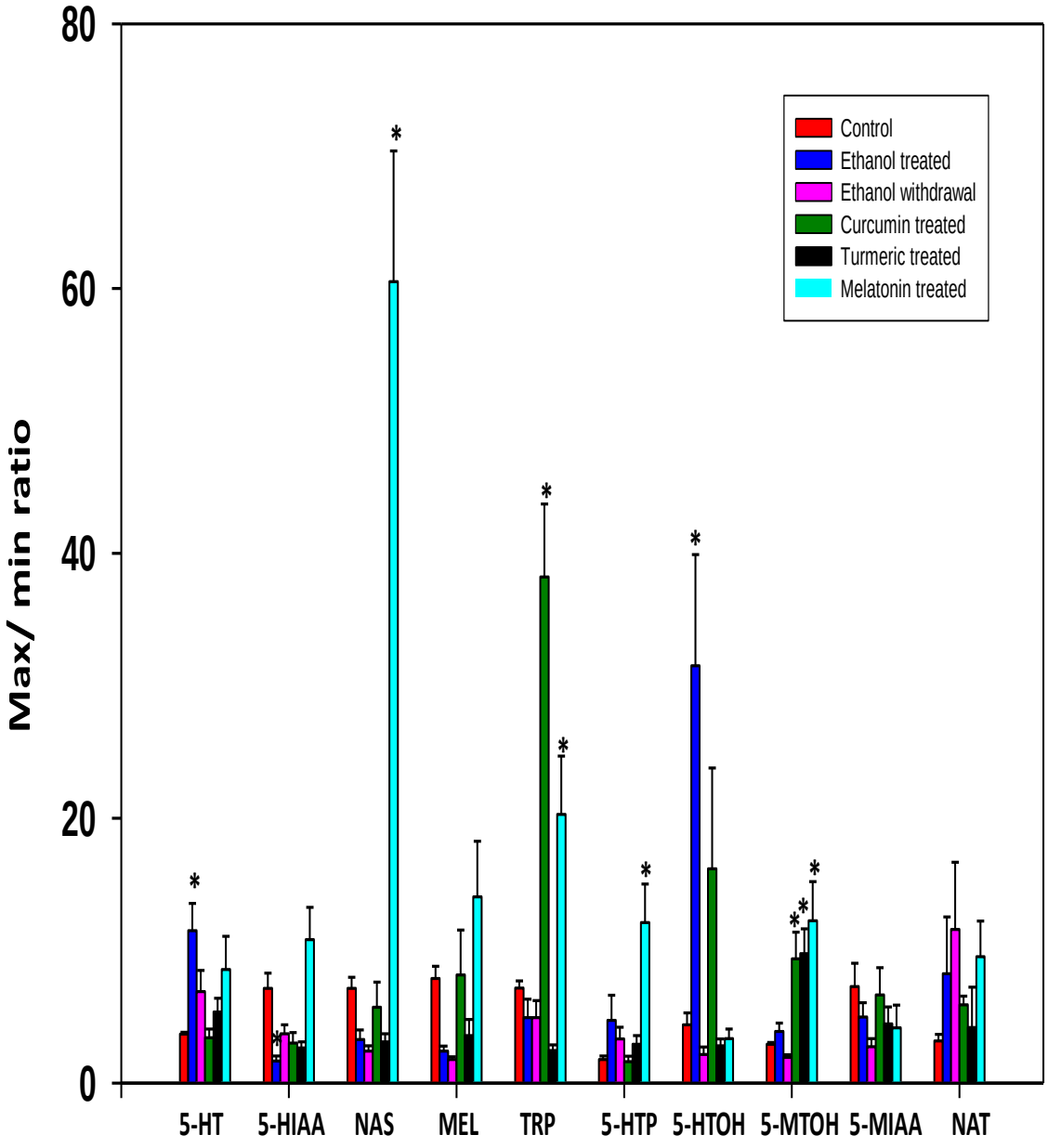

Fig. 45. Curcumin effect on ethanol induced changes in daily pulse levels of Serotonin chronometabolome in Pineal. Each value is mean ± SE, (n=6); One Way ANOVA: * Refers to comparison with control ($p < 0.05$).

Table 5. Curcumin effect on ethanol induced changes in daily mean and pulse levels of Serotonin chronometabolome in Pineal.

EXP		5-HT	5-HIAA	5-HTP	HTOH	NAS	TRP	MTOH	MEL	5-MIAA	NAT
C	Mean	142.4±18.7	10.8± 1.29	4.41±0.47	2.09 ±0.71	3.30±0.77	0.32±0.09	19.8±2.07	3.60± 0.67	1.74 ± 0.3	0.38±0.076
	Max:Min	3.697±0.14	7.1± 1.1	1.78±0.27	4.40 ± 0.9	7.14±0.84	7.17±0.53	2.93± 0.14	7.90±0.91	7.29±1.76	3.18 ± 0.50
	Rhythm	Persistent	Persistent	Persistent	Persistent	Persistent	Persistent	Persistent	Persistent	Persistent	Persistent
ET	Mean	57.0±10.9*	1.8±0.23*	7.5 ± 1.8	6.549±2.3	1.51±0.24	0.26±0.05	10.26± 2.0	0.3± 0.04*	0.4± 0.07*	0.19±0.097*
	Max : Min	11.5±2.07*	1.65±0.3*	4.72±1.91	31.5±8.4*	3.2± 0.7	4.9±1.4	3.89± 0.64	2.40± 0.38	4.99± 1.07	8.25 ± 4.29
	Rhythm	Persistent	Abolished	Persistent	Persistent	Persistent	Abolished	Abolished	Abolished	Persistent	Persistent
EW	Mean	18.9± 4.78*	17.0± 2.7*	17.9± 4.5*	12.3± 1.8*	1.08± 0.16	0.19± 0.04	5.30± 0.48	4.61± 0.35	1.01± 0.15	0.104±0.058
	Max:Min	6.89 ± 1.62	3.71± 0.69	3.33± 0.89	2.15± 0.57	2.4 ± 0.41	4.93± 1.30	1.92± 0.21	1.78± 0.19	2.74± 0.61	11.6 ± 5.07
	Rhythm	Persistent	Persistent	Persistent	Persistent	Abolished	Persistent	Persistent	Abolished	Persistent	Persistent
CT	Mean	99.8± 12.8*	7.29± 1.39	2.69± 0.31	4.77±2.37	0.52±0.15	1.19± 0.49	42.1±13.6*	3.25± 1.18	1.75± 0.42	0.28± 0.077
	Max: Min	3.42 ± 0.66	3.01± 0.79	1.62±0.38	16.18± 7.6	5.72± 1.89	38 ±10.5*	9.39±2.0*	8.16± 3.38	6.66± 2.04	5.90 ± 0.67
	Rhythm	Persistent	Persistent	Abolished	Persistent	Persistent	Persistent	Abolished	Persistent	Abolished	Abolished
TT	Mean	36.8±4.19*	8.02±0.91	1.68±0.22	5.00±0.7	41.9±6.0*	4.6±0.49*	7.32±1.16	0.5±0.1*	0.4±0.09*	0.03±0.01*
	Max:Min	5.367±1.04	2.65±0.46	2.92±0.65	2.82±0.52	3.11±0.6	2.44±0.44	9.763±1.8*	3.58±1.21	4.46±1.28	4.184±3.066
	Rhythm	Abolished	Persistent	Persistent	Persistent	Abolished	Abolished	Abolished	Abolished	Persistent	Persistent
MT	Mean	11.6±2.13*	7.16±1.62	16.0±3.2*	24.0±3.92	50 ±10.3*	2.1±0.4*	39.23±10.5	1.86±0.54	0.85±0.18	0.047±0.01*
	Max:Min	8.57±2.56	10.8±2.45	12.1±2.9*	3.36±0.7*	60.5±9.8*	20.2±4.4*	12.2±2.96*	14.05±4.2	4.15±1.73	9.545±2.69
	Rhythm	Abolished	Persistent	Persistent	Persistent	Abolished	Abolished	Abolished	Persistent	Persistent	Abolished

Each value is mean $\pm$ SE, (n=6); One Way ANOVA: * Refers to comparison with control ($p< 0.05$).

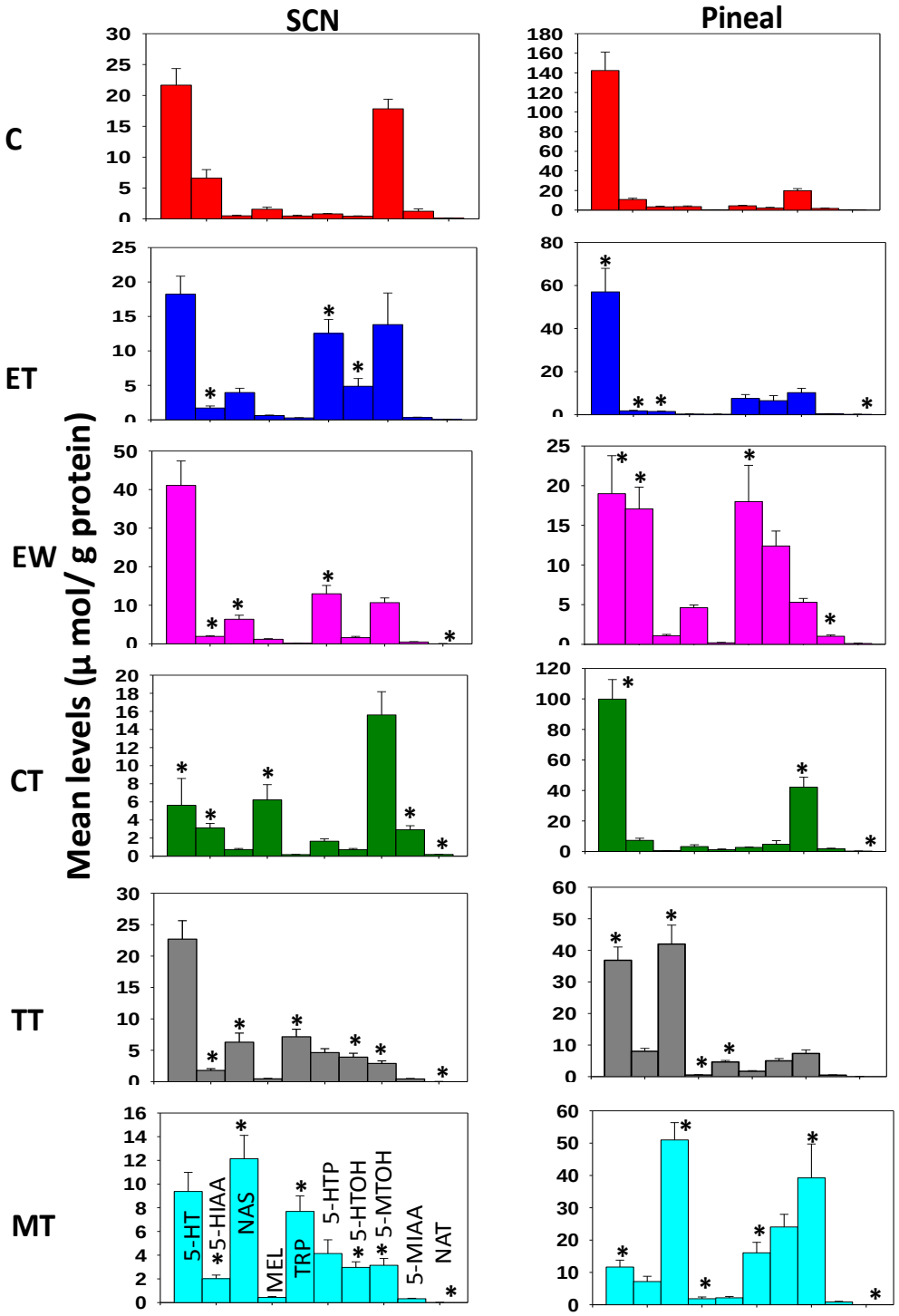

Fig. 46. Curcumin effect on ethanol induced changes in daily mean levels of Serotonin chronometabolome comparison in SCN and Pineal. Each value is mean ± SE, (n=6); One Way ANOVA: * Refers to comparison with control ($p< 0.05$). Column 1-10 represents 5-HT, 5-HIAA, NAS, MEL, TRP, 5-HTP, 5-HTOH, 5-MTOH, 5-MIAA, NAT

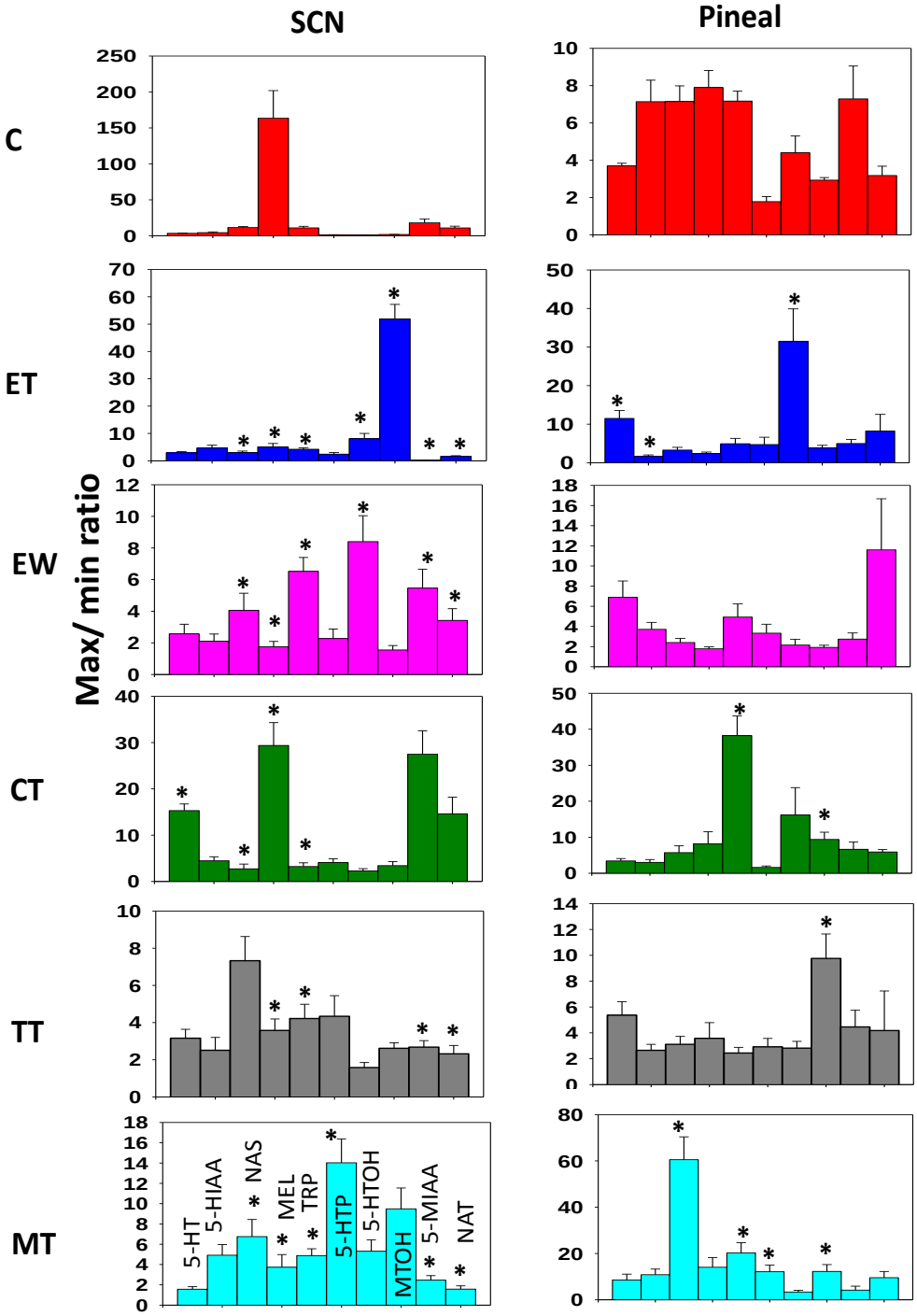

Fig. 47. Curcumin effect on ethanol induced changes in daily pulse levels of Serotonin chronometabolome comparison in SCN and Pineal. Each value is mean ± SE, (n=6); One Way ANOVA: * Refers to comparison with control ($p< 0.05$). Column 1-10 represents 5-HT, 5-HIAA, NAS, MEL, TRP, 5-HTP, 5-HTOH, 5-MTOH, 5-MIAA, NAT.

OBJECTIVE 2

To find Sensitivity of Curcumin treatment on Ethanol induced changes in daily rhythms of Serotonin Chronometabolome in aging

The impact of alcohol on aging is multifaceted which may contribute to the incidence of age related as well as alcohol related disorders. This study examines the relationship among alcoholism, serotonin chronometabolome and role of antioxidants in the aging.

1. 5-HTP:

In SCN, 5-HTP showed rhythmicity in 90 day and this was abolished upon aging. The mean levels were increased upon aging. Highest daily pulse levels were found in middle age group (1yr). Ethanol treatment causes abolition of rhythmicity in 90 day but not in other two age groups (1 and 2yr). ET caused elevation of mean levels in 90 day ($p \leq 0.05$) whereas the levels were decreased in 1 and 2yr ($p \leq 0.05$). Though daily pulse levels were increased in all three age groups, statistical significance was observed in 1yr only ($p \leq 0.05$). Upon EW, rhythmicity was observed in 1 and 2yr but not in 90 day. Mean levels increased in 90 day and 1yr ($p \leq 0.05$) whereas in 2yr, levels decreased ($p \leq 0.05$). Daily pulse levels were increased in 90 day and 2yr and levels were not affected in 2yr. CT was helpful in partial restoration of mean levels in 90 day as well as 1yr but not in 2yr. Curcumin caused increased levels in daily pulse in all three age groups ($p \leq 0.05$). Rhythmicity was restored in all three age groups. In TT, rhythmicity was observed in 90 day and l yr but not in 2 yr. Increased levels were found in 90 day whereas levels were decreased upon aging ($p \leq 0.05$). Increased daily pulse levels were observed in 90day as well as 1yr but not in 2yr. In MT, rhythmicity was observed in 90 day and 1yr but not in 2yr. Partial restoration of mean levels was observed in 1yr whereas in 90day increased levels were found and 2yr, decreased levels were observed ($p \leq 0.05$). Though daily pulse levels were increased in all three age groups, it was not statistically significant in 2yr (Table 6; Fig. 58 and 59).

In Pineal, 5-HTP showed rhythmicity in all three age groups. The mean levels decreased upon aging ($p \leq 0.05$). Highest daily pulse levels were found in middle age group (1yr). ET caused abolition of rhythmicity in 90 day and 1yr but not in 2yr. ET resulted in elevation of mean levels in all three age groups ($p \leq 0.05$). Daily pulse levels were increased in 90 day and decreased in 1 and 2yr.

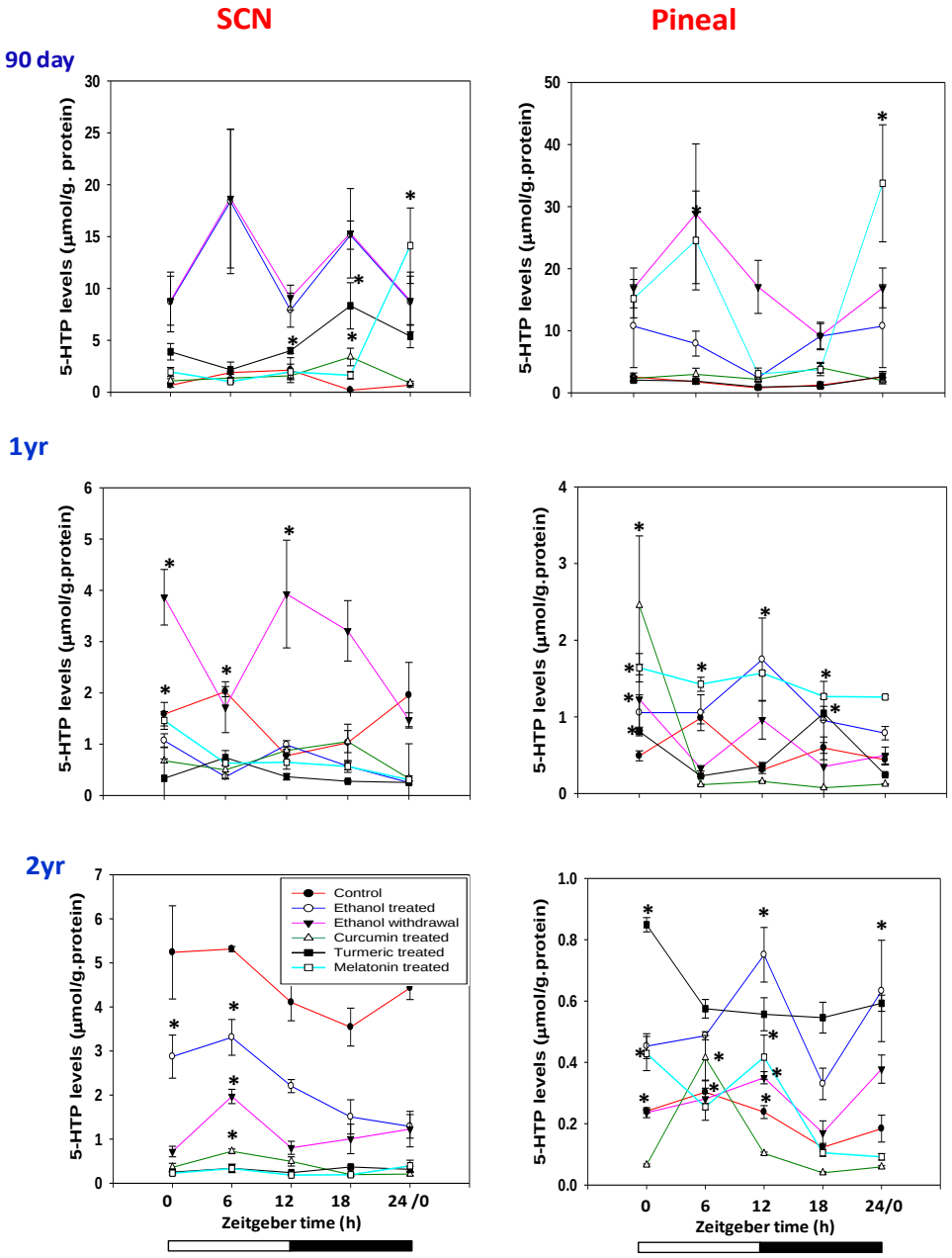

Fig. 48. Curcumin effect on ethanol induced changes in daily rhythms of 5-HTP. Each value is mean ± SE, (n=6); One Way ANOVA: * Refers to comparison with lowest value in each group ($p \leq 0.05$).

Table 6. Curcumin effect on ethanol induced changes in daily rhythms, mean and pulse levels of 5-HTP in SCN.

A. 90 day									
Exp. Group	Zeitgeber time (h)					Mean (24h)	Max	Min	Ratio
	0	6	12	18	24/0				
C	0.82±0.02	0.66±0.05	0.9±0.07*	0.80±0.07	0.82±0.02	0.8±0.05	0.9±0.07	0.66±0.05	1.41±.0.09
ET	8.69±2.88	18.3±6.95	7.91±1.64	15.1±1.3	8.69±2.88	12.5±2.04[a]	18.3±6.9	7.91±1.64	2.42 ± 0.61
EW	8.82±2.36	18.67±6.7	9.10±1.20	15.3±4.31	8.82±2.36	12.9 ±2.15[a]	18.6±6.7	8.82±2.36	2.27 ± 0.60
CT	1.08±0.22	1.35±0.38	1.58±0.28	3.3±0.84*	0.87±0.19	1.65 ±0.2	3.39±0.84	0.87±0.19	4.09 ± 0.79
TT	3.90±0.79	2.16±0.74	4.00±0.27	8.33±2.2*	5.39±1.11	4.62±0.63	8.33±2.23	2.16±0.74	4.34±1.11
MT	1.95±0.43	1.02±0.13	1.95±0.7	1.63±0.36	14.1±3.6*	4.13±1.16	14.1±3.64	1.02±0.13	14.02±2.3[b]
B. 1 year									
C	1.58±0.22	2.02±0.09	0.77±0.10	1.02±0.36	1.95±0.64	1.47±0.24	2.02±0.09	0.77±0.10	2.65±0.210
ET	1.06±0.13	0.36±0.04	0.98±0.08	0.57±0.07	3.25±0.7*	1.24±0.24	3.25±0.75	0.36±0.04	8.98±0.87[b]
EW	3.8±0.54*	1.72±0.49	3.9±1.05*	3.21±0.59	1.47±0.13	2.71±0.31[a]	3.92±1.05	1.47±0.13	2.66±0.257
CT	0.67±0.03	0.49±0.16*	0.87±0.07	1.05±0.21	0.32±0.05*	0.68±0.07[a]	1.05±0.21	0.32±0.05	3.27±0.501
TT	0.33±0.61	0.73±0.13*	0.36±0.06	0.27±0.02	0.25±0.03	0.39±0.04[a]	0.73±0.13	0.25±0.03	2.97±0.397
MT	1.4±0.17*	0.628±0.05	0.64±0.13	0.56±0.11	0.30±0.03	0.72±0.08[a]	1.46±0.17	0.30±0.03	4.83±0.44[b]
C. 2 year									
C	5.23±1.05	5.31±0.05	4.10±0.41	3.54±0.43	4.43±0.26	4.52±0.33	5.23±1.05	3.54±0.43	1.50±0.204
ET	2.87±0.48*	3.31±0.40*	2.20±0.14	1.50±0.38	1.29±0.26	2.23±0.20[a]	3.31±0.40	1.29±0.26	2.68±0.375
EW	0.71±0.11	1.96±0.16*	0.80±0.14	1.00±0.33	1.22±0.40	1.14±0.13[a]	1.96±0.16	0.71±0.11	2.81±0.30[b]
CT	0.361±0.09	0.72±0.05*	0.49±0.1*	0.19±0.04	0.20±0.03	0.39±0.04[a]	0.72±0.05	0.20±0.03	3.60±0.42[b]
TT	0.25±0.01	0.33±0.09	0.2±0.008	0.36±0.03	0.316±0.07	03.0±0.01[a]	0.36±0.03	0.2±0.008	1.528±0.09
MT	0.22±0.04	0.33±0.09	0.18±0.01	0.19±0.01	0.39±0.12	0.26±0.03[a]	0.39±0.12	0.18±0.01	2.147±0.42

Each value is mean ± SE, (n=6); One Way ANOVA: * Refers to comparison with lowest value in each experimental group with other time points ($p \leq 0.05$). [a] Refers to comparison with control in mean levels ($p \leq 0.05$). [b] Refers to comparison with control in daily pulse levels ($p \leq 0.05$).

Table 7. Curcumin effect on ethanol induced changes in daily rhythms, mean and pulse levels of 5-HTP in Pineal.

A. 90 day									
Exp. Group	Zeitgeber time (h)					Mean (24h)	Max	Min	Ratio
	0	6	12	18	24/0				
C	5.8±0.20*	4.5±0.59	3.96±0.23	3.53±0.92	5.8±0.27*	4.4±0.47	5.8±0.20	3.53±0.92	1.78±0.27
ET	10.7±6.68	7.92±2.01	2.46±0.73	9.11±2.03	10.7±6.68	7.5±1.82	10.7± 6.6	2.46 ± 0.7	4.72±1.91
EW	16.8±3.20	28.8±11.2	17.0±4.27	9.15±2.21	16.8±3.20	17.9±4.5[a]	28.8±11.2	9.15 ± 2.2	3.33± 0.89
CT	2.3±0.35	2.97±0.98	2.18±0.35	4.04±0.86	1.95±0.51	2.69±0.31	2.97 ± 0.9	1.95 ± 0.5	1.62± 0.38
TT	2.04±0.38	1.89±0.37	0.93±0.19	1.10±0.19	2.61±0.83	1.68±0.22	2.61±0.83	0.93±0.19	2.92±0.65
MT	15.1±3.09	24.5±7.9*	3.06±0.92	3.75±0.98	33.7±9.42*	16.0±3.24[a]	33.74±9.4	3.06±0.92	12.1±2.92[b]
B.1 year									
C	0.49±0.06	0.98±0.07*	0.30±0.04	0.59±0.07*	0.440±0.06	0.56±0.115	0.98±0.07	0.30±0.04	3.27±0.324
ET	1.05±0.23	1.05±0.234	1.74±0.54	0.952±0.511	0.787±0.08	1.12±0.16[a]	1.74±0.54	0.78±0.08	2.58±0.955
EW	1.23±0.45*	0.329±0.03	0.96±0.25	0.352±0.03	0.496±0.11	0.67±0.119	1.23±0.45	0.32±0.03	3.77±0.844
CT	2.45±0.90*	0.11±0.005	0.15±0.008	0.075±0.002	0.12±0.005	0.601±0.24	2.45±0.90	0.07±0.002	32.7±6.93[b]
TT	0.80±0.05*	0.229±0.01	0.353±0.05	1.047±0.09*	0.24±0.012	0.537±0.06	1.04±0.09	0.22±0.013	4.597±0.27
MT	1.64±0.18*	1.425±0.09	1.57±0.02*	1.265±0.029	1.258±0.02	1.43±0.048[a]	1.64±0.18	1.25±0.021	1.304±0.08
C. 2 year									
C	0.24±0.007*	0.30±0.03*	0.23±0.02*	0.12±0.013	0.184±0.04	0.212±0.029	0.30±0.03	0.12±0.013	2.49±0.208
ET	0.453±0.040	0.487±0.01	0.75±0.08*	0.33±0.051	0.63±0.16*	0.531±0.04[a]	0.75±0.08	0.33±0.051	2.32±0.252
EW	0.236±0.016	0.28±0.035	0.35±0.01*	0.17±0.039	0.37±0.04*	0.283±0.019	0.37±0.04	0.170±0.03	2.34±0.343
CT	0.065±0.003	0.41±0.07*	0.10±0.004	0.39±0.003	0.05±0.004	0.136±0.029	0.1±0.004	0.03±0.003	2.65±0.133
TT	0.84±0.023*	0.575±0.03	0.55±0.05	0.546±0.05	0.59±0.026	0.624±0.02[a]	0.84±0.02	0.546±0.05	1.568±0.08
MT	0.429±0.05*	0.25±0.04*	0.41±0.07*	0.105±0.011	0.09±0.010	0.259±0.033	0.42±0.05	0.092±0.01	4.96±0.42[b]

Each value is mean ± SE, (n=6); One Way ANOVA: * Refers to comparison with lowest value in each experimental group with other time points ($p \leq 0.05$). [a] Refers to comparison with control in mean levels ($p \leq 0.05$). [b] Refers to comparison with control in daily pulse levels ($p \leq 0.05$).

Upon EW, rhythmicity was not observed in 90 day and 1yr but not in 2yr. Though mean levels were increased in all age groups, statistical significance was observed in 90 day ($p \leq 0.05$). Daily pulse levels were increased in 90 day and not much change was observed upon aging. Curcumin treatment was helpful in partial restoration of mean levels in all three age groups but rhythmicity was not observed in 90 day. Curcumin caused restoration of daily pulse levels 90 day as well as 2yr whereas in 1yr robust increase in daily pulse level was observed ($p \leq 0.05$). Turmeric treatment was sensitive in restoration of rhythmicity in 1yr as well as 2yr but not in 90 day. Mean levels were decreased in 90 day whereas in 2yr increased levels were found ($p \leq 0.05$). Partial restoration of mean levels was observed in middle age group (1yr). Melatonin treatment, rhythmicity was observed in all three age groups. Partial restoration of mean levels was observed in 2yr only. Robust increases in mean levels were observed in 90 day ($p \leq 0.05$). Daily pulse levels were increased 90 day and 2yr ($p \leq 0.05$) (Table 7; Fig. 60 and 61).

Ethanol treatment causes robust change in the levels as well as rhythmicity of 5-HTP in SCN and Pineal. These changes were not restored in EW. Curcumin treatment was effective in restoration of levels in 90 day than other age groups and more sensitive in Pineal than SCN. Melatonin treatment causes partial restoration in 1yr age group of SCN and 2yr of Pineal. Turmeric was not helpful in restoration of levels in all three age groups in SCN as well as Pineal (Fig. 48).

2. 5-HT

In SCN, 5-HT showed rhythmicity in all three age groups. Elevated mean levels were found in middle age group (1yr) and not much significant change in daily pulse levels upon aging. Ethanol treatment caused abolition of rhythmicity in 90 day but not in other two age groups (1 and 2yr). ET resulted in decrease of mean levels in 90 day whereas robust increase was observed in 1 and 2yr ($p \leq 0.05$). Significant change was not observed in daily pulses of 90 day as well as 2yr whereas increased levels were observed in 1yr ($p \leq 0.05$). Upon EW, rhythmicity was lost in 90day. Mean levels were increased in 90 day and 1yr ($p \leq 0.05$) whereas in 2yr, levels were decreased ($p \leq 0.05$). Daily pulse levels were decreased in all three age groups ($p \leq 0.05$). Curcumin treatment was helpful in partial restoration of mean levels in 1yr as well as 2yr but not in 90 day but rhythmicity was observed in all three age groups. Significant increase in daily pulse of 90day and decrease in 1yr was observed ($p \leq 0.05$). Restoration of daily pulse was observed in 2yr. In Turmeric treatment, rhythmicity was observed in l and 2 yr. Restoration of mean levels were found in 90 day and levels were decreased in 1 and 2yr ($p \leq 0.05$). Increased daily pulse levels were observed in 2yr and decreased levels in 1yr. Restoration of daily pulse levels were observed in 90 day age group. Melatonin treatment, rhythmicity was observed in 1yr only. Mean levels were decreased in all three age groups ($p \leq 0.05$). Though daily pulse levels were decreased in all three age groups, statistically significance observed in 2yr only ($p \leq 0.05$) (Table 8; Fig. 58 and 59).

In Pineal, 5-HT showed rhythmicity in all three age groups. The mean levels were decreased upon aging ($p \leq 0.05$). Daily pulse levels did not change upon aging. Ethanol treatment didn't affect the rhythmicity in all three age groups. ET resulted elevation of mean levels in 1 and 2yr whereas decreased levels were observed in 90 day ($p \leq 0.05$). Significant increase in daily pulse levels were observed in 90 day and 1yr whereas decreased levels were observed in 2yr ($p \leq 0.05$). Upon EW, rhythmicity was not observed in 90 day only. Mean levels were decreased in 90 day and increased in 1 and 2yr ($p \leq 0.05$). Significant increase in daily pulse levels were observed in all age groups ($p \leq 0.05$). Curcumin treatment was sensitive in partial restoration of mean levels as well as rhythmicity in all three age groups. Curcumin caused partial restoration of daily pulse levels in 90 day and 1yr whereas increased in 2yr ($p \leq 0.05$). In Turmeric treatment, rhythmicity was observed in all three age groups.

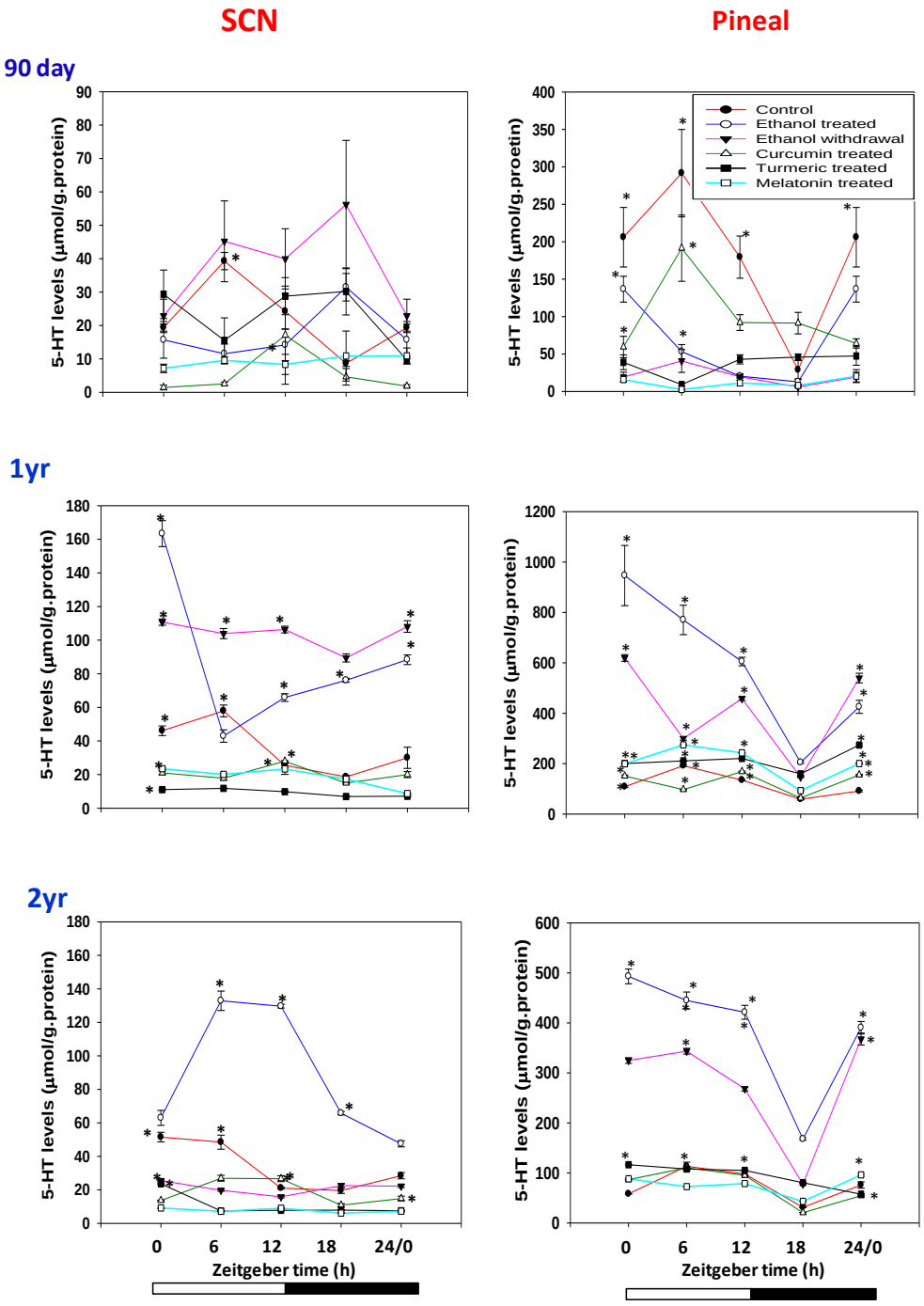

Fig. 49. Curcumin effect on ethanol induced changes in daily rhythms of 5-HT. Each value is mean ± SE, (n=6); One Way ANOVA: * Refers to comparison with lowest value in each group ($p \leq 0.05$).

Table 8. Curcumin effect on ethanol induced changes in daily rhythms, mean and pulse levels of 5-HT in SCN.

A. 90 day									
Exp. Group	Zeitgeber time (h)					Mean (24h)	Max	Min	Ratio
	0	6	12	18	24/0				
C	22.6±2.04*	34.1±1.73*	19.9±0.5*	9.9±1.127	22.6±2.04*	21.6±2.67	34.1±1.73	9.9±1.127	3.47±0.24
ET	15.7±5.49	11.5±2.91	14.1±4.7	31.4±4.1*	15.7±5.49	18.2 ±2.62	31.4 ±4.12	11.5 ± 2.9	2.90 ± 0.48
EW	22.8±5.01	45.2±12.09	39.9±9.01	56.2±19.2	22.8±5.0	41.0 ±6.33[a]	56.2 ±19.2	22.8 ± 5.0	2.57 ± 0.61
CT	1.45±0.7	2.5±0.537	17.1±14*	4.6±2.47	1.8±0.43	5.6 ±2.97	17.1 ±14.6	1.45 ± 0.7	15.3 ± 9.4[b]
TT	29.3±7.27	15.5±6.74	28.7±5.55	30.1±7.04	9.6±1.19	22.6±2.94	30.1±7.04	9.68±1.1	3.16±0.48
MT	7.1±1.26	9.5±1.03	8.3±3.009	10.8±7.49	10.9±2.38	9.3±1.61	10.9±2.38	7.17±1.2	1.56±0.25
B. 1yr									
C	46.0±2.8*	57.8±3.51*	25.5±1.39	18.6±0.92	29.9±6.28*	35.6±7.16	57.8±3.5	18.6±0.92	3.11±0.141
ET	163.3±7.7*	42.9±3.68	65.8±2.3*	76.0±1.2*	88.35±2.6*	87.2±7.78[a]	163.3±7.7	42.9±3.6	3.83±0.21[b]
EW	110.7±1.8*	103.9±3.0*	106±1.9*	89.4±2.4	108.1±3.4*	103.9±1.7[a]	110.7±1.8	89.42±2.4	1.22±0.01[b]
CT	21.0±1.13*	17.7±1.1	27.9±0.3*	14.9±0.33	20.0±1.78*	20.3±2.16	27.9±0.39	14.9±0.33	1.86±0.02[b]
TT	11.06±0.4*	11.7±0.17*	9.8±0.56*	6.95±0.13	7.15±0.31	9.35±0.39[a]	11.7±0.17	6.95±0.13	1.69±0.02[b]
MT	23.5±0.72*	20.0±0.81*	23.3±3.1*	17.2±0.7*	8.7±0.27	18.5±1.19[a]	23.5±0.72	8.72±0.77	2.69±0.068
C.2yr									
C	51.5±2.87*	48.4±4.18*	21.0±0.61	19.6±1.75	28.44±1.8*	29.81±5.16	48.4±4.18	19.6±1.75	2.49±0.179
ET	62.9±4.4*	132.8±5.7*	129.6±1*	65.7±1.0*	47.49±1.83	87.75±6.8[a]	132.8±5.7	47.4±1.83	2.79±0.094
EW	25.5±0.48*	19.7±0.55*	15.8±0.64	22.5±1.1*	22.2±0.79*	21.16±0.68	25.5±0.48	15.8±0.64	1.61±0.04[b]
CT	13.66±0.25	26.9±1.73*	26.6±1.7*	10.8±0.95	14.79±1.4	18.3±1.345	26.9±1.75	10.8±0.95	2.49±0.158
TT	23.6±0.42*	7.42±0.16	7.8±0.32	7.88±0.25	7.54±0.22	10.8±1.19[a]	23.6±0.42	7.42±0.16	3.18±0.05[b]
MT	9.18±0.39*	7.17±0.13	9.03±0.5*	6.09±0.17	7.21±0.44	7.73±0.28[a]	9.18±0.39	6.09±0.17	1.50±0.04[b]

Each value is mean ± SE, (n=6); One Way ANOVA: * Refers to comparison with lowest value in each experimental group with other time points ($p \leq 0.05$). [a] Refers to comparison with control in mean levels ($p \leq 0.05$). [b] Refers to comparison with control in daily pulse levels ($p \leq 0.05$).

Table 9. Curcumin effect on ethanol induced changes in daily rhythms, mean and pulse levels of 5-HT in Pineal.

Exp. Group	Zeitgeber time (h)					Mean (24h)	Max	Min	Ratio
	0	6	12	18	24/0				
A. 90 day									
C	133.7±15.94*	235.6±3.1*	136.2±4.1*	64.0±4.23	133.7±15.9*	142.4±18.7	235.6±3.19	64.0±4.23	3.6±0.10
ET	136.60±17.2*	53.1±9.42*	20.4±3.33	12.8±3.69	136.6±17.2*	57.0 ±10.9[a]	136.6±17.2	12.8 ±3.6	11.5±2.0[b]
EW	19.208±6.46	40.69±15.3	19.68±0.91	6.02±0.91	19.20±6.46	18.99±4.78[a]	40.6 ± 15.3	6.02±0.9	6.89±1.62
CT	59.35±14.31	191.4±44.2*	92.08±10.4	91.2±14.3	64.1±6.21	99.8±12.8[a]	191.4±44.2	59±14.3	3.42±0.66
TT	38.961±9.89*	9.224±1.90	42.0±6.11*	45.8±4.09*	47.4±12.4*	36.8±4.19[a]	47.41±12.4	9.22±1.9	5.36±1.04
MT	15.876±2.22	2.586±0.72	11.4±3.44	7.968±1.75	20.4±8.59	11.6±2.13[a]	20.448±8.5	2.58±0.7	8.57±2.5
B. 1 year									
C	108.3±3.17*	191.9±3.56*	134.6±2.3*	58.7±1.54	91.3±3.502*	117.0±22.4	191.9±3.56	58.7±1.5	3.26±0.06
ET	946.3±119.2*	770.2±58.5*	605±16.68*	204.7±4.03	425.3±25.8*	590±54.34[a]	946±119.2	204±4.03	4.62±0.3[b]
EW	619.4±13.18*	299.7±7.0*	457.9±6.5*	145.9±1.47	539.4±19.0*	412±31.9[a]	619.4±13.1	145±1.47	4.2±0.05[b]
CT	150.8±4.23*	97.04±1.47*	168.3±2.3*	63.4±2.06	155.3±1.88*	127.0±7.51	168.3±2.35	63.4±2.0	2.6±0.05[b]
TT	200.4±1.23*	211.0±3.28*	220.6±3.1*	159.4±6.79	273.8±4.74*	213.0±7.06	273.8±4.74	159±6.79	1.7±0.04[b]
MT	199.3±5.19*	274.1±3.27*	242.6±1.9*	92.6±3.85	200.4±2.22*	201.8±11.4	274.1±3.27	92.6±3.8	2.96±0.07
C. 2 year									
C	58.09±0.623*	113.3±8.15*	97.7±6.52*	31.20±0.65	75.7±6.25*	75.2±14.48	113.3±8.15	31.2±0.6	3.66±0.17
ET	492.9±14.6*	444.8±16.8*	421±13.88*	167.7±2.50	390.3±12.5*	383±21.6[a]	492.9±14.6	167±2.5	2.9±0.05[b]
EW	324.8±5.612*	343.4±5.81*	268.1±4.2*	77.69±1.19	367.8±11.8*	276±19.6[a]	367.8±11.8	77.6±1.1	4.7±0.09[b]
CT	86.67±0.858*	110.4±2.36*	95.2±0.93*	20.6±0.254	54.50±0.73*	73.49±5.99	110.4±2.36	20.6±0.2	5.3±0.07[b]
TT	116.37±1.44*	107.8±3.38*	105.1±1.1*	80.5±1.20*	57.425±1.32	93.60±4.10	116.37±.1.4	57.4±1.3	2.0±0.03[b]
MT	88.13±1.497*	72.50±1.02*	78.6±2.13*	43.1±0.70	96.14±2.62*	75.9±3.443	96.14±2.62	43.1±0.7	2.2±0.04[b]

Each value is mean ± SE, (n=6); One Way ANOVA: * Refers to comparison with lowest value in each experimental group with other time points ($p \leq 0.05$). [a] Refers to comparison with control in mean levels ($p \leq 0.05$). [b] Refers to comparison with control in daily pulse levels ($p \leq 0.05$).

Mean levels decreased in 90 day ($p \leq 0.05$) whereas in 1 and 2yr, increased levels were found. Daily pulse levels were increased in 90 day whereas in 1 and 2yr, decreased levels were found ($p \leq 0.05$). Melatonin treatment, rhythmicity was not observed 90 day only. Partial restoration of mean levels was observed in 2yr only. Robust decrease in mean levels was observed in 90 day and increase in levels was observed 1yr ($p \leq 0.05$). Daily pulse levels were increased 90 day and whereas decreased in 1 and 2yr (Table 9; Fig. 60 and 61).

Ethanol treatment causes change in mean, daily pulse levels and rhythmicity of 5-HT in SCN as well as Pineal. These changes were not restored in ethanol withdrawal. Curcumin treatment was partially helpful in restoration of levels in SCN as well as pineal. Melatonin treatment causes partial restoration in 2yr Pineal but not in SCN. Turmeric was helpful in restoration of levels in 90 day SCN only (Fig. 49).

3. 5-HIAA

In SCN, 5-HIAA showed rhythmicity in 90 day and 2yr but not in 1yr. Age related increase in mean levels were observed ($p \leq 0.05$). Significant change was not observed in daily pulse among all three age groups ($p \leq 0.05$). Ethanol treatment did not affect the rhythmicity in any of three age groups. ET caused decreased mean levels in 90 day whereas robust increase was observed in 1 and 2yr ($p \leq 0.05$). Significant change was not observed in daily pulses of 90 day as well as 2yr whereas decreased levels were observed in 1yr ($p \leq 0.05$). Upon EW, rhythmicity was lost in 90day only. Mean levels were decreased in 90 day ($p \leq 0.05$) whereas levels were increased in 1 ($p \leq 0.05$) and 2yr. Daily pulse levels were decreased in all three age groups. Curcumin treatment was helpful in partial restoration of mean levels in 90day as well as 1yr but not in 2yr but rhythmicity was not restored in 90 day. Partial restoration of daily pulse was observed in 90 day and 1yr whereas in 2yr robust increase was observed ($p \leq 0.05$). In Turmeric treatment, rhythmicity was not observed in 90 day. Mean levels were decreased in all three age groups significantly ($p \leq 0.05$). Daily pulse levels were decreased in all three age groups. Melatonin treatment, rhythmicity was observed in all three age groups. Significant decreases in mean levels were observed in all three age groups ($p \leq 0.05$). Restoration of daily pulse was observed in 90 day only and decreases in the levels were found in 1yr ($p \leq 0.05$) and 2yr (Table 10; Fig. 58 and 59).

In Pineal, 5-HIAA showed rhythmicity in all three age groups. The mean as well as daily pulse levels were decreased upon aging ($p \leq 0.05$). Ethanol treatment caused abolition of rhythm in 90 day only. Elevation of mean levels was observed in 1 and 2yr ($p \leq 0.05$) whereas decreased levels were observed in 90 day ($p \leq 0.05$). Significant decrease in daily pulse as observed in 90 day and levels were not affected in 1 and 2yr. Upon EW, rhythmicity was not affected in any of the three age groups. Mean levels were increased in all three age groups ($p \leq 0.05$). Significant decrease in daily pulse levels were observed in all age groups. Curcumin treatment was sensitive in partial restoration of mean levels in 90 day as well as in 2yr but not in 1yr.

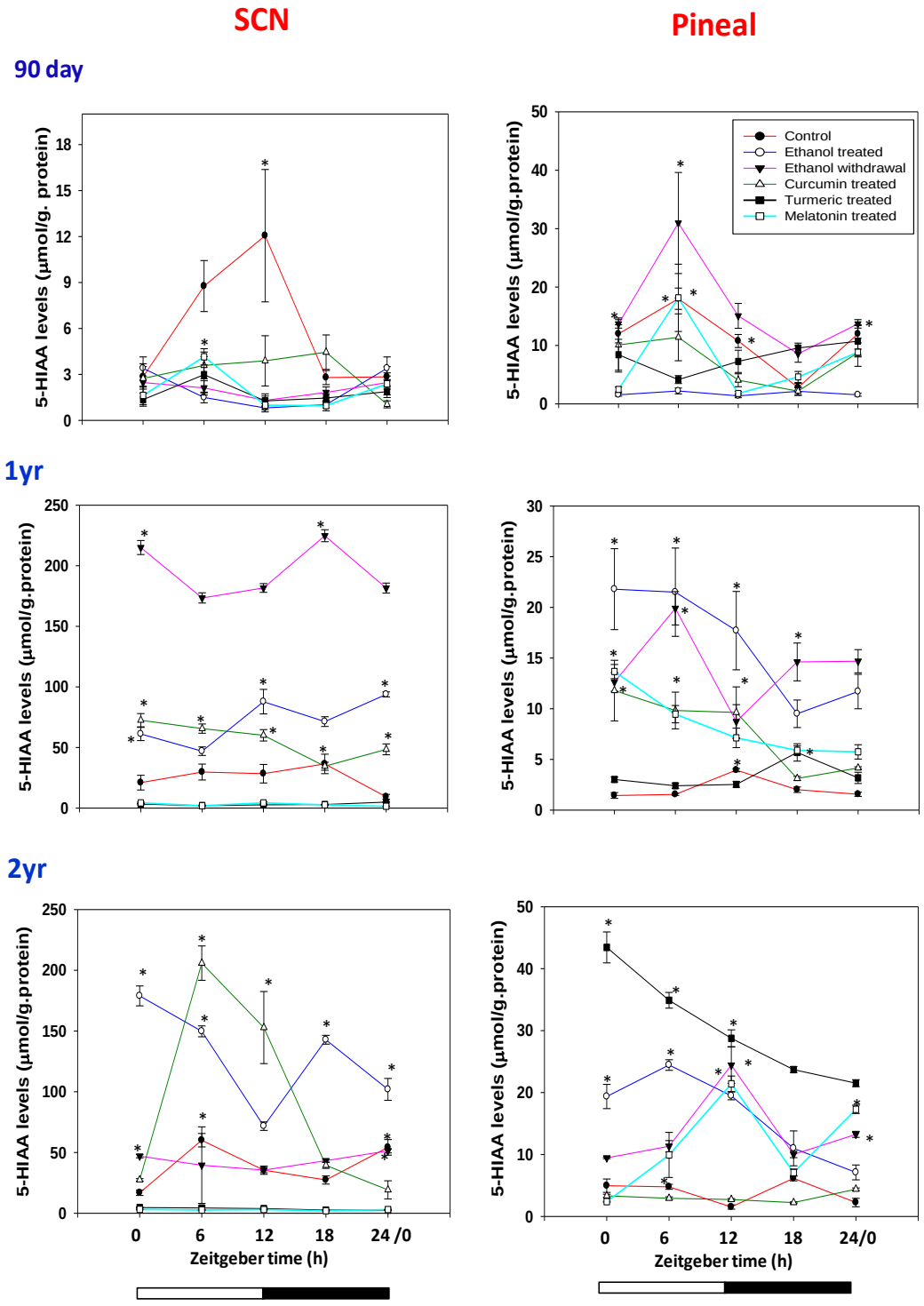

Fig. 50. Curcumin effect on ethanol induced changes in daily rhythms of 5-HIAA. Each value is mean ± SE, (n=6); One Way ANOVA: * Refers to comparison with lowest value in each group ($p \leq 0.05$).

Table 10. Curcumin effect on ethanol induced changes in daily rhythms, mean and pulse levels of 5-HIAA in SCN.

A .90 day									
Exp. Group	Zeitgeber time (h)					Mean (24h)	Max	Min	Ratio
	0	6	12	18	24/0				
C	2.83±0.67	8.76±1.66	12.0±4.3*	2.79±0.46	2.83±0.67	6.61 ± 1.37	12.05±4.3	2.79± 0.46	4.43 ± 1.01
ET	3.39±0.74*	1.49±0.35	0.81±0.26	1.02±0.21	3.39±0.74*	1.72± 0.29[a]	3.39 ±0.74	0.81± 0.26	4.65 ± 1.05
EW	2.46±0.43	2.11±0.47	1.30±0.42	1.82±0.36	2.46±0.43	1.92± 0.21[a]	2.46±0.43	1.30± 0.42	2.11 ± 0.45
CT	2.73±0.962	3.58±0.879	3.88±1.63	4.44±1.12	1.04±0.230	3.13±0.49[a]	4.44± 1.12	1.04± 0.23	4.46 ± 0.87
TT	1.35±0.43	2.98±1.183	1.26±0.32	1.45±0.27	1.87±0.39	1.78±0.28[a]	2.98±1.18	1.26±0.32	2.511±0.69
MT	1.62±0.60	4.14±0.53*	0.98±0.29	0.95±0.33	2.37±0.72	2.01±0.30[a]	4.14±0.53	0.95±0.33	4.916±1.05
B.1yr									
C	20.98±6.07	29.74±6.55	28.39±7.6	36.4±8.03	9.27±2.012	24.9±4.62	36.4±8.03	9.27±2.01	4.12±0.745
ET	61.15±5.44	46.97±3.51	87.8±10*	71.3±4.0*	93.81±2.1*	72.2±3.98[a]	93.8±2.10	46.97±3.5	2.00±0.09[b]
EW	215.0±5.7*	173.44±4.2	181.6±3.5	224±4.9*	181.5±4.13	195.3±4.2[a]	224.7±4.9	181.5±4.1	1.23±0.02[b]
CT	72.5±5.45*	65.58±3.8*	60.0±4.7*	34.55±3.3	48.46±4.4*	20.34±0.92	72.53±5.4	34.55±3.3	2.11±0.14[b]
TT	3.206±0.50	1.98±0.41	2.62±0.59	2.92±0.28	4.89±0.90*	3.12±0.30[a]	4.895±0.9	1.98±0.41	2.53±0.41[b]
MT	4.27±0.58*	1.93±0.27	4.28±0.8*	2.67±0.20	1.47±0.105	2.92±0.30[a]	4.28±0.88	1.47±0.10	2.922±0.37
C. 2yr									
C	16.88±2.22	60.1±5.56*	35.4±3.3*	27.37±3.3	54.2±6.43*	38.81±8.10	60.1±5.56	16.88±2.2	3.62±0.337
ET	178.9±8.1*	149.7±4.4*	71.7±3.48	143±3.6*	101.9±9.0*	131.4±7.3[a]	178.9±8.1	71.7±3.48	2.50±0.096
EW	47.0±1.38*	39.57±1.51	35.5±2.16	43.3±1.1*	51.36±1.6*	43.35±1.48	51.36±1.6	35.5±2.16	1.45±0.058
CT	27.4±0.987	205.8±14*	152.8±29*	39.3±2.4*	19.23±7.49	88.9±15.4[a]	205.8±14	19.23±7.4	12.6±2.88[b]
TT	4.71±0.371	4.31±1.66	3.84±0.67	2.61±0.29	2.582±0.38	3.61±0.38[a]	4.71±0.37	2.58±0.28	1.847±0.14
MT	3.12±0.28*	2.66±0.15	2.9±0.29*	1.99±0.27	3.02±0.21*	2.74±0.13[a]	3.12±0.28	1.91±0.27	1.663±0.16

Each value is mean ± SE, (n=6); One Way ANOVA: * Refers to comparison with lowest value in each experimental group with other time points ($p \leq 0.05$). [a] Refers to comparison with control in mean levels ($p \leq 0.05$). [b] Refers to comparison with control in daily pulse levels ($p \leq 0.05$).

Table 11. Curcumin effect on ethanol induced changes in daily rhythms, mean and pulse levels of 5-HIAA in Pineal.

A. 90 day									
Exp. Group	Zeitgeber time (h)					Mean (24h)	Max	Min	Ratio
	0	6	12	18	24/0				
C	11.9±1.54*	18.0±1.81*	10.7±1.12*	2.79±0.71	11.9±1.54*	10.8±1.29	18.0±1.8	2.7 ± 0.7	7.14±1.15
ET	1.54±0.24	2.21±0.50	1.37±0.32	2.15±0.71	1.54±0.24	1.82±0.23[a]	2.15±0.7	1.37±0.32	1.65±0.39[b]
EW	13.6±0.725	30.9±8.65*	15.06±2.13	8.56±1.43	13.69±0.72	17.07±2.74	30.9±8.6	8.56 ±1.4	3.71 ± 0.69
CT	10.08±4.62	11.39±4.01	4.04±1.103	2.18±0.63	8.77±0.61	7.29±1.39	11.3±4.01	4.04 ±1.1	3.01 ± 0.79
TT	8.403±2.65	4.155±0.68	7.27±1.91	9.56±0.83	10.72±2.68	8.02±0.91	10.7±2.68	4.15±0.68	2.65±0.461
MT	2.46±0.412	18.1±5.76*	1.763±0.39	4.60±0.95	8.835±2.41	7.16±1.62	18.1±5.76	1.76±0.39	10.82±2.45
B.1 year									
C	1.43±0.284	1.54±0.099	3.94±0.15*	2.00±0.27	1.56±0.209	2.09±0.47	3.94±0.15	1.43±0.284	2.84±0.331
ET	21.7±3.99*	21.5±4.35*	17.70±3.86	9.50±1.36	11.69±1.69	16.4±1.65[a]	21.7±3.99	9.50±1.36	2.34±0.316
EW	12.63±0.91	19.9±1.64*	8.73±1.672	14.6±1.87*	14.6±1.14*	12.3±0.75[a]	14.6±1.14	8.73±1.672	1.74±0.208
CT	11.78±2.99*	9.82±1.81*	9.63±2.52*	3.12±0.20	4.154±0.12	7.70±1.71[a]	11.7±2.99	3.12±0.20	3.76±0.573
TT	3.01±0.244	2.4±0.265	2.531±0.28	5.68±0.85*	3.17±0.55	3.55±0.30	5.68±0.85	2.4±0.265	2.29±0.181
MT	13.6±0.69*	9.46±0.85*	7.12±0.96	5.89±0.447	5.74±0.70	8.37±0.63[a]	13.6±0.69	5.74±0.70	2.41±0.185
C. 2 year									
C	4.96±1.08*	4.78±0.40*	1.53±0.345	6.16±0.41*	2.24±0.67	3.93±0.87	6.16±0.41	2.24±0.67	3.02±0.544
ET	19.3±1.95*	24.4±0.85*	19.4±0.63*	10.9±2.81	7.10±1.19	16.2±1.36[a]	24.4±0.85	7.10±1.19	3.53±0.350
EW	9.45±0.132	11.32±0.91	24.4±2.93*	10.01±0.51	13.24±0.48	13.6±1.18[a]	24.4±2.93	9.45±0.132	2.58±0.180
CT	3.32±0.24*	2.93±0.23*	2.73±0.14	2.23±0.108	4.38±0.20*	3.12±0.157	4.38±0.20	2.23±0.10	1.96±0.076
TT	43.4±2.49*	34.8±1.27*	28.7±1.36*	23.7±0.48	21.51±0.56	30.4±1.59[a]	43.4±2.49	21.5±0.56	2.02±0.073
MT	2.37±0.076	9.92±3.63*	21.4±1.26*	7.07±0.56	17.2±0.67*	11.6±1.47[a]	21.4±1.26	2.37±0.07	9.03±0.35[b]

Each value is mean ± SE, (n=6); One Way ANOVA: * Refers to comparison with lowest value in each experimental group with other time points ($p \leq 0.05$). [a] Refers to comparison with control in mean levels ($p \leq 0.05$). [b] Refers to comparison with control in daily pulse levels ($p \leq 0.05$).

Rhythmicity was not restored in 90 day. Curcumin caused decreased daily pulse levels in 90 day and 2yr whereas increased levels in 1yr. In Turmeric treatment, rhythmicity was not observed in 90 day only. Partial restoration of mean levels was observed in 90 day and 1yr but not in 2yr. Robust increases in mean levels were observed in 2yr ($p \leq 0.05$). Daily pulse levels were decreased in all three age groups. In Melatonin treatment, rhythmicity was observed in all three age groups. Mean levels were increased in 1 and 2yr ($p \leq 0.05$) and partial restoration was observed in 90 day. Daily pulse levels were increased in 90 day and 1yr ($p \leq 0.05$) whereas in 2yr, decreased levels were found (Table 11; Fig. 60 and 61).

Ethanol treatment causes change in mean, daily pulse levels and rhythmicity of 5-HIAA in SCN as well as Pineal. Restoration was not observed in ethanol withdrawal. Curcumin treatment was partially sensitive in restoration of levels 90 day and 1yr in SCN whereas 90 day and 2yr in pineal. Melatonin treatment causes partial restoration in 90 day Pineal only. Turmeric was sensitive in restoration of levels in 90 day and 1yr Pineal (Fig. 50).

4. NAS

In SCN, NAS showed rhythmicity in 90 day and lost upon aging. Higher levels were observed in middle age group ($p \leq 0.05$). Significant decrease in daily pulse was observed upon aging ($p \leq 0.05$). Ethanol treatment resulted in abolition of rhythmicity in 90 day only. Mean levels were increased in 90 day and 2yr ($p \leq 0.05$) whereas decrease was observed in 1yr ($p \leq 0.05$). Significant decrease was observed in daily pulses of 90 day ($p \leq 0.05$) as well as 2yr whereas increased levels were observed in 1yr. Upon EW, rhythmicity was lost in 90day only. Mean levels were increased in 90 day as well as 1yr ($p \leq 0.05$) and not much significant change observed in 2yr. Daily pulse levels were decreased in all three age groups but significant change observed in 90 day only ($p \leq 0.05$). Curcumin treatment was helpful in partial restoration of mean levels in 90day only and decreases in 1yr as well as 2yr ($p \leq 0.05$) but rhythmicity was restored in 1 and 2yr. Daily pulse levels were decreased in 90 day ($p \leq 0.05$) and increased in 2yr ($p \leq 0.05$) and not much significant change was found in 1yr. In Turmeric treatment, rhythmicity was not observed in 90 day. Mean levels were increased in 90 day ($p \leq 0.05$) whereas decreased in 1yr as well as 2yr ($p \leq 0.05$). Robust increase in daily pulse levels were observed in 1yr ($p \leq 0.05$) whereas decreased levels were observed in 90 day as well as 2yr. Melatonin treatment, rhythmicity was observed in 90 day but not in 1 and 2yr. Significant increases in mean levels were observed in 90 day and decrease in 1 as well as 2yr ($p \leq 0.05$). Restoration of daily pulse was observed in 1 and 2yr but levels were decreased in 90 day ($p \leq 0.05$) (Table 12; Fig. 58 and 59).

In Pineal, NAS showed rhythmicity in 90 day and abolition was observed upon aging. Higher mean levels were observed in 1yr than other age groups. Daily pulse levels were decreased in 1yr when compared with other age groups ($p \leq 0.05$). Ethanol treatment was not affected the rhythmicity in three age groups. Elevation of mean levels was observed in 1 and 2yr ($p \leq 0.05$) whereas decreased levels were observed in 90 day. Significant decrease in daily pulse levels were observed in 90 day and 2yr ($p \leq 0.05$) and not affected in 1yr. Upon EW, rhythmicity was not affected in 90 day only. Mean levels were increased in 1 and 2yr ($p \leq 0.05$) but decreased in 90 day. Significant decrease in daily pulse levels were observed in 2yr only. Curcumin treatment was sensitive in partial restoration of mean levels in 1yr whereas levels were decreased in 90 day and increased in 2yr. Restoration of rhythmicity was observed in 1 and 2yr.

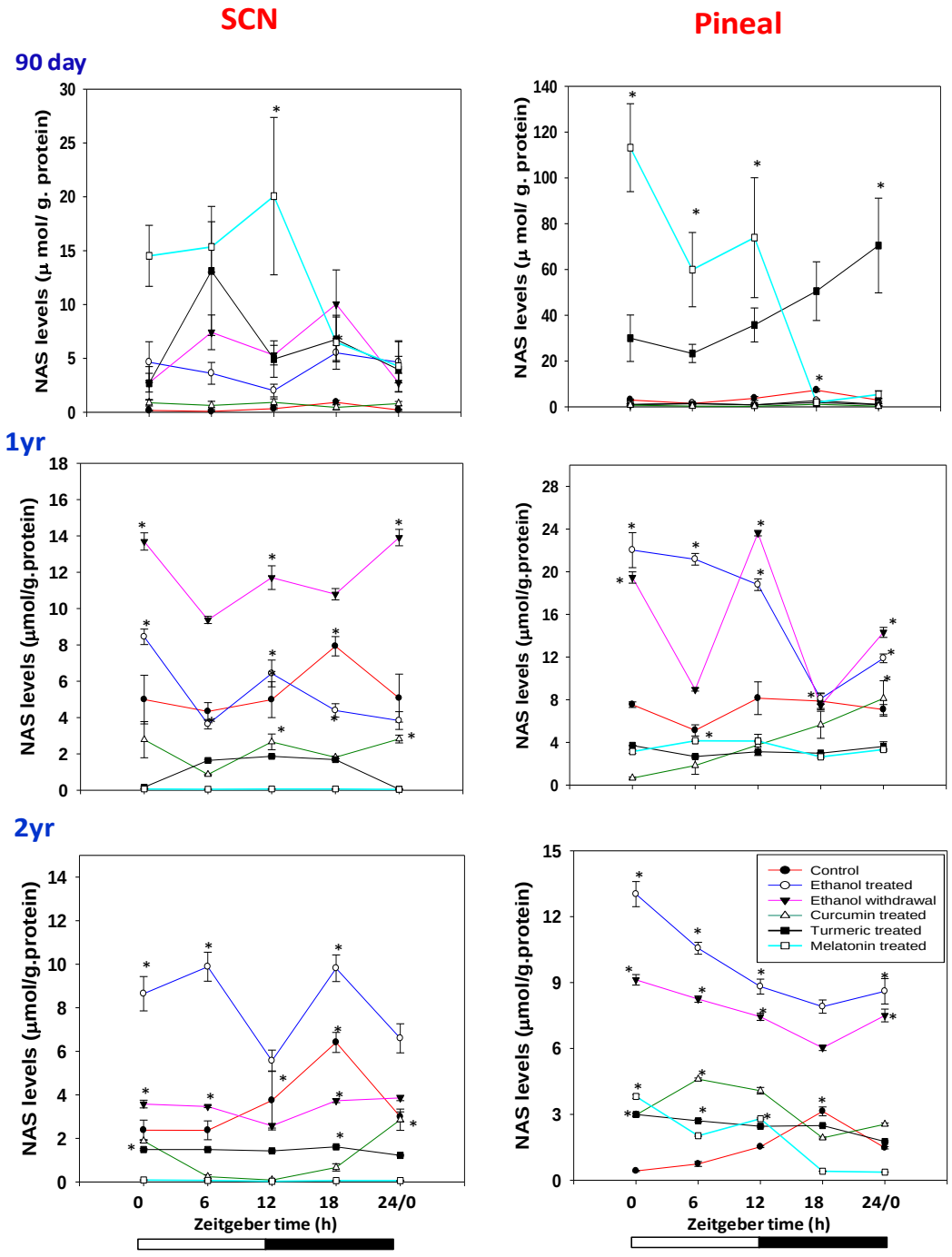

Fig. 51. Curcumin effect on ethanol induced changes in daily rhythms of NAS. Each value is mean ± SE, (n=6); One Way ANOVA: * Refers to comparison with lowest value in each group ($p \leq 0.05$).

Table 12. Curcumin effect on ethanol induced changes in daily rhythms, mean and pulse levels of NAS in SCN.

A .90 day									
Exp. Group	Zeitgeber time (h)					Mean (24h)	Max	Min	Ratio
	0	6	12	18	24/0				
C	0.16±0.056	0.07±0.0007	0.32±0.08	0.91±0.18*	0.210±0.07	0.49±0.112	0.91±0.18	0.08±0.0007	11.54±1.34
ET	4.641±1.9	3.61±1.01	2.01±0.59	5.51±0.71	4.641±1.9	3.961±0.62	5.51± 0.71	2.01± 0.59	2.99±0.56[b]
EW	2.74±0.87	7.41±1.61	5.30±0.91	10.0±3.18	2.740±0.87	6.37±1.04[a]	10.0±3.18	2.74± 0.87	4.0 ± 1.08[b]
CT	0.87±0.30	0.63±0.383	0.91±0.32	0.45±0.23	0.802±0.20	0.73±0.127	0.91±0.32	0.45± 0.23	2.73±1.02[b]
TT	2.68±1.55	13.12±5.99	4.93±1.69	6.75±2.08	3.95±1.233	6.29±1.44[a]	13.1±5.99	2.68±1.55	7.328±3.30
MT	14.52±2.83	15.34±2.34	20.0±7.3*	6.49±2.51	4.276±2.36	12.1±1.97[a]	20.0±7.31	4.27±2.36	6.747±2.6[b]
B.1yr									
C	4.991±1.34	4.335±0.49	4.98±0.98	7.92±0.53	5.070±1.32	5.461±0.63	7.928±0.53	4.33±0.49	1.85±0.141
ET	8.44±0.42*	3.634±0.26	6.4±0.73*	4.39±0.37	3.835±0.48	3.835±0.48[a]	8.44±0.426	3.63±0.260	2.33±0.118
EW	13.69±0.47*	9.37±0.201	11.7±0.65*	10.79±0.31*	13.91±0.45*	11.89±0.37[a]	13.6±0.477	9.376±0.20	1.45±0.034
CT	2.78±0.99*	0.865±0.02	2.66±0.43*	1.81±0.06	2.82±0.215*	2.18±0.249[a]	2.82±0.215	0.865±0.02	3.26±0.157
TT	0.166±0.05	1.64±0.05*	1.86±0.05*	1.68±0.05*	0.052±0.01	1.081±0.14[a]	1.864±0.05	0.052±0.01	37.4±4.93[b]
MT	0.066±0.01	0.05±0.012	0.068±0.017	0.068±0.01	0.046±0.008	0.06±0.005[a]	0.068±0.01	0.046±0.008	1.530±0.22
C.2yr									
C	2.37±0.463*	2.37±0.432	3.74±1.35	6.40±0.45*	2.98±0.229	3.37±0.814	6.408±0.45	2.37±0.432	2.79±0.315
ET	8.64±0.789*	9.88±0.665*	5.56±0.49	9.81±0.61*	6.59±0.667	8.10±0.421[a]	9.884±0.66	5.56±0.491	1.79±0.114
EW	3.57±0.177*	3.46±0.049*	2.58±0.10	3.73±0.08*	3.85±0.122*	3.44±0.096	3.73±0.088	2.58±0.104	1.44±0.039
CT	1.88±0.061*	0.24±0.087	0.085±0.01	0.66±0.17	2.85±0.488*	1.147±0.22[a]	2.85±0.488	0.24±0.087	13.5±3.18[b]
TT	1.48±0.044*	1.48±0.03**	1.42±0.049*	1.61±0.05*	1.22±0.033	1.44±0.029[a]	1.612±0.05	1.221±0.03	1.320±0.03
MT	0.086±0.018*	0.068±0.014*	0.029±0.004	0.061±0.01	0.06±0.0098	0.06±0.006[a]	0.086±0.01	0.029±0.004	3.052±0.47

Each value is mean ± SE, (n=6); One Way ANOVA: * Refers to comparison with lowest value in each experimental group with other time points ($p \leq 0.05$). [a] Refers to comparison with control in mean levels ($p \leq 0.05$). [b] Refers to comparison with control in daily pulse levels ($p \leq 0.05$).

Table 13. Curcumin effect on ethanol induced changes in daily rhythms, mean and pulse levels of NAS in Pineal.

A. 90 day									
Exp. Group	Zeitgeber time (h)					Mean (24h)	Max	Min	Ratio
	0	6	12	18	24/0				
C	1.00±0.20	0.86±0.34	4.46±0.43*	6.8±0.31*	1.00±0.20	3.3±0.77	6.89±0.31	0.86±0.34	7.14±0.84
ET	1.05±0.40	1.53±0.22	0.878±0.23	2.68±0.6*	1.05±0.40	1.51±0.24	2.68 ± 0.68	0.87± 0.23	3.29±0.71
EW	0.94±0.41	1.34±0.41	0.851±0.24	1.88±0.17	0.94±0.41	1.08± 0.16	1.88 ±0.17	0.85±0.24	2.4±0.41
CT	0.73±0.43	0.31±0.18	0.20±0.004	1.10±0.58	0.24±0.05	0.52± 0.15	1.1 ± 0.58	0.20±0.04	5.72±1.89
TT	29.9±10.1	23.31±3.9	35.753±7.43	50.5±12.8	70.4±20.6	41.9±6.0[a]	70.46±20.6	23.3±3.97	3.11±0.61
MT	113.1±19.1*	59.9±16.2*	73.8±26.1*	1.96±0.44	5.38±1.65	50.9±10.3[a]	113.1±19.1	1.96±0.44	60.5±9.8[b]
B. 1 year									
C	7.53±0.26	5.12±0.52	8.141±1.53	7.87±0.69	7.07±0.47	7.15±0.53	8.141±1.53	5.12±0.52	1.60±0.19
ET	22.0±1.64*	21.1±0.54*	18.7±0.54*	8.10±0.52	11.8±0.41*	16.3±1.07[a]	22.02±1.64	8.10±0.52	2.72±0.156
EW	19.4±0.53*	8.95±0.14*	23.6±0.25*	7.43±0.36	14.3±0.46*	14.7±1.14[a]	19.4±0.531	7.43±0.36	2.61±0.08
CT	0.65±0.09	1.83±0.82	3.76±0.99	5.6±1.26*	8.11±1.66*	7.70±1.02	8.11±1.664	0.65±0.09	12.6±1.85[b]
TT	3.71±0.02*	2.67±0.14	3.12±0.07	2.99±.0.05	3.62±0.44*	3.22±0.11[a]	3.71±0.025	2.67±0.14	1.39±0.044
MT	3.14±0.16*	4.15±0.07*	4.125±0.07*	2.64±0.10	3.33±0.05*	3.47±0.11[a]	4.15±0.072	2.64±0.10	1.575±0.03
C. 2 year									
C	0.42±0.029	0.74±0.111	1.52±0.03	3.13±0.2*	1.47±0.051	1.49±0.501	3.13±0.201	0.42±0.02	7.37±0.39[b]
ET	13.0±0.57*	10.5±0.27*	8.817±0.34	7.90±0.30	8.60±0.578	9.78±0.38[a]	13.02±0.57	7.90±0.30	1.65±0.05[b]
EW	9.12±0.24*	8.24±0.14*	7.44±0.16*	6.03±0.12	7.50±0.29*	7.66±0.20[a]	9.12±0.241	6.03±0.12	1.51±0.02[b]
CT	2.97±0.06*	4.60±0.05*	4.07±0.14*	1.92±0.04	2.54±0.05*	3.22±0.18[a]	4.60±0.055	1.92±0.04	2.39±0.03[b]
TT	2.99±0.05*	2.70±0.07*	2.45±0.03*	2.4±0.04*	1.76±0.03	2.48±0.078	2.997±0.05	1.76±0.03	1.69±0.02[b]
MT	3.81±0.08*	2.02±0.03*	2.80±0.03*	0.4±0.009	0.37±0.017	1.884±0.25	3.814±0.08	0.37±0.01	10.34±0.3[b]

Each value is mean ± SE, (n=6); One Way ANOVA: * Refers to comparison with lowest value in each experimental group with other time points ($p \leq 0.05$). [a] Refers to comparison with control in mean levels ($p \leq 0.05$). [b] Refers to comparison with control in daily pulse levels ($p \leq 0.05$).

Curcumin caused decrease in daily pulse levels in 90 day and 2yr whereas increased in 1yr. Turmeric treatment, rhythmicity was not observed 90 day only. Mean levels were increased in 90 day and 2yr whereas decreased levels were observed in 2yr. Daily pulse levels were decreased in all three age groups but significant change was observed in 2yr only ($p \leq 0.05$). In Melatonin treatment, rhythmicity was observed in all three age groups. Mean levels were increased in 90 day whereas decreased in 1yr ($p \leq 0.05$) and partial restoration was observed in 2yr. Daily pulse levels were increased in 90 day and 2yr ($p \leq 0.05$) whereas restoration was found in 1yr (Table 13; Fig. 60 and 61).

Ethanol treatment causes change in mean, daily pulse levels and rhythmicity of NAS in SCN as well as Pineal. Restoration was not observed in ethanol withdrawal. Curcumin treatment was partially sensitive in restoration of levels 90 day SCN and 1yr in pineal. Melatonin treatment causes partial restoration in 2yr Pineal only. Turmeric was not sensitive in restoration of levels all three age groups in SCN as well as Pineal (Fig. 51).

5. TRP

In SCN, TRP showed rhythmicity in 90 day and 2yr but abolition was observed in 1yr. Increased mean levels were observed upon aging ($p \leq 0.05$). Significant decrease in daily pulse was observed upon aging ($p \leq 0.05$). Ethanol treatment gives abolition of rhythmicity in 1 and 2yr. Mean levels were decreased in 90 day and 2yr ($p \leq 0.05$) whereas increase was observed in 1yr. Significant decrease was observed in daily pulses of 90 day ($p \leq 0.05$) as well as 2yr whereas increased levels were observed in 1yr. Upon EW, rhythmicity was observed in 90 day only. Mean levels were decreased in 2yr ($p \leq 0.05$) and no significant change observed in 90 day and 2yr. Daily pulse levels were decreased in 90 day ($p \leq 0.05$) and increased in 1yr as well as 2yr . Curcumin treatment was not helpful in partial restoration of mean levels and decreased levels were found in all three age groups but rhythmicity was restored in 90 day and 2yr. Daily pulse levels were decreased in 90 day ($p \leq 0.05$) and increased in 1 and 2yr. In Turmeric treatment, rhythmicity was not observed in all three age groups. Mean levels were increased in 90 day ($p \leq 0.05$) and 1yr whereas decreased in 2yr ($p \leq 0.05$). Robust increase in daily pulse levels were observed in 90 day ($p \leq 0.05$) whereas increased levels were observed in 1yr as well as 2yr. Melatonin treatment, rhythmicity was observed in all age groups. Significant increases in mean levels were observed in 90 day and 1yr ($p \leq 0.05$) but decrease in the levels were observed in 2yr ($p \leq 0.05$). Daily pulse levels were decreased in 90 day ($p \leq 0.05$) and increased 1yr as well as 2yr (Table 14; Fig. 58 and 59).

In Pineal, TRP showed rhythmicity in 90 day as well as 2yr. Age related increase in the mean as well as daily pulse levels were observed upon aging ($p \leq 0.05$). Ethanol treatment was not affected the rhythmicity in three age groups. Elevation of mean levels was observed in 1yr ($p \leq 0.05$) whereas decreased levels were observed in 2yr and not much significant change was observed in 90 day. Significant increase in daily pulse levels was observed in 1 and 2yr ($p \leq 0.05$) and decrease in 90day. Upon EW, rhythmicity was not restored in 90 day only. Mean levels were increased in 1yr ($p \leq 0.05$) whereas decreased in 2yr ($p \leq 0.05$) and 90 day. Decreases in daily pulse levels were observed in 90 day and 2yr only but in 1yr, levels were increased ($p \leq 0.05$).

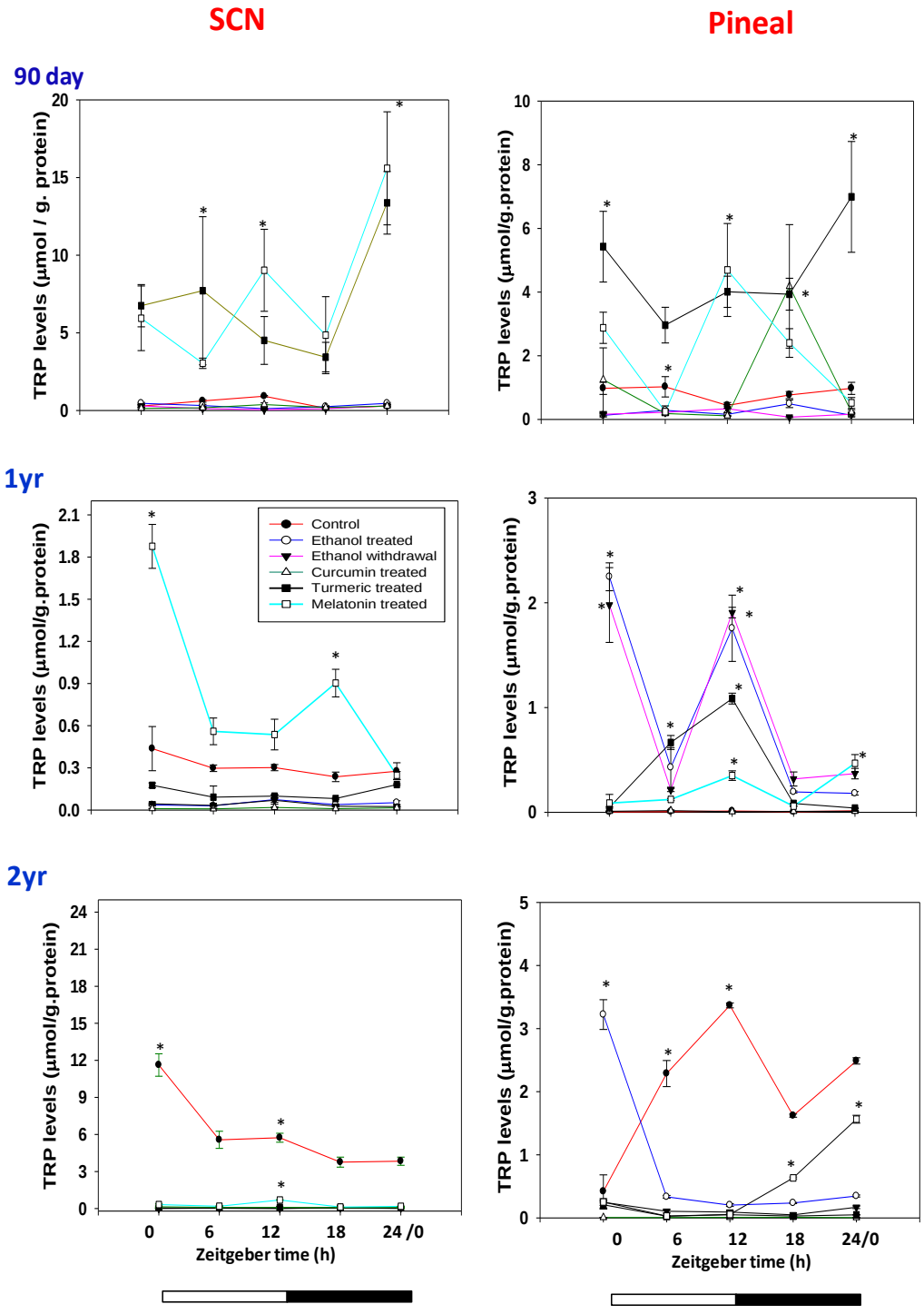

Fig. 52. Curcumin effect on ethanol induced changes in daily rhythms of TRP. Each value is mean ± SE, (n=6); One Way ANOVA: * Refers to comparison with lowest value in each group ($p \leq 0.05$).

Table 14. Curcumin effect on ethanol induced changes in daily rhythms, mean and pulse levels of TRP in SCN.

A .90 day									
Exp. Group	Zeitgeber time (h)					Mean (24h)	Max	Min	Ratio
	0	6	12	18	24/0				
C	0.283±0.08	0.61±0.08*	0.92±0.05*	0.14±0.09	0.283±0.09	0.468±0.14	0.91 ± 0.05	0.14 ± 0.09	11.07 ±4.13
ET	0.45±0.06*	0.32±0.07*	0.11±0.02	0.24±0.07	0.45±0.06*	0.28±0.038	0.45 ± 0.06	0.11 ± 0.02	4.22± 0.55[b]
EW	0.29±0.06*	0.124±0.02	0.05±0.007	0.115±0.06	0.29±0.06*	0.14±0.028	0.29 ± 0.06	0.05±0.007	6.52± 0.88[b]
CT	0.134±0.04	0.166±0.05	0.38±0.11*	0.19±0.04	0.28±0.008	0.18 ±0.127	0.38 ± 0.11	0.134±0.04	3.22 ±0.80[b]
TT	6.743±1.36	7.709±4.77	4.51±1.54	3.43±0.95	13.37±2.01	7.153±1.23[a]	13.37±2.01	3.433±0.95	4.21±0.77[b]
MT	5.932±2.08	3.038±1.03	9.02±2.64	4.84±2.48	15.59±3.64*	7.687±1.31[a]	15.59±3.64	3.038±0.33	4.88±0.67[b]
B.1yr									
C	0.43±0.157	0.29±0.023	0.3±0.022	0.236±0.03	0.27±0.06	0.039±0.03	0.437±0.15	0.23±0.034	1.89±0.410
ET	0.036±0.008	0.03±0.005	0.07±0.03	0.03±0.003	0.052±0.01	0.046±0.007	0.074±0.03	0.036±0.008	2.16±0.587
EW	0.044±0.007	0.032±0.004	0.069±0.01*	0.026±0.003	0.02±0.001	0.039±0.004	0.069±0.01	0.024±0.001	2.87±0.250
CT	0.01±0.0017	0.008±0.001	0.018±0.003	0.009±0.001	0.01±0.004	0.012±0.001	0.01±0.003	0.008±0.001	2.28±0.338
TT	0.17±0.016	0.091±0.07	0.099±0.011	0.081±0.004	0.18±0.006	0.126±0.009	0.18±0.006	0.081±0.004	2.22±0.075
MT	1.87±0.16*	0.56±0.095	0.53±0.109	0.9±0.098*	0.24±0.02	0.82±0.11[a]	1.876±0.15	0.24±0.028	7.69±0.62[b]
C.2yr									
C	11.62±0.9*	5.56±0.692*	5.73±0.36*	3.75±0.407	3.834±0.33	6.104±1.44	11.62±0.90	3.75±0.407	3.12±0.241
ET	0.025±0.007	0.035±0.007	0.029±0.004	0.03±0.001	0.02±0.004	0.03±0.002[a]	0.03±0.007	0.02±0.007	1.34±0.296
EW	0.012±0.001	0.028±0.003*	0.016±0.003	0.07±0.005*	0.04±4e-3*	0.03±0.003[a]	0.06±0.005	0.012±0.001	5.30±0.475
CT	0.062±0.034	0.023±0.014	0.012±0.003	0.04±0.011	0.011±2e-3	0.03±0.008[a]	0.062±0.03	0.011±0.002	6.08±2.007
TT	0.14±0.004*	0.098±0.002*	0.095±0.01*	0.08±0.006	0.08±6e-3*	0.10±0.005[a]	0.14±0.005	0.08±0.006	1.822±0.08
MT	0.32±0.083*	0.18±0.045	0.69±0.04*	0.124±0.02	0.18±0.004	0.30±0.043[a]	0.696±0.04	0.12±0.022	5.794±0.63

Each value is mean $\pm$ SE, (n=6); One Way ANOVA: * Refers to comparison with lowest value in each experimental group with other time points ($p \leq 0.05$). [a] Refers to comparison with control in mean levels ($p \leq 0.05$). [b] Refers to comparison with control in daily pulse levels ($p \leq 0.05$).

Table 15. Curcumin effect on ethanol induced changes in daily rhythms, mean and pulse levels of TRP in Pineal.

A. 90 day									
Exp. Group	Zeitgeber time (h)					Mean (24h)	Max	Min	Ratio
	0	6	12	18	24/0				
C	0.11±0.006	0.14±0.03	0.17±0.04	0.84±0.10*	0.11±0.006	0.32±0.09	0.84±0.10	0.11±0.0061	7.17±0.53
ET	0.126±0.05	0.28±0.14	0.15±0.06	0.49±0.12*	0.126±0.05	0.26±0.05	0.49±0.12	0.12 ± 0.05	4.93±1.40
EW	0.161±0.05	0.22±0.09	0.33±0.13	0.07±0.015	0.161±0.05	0.19±0.04	0.33±0.13	0.07 ± 0.015	4.93±1.30
CT	1.247±1.00	0.184±0.03	0.11±0.01	4.17±1.94*	0.233±0.06	1.19 ± 0.49	4.17±1.94	0.11 ± 0.01	38.2±10.5[b]
TT	5.426±1.11	2.96±0.56	4.01±0.49	3.93±0.50	6.989±1.74	4.63±0.49[a]	6.98±1.74	2.962±0.56	2.44±0.44
MT	2.87±0.49*	0.24±0.05	4.69±1.46*	2.401±0.45	0.518±0.17	2.14±0.42[a]	4.69±1.46	0.242±0.05	20.29±4.4
B.1 year									
C	0.004±7e-4	0.006±1e-4	0.01±0.002	0.005±9e-4	0.01±1e-4	0.008±1e-3	0.01±1e-4	0.004±7e-4	2.81±0.19
ET	2.24±0.13*	0.428±0.18	1.75±0.31*	0.19±0.009	0.18±0.016	0.96±0.17[a]	2.24±0.13	0.181±0.016	12.4±0.76[b]
EW	1.97±0.35*	0.213±0.01	1.90±0.05*	0.318±0.06	0.36±0.04	0.95±0.16[a]	1.97±0.35	0.213±0.015	10.1±0.61[b]
CT	0.009±3e-4	0.015±7e-3	0.005±1e-4	0.008±1e-3	0.009±1e-3	0.009±1e-3	0.01±7e-3	0.005±0.001	2.08±0.891
TT	0.04±0.006	0.66±0.06*	1.08±0.05*	0.08±0.003	0.04±0.005	0.384±0.08	1.08±0.05	0.040±0.005	27.57±2.2[b]
MT	0.08±0.08	0.122±0.01	0.35±0.04*	0.05±0.003	0.46±0.08*	0.21±0.035	0.46±0.08	0.058±0.003	8.07±0.87[b]
C. 2 year									
C	0.420±0.26	2.28±0.20*	3.36±0.03*	1.62±0.02*	2.48±0.05*	2.03±0.49	3.36±0.03	0.420±0.263	13.15±4.75
ET	3.22±0.23*	0.334±0.02	0.20±0.02	0.23±0.009	0.34±0.016	0.86±0.22[a]	3.22±0.23	0.203±0.022	16.05±1.21
EW	0.24±0.01*	0.10±0.02*	0.092±0.01	0.04±0.009	0.16±0.019*	0.13±0.01[a]	0.24±0.01	0.048±0.009	5.18±0.581
CT	0.006±1e-3	0.006±2e-3	0.009±3e-3	0.01±0.005	0.004±0.001	0.007±1e-3[a]	0.01±5e-3	0.004±0.001	2.93±0.89[b]
TT	0.20±0.06*	0.028±1e-3	0.049±3e-3	0.02±0.001	0.048±0.001	0.072±0.01[a]	0.20±0.06	0.028±0.001	7.424±1.42
MT	0.25±0.05*	0.02±0.002	0.05±0.004	0.63±0.01*	1.564±0.06*	0.50±0.10[a]	1.56±0.06	0.028±0.002	55.45±2.6[b]

Each value is mean ± SE, (n=6); One Way ANOVA: * Refers to comparison with lowest value in each experimental group with other time points ($p \leq 0.05$). [a] Refers to comparison with control in mean levels ($p \leq 0.05$). [b] Refers to comparison with control in daily pulse levels ($p \leq 0.05$).

Curcumin treatment was sensitive in partial restoration of mean levels in 1yr whereas levels were increased in 90 day and decreased in 2yr. Restoration of rhythmicity was observed in 90 day and 2yr. Curcumin caused increased daily pulse levels in 90 day and decreased in 2yr ($p \leq 0.05$) whereas restoration was observed in 1yr. Turmeric treatment, rhythmicity was not observed in 90 day only. Mean levels were increased in 90 day ($p \leq 0.05$) and 1yr whereas decreased levels were observed in 2yr. Daily pulse levels were decreased in 90 day and 2yr whereas increased levels were observed in 1yr ($p \leq 0.05$). In Melatonin treatment, rhythmicity was observed in all three age groups. Mean levels were increased in 90 day ($p \leq 0.05$) and 1yr whereas decreased in 2yr ($p \leq 0.05$). Daily pulse levels were increased in all age groups ($p \leq 0.05$) (Table 15; Fig. 60 and 61).

Ethanol treatment causes change in mean, daily pulse levels and rhythmicity of TRP in SCN as well as Pineal. Restoration was not observed in ethanol withdrawal. Curcumin treatment was partially sensitive in restoration of levels in 1yr pineal. Melatonin treatment was not helpful in both SCN and Pineal. Turmeric was also not sensitive in restoration of levels all three age groups in SCN as well as Pineal (Fig. 52).

6. 5-HTOH

In SCN, 5-HTOH showed rhythmicity in 90 day and 2yr but abolition was observed in 1yr. Increased mean as well as daily pulses were observed upon aging ($p \leq 0.05$). Ethanol treatment gives abolition of rhythmicity in 1 and 2yr. Mean levels were increased in 90 day ($p \leq 0.05$) and 1yr whereas decrease was observed in 2yr ($p \leq 0.05$). Significant increase was observed in daily pulses of 90 day ($p \leq 0.05$) and increased levels were observed in 2yr ($p \leq 0.05$) and no change in 1yr. Upon EW, rhythmicity was observed in 90day only. Mean levels were decreased in 1 and 2yr ($p \leq 0.05$) and increased in 1yr. Daily pulse levels were increased in 90 day ($p \leq 0.05$) and decreased in 1yr as well as 2yr ($p \leq 0.05$). Curcumin treatment was helpful in partial restoration of mean levels in 90 day and decreased in 1yr as well as 2yr ($p \leq 0.05$) but rhythmicity was restored in 90 day only. Daily pulse levels were decreased in 2yr ($p \leq 0.05$) and increased in 90 day. Restoration of daily pulse was observed in 1yr age group. In Turmeric treatment, rhythmicity was not observed in all three age groups. Mean levels were increased in 90 day ($p \leq 0.05$) and decreased in 1 and 2yr ($p \leq 0.05$). Robust decrease in daily pulse levels were observed in 2yr ($p \leq 0.05$) whereas increased levels were observed in 1yr ($p \leq 0.05$). Restoration of daily pulse was observed in 90 day. Melatonin treatment, rhythmicity was not observed in 90 day as well as 1yr. Significant increases in mean levels were observed in 90 day ($p \leq 0.05$) and 1yr but decrease in the levels were observed in 2yr ($p \leq 0.05$). Daily pulse levels were decreased in 1 and 2yr ($p \leq 0.05$) and increased in 90 day ($p \leq 0.05$) (Table 16; Fig. 58 and 59).

In Pineal, 5-HTOH showed rhythmicity in 90 day as well as 2yr. Age related decrease in the mean as well as daily pulse levels were observed upon aging ($p \leq 0.05$). Ethanol treatment affected rhythmicity in 1yr as well as 2yr. Elevation of mean levels was observed in all three age groups ($p \leq 0.05$). Significant increase in daily pulse levels were observed in 90 day only ($p \leq 0.05$). Upon EW, rhythmicity was not restored in 90 day only. Mean levels were increased significantly in all three age groups ($p \leq 0.05$). Decreases in daily pulse levels were observed in 90 day and significant increase in 1 and 2yr ($p \leq 0.05$). Curcumin treatment was sensitive in partial restoration of mean levels in all age groups. Restoration of rhythmicity was observed in 2yr only.

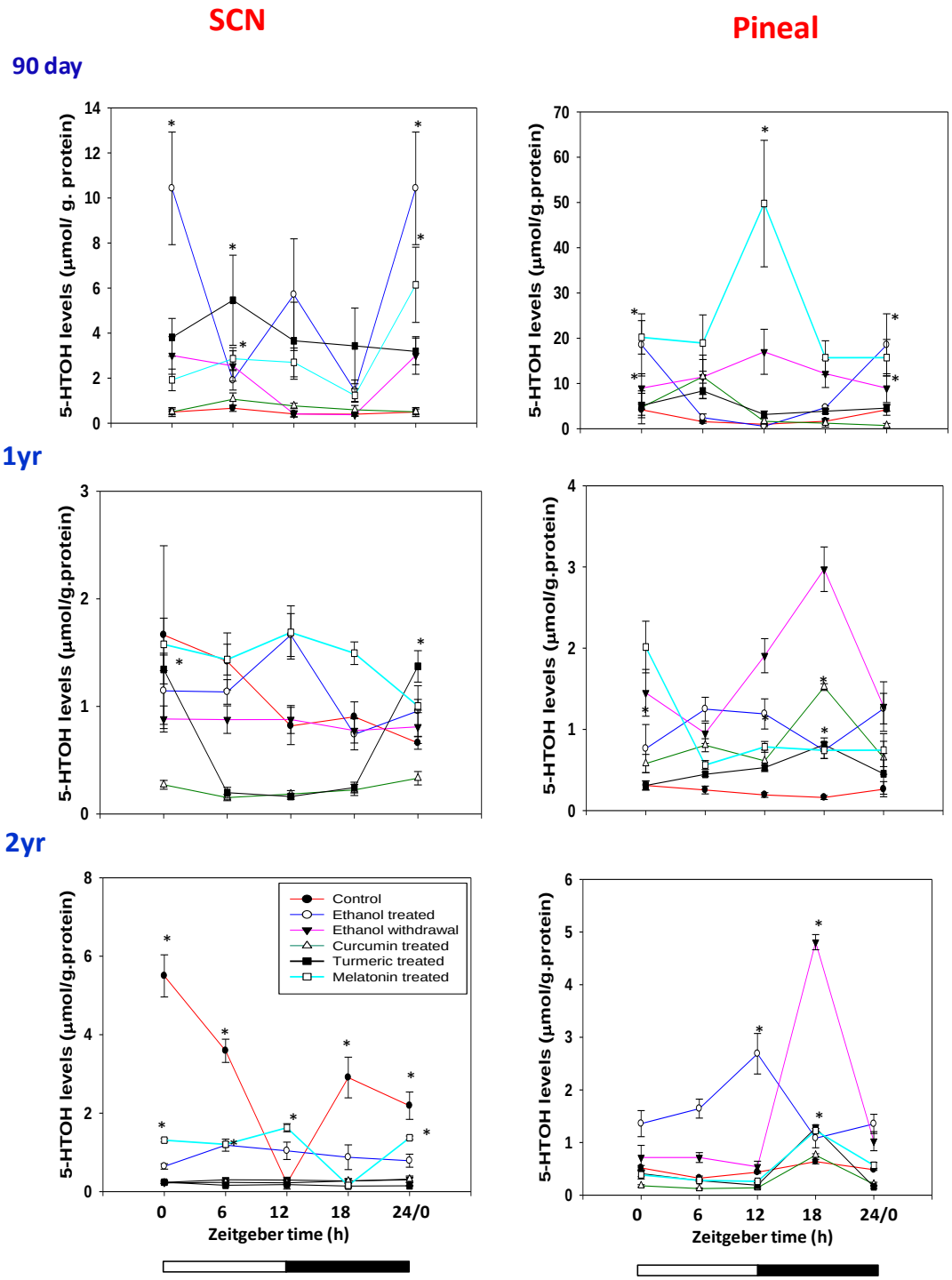

Fig. 53. Curcumin effect on ethanol induced changes in daily rhythms of 5-HTOH. Each value is mean ± SE, (n=6); One Way ANOVA: * Refers to comparison with lowest value in each group ($p \leq 0.05$).

Table 16. Curcumin effect on ethanol induced changes in daily rhythms, mean and pulse levels of 5-HTOH in SCN.

A .90 day									
Exp. Group	Zeitgeber time (h)					Mean (24h)	Max	Min	Ratio
	0	6	12	18	24/0				
C	0.443±0.01	0.38±0.018	0.49±0.015*	0.47±0.019*	0.443±0.01	0.44±0.042	0.49±0.015	0.38±0.018	1.293±0.04
ET	10.42±2.50	1.903±0.43	5.705±2.48	1.44±0.47	10.42±2.5*	4.86±1.124[a]	10.42 ± 2.5	1.44 ± 0.47	8.09 ± 1.9[b]
EW	3.008±0.83	2.54±0.675	0.409±0.12	0.37±0.07	3.008±0.83*	1.58 ±0.356	3.00 ± 0.83	0.37 ± 0.07	8.4 ± 1.64[b]
CT	0.502±0.13	1.063±0.28*	0.767±0.12	0.59±0.19	0.502±0.13	0.73 ±0.123	1.063±0.28	0.50 ± 0.13	2.27 ± 0.49
TT	3.809±0.84	5.460±2.00	3.660±1.71	3.43±1.68	3.187±0.59	3.9±0.629[a]	5.460±2.00	3.187±0.59	1.581±0.27
MT	1.921±0.48	2.863±0.49	2.698±0.64	1.232±0.3	6.14±1.67*	2.97±0.47[a]	6.14±1.670	1.232±0.30	5.304±1.13
B.1yr									
C	1.663±0.83	1.418±0.265	0.818±0.173	0.903±0.13	0.660±0.058	1.092±0.191	1.663±0.83	0.66±0.058	2.53±0.745
ET	1.144±0.350	1.13±0.1144	1.663±0.199	0.740±0.14	0.957±0.235	1.128±0.109	1.663±0.19	0.740±0.14	2.33±0.306
EW	0.883±0.121	0.877±0.128	0.878±0.130	0.775±0.11	0.810±0.155	0.845±0.055	0.88±0.121	0.77±0.116	1.16±0.138
CT	0.271±0.041	0.15±0.0288	0.18±0.0319	0.22±0.051	0.332±0.062	0.23±0.022[a]	0.332±0.06	0.15±0.028	2.26±0.339
TT	1.34±0.134*	0.198±0.050	0.16±0.0257	0.245±0.05	1.37±0.146*	0.66±0.112[a]	1.37±0.146	0.16±0.025	8.67±0.94[b]
MT	1.576±0.244	1.435±0.143	1.687±0.247	1.49±0.105	1.003±0.061	1.439±0.085	1.68±0.247	1.003±0.06	1.68±0.155
C.2yr									
C	5.49±0.537*	3.58±0.296*	0.216±0.152	2.90±0.51*	2.19±0.349*	2.88±0.864	5.498±0.53	0.216±0.15	50.42±20.7
ET	0.64±0.0692	1.18±0.153	1.039±.0.221	0.87±0.314	0.788±0.166	0.906±0.09[a]	1.18±0.153	0.64±0.069	1.85±0.18[b]
EW	0.23±0.0243	0.301±0.025	0.294±0.027	0.27±0.052	0.301±0.067	0.28±0.018[a]	0.301±0.02	0.23±0.024	1.28±0.09[b]
CT	0.22±0.0158	0.237±0.022	0.224±0.045	0.26±0.055	0.31±0.0809	0.25±0.021[a]	0.318±0.08	0.22±0.015	1.43±0.21[b]
TT	0.240±0.040	0.162±0.034	0.179±0.026	0.14±0.027	0.147±0.013	0.17±0.014[a]	0.24±0.047	0.14±0.027	1.76±0.28[b]
MT	1.31±0.044*	1.20±0.058*	1.628±0.10*	0.156±0.06	1.37±0.045*	1.29±0.049[a]	1.62±0.103	0.95±00.06	1.70±0.08[b]

Each value is mean ± SE, (n=6); One Way ANOVA: * Refers to comparison with lowest value in each experimental group with other time points ($p \leq 0.05$). [a] Refers to comparison with control in mean levels ($p \leq 0.05$). [b] Refers to comparison with control in daily pulse levels ($p \leq 0.05$).

Table 17. Curcumin effect on ethanol induced changes in daily rhythms, mean and pulse levels of 5-HTOH in Pineal.

A . 90 day									
Exp. Group	Zeitgeber time (h)					Mean (24h)	Max	Min	Ratio
	0	6	12	18	24/0				
C	4.18±1.21*	1.55±0.33	0.99±0.23	1.64±0.63	4.18±1.21*	2.09±0.71	4.18±1.2	0.99 ± 0.2	4.40±0.90
ET	18.5±6.87*	2.44±0.82	0.55±0.10	4.67±0.35	18.5±6.87*	6.54±2.33	18.5±6.87	0.55 ± 0.1	31.5±8.4[b]
EW	8.976±3.18	11.43±3.85	16.9±4.97	12.1±3.07	8.976±3.18	12.3±1.89[a]	16.9±4.9	8.9 ± 3.1	2.15±0.57
CT	4.73±3.64	11.4±4.77	1.62±0.61	1.23±0.87	0.714±0.45	4.77 ±2.3	11.49±4.7	1.23 ± 0.8	16.18±7.6
TT	5.13±2.69	8.35±1.71	3.14±0.77	3.86±0.52	4.54±0.65	5.00±0.712	8.35±1.71	3.14±0.77	2.82±0.52
MT	20.19±3.70	18.9±6.22	49.7±13.9*	15.6±3.75	15.7±4.02	24.05±3.9[a]	49.7±13.9	15.6±3.75	3.36±0.72
B .1 year									
C	0.30±0.05	0.25±0.04	0.19±0.02	0.16±0.02	0.26±0.09	0.23±0.026	0.30±0.05	0.16±0.02	1.97±0.26
ET	0.76±0.29	1.24±0.14	1.19±0.18	0.74±0.09	1.25±0.188	1.04±0.09[a]	1.255±0.18	0.74±0.09	1.72±0.19
EW	1.45±0.28	0.95±0.12	1.90±0.21*	2.9±0.27*	1.28±0.305	1.71±0.166[a]	2.97±0.27	0.95±0.12	3.17±0.29[b]
CT	0.57±0.11	0.80±0.08	0.61±0.13	1.52±0.04*	0.65±0.203	0.83±0.08[a]	1.52±0.04	0.57±0.11	2.74±0.299
TT	0.30±0.05	0.44±0.02	0.52±0.029	0.811±0.08	0.453±0.25	0.51±0.059	0.81±0.08	0.30±0.05	2.716±0.33
MT	2.01±0.31*	0.56±0.05	0.78±0.067	0.74±0.101	0.74±0.20	0.96±0.12[a]	2.01±0.31	0.56±0.05	3.62±0.39[b]
C. 2 year									
C	0.51±0.04*	0.32±0.04	0.43±0.022*	0.63±0.04*	0.48±0.01*	0.47±0.051	0.63±0.04	0.32±0.04	2.01±0.167
ET	1.35±0.24	1.64±0.18	2.68±0.38*	1.08±0.181	1.35±0.183	1.62±0.14[a]	2.68±0.38	1.08±0.18	2.54±0.32
EW	0.71±0.23	0.71±0.09	0.53±0.104	4.80±0.14*	1.026±0.18	1.56±0.31[a]	4.80±0.14	0.53±0.10	9.26±1.04[b]
CT	0.17±0.02	0.12±0.01	0.140±0.02	0.76±0.02*	0.20±0.03*	0.28±0.04	0.76±0.02	0.12±0.01	6.17±0.39[b]
TT	0.40±0.06*	0.27±0.02	0.189±0.04	1.27±0.05*	0.158±0.02	0.46±0.07	1.27±0.05	0.15±0.02	8.34±0.88[b]
MT	0.38±0.07	0.28±0.01	0.262±0.02	1.22±0.03*	0.56±0.05*	0.544±0.06	1.22±0.03	0.26±0.02	4.75±0.27[b]

Each value is mean $\pm$ SE, (n=6); One Way ANOVA: * Refers to comparison with lowest value in each experimental group with other time points ($p \leq 0.05$). [a] Refers to comparison with control in mean levels ($p \leq 0.05$). [b] Refers to comparison with control in daily pulse levels ($p \leq 0.05$).

Curcumin caused increased daily pulse levels in 90 day and 2yr ($p \leq 0.05$) whereas restoration was observed in 1yr. Turmeric treatment, rhythmicity was observed in 2yr only. Mean levels were increased in 90 day ($p \leq 0.05$) and restoration was observed in 1yr as well as 2yr. Daily pulse levels were decreased in 90 day whereas increased levels were observed in 2yr ($p \leq 0.05$). Partial restoration of daily pulse levels were observed in 1yr. In Melatonin treatment, rhythmicity was observed in all three age groups. Mean levels were increased in 90 day ($p \leq 0.05$) and 1yr. Restoration of mean levels were observed in 2yr. Daily pulse levels were increased in all age groups ($p \leq 0.05$) (Table 17; Fig. 60 and 61).

Ethanol treatment causes change in mean, daily pulse levels and rhythmicity of 5-HTOH in SCN as well as Pineal. Changes caused by ET were not reversed in ethanol withdrawal. Curcumin treatment was partially sensitive in restoration of levels in 90 day SCN and all three age groups in pineal. Melatonin treatment was helpful in restoration of levels in 2yr Pineal only. Turmeric was also sensitive in restoration of levels in 1 and 2yr in Pineal but not in SCN (Fig. 53).

7. 5-MIAA

In SCN, 5-MIAA showed rhythmicity in 90 day only. Decreased mean as well as daily pulses were observed upon aging ($p \leq 0.05$). Ethanol treatment gives abolition of rhythmicity in all three age groups. Mean levels were decreased in all age groups but significant change was observed in 2yr only ($p \leq 0.05$). Significant decrease was observed in daily pulses of all age groups ($p \leq 0.05$). Upon EW, rhythmicity was observed in 90day only. Mean levels were decreased in all age groups but significant change was observed in 2yr only ($p \leq 0.05$). Significant decrease was observed in daily pulses of all age groups ($p \leq 0.05$). Curcumin treatment was not helpful in partial restoration of mean levels, caused elevation in 90 day ($p \leq 0.05$) and decreased in 1yr as well as 2yr ($p \leq 0.05$) but rhythmicity was restored in all age groups. Daily pulse levels were increased in 90 day and decreased in 1yr ($p \leq 0.05$). Restoration of daily pulse was observed in 2yr age group. In Turmeric treatment, rhythmicity was not observed in all three age groups. Mean levels were decreased in all age groups ($p \leq 0.05$). Decrease in daily pulse levels were observed in all age groups ($p \leq 0.05$). Melatonin treatment, rhythmicity was not observed in all age groups. Decreases in mean levels were observed in all age groups ($p \leq 0.05$). Daily pulse levels decreased in all age groups ($p \leq 0.05$) (Table 18; Fig. 58 and 59).

In Pineal, 5-MIAA showed rhythmicity in all age groups. Age related decrease in the mean as well as daily pulse levels were observed upon aging ($p \leq 0.05$). Ethanol treatment was affected the rhythmicity in 1yr as well as 2yr. Mean levels were decreased in all three age groups but significant change was observed in 90 day only ($p \leq 0.05$). Decrease in daily pulse levels were observed in 90 day and 1yr whereas significant increase was observed in 2yr ($p \leq 0.05$). Upon EW, rhythmicity was not restored in 1yr only. Mean levels were decreased in 90 day as well as 2yr whereas increased in 1yr. Decreased daily pulse levels were observed in 90 day ($p \leq 0.05$) and increased levels were observed in 2yr. Partial restoration was observed in 1yr only. Curcumin treatment was sensitive in partial restoration of mean levels in 90 day and 1yr. Restoration of rhythmicity was not observed in all three age groups.

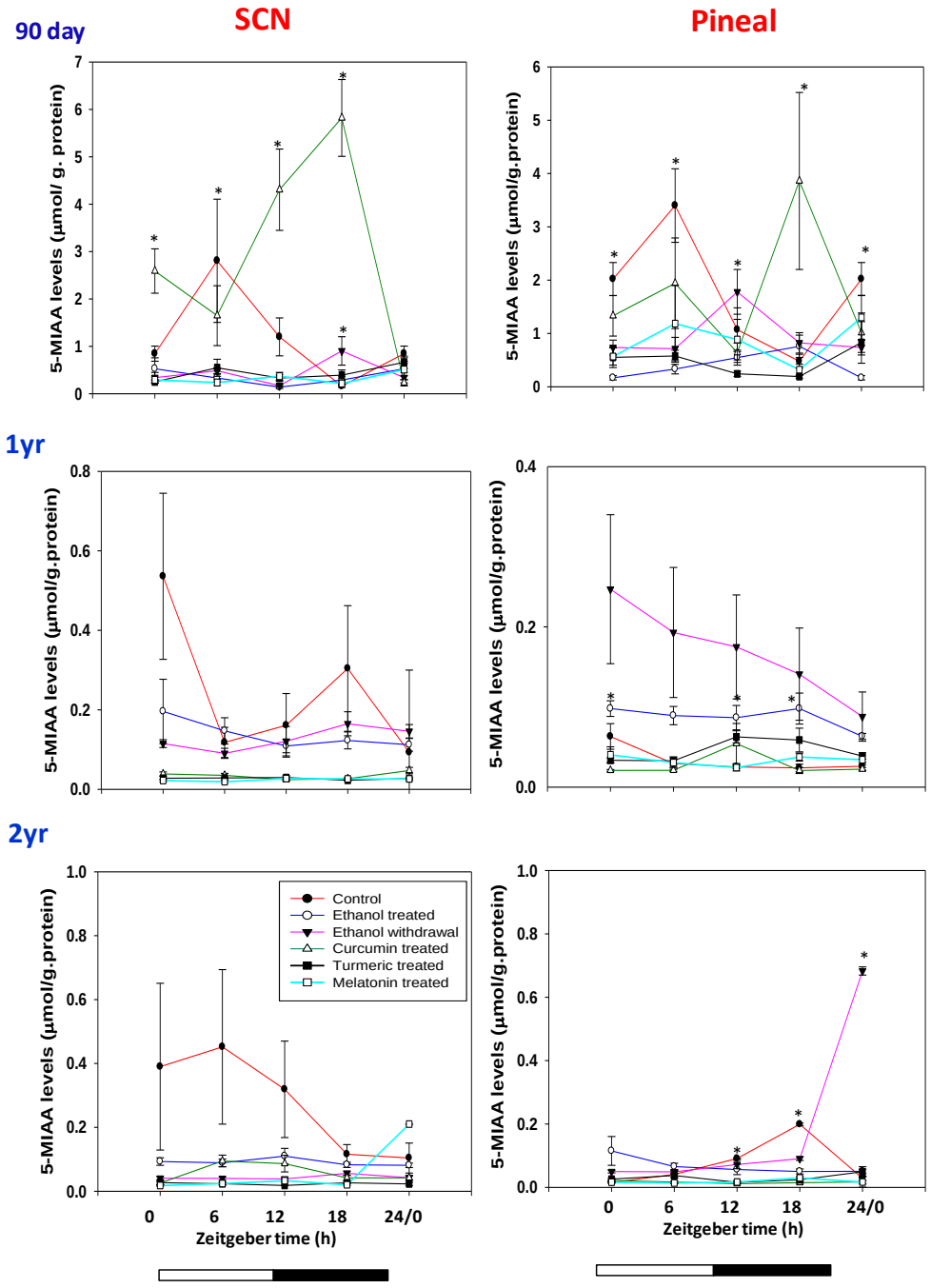

Fig. 54. Curcumin effect on ethanol induced changes in daily rhythms of 5-MIAA. Each value is mean ± SE, (n=6); One Way ANOVA: * Refers to comparison with lowest value in each group ($p \leq 0.05$).

Table 18. Curcumin effect on ethanol induced changes in daily rhythms, mean and pulse levels of 5-MIAA in SCN.

A. 90 day									
Exp. Group	Zeitgeber time (h)					Mean (24h)	Max	Min	Ratio
	0	6	12	18	24/0				
C	0.845±0.16	2.8058±1.3*	1.20±0.40	0.16±0.03	0.845±0.16	1.237 ±037	2.80 ± 1.3	0.16 ± 0.03	18.09 ±5.31
ET	0.528±0.23	0.331±0.08	0.140±0.03	0.29±0.08	0.528±0.23	0.32±0.069	1.23± 0370	0.52 ± 0.23	0.14± 0.03[b]
EW	0.339±0.11	0.485±0.06	0.170±0.03	0.903±0.3*	0.339±0.11	0.47 ±0.096	0.90 ± 0.3	0.17 ± 0.03	5.46 ± 1.2[b]
CT	2.58±0.468*	1.648±0.63	4.305±0.85*	5.82±0.81*	0.23±0.065	2.918±0.45[a]	5.82 ± 0.81	0.23±0.065	27.49 ±5.04
TT	0.251±0.03	0.548±0.18	0.332±0.07	0.391±0.11	0.663±0.13	0.43±0.057	0.663±0.13	0.251±0.03	2.67±0.35[b]
MT	0.292±0.06	0.236±0.06	0.370±0.08	0.21±0.04	0.510±0.13	0.32±0.040	0.510±0.13	0.215±0.04	2.45±0.45[b]
B.1yr									
C	0.53±0.209	0.118±0.03	0.161±0.08	0.304±0.15	0.093±0.02	0.24±0.082	0.53±0.209	0.093±0.02	5.97±1.577
ET	0.190.0809	0.14±0.033	0.109±0.017	0.12±0.021	0.11±0.016	0.137±0.01	0.147±0.03	0.109±0.01	1.38±0.20[b]
EW	0.115±0.01	0.09±0.012	0.121±0.037	0.165±0.03	0.14±0.017	0.128±0.01	0.165±0.03	0.090±0.01	1.86±0.24[b]
CT	0.038±0.005	0.035±0.005	0.024±0.002	0.026±0.003	0.047±0.008*	0.034±0.002[a]	0.04±0.008	0.024±0.002	1.97±0.21[b]
TT	0.027±0.004	0.028±0.001	0.029±0.005	0.022±0.002	0.027±0.002	0.02±0.0017[a]	0.02±0.005	0.022±0.002	1.31±0.15[b]
MT	0.02±0.0036	0.019±0.003	0.02±0.0056	0.026±0.002	0.026±0.003	0.024±0.001[a]	0.02±0.005	0.019±0.003	1.48±0.23[b]
C.2yr									
C	0.39±0.261	0.45±0.242	0.319±0.151	0.116±0.03	0.104±0.047	0.276±0.07	0.452±0.24	0.104±0.04	4.15±1.482
ET	0.093±0.011	0.089±0.012	0.11±0.0242	0.082±0.008	0.081±0.006	0.09±0.006[a]	0.11±0.024	0.081±0.006	1.36±0.18[b]
EW	0.04±0.004	0.04±0.005	0.038±0.004	0.056±0.007	0.042±0.006	0.04±0.002[a]	0.05±0.007	0.038±0.004	1.35±0.14[b]
CT	0.025±0.004	0.094±0.018*	0.086±0.026*	0.042±0.008	0.041±0.007	0.058±0.008[a]	0.094±0.01	0.025±0.004	3.85±0.429
TT	0.027±0.006	0.023±0.003	0.018±0.0005	0.026±0.004	0.023±0.005	0.023±0.001[a]	0.02±0.006	0.018±0.0005	1.51±0.21[b]
MT	0.017±0.002	0.024±0.005	0.032±0.004	0.02±0.003	0.021±0.002	0.02±0.0018[a]	0.03±0.004	0.017±0.002	1.87±0.209

Each value is mean ± SE, (n=6); One Way ANOVA: * Refers to comparison with lowest value in each experimental group with other time points ($p \leq 0.05$). [a] Refers to comparison with control in mean levels ($p \leq 0.05$). [b] Refers to comparison with control in daily pulse levels ($p \leq 0.05$).

Table 19. Curcumin effect on ethanol induced changes in daily rhythms, mean and pulse levels of 5-MIAA in Pineal.

A . 90 day									
Exp. Group	Zeitgeber time (h)					Mean (24h)	Max	Min	Ratio
	0	6	12	18	24/0				
C	2.02±0.31*	3.4±0.69*	1.07±0.41	0.47±0.13	2.02±0.31*	1.74±0.30	3.4±0.69	0.47±0.13	7.29±1.76
ET	0.168±0.04	0.33±0.09	0.54±0.14	0.75±0.20*	0.168±0.04	0.45±0.07[a]	0.75±0.2	0.16±0.04	4.99±1.07
EW	0.736±0.14	0.71±0.21	1.78±0.42*	0.82±0.191	0.168±0.04	1.014±0.15	1.78±0.4	0.71±0.21	2.74±0.61[b]
CT	1.331±0.38	1.94±0.85	0.63±0.181	3.861±1.66	1.014±0.37	1.757±0.42	3.86±1.6	0.63±0.18	6.66± 2.04
TT	0.551±0.19	0.57±0.10	0.243±0.05	0.191±0.03	0.83±0.39	0.47±0.09[a]	0.83±0.3	0.19±0.03	4.46±1.286
MT	0.567±0.16	1.18±0.72	0.885±0.38	0.32±0.106	1.30±0.415	0.851±0.18	1.18±0.7	0.32±0.10	4.155±1.73
B. 1 year									
C	0.06±0.01*	0.02±0.004	0.02±0.002	0.02±0.004	0.02±0.005	0.03±0.007	0.06±0.01	0.02±4e-3	2.72±0.394
ET	0.09±0.009	0.08±0.011	0.08±0.015	0.09±0.019	0.06±0.003	0.08±5e-3[a]	0.09±9e-3	0.06±3e-3	1.55±0.104
EW	0.24±0.09	0.193±0.08	0.17±0.064	0.14±0.057	0.088±0.03	0.16±0.03[a]	0.24±0.09	0.14±0.05	2.03±0.679
CT	0.02±0.001	0.02±0.001	0.054±0.02	0.02±0.003	0.02±0.002	0.02±0.005	0.05±0.02	0.02±3e-3	2.49±0.775
TT	0.03±0.003	0.03±0.005	0.06±0.007*	0.05±0.01*	0.03±0.004	0.04±0.004	0.06±7e-3	0.03±5e-3	1.956±0.22
MT	0.04±0.010	0.03±0.006	0.024±0.004	0.03±0.005	0.03±0.006	0.03±0.003	0.04±0.01	0.02±4e-3	1.69±0.295
C. 2 year									
C	0.01±0.001	0.041±0.01*	0.09±1e-4*	0.01±0.003	0.02±0.007	0.074±0.05	0.04±0.01	0.09±1e-4	0.45±0.063
ET	0.115±0.04	0.065±0.010	0.056±0.016	0.05±0.007	0.05±0.006	0.06±0.010	0.11±0.04	0.05±7e-3	2.35±0.52[b]
EW	0.04±0.004	0.049±0.006	0.072±0.013	0.09±5e-3*	0.06±0.013	0.06±0.004	0.09±5e-3	0.04±4e-3	1.85±0.112
CT	0.02±0.002	0.016±0.004	0.011±0.001	0.01±0.002	0.017±0.01	0.01±0.001	0.02±2e-3	0.01±1e-3	2.03±0.19[b]
TT	0.02±0.003	0.037±0.007	0.016±0.002	0.02±0.005	0.049±0.01	0.03±0.004	0.04±0.01	0.01±2e-3	3.15±0.66[b]
MT	0.01±0.002	0.013±0.002	0.016±0.003	0.02±0.005	0.01±0.004	0.01±0.001	0.02±5e-3	0.01±2e-3	2.23±0.31[b]

Each value is mean ± SE, (n=6); One Way ANOVA: * Refers to comparison with lowest value in each experimental group with other time points ($p \leq 0.05$). [a] Refers to comparison with control in mean levels ($p \leq 0.05$). [b] Refers to comparison with control in daily pulse levels ($p \leq 0.05$).

Curcumin caused restoration of daily pulse levels in 90 day and 1yr whereas levels were increased significantly in 2yr. Turmeric treatment, rhythmicity was observed in 1yr only. Mean levels were decreased in 90 day ($p \leq 0.05$) and 2yr. Restoration was observed in 1yr. Partial restoration of daily pulse levels were observed in 90 day and 1yr whereas significant increase was observed in 2yr ($p \leq 0.05$). In Melatonin treatment, rhythmicity was not observed in all three age groups. Mean levels were increased in 90 day and 2yr. Restoration of mean levels were observed in 1yr. Partial restoration of daily pulse levels were observed in 90 day and 1yr whereas significant increase was observed in 2yr ($p \leq 0.05$) (Table 19; Fig. 60 and 61).

Ethanol treatment causes change in mean, daily pulse levels and rhythmicity of 5-MIAA in SCN as well as Pineal. Changes caused by ET were not restored in ethanol withdrawal. Curcumin treatment was partially sensitive in restoration of levels in 90 day and 1yr of pineal. Melatonin treatment was helpful in restoration of levels in 1yr Pineal only. Turmeric was also sensitive in restoration of levels in 1yr Pineal only (Fig. 54).

5-MTOH

In SCN, 5-MTOH showed rhythmicity in 90 day only. Decreased mean levels were observed upon aging ($p \leq 0.05$) and there was no significant change was observed in daily pulses upon aging. Ethanol treatment gives abolition of rhythmicity in 1 and 2yr. Mean levels were decreased in 90 day whereas increased in 1 and 2yr. Increase in daily pulse was observed in all age groups but significant change was observed in 90 day only ($p \leq 0.05$). Upon EW, rhythmicity was abolished in all age groups. Mean levels were decreased in all age groups. Decrease was observed in daily pulses in 90 day as well as 1yr but increased levels were observed in 2yr. Curcumin treatment was helpful in partial restoration of mean levels in 90 day and 2yr whereas decreased levels were observed in 1yr but rhythmicity was restored 90 day only. Daily pulse levels were restored partially in all age groups. In Turmeric treatment, rhythmicity was not observed in all three age groups. Mean levels were decreased in all age groups. Daily pulse levels were restored partially in all age groups. Melatonin treatment, rhythmicity was observed in 90 day only. Decreases in mean levels were observed in all age groups ($p \leq 0.05$). Daily pulse levels were decreased in 1yr as well as 2yr whereas increased in 90 day (Table 20; Fig. 58 and 59).

In Pineal, 5-MTOH showed rhythmicity in 90 day only. Age related decrease in the mean levels was observed upon aging ($p \leq 0.05$). Elevated levels were observed in 1yr. Ethanol treatment did not affect the rhythmicity in any of the age groups. Mean levels were decreased in 90 day whereas increased in 1yr as well as 2yr. Decrease in daily pulse levels were observed in 1 and whereas increase was observed in 90 day as well as 2yr . Upon EW, rhythmicity was not restored in 2yr only. Mean levels were decreased in 90 day whereas increased in 1 and 2yr. Decreased daily pulse levels were observed in 90 day and 2yr and increased levels were observed in 1yr ($p \leq 0.05$). Curcumin treatment was sensitive in partial restoration of mean levels in 1 and 2yr. Restoration of rhythmicity was observed in 90 day only. Curcumin caused decreased daily pulse levels in 1 and 2yr whereas significantly increased levels were observed in 90 day ($p \leq 0.05$). Turmeric treatment, rhythmicity was not observed in 2yr only. Mean levels were decreased in all age groups. Turmeric caused decreased daily pulse levels in 1 and 2yr whereas significantly increased levels were observed in 90 day ($p \leq 0.05$).

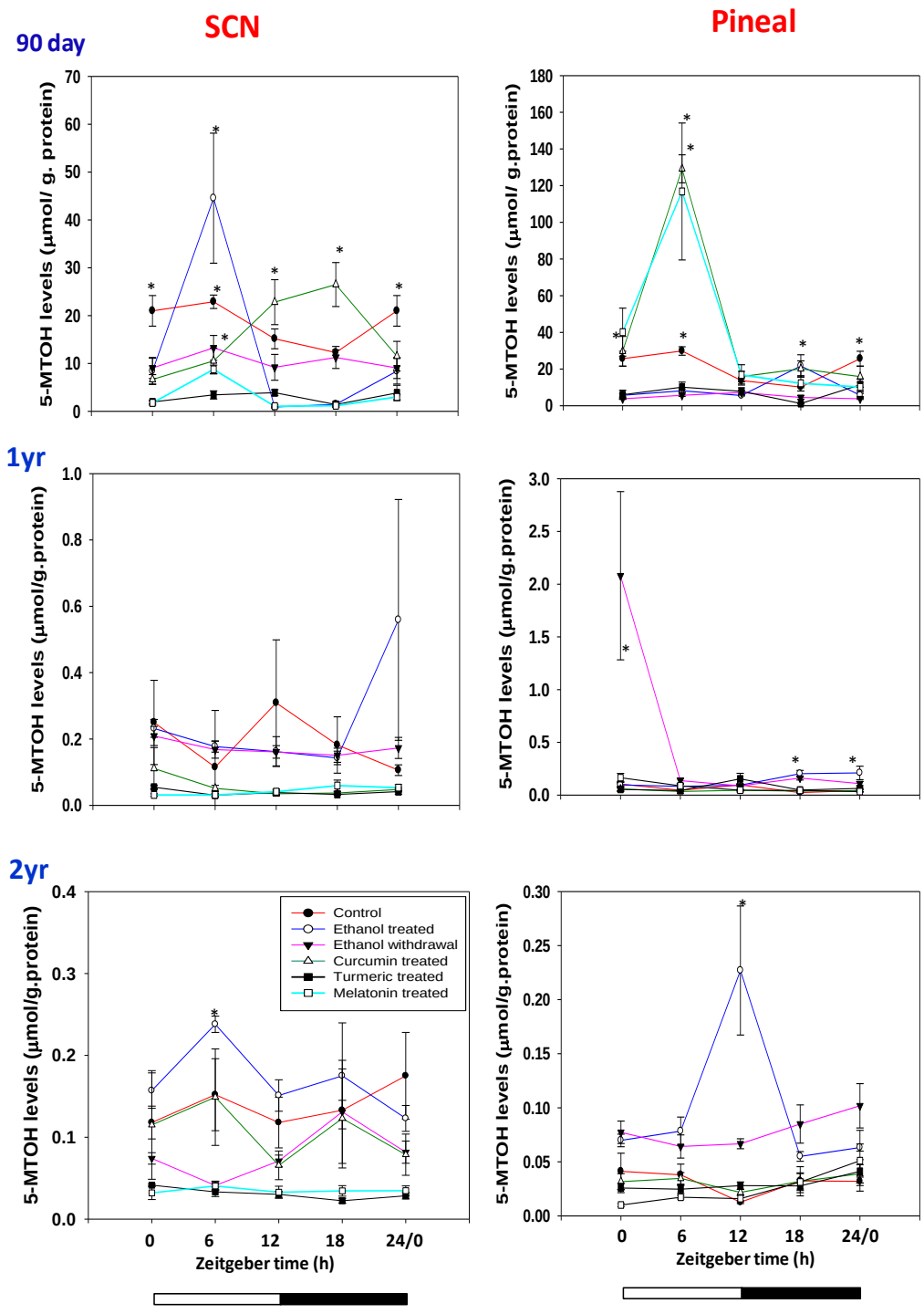

Fig. 55. Curcumin effect on ethanol induced changes in daily rhythms of 5-MTOH. Each value is mean ± SE, (n=6); One Way ANOVA: * Refers to comparison with lowest value in each group ($p \leq 0.05$).

Table 20. Curcumin effect on ethanol induced changes in daily rhythms, mean and pulse levels of 5-MTOH in SCN.

A. 90 day									
Exp. Group	Zeitgeber time (h)					Mean (24h)	Max	Min	Ratio
	0	6	12	18	24/0				
C	20.993±3.2*	22.893±1.4*	15.15±2.07	12.27±0.5	20.99±3.2*	17.82±1.55	22.89 ± 1.4	12.27 ± 0.55	1.865± 0.08
ET	8.391±2.7	44.54±13.5*	0.888±0.14	1.44±0.6	8.391±2.7	13.81±4.59	44.5 ± 13.5	0.88 ± 0.14	51.9±10.3[b]
EW	9.049±2.2	13.25±2.6	9.185±2.7	11.25±2.3	9.049±2.2	10.66±1.22	13.25 ± 2.6	9.04 ± 2.2	1.55 ± 0.28
CT	6.642±1.03	10.53±2.7	22.81±4.7*	26.49±4.6*	11.507±3.1	15.6±2.57	26.49 ± 4.6	6.64 ± 1.03	3.42 ± 0.86
TT	1.954±0.35	3.424±0.85	3.889±0.62	1.40±0.26	3.82±1.60	2.90±0.41[a]	3.889±0.62	1.403±0.266	2.619±0.29
MT	1.750±0.44	8.746±0.90*	1.058±0.38	1.17±0.28	2.99±0.55	3.146±0.58[a]	8.746±0.90	1.058±0.38	9.490±2.05
B. 1yr									
C	0.250±0.12	0.115±0.17	0.309±0.19	0.18±0.085	0.10±0.016	0.19±0.039	0.25±0.12	0.106±0.016	2.40±0.712
ET	0.23±0.027	0.17±0.016	0.162±0.045	0.143±0.02	0.55±0.363	0.25±0.074	0.559±0.36	0.143±0.020	3.98±1.531
EW	0.20±0.028	0.168±0.02	0.161±0.019	0.15±0.021	0.173±0.031	0.172±0.01	0.209±0.02	0.151±0.021	1.41±0.138
CT	0.111±0.063	0.051±0.009	0.034±0.005	0.037±0.003	0.047±0.007	0.056±0.013[a]	0.11±0.063	0.034±0.005	3.30±1.140
TT	0.054±0.005	0.03±0.005	0.038±0.006	0.033±0.002	0.041±0.007	0.04±0.0029[a]	0.05±0.005	0.030±0.005	1.851±0.21
MT	0.031±0.003	0.031±0.003	0.041±0.008	0.059±0.016	0.053±0.009	0.043±0.004[a]	0.05±0.012	0.031±0.003	1.933±0.27
C. 2yr									
C	0.118±0.02	0.15±0.044	0.118±0.03	0.13±0.012	0.17±0.053	0.13±0.010	0.175±0.05	0.118±0.02	1.52±0.296
ET	0.157±0.02	0.238±0.01	0.15±0.0192	0.17±0.064	0.12±0.015	0.169±0.02	0.238±0.10	0.123±0.015	1.96±0.499
EW	0.074±0.071	0.041±0.005	0.071±0.007	0.13±0.063	0.081±0.013	0.08±0.013	0.131±0.06	0.041±0.005	2.98±0.854
CT	0.115±0.066	0.149±0.058	0.065±0.017	0.123±0.06	0.078±0.025	0.106±0.02	0.14±0.058	0.065±0.017	2.31±0.669
TT	0.041±0.01	0.033±0.005	0.030±0.003	0.022±0.003	0.028±0.003	0.03±0.002[a]	0.04±0.010	0.022±0.003	1.877±0.30
MT	0.032±0.008	0.04±0.0049	0.032±0.007	0.034±0.006	0.034±0.005	0.034±0.02[a]	0.04±0.004	0.032±0.008	1.351±0.22

Each value is mean ± SE, (n=6); One Way ANOVA: * Refers to comparison with lowest value in each experimental group with other time points ($p \leq 0.05$). [a] Refers to comparison with control in mean levels ($p \leq 0.05$). [b] Refers to comparison with control in daily pulse levels ($p \leq 0.05$).

Table 21. Curcumin effect on ethanol induced changes in daily rhythms, mean and pulse levels of 5-MTOH in Pineal.

A . 90 day									
Exp. Group	Zeitgeber time (h)					Mean (24h)	Max	Min	Ratio
	0	6	12	18	24/0				
C	25.6±4.09*	29.8±2.32*	13.7±1.87	10.1±0.35	25.6±4.09*	19.8±2.07	29.86 ± 2.3	10.1± 0.35	2.93±0.14
ET	5.659±1.02	8.14±0.96	5.57±0.28	21.6±6.12*	5.659±1.02	10.2±2.02	21.66± 6.12	5.57± 0.28	3.89± 0.64
EW	3.782±0.35	5.698±0.61	7.22±1.24*	4.512±0.94	3.78±0.35	5.3 ±0.484	7.22 ± 1.24	3.78± 0.35	1.92± 0.21
CT	29.70±8.08	129.1±7.64*	15.85±2.88	20.12±4.31	15.84±5.78	42.1±13.6[a]	129.1± 7.64	15.8± 5.78	9.39±2.01[b]
TT	6.017±2.46	10.08±2.82*	7.853±1.89	1.218±0.25	11.39±2.96	7.32±1.16	11.39±2.96	1.21±0.25	9.76±1.89[b]
MT	40.1±13.07	116.8±37.3*	16.80±5.55	12.2±4.19	10.21±2.64	39.2±10.5[a]	116.8±37.3	10.2±2.64	12.2±2.96[b]
B. 1 year									
C	0.105±0.02	0.045±0.005	0.095±0.05	0.02±0.002	0.04±0.009	0.06±0.016	0.131±0.02	0.02±0.002	6.16±0.418
ET	0.096±0.02	0.082±0.019	0.093±0.01	0.20±0.038*	0.21±0.06*	0.13±0.017	0.21±0.064	0.082±0.01	2.35±0.413
EW	2.08±0.79*	0.137±0.025	0.08±0.009	0.16±0.018	0.10±0.019	0.52±0.21[a]	2.080±0.79	0.08±9e-4	24.82±5.8[b]
CT	0.058±0.02	0.034±0.002	0.045±0.01	0.037±0.006	0.042±0.01	0.04±0.006	0.058±0.02	0.03±0.002	2.60±1.071
TT	0.05±0.013	0.04±0.005	0.15±0.05*	0.048±0.009	0.06±0.008	0.07±0.012	0.15±0.052	0.04±0.005	3.672±0.78
MT	0.16±0.04*	0.0878±0.02	0.04±0.005	0.043±0.009	0.03±0.001	0.07±0.012	0.165±0.04	0.03±0.001	5.143±0.72
C. 2 year									
C	0.04±0.016	0.038±0.009	0.01±0.001	0.032±0.007	0.03±0.003	0.03±0.004	0.041±0.01	0.01±0.001	3.45±0.535
ET	0.06±0.005	0.078±0.013	0.22±0.05*	0.05±0.004	0.06±0.003	0.09±0.016	0.22±0.059	0.05±0.004	4.02±0.653
EW	0.07±0.010	0.064±0.010	0.06±0.004	0.085±0.017	0.101±0.02	0.07±0.006	0.101±0.02	0.064±0.01	1.60±0.240
CT	0.03±0.007	0.034±0.005	0.02±0.002	0.032±0.013	0.03±0.008	0.03±0.003	0.038±0.02	0.02±0.002	1.80±0.564
TT	0.02±0.004	0.024±0.004	0.02±0.003	0.027±0.006	0.04±0.010	0.02±0.002	0.04±0.010	0.02±0.004	1.719±0.30
MT	0.01±0.001	0.017±0.001	0.01±0.003	0.031±0.007	0.051±0.02	0.02±0.006	0.051±0.02	0.01±0.001	5.201±1.76

Each value is mean ± SE, (n=6); One Way ANOVA: * Refers to comparison with lowest value in each experimental group with other time points ($p \leq 0.05$). [a] Refers to comparison with control in mean levels ($p \leq 0.05$). [b] Refers to comparison with control in daily pulse levels ($p \leq 0.05$).

In Melatonin treatment, rhythmicity was observed in 90 day only. Mean levels increased in 90 day ($p \leq 0.05$) and 1yr whereas decreased in 2yr. Increased daily pulse levels were observed in 90 day ($p \leq 0.05$) and 2yr whereas partial restoration was observed in 1yr (Table 21; Fig. 60 and 61).

Ethanol treatment causes change in mean, daily pulse levels and rhythmicity of 5-MTOH in SCN as well as Pineal. Changes caused by ET were not restored in ethanol withdrawal. Curcumin treatment was partially sensitive in restoration of levels in 90 day and 2yr of SCN whereas 1 and 2yr in Pineal. Melatonin treatment was helpful in restoration of levels in 90 day SCN as well as Pineal. Turmeric was not sensitive in restoration of levels in SCN as well as Pineal (Fig. 55).

9. MEL

In SCN, MEL showed rhythmicity in all age groups. Decreased mean as well as daily pulse levels were observed upon aging ($p \leq 0.05$). Ethanol treatment gives abolition of rhythmicity in 1yr only. Mean levels were decreased in all age groups. Decrease in daily pulse was observed in all age groups $p \leq 0.05$). Upon EW, rhythmicity was abolished in all age groups. Mean levels were decreased in 90 day and 1yr whereas decreased in 2yr ($p \leq 0.05$). Decrease was observed in daily pulses in all age groups ($p \leq 0.05$). Curcumin treatment caused increase in mean levels in 90 day whereas decreased levels were observed in 1 and 2yr ($p \leq 0.05$) but rhythmicity was restored 90 day only. Daily pulse levels were decreased in all age groups ($p \leq 0.05$). In Turmeric treatment, rhythmicity was observed in 90 day only. Mean levels were decreased in all age groups ($p \leq 0.05$). Daily pulse levels were decreased in all age groups ($p \leq 0.05$). Melatonin treatment, rhythmicity was observed in 90 day only. Decreases in mean levels were observed in all age groups ($p \leq 0.05$). Daily pulse levels were decreased in 90 day as well as 2yr ($p \leq 0.05$) whereas restored in 1yr (Table 22; Fig. 58 and 59).

In Pineal, MEL showed rhythmicity in 90 day and 1yr. Age related decrease in the mean levels was observed upon aging ($p \leq 0.05$). Age related decrease in the daily pulse levels was observed upon aging ($p \leq 0.05$). Ethanol treatment was abolished the rhythmicity in all age groups. Mean levels were decreased in 90 day whereas increased in 1yr as well as 2yr ($p \leq 0.05$). Decrease in daily pulse levels were observed in 90 day 2yr whereas restoration was observed in 1yr. Upon EW, rhythmicity was restored in 90 day only. Mean levels were increased in all age groups. Decreased daily pulse levels were observed in 90 day and 2yr ($p \leq 0.05$) and increased levels were observed in 1yr. Curcumin treatment was sensitive in partial restoration of mean levels in 90 day and 1yr. Restoration of rhythmicity was not observed in all age groups. Curcumin caused restoration of daily pulse levels in 90 day and 1yr whereas significantly decreased levels were observed in 2yr ($p \leq 0.05$). Turmeric treatment, rhythmicity was not observed in all age groups. Mean levels were decreased in 90 day ($p \leq 0.05$) and 2yr. Restoration was observed in 1yr age group. Daily pulse levels were decreased in 90 day and 2yr whereas restoration was observed in 1yr age group.

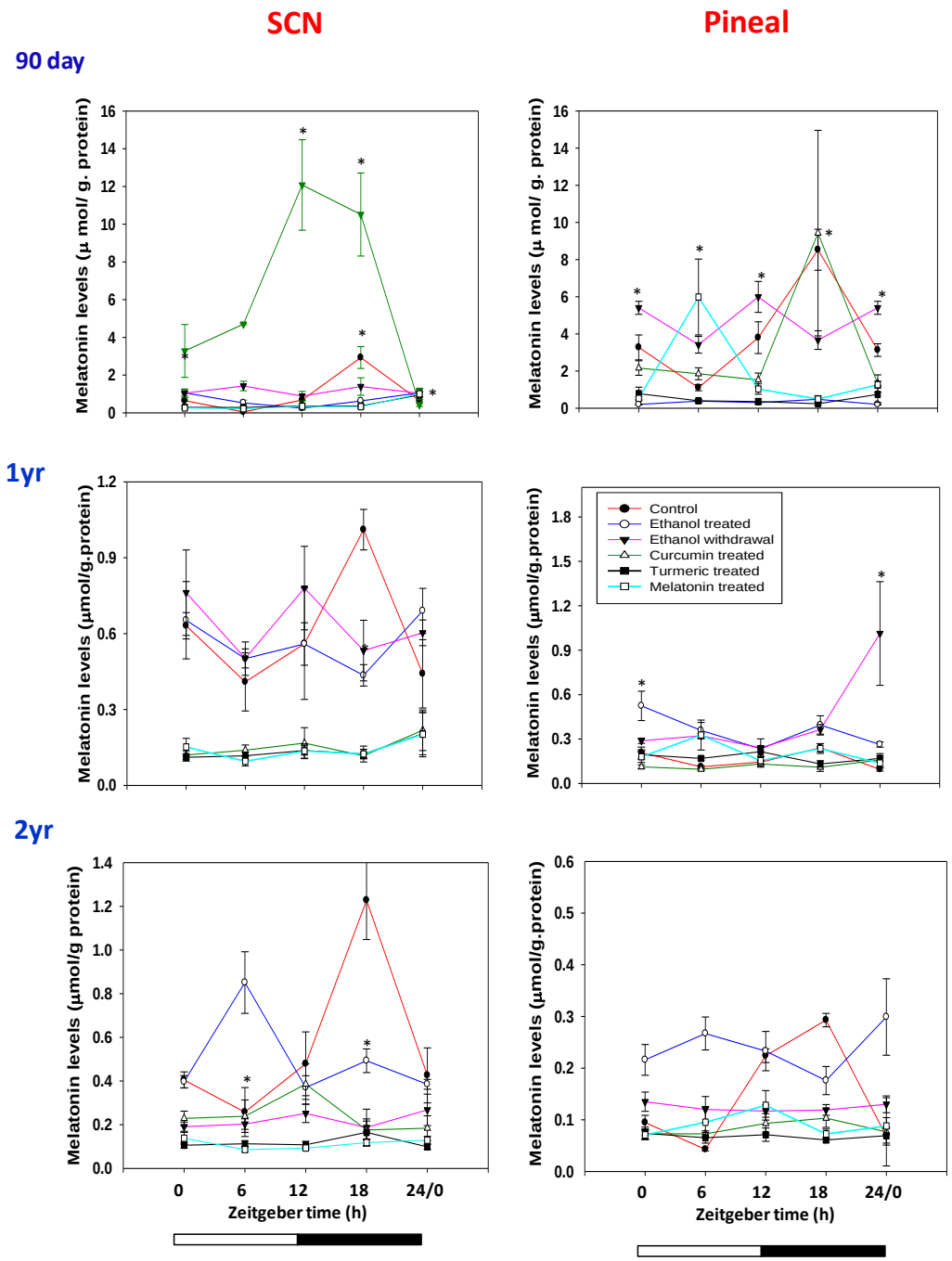

Fig. 56. Curcumin effect on ethanol induced changes in daily rhythms of MEL. Each value is mean ± SE, (n=6); One Way ANOVA: * Refers to comparison with lowest value in each group ($p \leq 0.05$).

Table 22. Curcumin effect on ethanol induced changes in daily rhythms, mean and pulse levels of MEL in SCN.

A . 90 day									
Exp. Group	Zeitgeber time (h)					Mean (24h)	Max	Min	Ratio
	0	6	12	18	24/0				
C	0.648±0.04	0.048±0.038	0.680±0.024	2.93±0.57*	0.648±0.056	1.562±0.321	2.930±0.57	0.048 ±0.038	163.5±78.4
ET	1.064±0.18*	0.523±0.15	0.245±0.09	0.630±0.05	1.064±0.18*	0.615±0.087	1.06 ± 0.18	0.24 ± 0.09	5.10 ±1.24[b]
EW	1.038±0.22	1.423±0.25	0.888±0.25	1.392±0.46	1.038±0.22	1.185±0.154	1.42 ± 0.25	0.88 ± 0.25	1.75± 0.34[b]
CT	3.282±1.4	4.702±0.045	12.080±2.4*	10.51±2.2*	0.432±0.09	6.22 ± 1.68[a]	12.08 ± 2.4	0.43 ± 0.09	29.3± 4.94[b]
TT	0.289±0.07	0.288±0.08	0.359±0.103	0.362±0.08	0.95±0.106*	0.450±0.059	0.95±0.106	0.288±0.08	3.57±0.62[b]
MT	0.257±0.06	0.212±0.03	0.341±0.09	0.327±0.12	1.002±0.30*	0.428±0.084	1.002±0.30	0.212±0.3	3.73±1.25[b]
B.1yr									
C	0.631±0.052	0.409±0.115	0.560±0.22	1.01±0.08*	0.441±0.135	0.610±0.107	1.011±0.08	0.409±0.115	2.68±0.455
ET	0.653±0.153	0.501±0.066	0.559±0.084	0.43±0.042	0.690±0.089	0.568±0.042	0.690±0.08	0.435±0.042	1.60±0.140
EW	0.762±0.169	0.50±0.0376	0.780±0.165	0.533±0.12	0.603±0.050	0.636±0.055	0.780±0.16	0.502±0.037	1.55±0.235
CT	0.120±0.016	0.139±0.020	0.167±0.061	0.11±0.011	0.218±0.080	0.152±0.02[a]	0.218±0.08	0.117±0.0116	1.87±0.412
TT	0.111±0.014	0.117±0.014	0.138±0.018	0.12±0.018	0.204±0.083	0.13±0.017[a]	0.204±0.08	0.111±0.0149	1.878±0.46
MT	0.152±0.034	0.095±0.018	0.136±0.028	0.12±0.031	0.202±0.089	0.142±0.02[a]	0.202±0.08	0.095±0.018	2.205±0.61
C. 2yr									
C	0.405±0.037	0.2580±0.11	0.479±0.146	1.22±0.18*	0.426±0.126	0.559±0.171	1.228±0.18	0.258±0.112	5.86±1.566
ET	0.397±0.028	0.85±0.141*	0.370±0.054	0.493±0.05	0.38±0.0226	0.499±0.046	0.851±0.14	0.370±0.054	2.34±0.30[b]
EW	0.191±0.021	0.203±0.024	0.252±0.042	0.18±0.084	0.268±0.072	0.22±0.018[a]	0.26±0.072	0.187±0.084	1.74±0.54[b]
CT	0.229±0.033	0.239±0.074	0.386±0.092	0.17±0.040	0.184±0.056	0.24±0.029[a]	0.386±0.09	0.184±0.056	2.31±0.52[b]
TT	0.106±0.013	0.113±0.011	0.108±0.016	0.16±0.061	0.098±0.011	0.11±0.013[a]	0.165±0.06	0.098±0.011	1.70±0.38[b]
MT	0.138±0.027	0.086±0.010	0.093±0.00	0.11±0.012	0.129±0.017	0.11±0.007[a]	0.138±0.02	0.0868±0.09	1.62±0.20[b]

Each value is mean ± SE, (n=6); One Way ANOVA: * Refers to comparison with lowest value in each experimental group with other time points ($p \leq 0.05$). [a] Refers to comparison with control in mean levels ($p \leq 0.05$). [b] Refers to comparison with control in daily pulse levels ($p \leq 0.05$).

Table 23. Curcumin effect on ethanol induced changes in daily rhythms, mean and pulse levels of MEL in Pineal.

A . 90 day									
Exp. Group	Zeitgeber time (h)					Mean (24h)	Max	Min	Ratio
	0	6	12	18	24/0				
C	3.27±0.66	1.10±0.16	3.79±0.8*	8.53±1.1*	3.12±0.34	3.60±0.6	8.5±1.10	1.10±0.16	7.90± 0.91
ET	0.20±0.03	0.38±0.10	0.30±0.06	0.47±0.1	0.20±0.03	0.34±0.04[a]	0.47±0.11	0.20±0.03	2.40 ± 0.38
EW	5.40±0.3*	3.41±0.45	5.99±0.8*	3.66±0.50	5.4±0.35*	4.61±0.35	5.99 ± 0.8	3.41±0.45	1.78 ±0.19[b]
CT	2.16±0.39	1.85±0.32	1.52±0.37	9.42±5.53	1.32±0.47	3.25 ± 1.18	9.42±5.53	1.32 ±0.47	8.16 ± 3.38
TT	0.78±0.35	0.39±0.08	0.35±0.15	0.24±0.08	0.74±0.43	0.50±0.11[a]	0.78±0.35	0.24±0.08	3.58±1.218
MT	0.52±0.07	5.9±2.04*	1.03±0.28	0.49±0.18	1.26±0.18	1.86±0.54	5.98±2.04	0.49±0.18	14.05±4.20
B.1 year									
C	0.2±0.03*	0.1±0.008	0.14±0.01	0.2±0.02*	0.09±0.01	0.16±0.02	0.24±0.02	0.09±0.01	2.57±0.212
ET	0.5±0.09*	0.35±0.05	0.23±0.01	0.39±0.06	0.26±0.01	0.35±0.03[a]	0.52±0.09	0.23±0.01	2.25±0.249
EW	0.28±0.01	0.32±0.01	0.24±0.06	0.36±0.03	1.01±0.3*	0.44±0.08[a]	1.01±0.35	0.28±0.01	3.52±0.717
CT	0.11±0.01	0.09±0.007	0.13±0.02	0.10±0.02	0.15±0.04	0.12±0.01	0.15±0.04	0.09±0.007	1.67±0.255
TT	0.19±0.03	0.16±0.011	0.21±0.03	0.13±0.02	0.16±0.01	0.17±0.01	0.21±0.03	0.13±0.029	1.706±0.25
MT	0.18±0.03	0.32±0.10	0.15±0.02	0.23±0.03	0.13±0.03	0.20±0.02	0.32±0.10	0.13±0.034	2.522±0.58
C. 2 year									
C	0.09±0.01	0.04±0.003	0.22±0.13	0.29±0.12	0.06±0.01	0.14±0.04	0.29±0.12	0.06±0.016	4.59±1.298
ET	0.21±0.02	0.26±0.031	0.23±0.03	0.17±0.02	0.29±0.07	0.23±0.01[a]	0.29±0.07	0.17±0.027	1.74±0.86[b]
EW	0.13±0.01	0.12±0.025	0.11±0.01	0.11±0.01	0.13±0.01	0.12±0.007	0.13±0.01	0.11±0.012	1.17±0.08[b]
CT	0.07±0.009	0.07±0.006	0.09±0.01	0.10±0.01	0.07±0.06	0.08±2.05	0.10±0.01	0.07±0.006	1.44±0.10[b]
TT	0.07±0.012	0.06±0.01	0.07±0.01	0.06±4e-3	0.06±0.01	0.06±0.004	0.07±0.01	0.06±0.004	1.21±0.12[b]
MT	0.07±0.008	0.095±0.03	0.12±0.02	0.07±9e-3	0.08±0.01	0.09±0.009	0.12±0.02	0.07±0.008	1.81±0.25[b]

Each value is mean ± SE, (n=6); One Way ANOVA: * Refers to comparison with lowest value in each experimental group with other time points ($p \leq 0.05$). [a] Refers to comparison with control in mean levels ($p \leq 0.05$). [b] Refers to comparison with control in daily pulse levels ($p \leq 0.05$).

In Melatonin treatment, rhythmicity was observed in 90 day only. Mean levels were decreased in 90 day and 2yr whereas increased in 1yr. Increased daily pulse levels were observed in 90 day ($p \leq 0.05$) whereas decreased daily pulse levels observed in 2yr and partial restoration was observed in 1yr (Table 23; Fig. 60 and 61).

Ethanol treatment causes change in mean, daily pulse levels and rhythmicity of MEL in SCN as well as Pineal. Changes caused by ET were not restored in ethanol withdrawal. Curcumin treatment was partially sensitive in restoration of levels in 90 day and 1yr of Pineal. Melatonin treatment was not helpful in restoration of levels in SCN as well as Pineal. Turmeric was sensitive in restoration of levels in 1yr Pineal (Fig. 56).

10. NAT

In SCN, NAT showed rhythmicity in all age groups. Decreased mean as well as daily pulse levels were observed upon aging ($p \leq 0.05$). Ethanol treatment gives abolition of rhythmicity in 90 day only. Mean levels were decreased in 90 day ($p \leq 0.05$) and increased in 2yr ($p \leq 0.05$) whereas levels were not affected in 1yr. Decrease in daily pulse was observed 90 day and whereas in other age groups, these levels were not affected due to ET. Upon EW, rhythmicity was observed in 90 day only. Mean levels were decreased in 90 day and 2yr and affected in 1yr. Decrease was observed in daily pulses in all age groups but significant change was observed in 90day only ($p \leq 0.05$). Curcumin treatment caused increase in mean levels in 90 day ($p \leq 0.05$) whereas decreased levels were observed in 1yr ($p \leq 0.05$) and 2yr but rhythmicity was restored 90 day only. Daily pulse levels were increased in 90 day and decreased in 2yr. Restoration was observed in 1yr age group. In Turmeric treatment, rhythmicity was not observed in all age groups. Mean levels were decreased in all age groups ($p \leq 0.05$). Daily pulse levels were decreased in all age groups ($p \leq 0.05$). Melatonin treatment, rhythmicity was observed in 1yr day only. Decreases in mean levels were observed in all age groups. Daily pulse levels were decreased in all age groups (Table 24; Fig. 58 and 59).

In Pineal, NAT showed rhythmicity in 90 day and 2yr. Age related decrease in the mean levels was observed upon aging ($p \leq 0.05$). Higher daily pulse levels were observed in middle age group (1yr) ($p \leq 0.05$). Ethanol treatment was abolished the rhythmicity in 1y and 2yr. Mean levels were decreased in 90 day whereas increased in 1yr as well as 2yr . Increase in daily pulse levels were observed in 90 day and whereas decrease in levels were observed in 1 ($p \leq 0.05$) and 2yr. Upon EW, rhythmicity was abolished in all age groups. Mean levels were decreased in 90 day ($p \leq 0.05$) whereas increased in 1 ($p \leq 0.05$) as well as 2yr ($p \leq 0.05$). Increase in daily pulse levels were observed in 90 day and 2yr whereas decreases in levels were observed in 1yr ($p \leq 0.05$). Curcumin treatment was sensitive in partial restoration of mean levels in all age groups. Restoration of rhythmicity was observed in 90 day only. Curcumin caused increased daily pulse levels in 90 day whereas significantly decreased levels were observed in 1 and 2yr ($p \leq 0.05$).

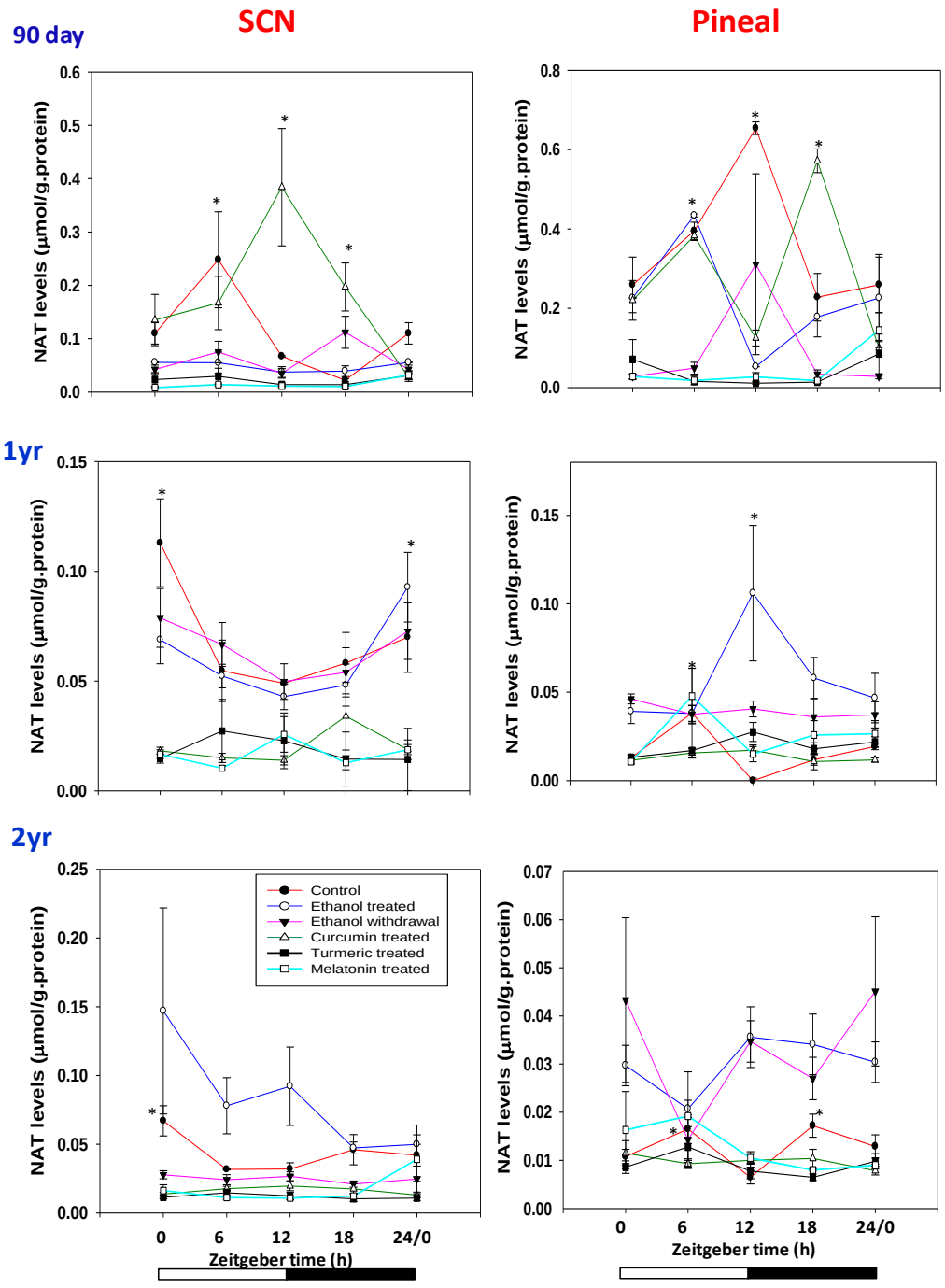

Fig. 57. Curcumin effect on ethanol induced changes in daily rhythms of NAT. Each value is mean ± SE, (n=6); One Way ANOVA: * Refers to comparison with lowest value in each group ($p \leq 0.05$).

Table 24. Curcumin effect on ethanol induced changes in daily rhythms, mean and pulse levels of NAT in SCN.

A . 90 day									
Exp. Group	Zeitgeber time (h)					Mean (24h)	Max	Min	Ratio
	0	6	12	18	24/0				
C	0.110±0.02	0.248±0.09*	0.067±0.001	0.02±0.002	0.110±0.02	0.111±0.028	0.24 ± 0.09	0.02 ± 0.002	11.00± 2.46
ET	0.055±0.001	0.055±0.02	0.0375±0.01	0.039±0.01	0.055±0.001	0.03±0.008[a]	0.05± 0.001	0.037 ± 0.01	1.60± 0.25[b]
EW	0.042±0.008	0.075±0.02	0.035±0.009	0.11±0.03*	0.042±0.008	0.053±0.011	0.112± 0.03	0.035± 0.009	3.42± 0.74[b]
CT	0.135±0.048	0.167±0.05*	0.384±0.11	0.197±0.04	0.028±0.008	0.192 ±0.03[a]	0.384± 0.11	0.028 ±0.008	14.62± 3.60
TT	0.023±0.013	0.029±0.015	0.014±0.003	0.014±0.002	0.031±0.008	0.02±0.004[a]	0.03±0.008	0.014±0.003	2.32±0.45[b]
MT	0.008±0.001	0.014±0.002	0.011±0.002	0.01±0.002	0.03±0.008*	0.01±0.002[a]	0.01±0.002	0.008±0.001	1.57±0.32[b]
B. 1yr									
C	0.113±0.02*	0.054±0.014	0.049±0.0005	0.058±0.014	0.070±0.016	0.068±0.011	0.113±0.02	0.049±0.0005	2.32±0.238
ET	0.0689±0.01	0.052±0.005	0.042±0.005	0.048±0.009	0.092±0.015*	0.061±0.005	0.09±0.015	0.042±0.0059	2.17±0.258
EW	0.078±0.013	0.0667±0.01	0.049±0.008	0.054±0.011	0.072±0.012	0.064±0.005	0.078±0.01	0.049±0.0081	1.6380.197
CT	0.018±0.001	0.015±0.002	0.013±0.002	0.034±0.015	0.018±0.002	0.02±0.003[a]	0.03±0.015	0.0139±0.002	2.67±0.730
TT	0.014±0.00	0.027±0.014	0.022±0.012	0.014±0.012	0.014±0.014	0.018±0.003[a]	0.027±0.01	0.014±0.0142	1.935±0.60
MT	0.016±0.002	0.010±0.001	0.025±0.008*	0.01±0.003*	0.018±0.004	0.01±0.002[a]	0.02±0.008	0.010±0.0012	2.539±0.50
C. 2yr									
C	0.067±0.011*	0.03±0.0004	0.032±0.004	0.046±0.01	0.04±0.0079	0.043±0.006	0.067±0.01	0.031±0.0004	2.16±0.186
ET	0.147±0.075	0.078±0.02	0.092±0.028	0.047±0.004	0.049±0.006	0.083±0.016[a]	0.147±0.07	0.047±0.0043	3.14±0.883
EW	0.027±0.002	0.024±0.003	0.026±0.003	0.021±0.002	0.024±0.001	0.024±0.001	0.02±0.002	0.021±0.0023	1.29±0.09
CT	0.013±0.001	0.017±0.001	0.019±0.003	0.017±0.003	0.013±0.002	0.016±0.001	0.01±0.003	0.013±0.0021	1.49±0.191
TT	0.011±0.001	0.014±0.002	0.012±0.002	0.010±0.002	0.01±0.0018	0.01±0.0009	0.01±0.002	0.0103±0.002	1.488±0.23
MT	0.016±0.004	0.0112±0.001	0.0108±0.001	0.012±0.002	0.039±0.025	0.017±0.005	0.039±0.02	0.0108±0.001	3.66±1.389

Each value is mean $\pm$ SE, (n=6); One Way ANOVA: * Refers to comparison with lowest value in each experimental group with other time points ($p \leq 0.05$). [a] Refers to comparison with control in mean levels ($p \leq 0.05$). [b] Refers to comparison with control in daily pulse levels ($p \leq 0.05$).

Table 25. Curcumin effect on ethanol induced changes in daily rhythms, mean and pulse levels of NAT in Pineal.

Exp	Zeitgeber time (h)					Mean (24h)	Max	Min	Ratio
	0	6	12	18	24/0				
A. 90 day									
C	0.25±0.07	0.39±0.02	0.6±0.01*	0.22±0.06	0.25±0.07	0.3±0.076	0.65± 0.01	0.22± 0.06	3.18±0.5
ET	0.22±0.11	0.43±0.3*	0.05±0.007	0.17±0.05	0.22±0.11	0.197 ±0.09	0.43 ± 0.38	0.05±0.007	8.25± 4.29
EW	0.02±0.006	0.04±0.01	0.31±0.228	0.03±0.01	0.02±0.006	0.10 ±0.05[a]	0.31±0.22	0.02±0.006	11.6 ± 5.07
CT	0.22±0.05*	0.3±0.01*	0.125±0.02	0.57±0.03*	0.10±0.019	0.28±0.077	0.57 ± 0.03	0.10 ±0.019	5.90 ± 0.67
TT	0.071±0.05	0.01±2e^{-3}	0.01±0.002	0.01±0.003	0.085±0.05	0.03±0.016[a]	0.085±0.05	0.01±0.002	4.184±3.06
MT	0.02±0.006	0.01±1e^{-3}	0.027±0.01	0.01±0.006	0.14±0.04*	0.04±0.012[a]	0.145±0.04	0.01±0.006	9.545±2.69
B. 1 year									
C	0.01±0.001	0.03±0.02	0.01±0.001	0.01±0.003	0.008±1e^{-3}	0.018±0.005	0.038±0.02	0.008±8e^{-4}	6.35±1.168
ET	0.03±0.006	0.03±4e^{-3}	0.10±0.038	0.05±0.011	0.04±0.014	0.05±0.009[a]	0.10±0.038	0.03±0.006	2.79±0.54[b]
EW	0.04±0.002	0.03±5e^{-3}	0.04±0.004	0.036±0.01	0.03±7e^{-3}	0.039±0.002	0.04±0.002	0.03±0.010	1.39±0.23[b]
CT	0.01±0.001	0.01±2e^{-3}	0.017±0.00	0.01±0.004	0.011±1e^{-3}	0.01±0.0009	0.01±0.003	0.01±0.004	1.94±0.53[b]
TT	0.01±0.001	0.01±1e^{-3}	0.02±0.005	0.01±0.003	0.021±3e^{-3}	0.019±0.001	0.02±0.005	0.01±0.001	2.09±0.25[b]
MT	0.01±0.001	0.04±0.01	0.01±4e^{-3}*	0.02±0.008	0.026±7e^{-3}	0.025±0.004	0.04±0.015	0.01±0.001	4.556±0.94
C. 2 year									
C	0.01±0.001	0.01±3e^{-3}*	0.006±1e^{-3}	0.01±0.002*	0.012±2e^{-3}	0.012±0.001	0.01±0.002	0.006±1e^{-4}	2.74±0.390
ET	0.02±0.004	0.02±1e^{-3}	0.035±6e^{-3}	0.03±0.0063	0.030±4e^{-3}	0.03±0.002[a]	0.03±0.006	0.02±0.001	1.45±0.185
EW	0.043±0.17	0.01±4e^{-3}	0.03±4e^{-3}	0.027±0.004	0.045±0.01	0.032±5e^{-4}[a]	0.045±0.01	0.01±0.001	3.20±0.472
CT	0.01±0.002	9e^{-3}±1e^{-3}	0.01±1e^{-3}	0.01±0.0019	0.007±8e^{-4}	0.009±7e-4	0.01±0.002	0.007±8e^{-4}	1.48±0.216
TT	0.008±1e^{-3}	2e^{-3}±4e^{-3}	0.007±1e^{-3}	0.006±0.006	0.009±1e^{-3}	0.009±0.001	0.01±0.004	0.006±6e^{-4}	2.01±0.404
MT	0.016±8e^{-3}	9e^{-3}±9e^{-3}	0.01±1e^{-3}	0.008±2e^{-4}	0.009±2e^{-4}	0.012±0.002	0.01±0.009	0.008±2e^{-4}	2.401±0.66

Each value is mean ± SE, (n=6); One Way ANOVA: * Refers to comparison with lowest value in each experimental group with other time points ($p \leq 0.05$). [a] Refers to comparison with control in mean levels ($p \leq 0.05$). [b] Refers to comparison with control in daily pulse levels ($p \leq 0.05$).

Turmeric treatment, rhythmicity was not observed in all age groups. Mean levels were decreased in 90 day ($p \leq 0.05$) and restoration was observed in 1 as well as 2yr. Daily pulse levels were restored in 90 day and 2yr whereas significantly decreased levels were observed in 1yr ($p \leq 0.05$). Melatonin treatment, rhythmicity was observed in 90 day and 1yr. Mean levels were increased in 90 day ($p \leq 0.05$) and 1yr whereas restored in 2yr. Increased daily pulse levels were observed in 90 day ($p \leq 0.05$) whereas decreased daily pulse levels observed in 1 and 2yr (Table 25; Fig. 60 and 61).

Ethanol treatment causes change in mean, daily pulse levels and rhythmicity of NAT in SCN as well as Pineal. Changes caused by ET were not restored in ethanol withdrawal. Curcumin treatment was partially sensitive in restoration of levels in all age groups in Pineal. Melatonin treatment was helpful in restoration of levels in 2yr Pineal only. Turmeric was also sensitive in restoration of levels in 1 and 2yr Pineal but not in SCN (Fig. 57).

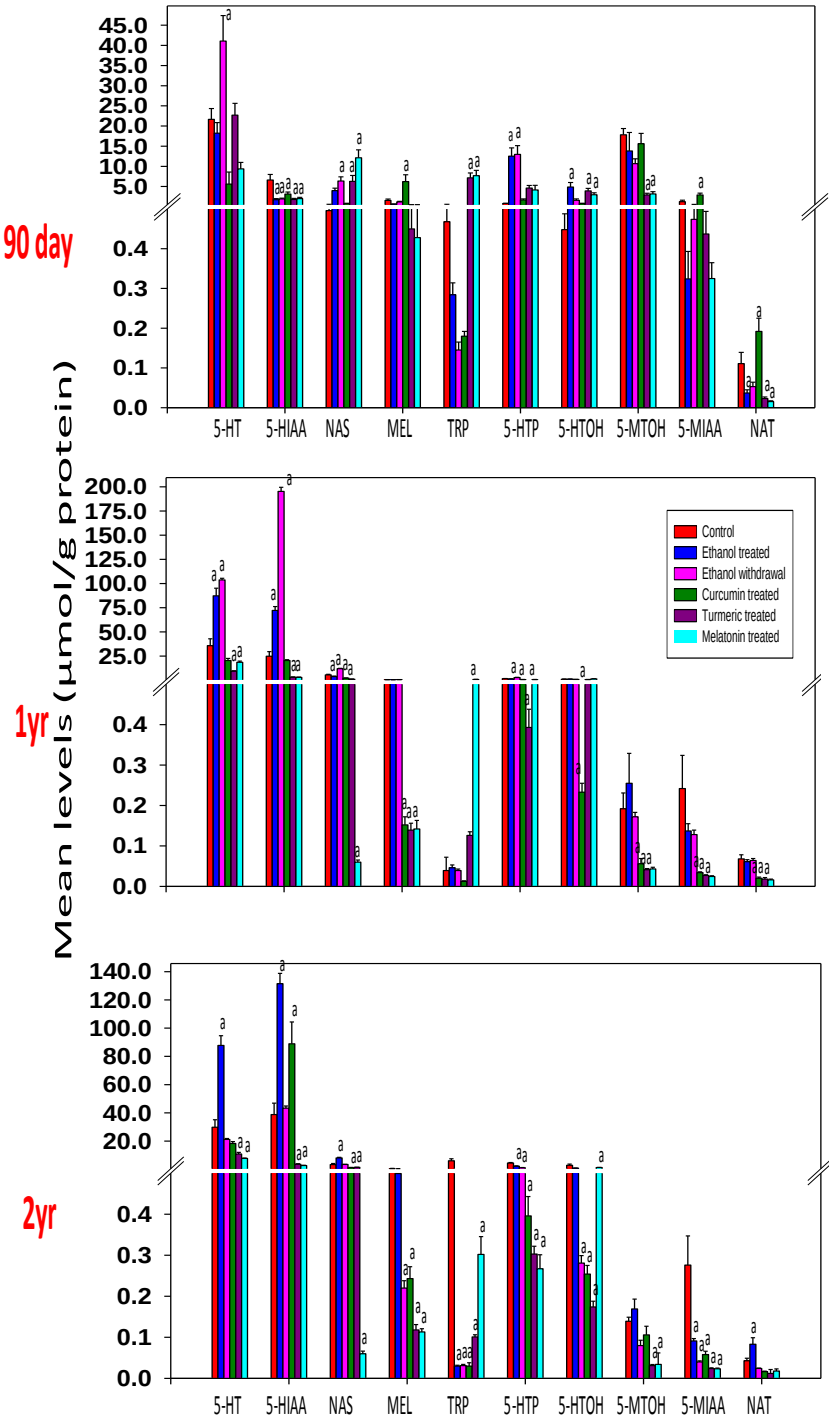

Fig. 58. Curcumin effect on ethanol induced changes in mean levels/24h in SCN. Each value is mean ± SE, (n=6); One Way ANOVA: [a] Refers to comparison with control value in each group ($p \leq 0.05$).

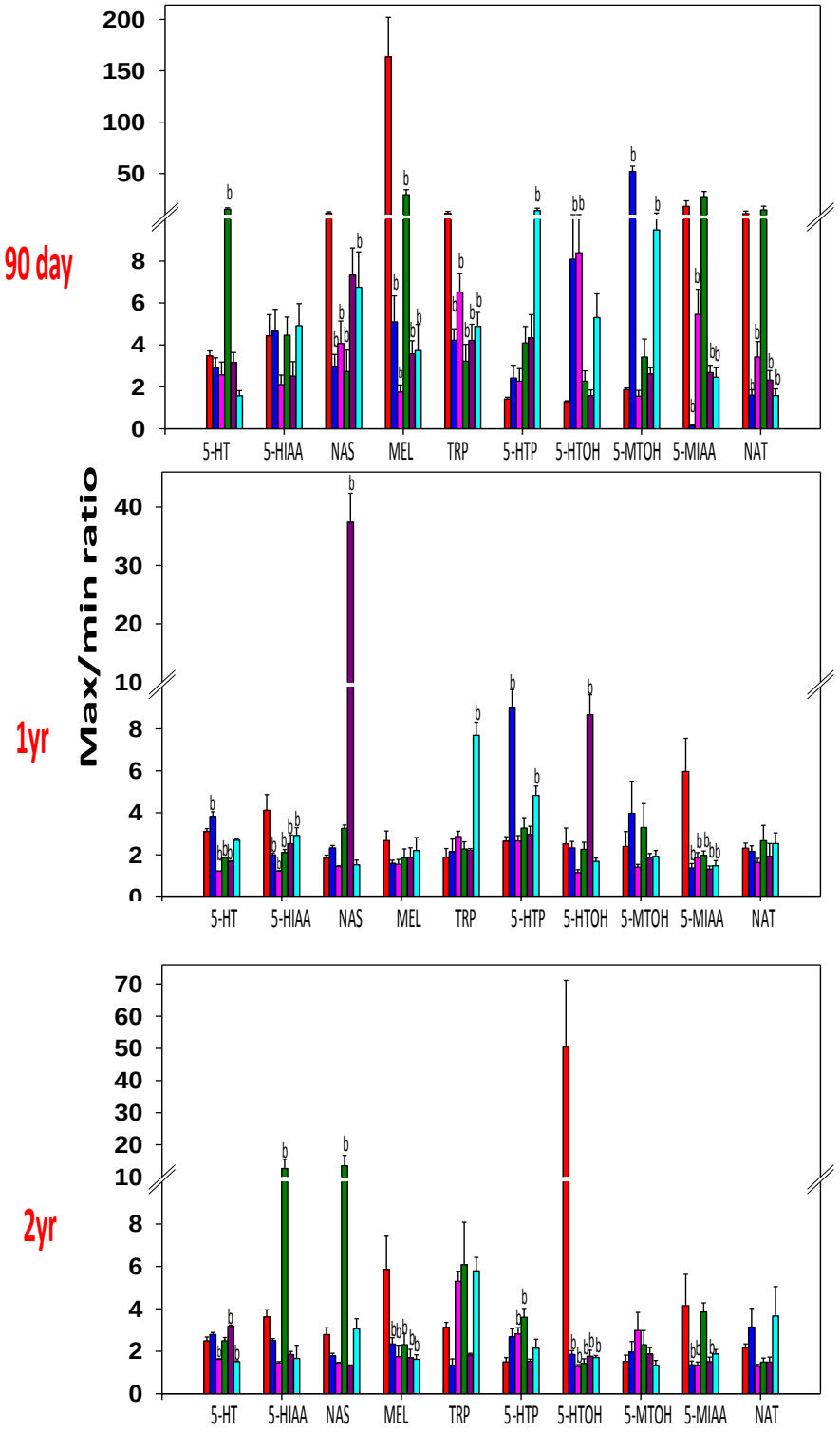

Fig. 59. Curcumin effect on ethanol induced changes in daily pulse levels in SCN. Each value is mean ± SE, (n=6); One Way ANOVA: [b] Refers to comparison with control value in each group ($p \leq 0.05$).

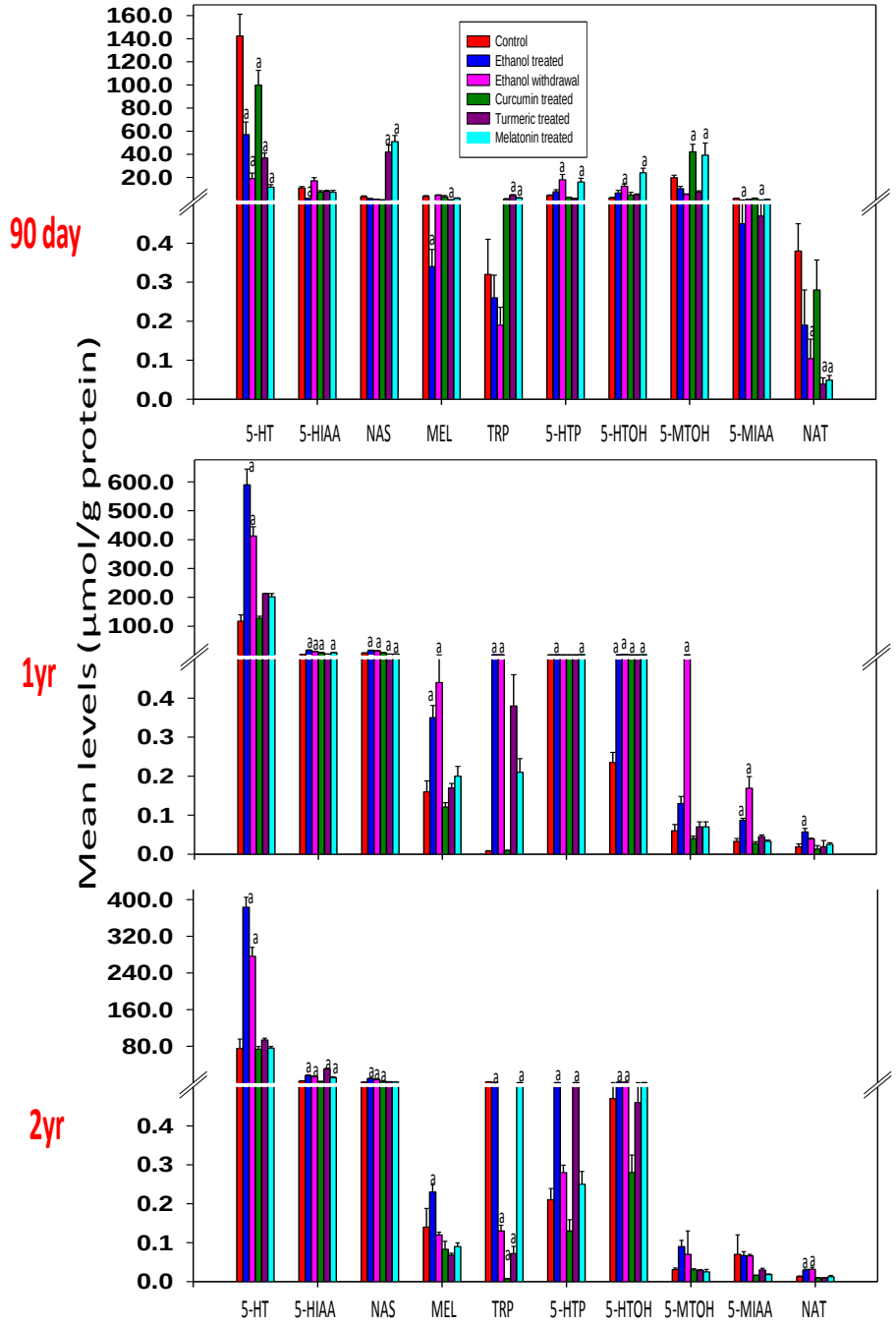

Fig. 60. Curcumin effect on ethanol induced changes in mean levels/24h in Pineal. Each value is mean ± SE, (n=6); One Way ANOVA: [a] Refers to comparison with control value in each group ($p \leq 0.05$).

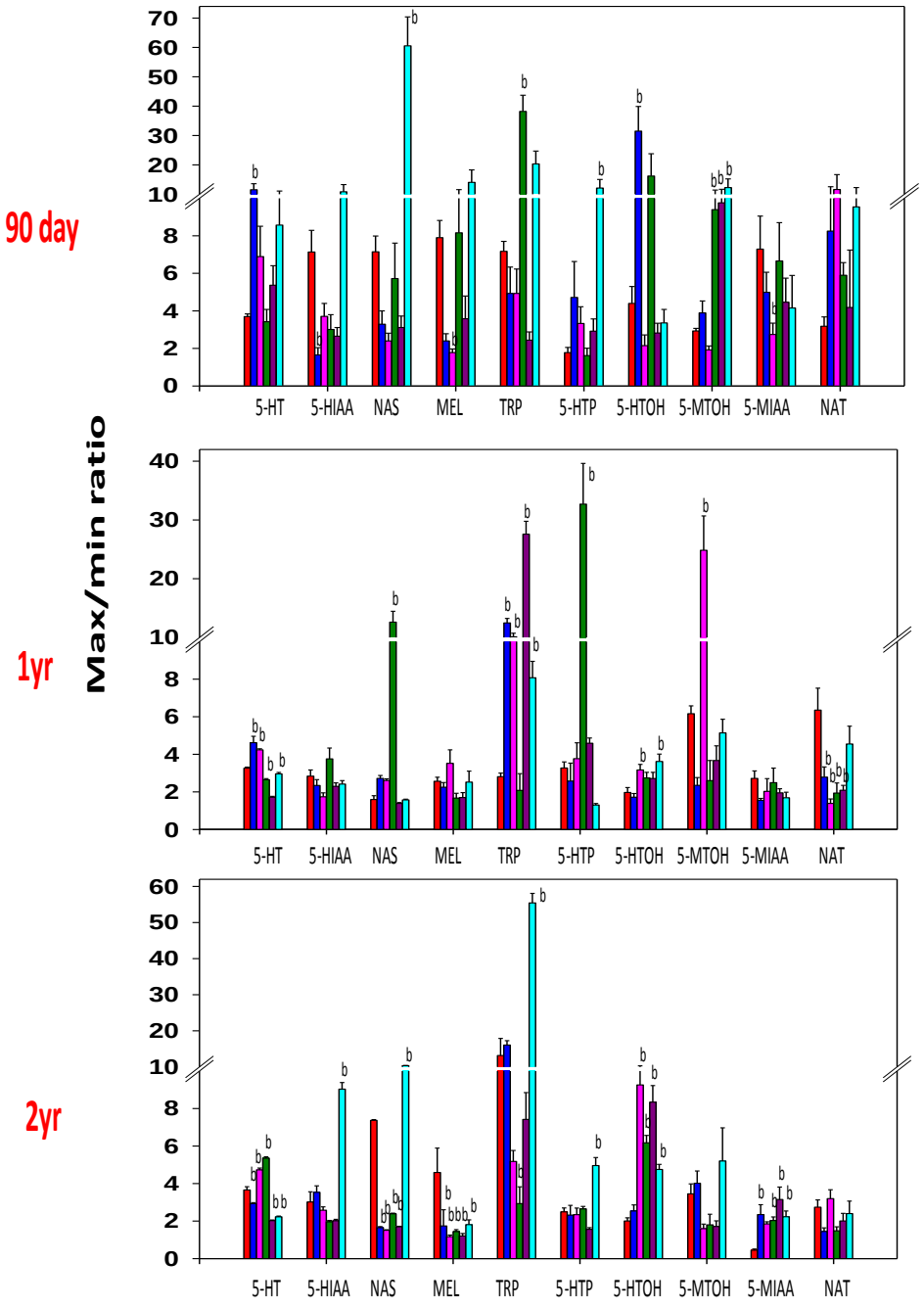

Fig. 61. Curcumin effect on ethanol induced changes in daily pulse levels in Pineal. Each value is mean ± SE, (n=6); One Way ANOVA: [b] Refers to comparison with control value in each group ($p \leq 0.05$).

OBJECTIVE 3

Effect of Curcumin on ethanol induced changes on *per1* and *per2* expression in SCN and Pineal.

1. Effect of Curcumin on ethanol induced changes in *per* gene expression in the SCN

(i). *rper1* expression:

Daily rhythms in *rper1* expression with maximum levels at ZT-6 ($p \leq 0.05$) and then nadir (minimum) at ZT-18 in SCN of 3 month old rat were found in present study. Upon ethanol treatment caused 1.3 fold increases in amplitude of *rper1* expression as compared to control ($p \leq 0.05$).

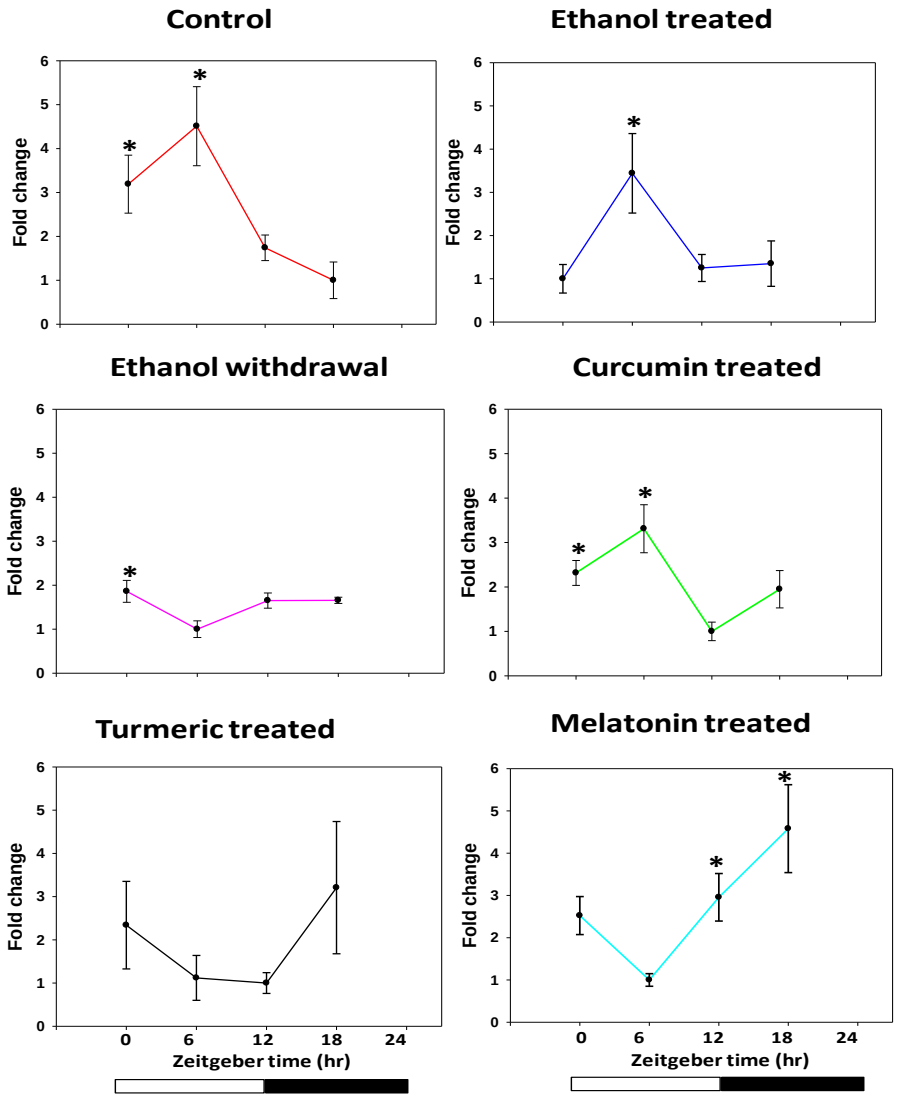

Fig. 62. Effect of curcumin on ethanol induced changes on *rper1* in SCN of male Wistar rat. Each value is mean ± SE (n=4) and values are expressed in fold change. * refers to comparison with lowest value of time point with other time points in each group ($p \leq 0.05$).

Phase shift was not observed but rhythmicity was maintained. There was decrease in amplitude of *rper1* mRNA expression in EW animals by 4 fold ($p \leq 0.05$). Phase advance by 6h was observed and rhythmicity also persisted. Curcumin treatment caused partial restoration in phase, levels and rhythmicity but phase was not restored completely (1.3 fold change). Statistical analysis showed that mean value of curcumin did not affect as compared with control group. Turmeric treatment was not sensitive in restoration of the levels, rhythmicity and phase. Melatonin treatment caused partial restoration in the levels but not in phase and amplitude. The level of *per1* peaked at ZT-18 and 12h phase delay was observed upon melatonin treatment. Melatonin treatment did not show statistically significant difference as compared to control in mean levels. Daily pulse levels showed that ethanol treatment as well as withdrawal caused reduction ($p \leq 0.05$) whereas curcumin (80%), turmeric (75%) and melatonin (96%) treatments showed restoration (Fig. 62, 63: Table 26).

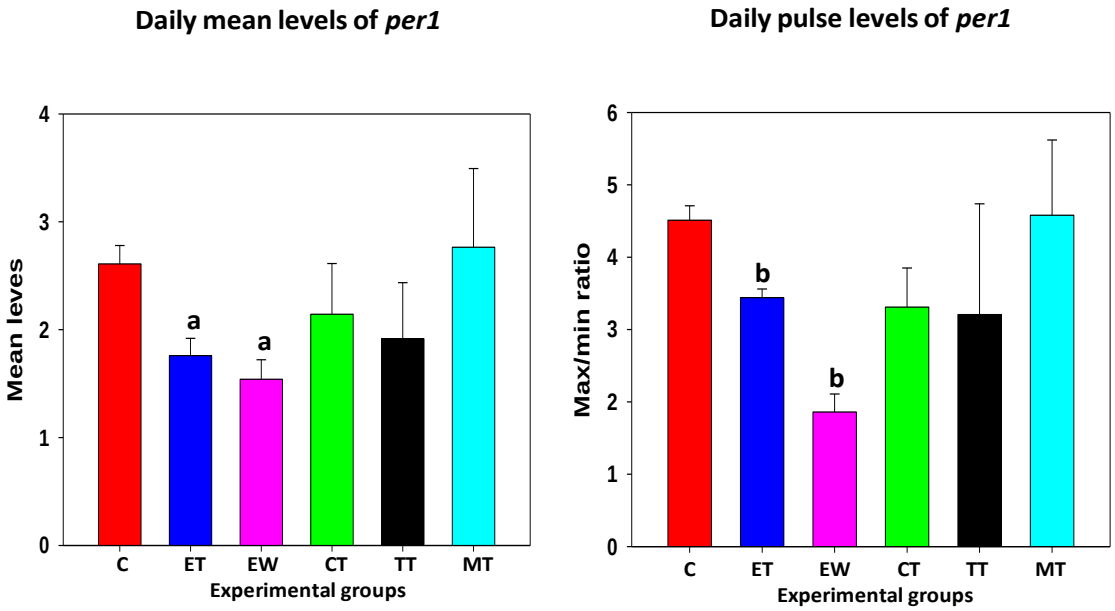

Fig. 63. Effect of curcumin on ethanol induced changes on daily mean and pulse levels of *rper1* in SCN. **a**, refers to comparison between control and treated groups in mean levels whereas **b**, refers to comparison between control and treated groups in daily pulse levels ($p \leq 0.05$).

(ii). *rper2* expression:

Daily rhythms in *rper2* expression with maximum levels at ZT-12 ($p \leq 0.05$) and minimum at ZT-18 in SCN were found in present study. Upon ethanol treatment caused robust decrease in the amplitude, abolition of rhythm and 2.5 fold decreases in the mean value ($p \leq 0.05$).

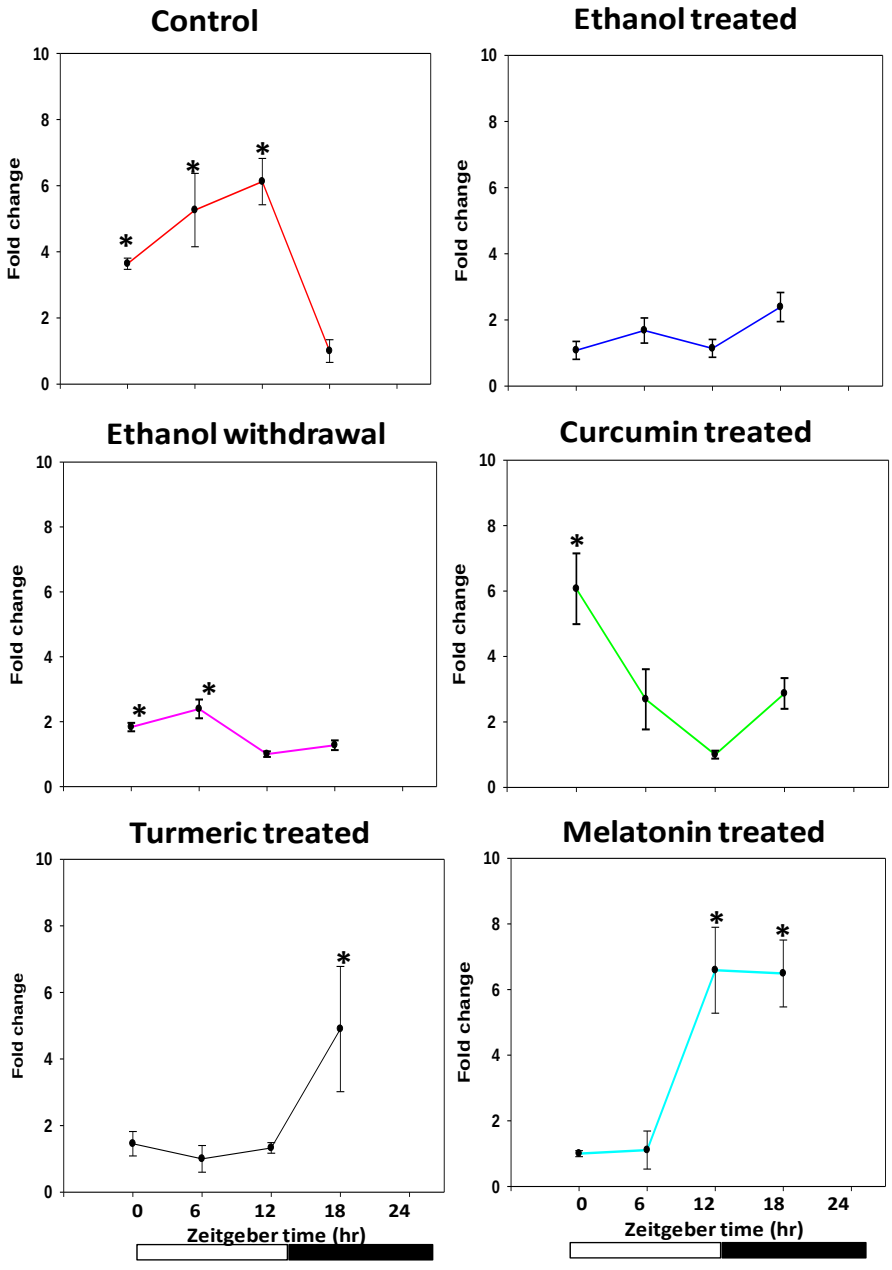

Fig. 64. Effect of curcumin on ethanol induced changes on *rper2* in SCN of male Wistar rat. Each value is mean ± SE (n=4) and values are expressed in fold change. * refers to comparison with lowest value of time point with other time points in each group ($p \leq 0.05$).

But in EW, rhythmicity was observed even though levels were reduced like ethanol treatment. Maximum levels were observed at ZT-6 and minimum was observed at ZT-12 respectively. Phase advance by 6h was observed and 2.4 fold decreases in mean value was found as compared to control ($p \leq 0.05$). Curcumin treatment resulted in complete restoration in amplitude and rhythmicity whereas partial restoration observed in levels. But 12h advance in phase and partial restoration in mean value were found. Turmeric treatment caused partial restoration in amplitude and levels but 6h phase delay was observed. Mean value also decreased due to turmeric treatment (1.8 fold) ($p \leq 0.05$). Melatonin treatment gives partial restoration in levels, amplitude, phase and mean value. Daily pulse levels showed that both ET and EW caused reduction in levels. Curcumin and melatonin showed complete restoration but turmeric showed partial restoration in daily pulse levels (Fig. 64, 65; Table 26).

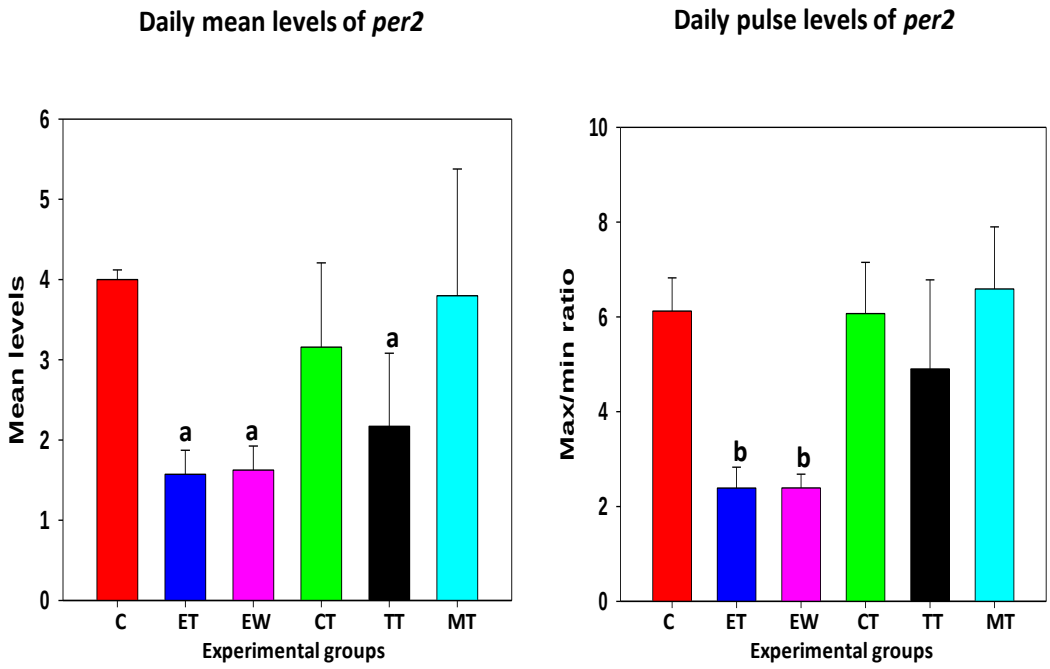

Fig. 65. Effect of curcumin on ethanol induced changes on daily mean and pulse levels of *rper2* in SCN. **a**, refers to comparison between control and treated groups in mean levels whereas **b**, refers to comparison between control and treated groups in daily pulse levels ($p \leq 0.05$).

Table 26. Effect of Curcumin on ethanol induced changes on *rper1* and *rper2* in SCN.

S.No	Experimental groups	gene	Zeitgeber time (h)				Mean	Max	Min	Ratio
			0	6	12	18				
1	Control	*per1*	3.19±0.66*	4.51±0.9*	1.74±0.2	1.00±0.417	2.61±0.77	4.51±0.90	1.00±0.4	4.51±0.90
		per2	3.64±0.17*	5.26±1.1*	6.1±0.7*	1.00±0.343	4.00±1.12	6.12±0.70	1.00±0.3	6.124±0.70
2	Ethanol treated	*per1*	1.00±0.33	3.44±0.9*	1.25±0.3	1.35±0.525	1.76±0.5[a]	3.44±0.92	1.00±0.3	3.44±0.92[b]
		per2	1.08±0.27	1.68±0.38	1.14±0.2	2.39±0.44	1.572±0.3	2.39±0.44	1.08±0.2	2.39±0.44
3	Ethanol withdrawal	*per1*	1.86±0.24*	1.00±0.19	1.65±0.1	1.656±0.07	1.54±0.1[a]	1.86±0.24	1.00±0.1	1.86±0.249[b]
		per2	1.83±0.13*	2.39±0.2*	1.0±0.08	1.275±0.15	1.62±0.30	2.39±0.29	1.0±0.08	2.393±0.29
4	Curcumin treated	*per1*	2.31±0.28*	3.31±0.5*	1.00±0.2	1.948±0.42	2.14±0.47	3.31±0.54	1.00±0.2	3.31±0.54
		per2	6.07±1.08*	2.69±0.92	1.00±0.1	2.87±0.47	3.15±1.05	6.07±1.08	1.00±0.1	6.07±1.08
5	Turmeric treated	*per1*	2.33±1.013	1.11±0.51	1.00±0.2	3.207±1.53	1.91±0.52	3.20±1.53	1.00±0.2	3.207±1.53
		per2	1.45±0.368	1.00±0.40	1.32±0.1	4.90±1.88*	2.17±0.91	4.90±1.88	1.00±0.4	4.90±1.88
6	Melatonin treated	*per1*	2.523±0.45	1.00±0.14	2.9±0.5*	4.5±1.04*	2.76±0.73	4.58±1.04	1.00±0.14	4.58±1.04
		per2	1.00±0.092	1.10±0.58	6.5±1.3*	6.49±1.02*	3.79±1.58	6.59±1.31	1.0±0.09	6.59±1.31

a, refers to comparison between control and treated groups in mean levels whereas **b**, refers to comparison between control and treated groups in daily pulse levels ($p \leq 0.05$). ***** refers to comparison with lowest value of time point with other time points in each group ($p \leq 0.05$).

2. Effect of curcumin on ethanol induced changes in *per* gene expression in the Pineal

(i). *rper1* expression:

In our study, daily rhythms of *rper1*expression with maximum levels at ZT-18 ($p \leq 0.05$) and then a nadir at ZT-6 were found in pineal. Upon ethanol treatment caused 3 fold decreases in amplitude of *rper1* expression as compared to control ($p \leq 0.05$). Rhythmicity was abolished due to ET. The daily peak amplitude of *rper1* expression further decreased upon EW (2.4 fold). Rhythmicity was also abolished in EW.

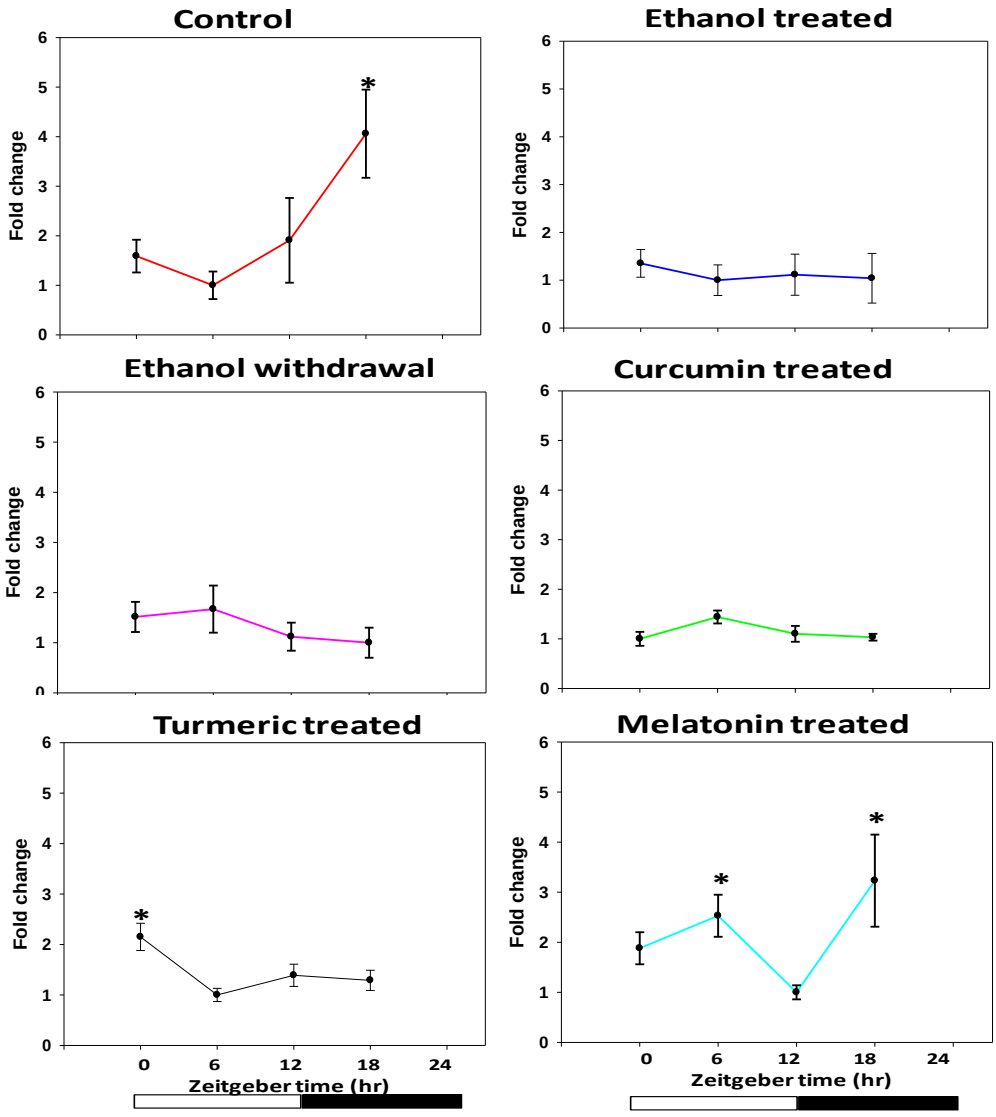

Fig. 66. Effect of curcumin on ethanol induced changes on *rper1* in pineal of male Wistar rat. Each value is mean ± SE (n=4) and values are expressed in fold change. * refers to comparison with lowest value of time point with other time points in each group ($p \leq 0.05$).

Curcumin treatment was not sensitive in restoration of phase, levels, rhythmicity and phase (2.8 fold change). Statistical analysis showed that mean value of curcumin was significant with control group ($p \leq 0.05$) (1.8 fold). Turmeric treatment was also not sensitive in restoration of the levels, rhythmicity and phase. Melatonin treatment resulted in partial restoration in the levels, phase and amplitude. The levels of *per1* peaked at ZT-18 in melatonin treatment. Melatonin treatment did not show statistically significant difference as compared to control in mean levels. Daily pulse levels showed that decreased levels in ET, EW and CT ($p \leq 0.05$). Melatonin treatments showed partial restoration in daily pulse levels (Fig 66, 67; Table 27).

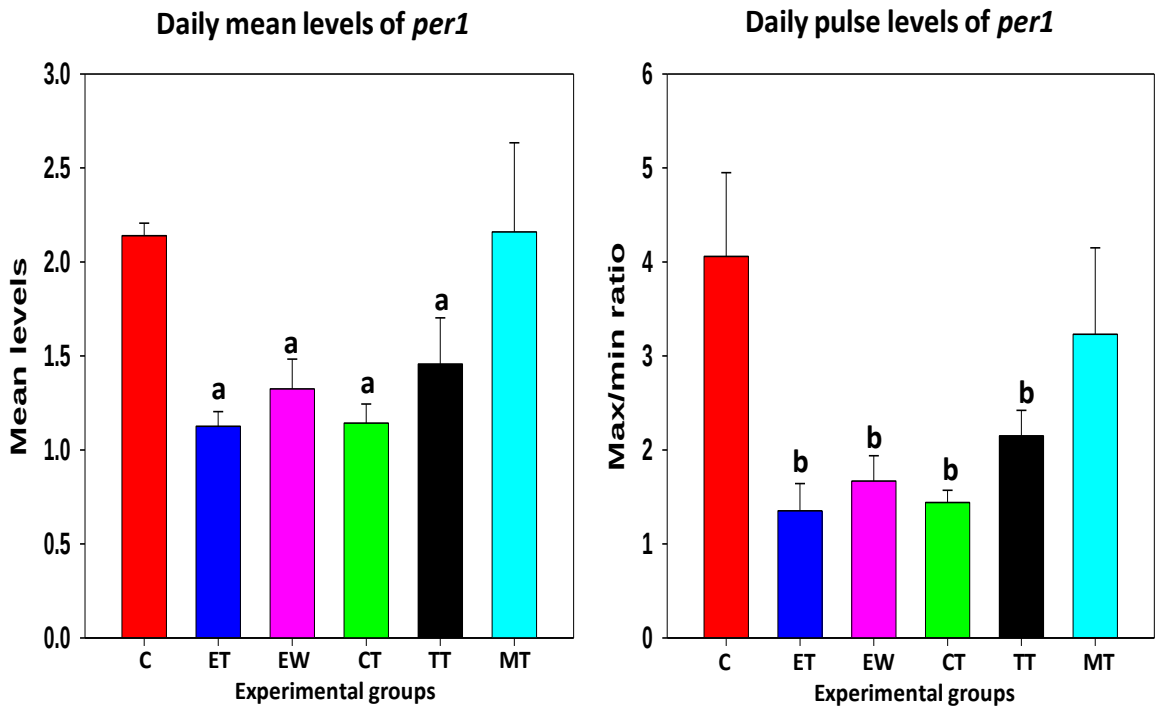

Fig. 67. Effect of curcumin on ethanol induced changes on daily mean and pulse levels of *rper1* in pineal. **a**, refers to comparison between control and treated groups in mean levels whereas **b**, refers to comparison between control and treated groups in daily pulse levels ($p \leq 0.05$).

(ii). *rper2* expression:

Daily rhythms of *rper2* with maximum levels at ZT-0 ($p \leq 0.05$) and nadir at ZT-12 were found in pineal. Ethanol treatment caused decrease in the amplitude though persistent in rhythm and 2.56 fold decreases in the mean value was found ($p \leq 0.05$). Phase advance was observed by 6h in ET.

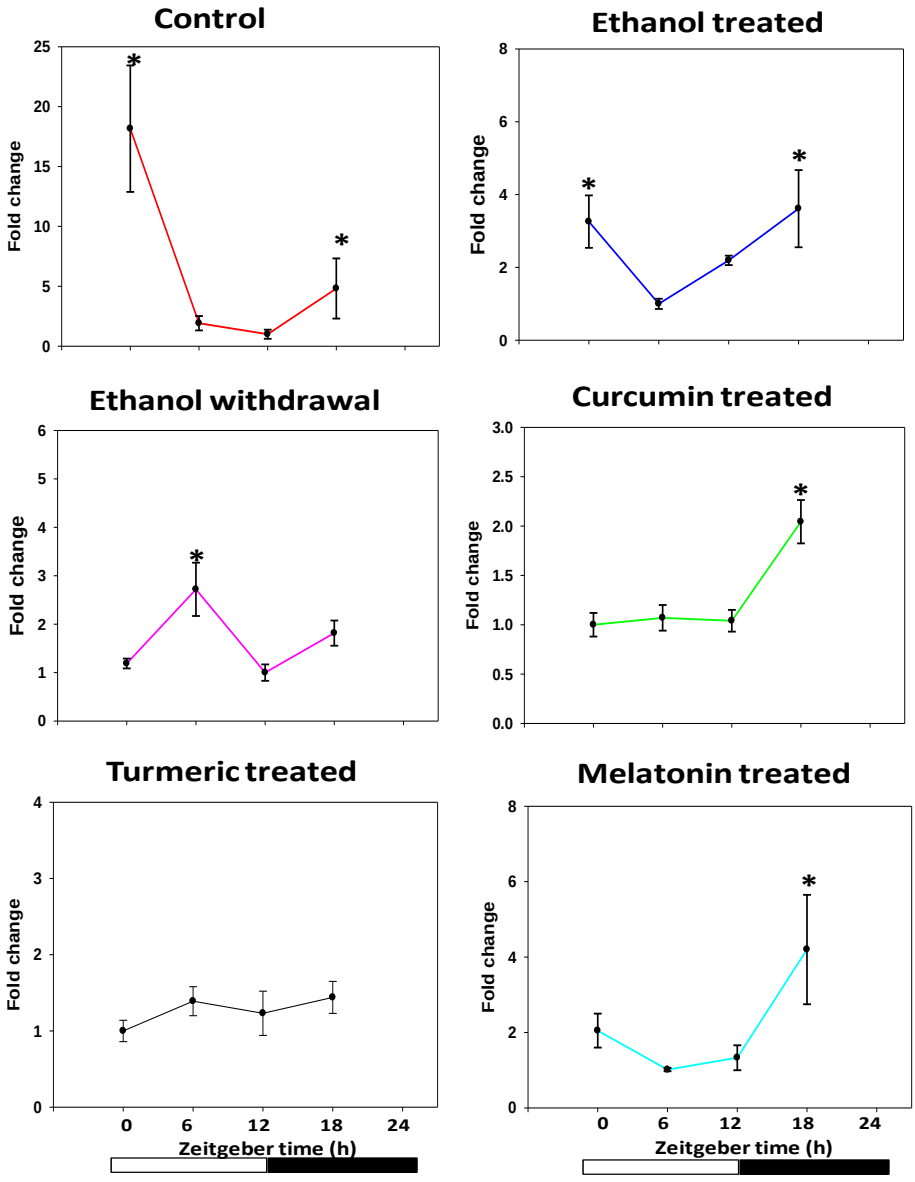

Fig. 68. Effect of curcumin on ethanol induced changes on *rper2* in pineal of male Wistar rat. Each value is mean ± SE (n=4) and values are expressed in fold change. * refers to comparison with lowest value of time point with other time points in each group ($p \leq 0.05$).

But in ethanol withdrawal rhythmicity was observed even though levels were reduced like ethanol treatment. Maximum levels were observed at ZT-6 and minimum was observed at ZT-12. 6 h phase delay was observed and 3.8 fold decreases in mean value was found, compared with control ($p \leq 0.05$). Curcumin treatment was not sensitive in restoration of amplitude though rhythmicity was observed. Whereas 6h advance in phase and 5 fold decrease in mean value was found ($p \leq 0.05$). Turmeric treatment was also not sensitive in restoration of amplitude, phase and rhythm. Mean value was decreased due to turmeric treatment (5 fold) as compared to control ($p \leq 0.05$). Melatonin treatment was sensitive in partial restoration of levels, amplitude, rhythm and mean value. Phase advance was observed by 6h. Daily pulse levels showed that decreased levels in ET, EW, CT and TT ($p \leq 0.05$). Melatonin treatment showed partial restoration in daily pulse levels (Fig 68, 69; Table 27).

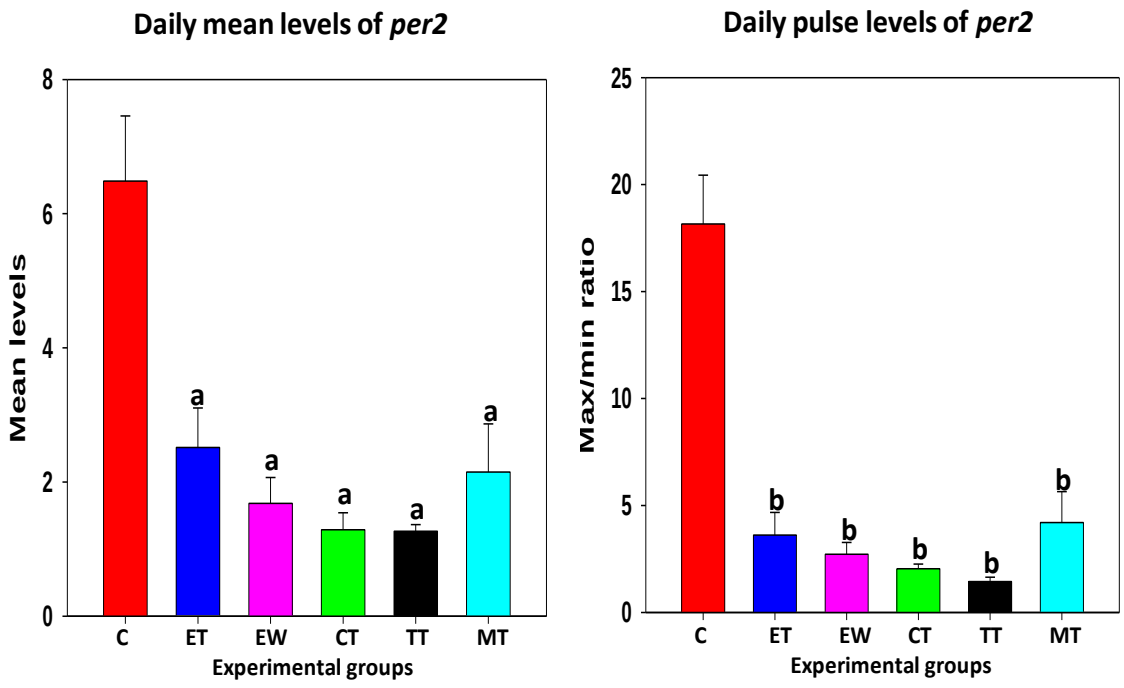

Fig. 69. Effect of curcumin on ethanol induced changes on daily mean and pulse levels of *rper2* in Pineal. **a**, refers to comparison between control and treated groups in mean levels whereas **b**, refers to comparison between control and treated groups in daily pulse levels ($p \leq 0.05$).

Table 27. Effect of Curcumin on ethanol induced changes on *rper1* and *rper2* in Pineal.

S.No	Experimental groups	gene	Zeitgeber time (h)				Mean	Max	Min	Ratio
			0	6	12	18				
1	Control	*Per1*	1.589±0.33	1.00±0.27	1.90±0.85	4.05±0.8*	2.13±0.06	4.05±0.89	1.00±0.27	4.05±0.8
		Per2	18.1±5.28*	1.91±0.60	1.00±0.38	4.86±2.5*	6.48±0.97	18.16±5.2	1.00±0.38	18.1±2.2
2	Ethanol treated	*Per1*	1.352±0.29	1.00±0.32	1.115±0.43	1.04±0.52	1.12±0.07[a]	1.35±0.29	1.00±0.32	1.35±0.2[b]
		Per2	3.25±0.72*	1.00±0.14	2.193±0.13	3.6±1.06*	2.51±0.58[a]	3.61±1.06	1.00±0.14	3.6±1.06[b]
3	Ethanol withdrawal	*Per1*	1.514±0.30	1.66±0.47	1.120±0.28	1.00±0.30	1.32±0.15[a]	1.66±0.47	1.00±0.30	1.6±0.27[b]
		Per2	1.18±0.101	2.71±0.5*	1.00±0.17	1.81±0.26	1.68±0.38[a]	2.71±0.55	1.00±0.17	2.7±0.55[b]
4	Curcumin treated	*Per1*	1.00±0.14	1.44±0.13	1.101±0.16	1.03±0.06	1.14±0.10[a]	1.44±0.13	1.00±0.14	1.44±0.1[b]
		Per2	1.00±0.12	1.07±0.13	1.04±0.11	2.04±0.2*	1.28±0.25[a]	2.04±0.22	1.00±0.12	2.04±0.2[b]
5	Turmeric treated	*Per1*	2.15±0.27*	1.00±0.13	1.39±0.22	1.29±0.20	1.45±0.24[a]	2.15±0.27	1.00±0.13	2.15±0.2
		Per2	1.00±0.14	1.39±0.19	1.231±0.29	1.44±0.21	1.26±0.09[a]	1.44±0.21	1.00±0.14	1.44±0.2[b]
6	Melatonin treated	*Per1*	1.88±0.32	2.53±0.4*	1.00±0.14	3.23±0.9*	2.16±0.47	3.23±0.92	1.00±0.14	3.23±0.9
		Per2	2.05±0.45	1.01±0.04	1.33±0.33	4.20±1.4*	2.14±0.71[a]	4.20±1.45	1.01±0.04	4.20±1.4[b]

a, refers to comparison between control and treated groups in mean levels whereas **b**, refers to comparison between control and treated groups in daily pulse levels ($p \leq 0.05$). ***** refers to comparison with lowest value of time point with other time points in each group ($p \leq 0.05$).

OBJECTIVE 4

Effect of Curcumin on ethanol induced changes in daily locomotor activity rhythms.

1. Effect of ethanol treatment and its withdrawal on gross locomotor activity of rat:

All control rats showed nocturnal activity with phase (~17:30h) and ~80-90 percentage of nocturnality. In Rat1, significant difference was found between experimental groups ($p \leq 0.05$). ET caused 2 fold increases in mean and slight decrease in amplitude with ~ 06:30h phase advance. Upon ET, day activity was increased by 5 fold where as nocturnal activity decreased (50%) ($p \leq 0.05$). In ethanol withdrawal period, further increase in mean (2.5 fold) and amplitude (1.5 fold) with ~ 05:50h phase advance was observed. Even night activity is less than day activity and statistically significant with control ($p \leq 0.05$). Slight restoration was observed in percentage of nocturnal activity when compared with ET (Fig. 70; Table 28).

In Rat2, significant difference was found between experimental groups ($p \leq 0.05$). Ethanol treatment resulted in increase of mean (4.5 fold) and amplitude (3 fold) with change of phase (advance ~09:00h). Day activity was increased than night activity compared with control. Percentage of nocturnal activity was decreased (60%) ($p \leq 0.05$). In ethanol withdrawal, the mean and amplitude were decreased when compared with ET but levels of mean, amplitude were higher when compared with control with phase shift (advance ~12:00h). Night activity is further reduced and day activity increased when compared with control ($p \leq 0.05$). The percentage of nocturnal activity further decreased when compared with ET (Fig. 70; Table 28).

In Rat3, significant difference was found between experimental groups ($p \leq 0.05$). ET increased the mean value (2.6 fold) and decreased the amplitude (2.3 fold) with ~09:40h phase advance. Day activity was increased than night activity compared with control and percentage of night activity was decreased when compared control (40%) ($p \leq 0.05$). Ethanol withdrawal caused further increase in the mean and amplitude and ~07:00h phase advance compared with control ($p \leq 0.05$). Day activity was more than night activity. The percentage of nocturnality was further reduced by 10% (Fig. 70; Table 28).

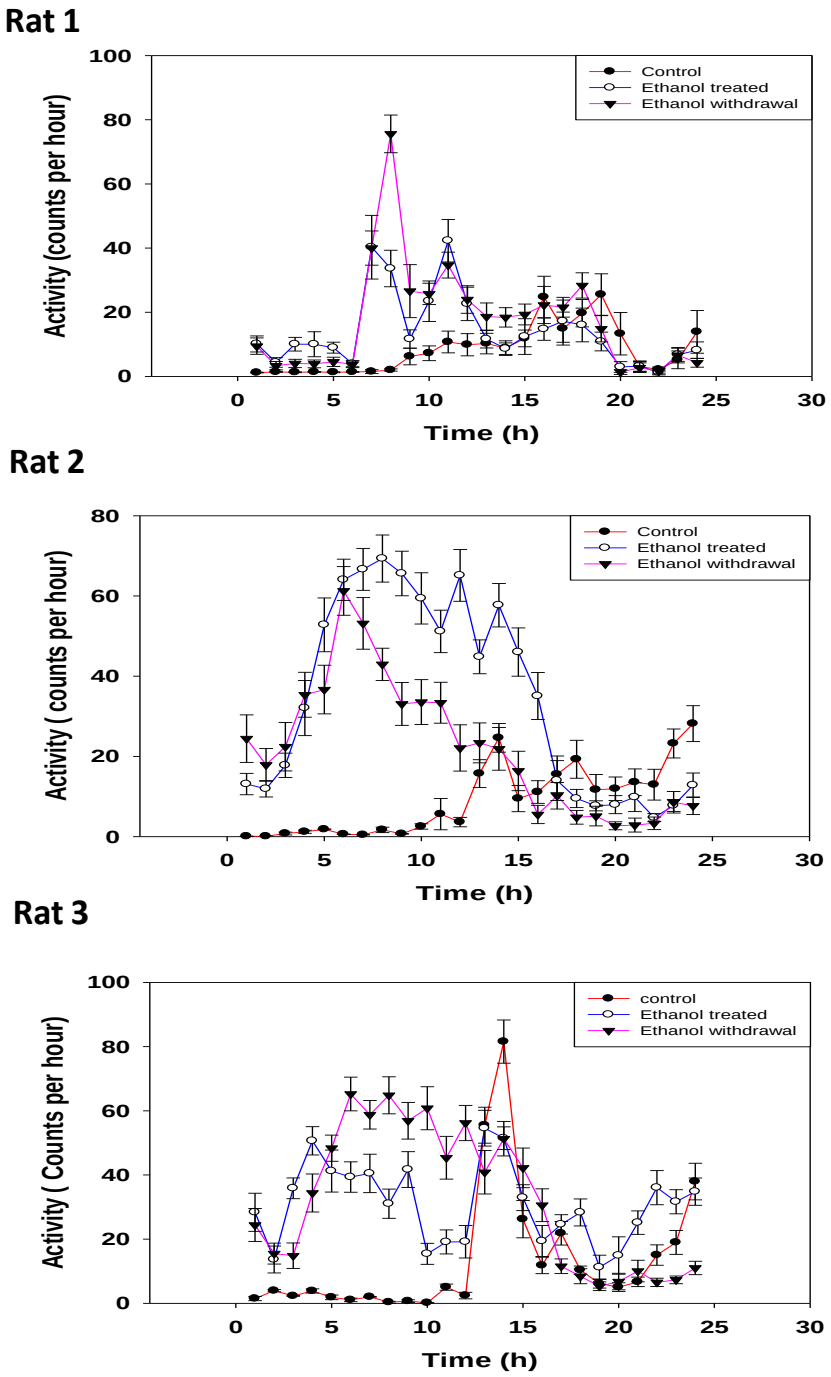

Fig. 70. Effect of ethanol withdrawal on ethanol induced changes in gross locomotor activity (n=3). Each value is activity per hour (mean ± SEM) calculated over 15 days for each experimental group.

The average values were taken from above data and summarized that ethanol treatment causes robust change in circadian activity and resulted in phase advance by ~08:00-09:00h. Activity was shifted from night to day. These changes were not reversed even in ethanol withdrawal (Fig. 71; Table 28).

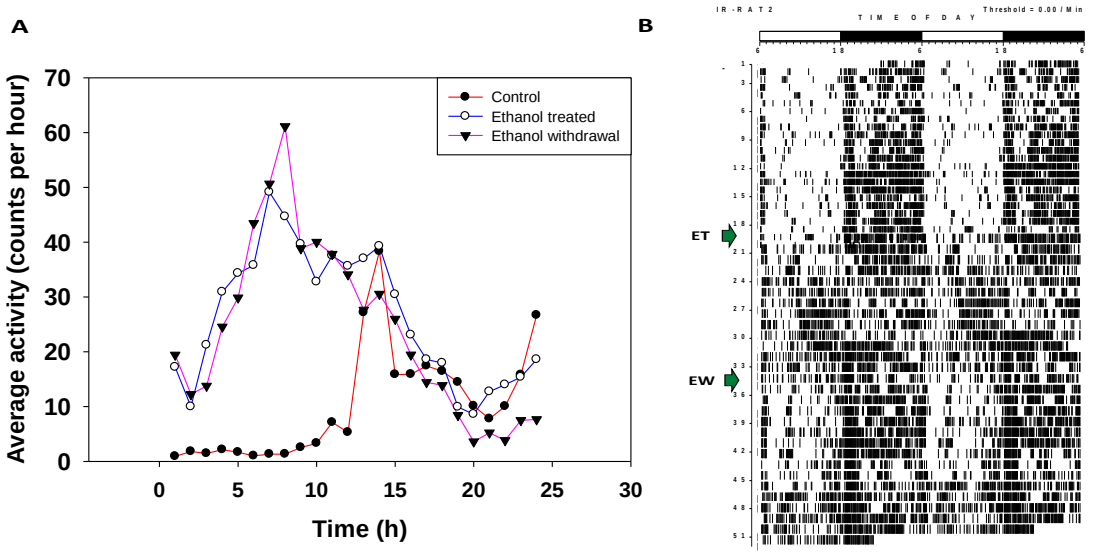

Fig. 71. Effect of ethanol withdrawal on ethanol induced changes in gross locomotor activity. A. Graph plotted by using average values taken from individuals from each experimental group. B. Representative double plotted actogram taken by using Chronobiology kit software.

Table 28. Effect of Ethanol withdrawal on ethanol induced changes in gross locomotor activity.

Rat 1

Exp group	Acrophase (h)	Day activity/hr	Night activity/hr	Mean activity/24hr	% nocturnal
Control	16:26	3.79±1.05	12.78±2.2	8.29±1.5	76.9
ET	9:49	18.45±3.96*	9.50±1.52	13.97±2.27*	34.0
EW	10:37	21.3±6.26*	13.3±2.73	17.32±3.44*	38.4

Rat 2

Exp group	Acrophase (h)	Day activity/hr	Night activity/hr	Mean activity/24hr	% nocturnal
Control	18:30	1.59±0.47	16.43±1.74	9.01±1.78	91.1
ET	9:20	47.41±6.46*	21.49±5.43	34.45±4.93*	31.0
EW	6:23	34.68±3.71*	9.36±2.10*	22.02±3.36*	21.25

Rat 3

Exp group	Acrophase (h)	Day activity/hr	Night activity/hr	Mean activity/24hr	% nocturnal
Control	15:40	2.09±0.43	24.76±6.69	13.42±4.04	92.3
ET	5:58	31.33±3.50*	30.42±3.77	30.87±2.51*	49.3
EW	8:39	45.46±5.39*	19.33±4.87	32.40±4.47*	29.82

Average

Exp group	Acrophase (h)	Day activity/hr	Night activity/hr	Mean activity/24hr	% nocturnal
Control	16:34	2.49±0.54	17.99±2.51	16.24±2.04	88.1
ET	8:47	32.4±3.25*	20.47±2.92	26.43±2.47*	38.7
EW	8:23	33.82±4.24*	14.03±2.79	23.9±3.23	29.3

Day activity, Night activity and Mean activity and acrophase calculated by kit analyze (Chronobiology software). * refers to comparison between control and treated groups ($p \leq 0.05$).

2. Effect of curcumin treatment on ethanol induced gross locomotor activity of rat:

In Rat1, significant difference was observed between experimental groups ($p \leq 0.05$). ET caused 4.5 fold increases in mean and 2.1fold decrease in amplitude with ~ 09:30h phase advance. Due to ethanol treatment, 4 fold increases in total daily activity, 20 fold in day activity, 2 fold in nocturnal activity was observed. The percentage of nocturnal activity was decreased by 50% ($p \leq 0.05$). In Curcumin treatment, further increase in mean (6.8 fold) and amplitude (1.2 fold) with ~ 09:00h phase advance was observed. Night activity was reduced and statistically significant with control ($p \leq 0.05$). Further decrease in percentage of nocturnal activity was observed when compared with ET (Fig. 72; Table 29).

In Rat2, significant difference was found between experimental groups ($p \leq 0.05$). Ethanol treatment resulted no change in mean and decrease in amplitude (3 fold) with change in phase (advance ~03:20h). Night activity was more than day activity when compared with control. Percentage of nocturnal activity was decreased by 30% ($p \leq 0.05$). Curcumin treatment increased the mean (1.39 fold) and no change in amplitude when compared with ET. But mean level was higher and amplitude level was lower when compared with control with phase shift (advance ~05:00h). Night activity was observed and slight increase in the day activity was observed compared with control ($p \leq 0.05$). The percentage of nocturnal activity was further decreased compared with ET (Fig. 72; Table 29).

In Rat3, experimental groups showed statistically significant difference ($p \leq 0.05$). Ethanol treatment increased the mean value (4.4 fold) and amplitude (1.5 fold) with no change in phase. Night activity was more than day activity, compared with control. But mean activity was increased (4 fold) and percentage of night activity was decreased by 30% ($p \leq 0.05$). Curcumin caused further increase in the mean (1.6 fold) and amplitude (1.6 fold) with ~03:20h phase advance compared with ET. Night activity was still maintained like ET but percentage of nocturnality was further reduced by 8) (Fig. 72; Table 29).

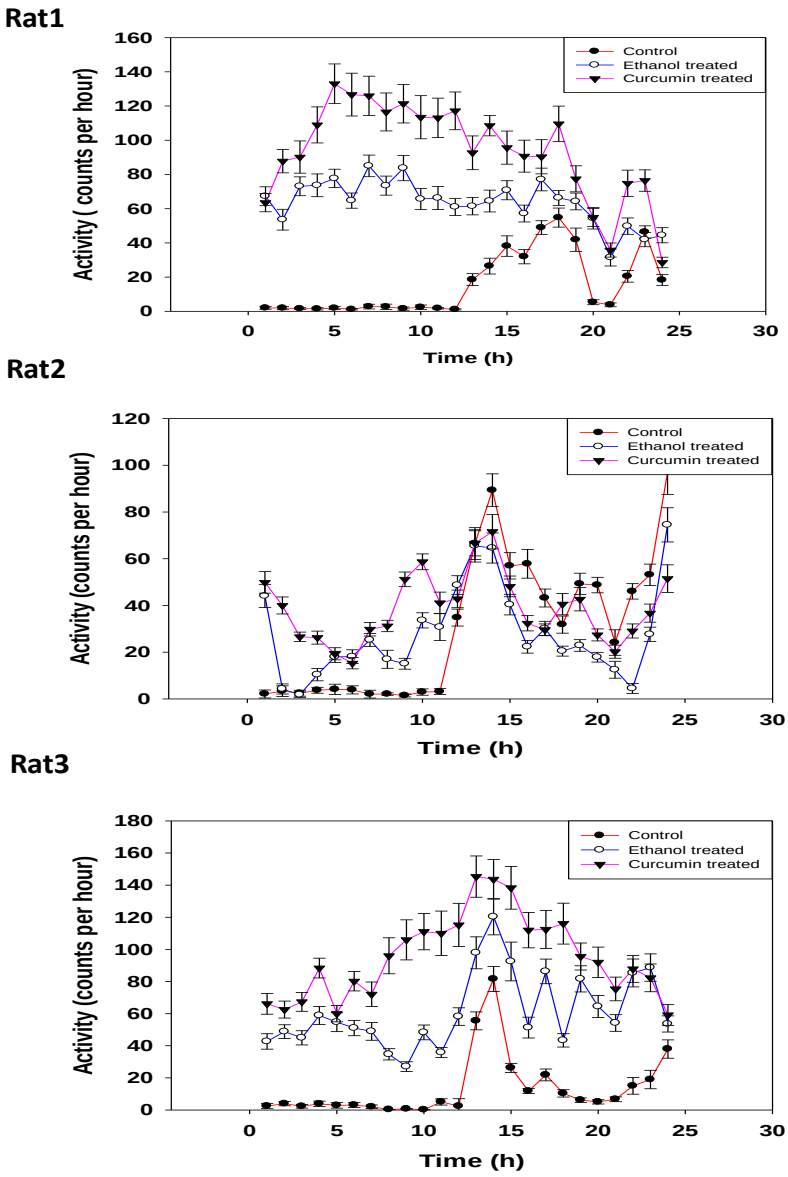

Fig. 72. Effect of curcumin on ethanol induced changes in gross locomotor activity (n=3). Each value is activity per hour (mean ± SEM) calculated over 15 days for each experimental group.

The average values were taken from above data in each experimental group. This summarizes that ethanol treatment cause change in circadian activity. Two animals showed change in phase and another animal didn't show phase shift. Nocturnal behavior maintained in all animals. Results shows that mean activity increased due to ET. In CT, further increases in mean activity and decrease in percentage of nocturnality was observed. Curcumin was not helpful in restoration of changes caused by ethanol (Fig. 73; Table 29).

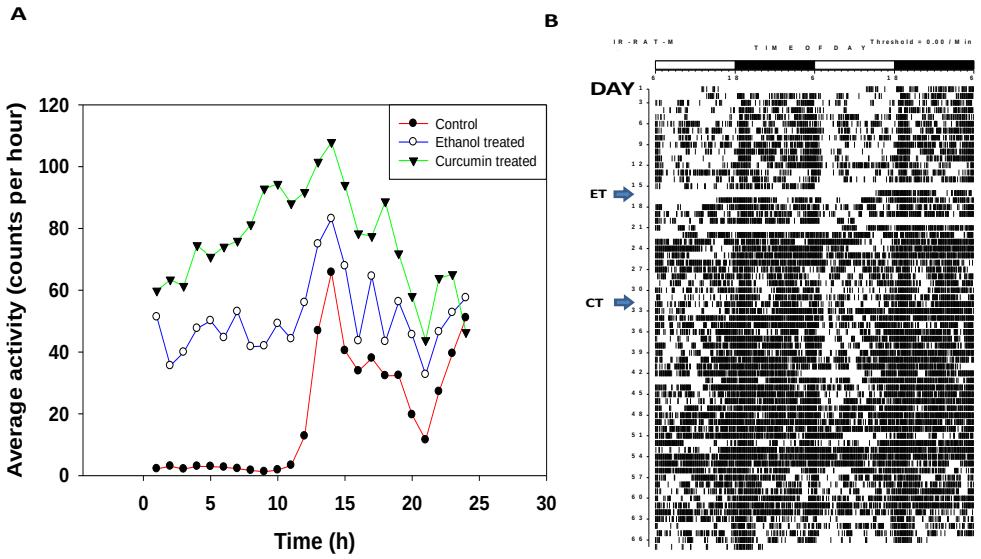

Fig. 73. Effect curcumin on ethanol induced changes in gross locomotor activity. A. Graph plotted by using average values taken from individuals from each experimental group. B. Representative double plotted actogram taken by using Chronobiology kit software.

Table 29. Effect of curcumin on ethanol induced changes in gross locomotor activity.

Rat 1					
Exp group	**Acrophase (h)**	**Day activity/hr**	**Night activity/hr**	**Mean activity/24hr**	**% nocturnal**
Control	**17:40**	**1.84±0.15**	**29.56±4.86**	**15.7±3.74**	**93.9**
ET	**8:16**	**70.42±2.64***	**56.95±3.79***	**63.68±2.66***	**44.7**
CT	**8:45**	**109.8±5.74***	**77.95±7.56***	**93.89±5.70***	**41.5**
Rat 2					
Exp group	**Acrophase (h)**	**Day activity/hr**	**Night activity/hr**	**Mean activity/24hr**	**% nocturnal**
Control	**17:24**	**5.49±2.67**	**55.31±6.07**	**30.40±6.12**	**90.9**
ET	**14:00**	**22.23±4.24***	**33.59±6.57***	**27.94±4.00**	**60.3**
CT	**12:24**	**36.0±3.87***	**41.36±4.55***	**38.68±2.97**	**53.2**
Rat 3					
Exp group	**Acrophase (h)**	**Day activity/hr**	**Night activity/hr**	**Mean activity/24hr**	**% nocturnal**
Control	**15:42**	**2.42±0.43**	**24.76±6.69**	**13.59±4.02**	**91.0**
ET	**17:15**	**46.15±2.80***	**76.62±6.67***	**61.39±4.75***	**62.7**
CT	**13:52**	**86.23±6.00***	**104.9±8.02***	**95.6±5.27***	**54.8**
Average					
Exp group	**Acrophase (h)**	**Day activity/hr**	**Night activity/hr**	**Mean activity/24hr**	**% nocturnal**
Control	**17:10**	**3.25±0.88**	**36.54±4.11**	**19.90±4.03**	**92.0**
ET	**14:44**	**46.27±1.72***	**55.72±4.24***	**50.99±2.44***	**54.6**
CT	**11:48**	**77.35±3.57***	**74.77±5.9***	**76.06±3.40***	**49.1**

Day activity, Night activity and Mean activity and acrophase calculated by kit analyze (Chronobiology software). * refers to comparison between control and treated groups ($p \leq 0.05$).

3. Effect of turmeric treatment on ethanol induced gross locomotor activity of rat:

In Rat1, significant difference was observed between experimental groups ($p \leq 0.05$). Ethanol treatment caused 3 fold decrease in mean and 9 fold decrease in amplitude with ~ 07:50h phase advance. Ethanol treatment caused 6.5 fold decreases in nocturnal activity and 1.5 fold increase in day activity but percentage of nocturnal activity was greatly decreased by 40% when compared with control ($p \leq 0.05$). In turmeric treatment, further decrease in mean (2 fold) and amplitude (1.3 fold) with~ 11:00h phase advance was observed, compared with ET. Even night activity is less than day activity and statistically significant with control ($p \leq 0.05$). Further decrease in percentage of nocturnal activity was observed when compared with ET (12%) (Fig. 74; Table 30).

In Rat2, significant difference was found between experimental groups compared with control ($p \leq 0.05$). Ethanol treatment resulted decrease in mean (7 fold) and amplitude (9.4 fold) with change in phase (advance ~00:30h). Night activity was observed more than day activity when compared with control. Percentage of nocturnal activity was decreased by 15% ($p \leq 0.05$). In turmeric treatment didn't affect in mean as well as amplitude compared with ET. But mean as well as amplitude level was lower when compared with control with phase shift (advance ~01:52h). Night activity was maintained as control and ET ($p \leq 0.05$) but percentage of nocturnal activity was restored compared with control (Fig. 74; Table 30).

In Rat3, experimental groups showed statistically significant difference ($p \leq 0.05$). Ethanol treatment decreased the mean value (3.7 fold) and amplitude (14 fold) with small change in phase (~01:40h). Night activity was more than day activity as compared with control. But total daily activity was decreased by 3.5 fold. Percentage of night activity was also decreased (30%) ($p \leq 0.05$). Turmeric caused increase in the mean (8.5 fold) and amplitude (12 fold) compared with ET. But phase advance (~10:40h) was observed when compared with control ($p \leq 0.05$). More day activity was observed and percentage of nocturnality was further reduced by 25% ($p \leq 0.05$) (Fig. 74; Table 30).

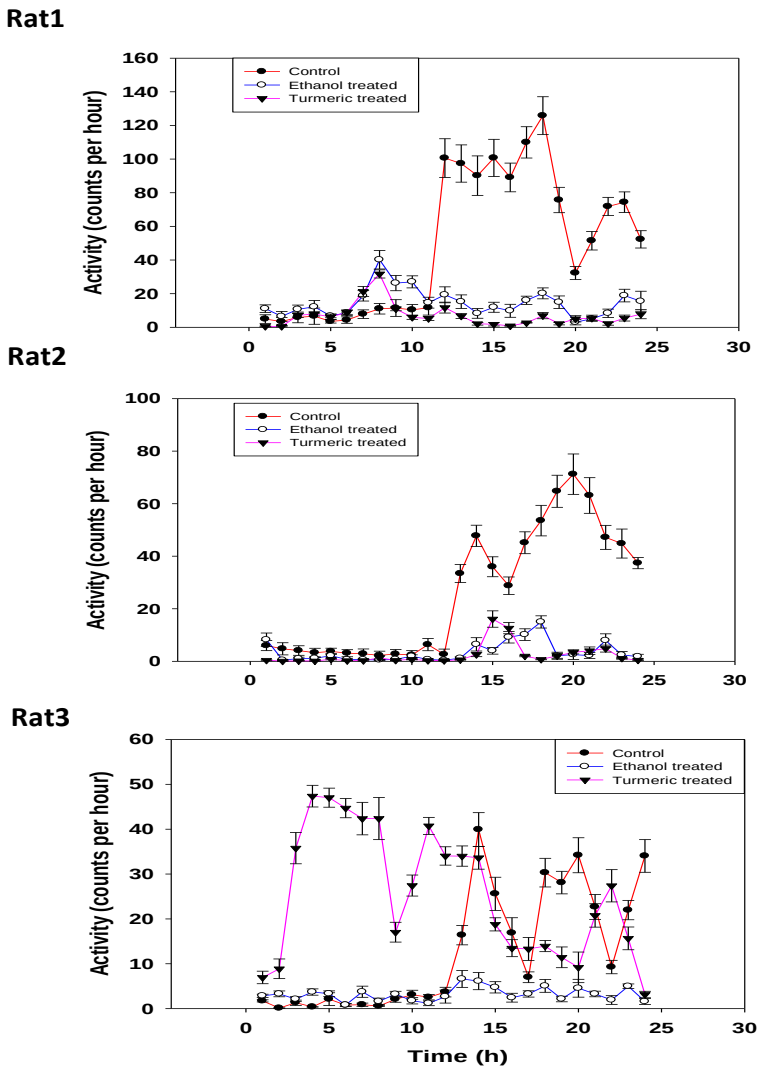

Fig. 74. Effect of turmeric on ethanol induced changes in gross locomotor activity (n=3). Each value is activity per hour (mean ± SEM) calculated over 15 days for each experimental group.

The average values were taken from above data in each experimental group. This summarizes that ethanol treatment cause change in circadian activity. Three animals were showed decrease in mean as well as amplitude. Two animals showed small change in phase shift (~00:30-01:00h), another animal showed significant phase shift (~07:30h). Nocturnal behavior was maintained in majority of animals. Results gives that mean activity was decreased due to ET. Slight increase in mean activity was observed in TT but percentage of nocturnality was decreased. Turmeric was not helpful in restoration of changes caused by ethanol (Fig. 75; Table 30).

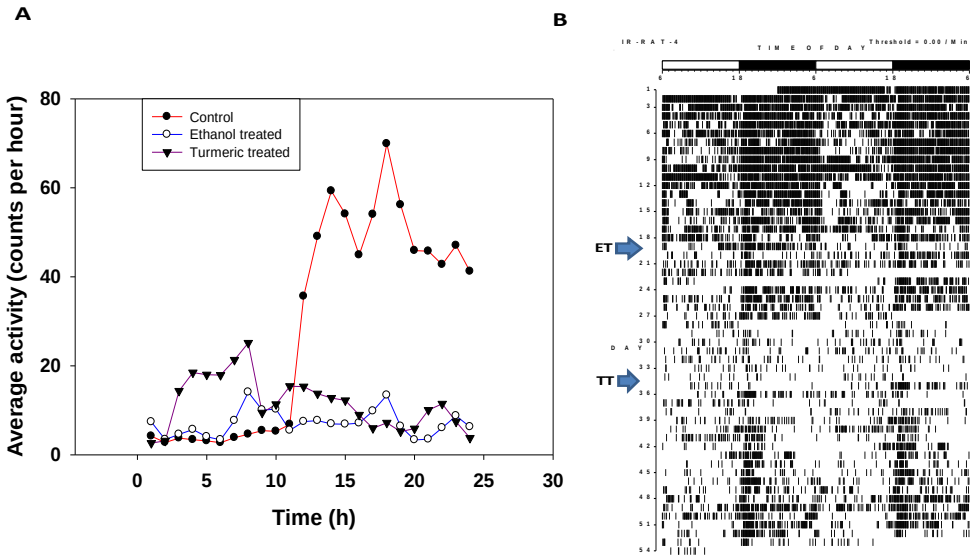

Fig. 75. Effect turmeric on ethanol induced changes in gross locomotor activity. A. Graph plotted by using average values taken from individuals from each experimental group. B. Representative double plotted actogram taken by using Chronobiology kit software.

Table 30. Effect of turmeric on ethanol induced changes in gross locomotor activity.

Rat1					
Exp group	**Acrophase (h)**	**Day activity/hr**	**Night activity/hr**	**Mean activity/24h**	**% nocturnal**
Control	16:40	15.12±7.82	80.9±7.72	48.0±8.71	84.2
ET	9:50	16.82±2.91	12.25±1.55*	14.53±1.68*	42.93
TT	6:37	9.81±2.53*	4.07±0.68*	6.94±1.41*	29.32
Rat2					
Exp group	**Acrophase (h)**	**Day activity/hr**	**Night activity/hr**	**Mean activity/24h**	**% nocturnal**
Control	18:30	3.67±0.39	47.75±3.83	25.71±4.96	92.9
ET	17:56	1.54±0.62*	5.41±1.25*	3.48±0.79*	77.8
TT	16:38	0.42±0.10*	4.20±1.44*	2.31±0.80*	90.9
Rat 3					
Exp group	**Acrophase (h)**	**Day activity/hr**	**Night activity/hr**	**Mean activity/24h**	**% nocturnal**
Control	17:56	1.60±0.32	23.85±2.93	12.72±2.73	93.7
ET	16:18	2.52±0.28*	3.88±0.48*	3.20±0.31*	60.2
TT	7:12	32.8±4.20*	17.89±2.75*	25.38±2.91*	35.1
Average					
Exp group	**Acrophase (h)**	**Day activity/hr**	**Night activity/hr**	**Mean activity/24h**	**% nocturnal**
Control	17:26	6.79±2.64	50.83±2.37	28.81±4.9	88.2
ET	12:10	6.96±0.93	7.18±0.77*	7.07±0.59*	50.7
TT	7:32	14.37±1.95*	8.72±0.95*	11.55±1.21*	37.7

Day activity, Night activity and Mean activity and acrophase calculated by kit analyze (Chronobiology software). * refers to comparison between control and treated groups ($p \leq 0.05$).

4. Effect of melatonin administration on ethanol induced gross locomotor activity of rat:

In Rat1, significant difference was observed between experimental groups with control ($p \leq 0.05$). Ethanol treatment resulted decrease in mean (1.5 fold) and amplitude (1.7fold) without phase shift. Due to ethanol treatment, 1.5 fold decreases in total daily activity and 1.6 fold in nocturnal activity were observed without changing the day time activity. But slight decrease in percentage of nocturnal activity was observed (5%). In melatonin treatment, restoration in mean and amplitude was observed with phase shift (~ 02:00h phase delay). Restoration was also observed in mean activity and percentage of nocturnal activity. But night activity was less than day activity and statistically significant with control ($p \leq 0.05$) (Fig. 76; Table 31).

In Rat2, significant difference was observed between experimental groups when compared with control ($p \leq 0.05$). Ethanol treatment resulted decrease in mean (2.7 fold) and amplitude (5.8 fold) with change in phase (advance ~03:00h). More night activity was observed than day activity when compared with control. Percentage of nocturnal activity was decreased by 30% ($p \leq 0.05$). In melatonin treatment restoration in mean and amplitude were observed when compared with control but phase is not restored (advance ~03:00h). Night activity was more than day time activity and restoration in mean activity was observed. Partial restoration in percentage of nocturnal activity was also observed (Fig. 76; Table 31).

In Rat3, experimental groups showed statistically significant difference ($p \leq 0.05$). Ethanol treatment increased mean (1.8 fold) and amplitude (3.6 fold) with change in phase (advance ~04:40h). Night activity was equal to day activity in ET. But mean activity was increased by 2 fold and percentage of night activity was decreased by 30% compared with control ($p \leq 0.05$). Melatonin didn't affect mean but partial restoration in amplitude was observed with phase shift (~03:00h phase advance) compared with control ($p \leq 0.05$). Night activity was more as compared with day time activity. Percentage of nocturnality was partially restored ($p \leq 0.05$) (Fig. 76; Table 31).

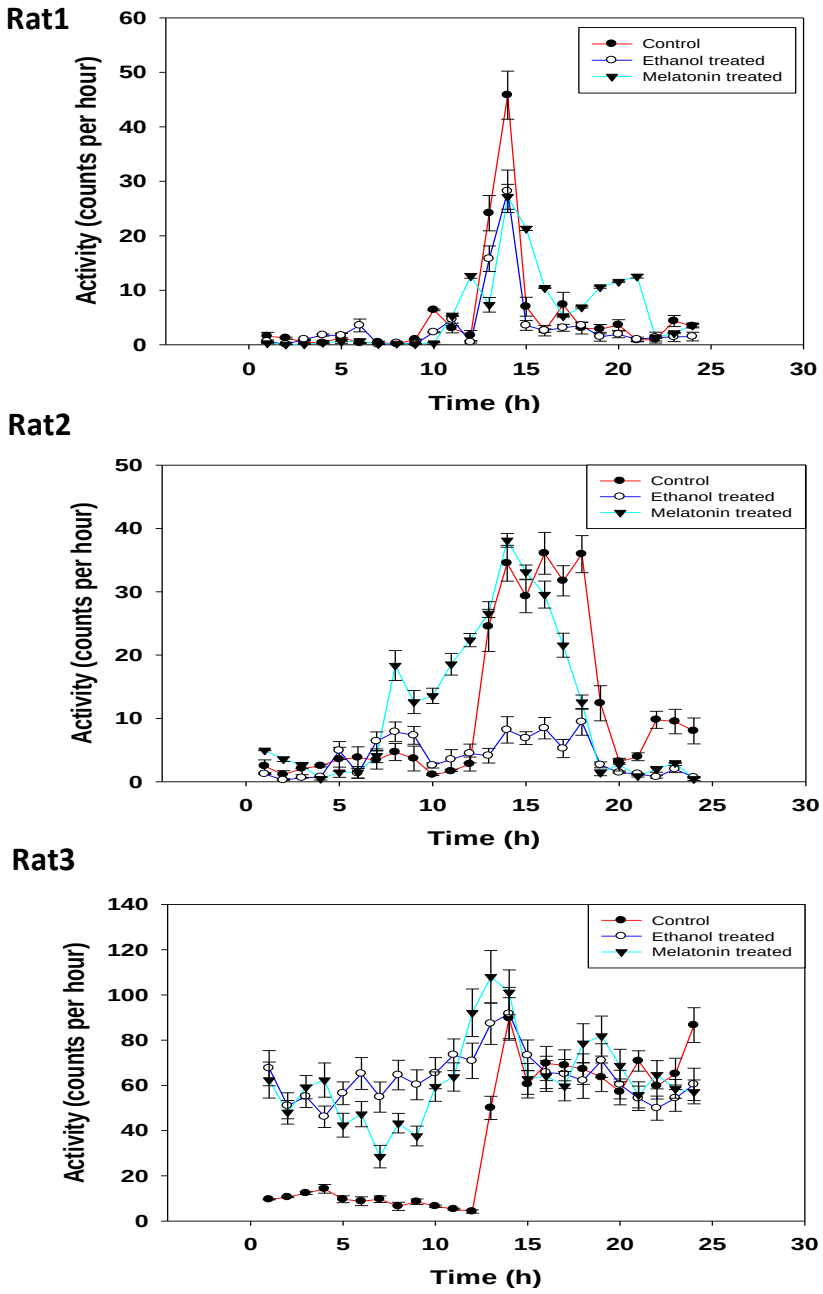

Fig. 76. Effect of melatonin on ethanol induced changes in gross locomotor activity (n=3). Each value is activity per hour (mean ± SEM) calculated over 15 days for each experimental group.

The average values were taken from above data in each experimental group and found that ethanol treatment caused change in circadian activity. ET caused decrease in mean activity, nocturnal activity and amplitude with phase shift. Melatonin treatment was sensitive in restoration of mean, amplitude, percentage of nocturnal activity and partial restoration in phase. Melatonin was partially sensitive in restoration of changes caused by ethanol than curcumin and turmeric (Fig. 77; Table 31).

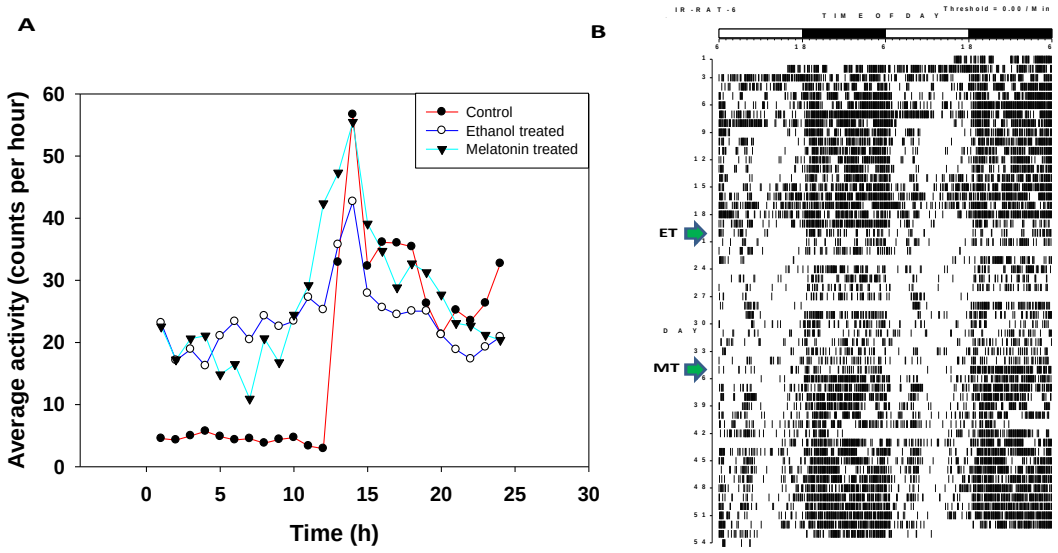

Fig. 77. Effect melatonin on ethanol induced changes in gross locomotor activity. A. Graph plotted by using average values taken from individuals from each experimental group. B. Representative double plotted actogram taken by using Chronobiology kit software.

Table 31. Effect of melatonin on ethanol induced changes in gross locomotor activity.

Rat 1					
Exp group	**Acrophase (h)**	**Day activity/hr**	**Night activity/hr**	**Mean activity/24h**	**% nocturnal**
Control	14:70	1.50±0.50	8.83±0.81	5.16±2.02	85.4
ET	14:00	1.37±0.42	5.44±0.36*	3.41±1.25	79.8
MT	15:55	1.72±1.07	10.13±2.21	5.87±1.48	85.4
Rat 2					
Exp group	**Acrophase (h)**	**Day activity/hr**	**Night activity/hr**	**Mean activity/24h**	**% nocturnal**
Control	16:18	2.37±0.32	19.90±3.81	11.32±2.59	87.6
ET	13:19	3.43±0.78	4.26±0.94*	3.85±0.60*	55.3
MT	13:16	8.67±2.28*	14.32±4.18	11.49±2.40	62.5
Rat 3					
Exp group	**Acrophase (h)**	**Day activity/hr**	**Night activity/hr**	**Mean activity/24h**	**% nocturnal**
Control	18:16	8.82±0.83	67.41±3.28	38.12±6.32	88.4
ET	13:40	60.9±2.40*	66.2±3.69	63.6±2.22*	52.1
MT	15:18	53.8±4.75*	71.7±5.00	62.8±3.85*	57.1
Average					
Exp group	**Acrophase (h)**	**Day activity/hr**	**Night activity/hr**	**Mean activity/24h**	**% nocturnal**
Control	17:22	4.35±0.21	32.05±2.68	18.2±3.17	88.04
ET	13:42	21.9±0.94*	25.3±2.12*	23.6±1.19*	53.6
MT	14:43	21.42±2.34*	32.03±3.12	26.72±2.20*	60.3

Day activity, Night activity and Mean activity and acrophase calculated by kit analyze (Chronobiology software). * refers to comparison between control and treated groups ($p \leq 0.05$).

Discussion

1. Effect of Curcumin on Ethanol induced changes in daily rhythms of Serotonin chronometabolome in SCN and pineal.

In the present study, role of serotonin and its related metabolites at various time points in a day (serotonin chronometabolome) were studied in alcoholism. In Control group, serotonin chronometabolome showed rhythmicity in SCN and as well as in pineal. Ethanol treatment caused decrease levels in 5-HT, 5-HIAA, TRP, 5-MTOH, MEL, 5-MIAA, NAT in SCN and 5-HT, 5-HIAA, TRP, NAS, MEL, 5-MTOH, 5-MIAA, NAT in pineal. Decreased levels of these metabolites may lead to change in numerous behavioral and physiological functions including circadian disturbances, mood as well as sleep disorders. These results suggested that alcoholism causes decrease in serotonin and its related metabolites, which could be responsible for change in circadian function of SCN. Increased levels were also observed in 5-HTP, NAS, 5-HTOH in SCN and 5-HTP, 5-HTOH in pineal. Increase of these metabolite levels may be excreted in urine or may act as biological markers in alcoholism. Rhythmicity was abolished by ET in NAS, 5-HTP, 5-HTOH in SCN and 5-HIAA, TRP, 5-MTOH, MEL in pineal. Ethanol withdrawal was not helpful to restore levels in any compound but rhythmicity was observed in 5-HIAA, 5-HTOH, TRP in SCN and all compounds except NAS and MEL in pineal. It has been reported earlier that acute alcohol consumption enhances the release of serotonin, GABA and taurine and decreases neuronal excitability in rat brain (Yoshimoto *et al.,* 1992; Dahchour *et al.,* 1994; Le Marquand *et al*., 1994). In addition dopamine, noradrenaline, γ-amino butyric acid (GABA) concentration also gets affected (Gewiss *et al*., 1991; Littleton, 1998). However, the decrease in serotonin levels in present study upon ET is in agreement with earlier studies where it has been shown that chronic alcohol consumption decreases serotonin release and increases concentrations of endogenous opioid peptides, while increasing the number of glutamate binding sites in synaptosomal membranes (Carmichael and Israel, 1975; Michaelis *et al.,* 1978). The long lasting alcohol tolerance has been related to multifunctional neurotransmitters like serotonin, norepinephrine, dopamine (Tabakoff and Hoffman, 1996; Valenzuela and Harris, 1997). Some of them reported that ethanol consumption altered sleep (Ehlers and Slawecki, 2000), food intake (Barr, 1988), corticosterone secretion and other functions (Rajakrishnan *et al*., 1999; El-Mas and Abdel-Rahman, 2000). Acute ethanol administration activates liver Trp pyrrolase and exerts a biphasic effect on brain 5-HT synthesis, whereas chronic ethanol

administration and subsequent withdrawal exert opposite effects on 5-HT synthesis mediated by corresponding changes in liver Trp pyrrolase activity in rats (Badawy, 1996).

As curcumin have beneficial properties like antioxidant, anti-inflammatory, anti-carcinogenic etc. (Jagota and Reddy, 2007), we have studied its protective effect on alcohol induced alteration in 5-HT and its metabolite levels and rhythms in SCN as well as pineal. The comparative antioxidant activity of curcumin, turmeric and melatonin were also studied using DPPH (1, 1-diphenyl-2-picrylhydrazyl) method by spectrophotometry and found that curcumin has more antioxidant activity than melatonin and turmeric. Similar results were found in *in vivo* also. Curcumin was effective in the partial restoration of levels in 5-HT, 5-HIAA, TRP, 5-HTOH, 5-HTP, NAS, 5-MTOH in SCN and 5-HT, 5-HIAA, 5-HTP, NAS, 5-HTOH, NAT in pineal. Rhythmicity was observed in all compounds except NAS in SCN and 5-HT, 5-HIAA, 5-HTOH, NAS, TRP, MEL in pineal. Turmeric was also effective in the partial restoration of levels in 5-HT of SCN and 5-HTP of pineal but turmeric was not sensitive as compared with curcumin. More experiments are in progress in our laboratory for confirmation of these results. But present studies suggest that curcumin may act as a viable food based as well as chrono-pharmacologic approach to ET induced alteration in 5-HT and its metabolite levels.

In our study, administration of melatonin did not result insignificant restoration of serotonin chronometabolome levels except 5-HT and 5-HIAA. Other metabolites were not effective with melatonin treatment. Some studies demonstrated that alcoholics had diminished melatonin levels in comparison to a control group (Reiter and Robinson, 1995) and undergoing delirium tremens during their abstinence period, manifested by abnormal circadian rhythms due to their low melatonin hormone levels (Mukai *et al.,* 1998).So external administration of melatonin is necessary in alcoholics. But in present study, external administration of melatonin did not prove to restore normally. It indicates that variable dose, duration and frequency need to be experimented to get beneficial results. An interdependent relationship exists between melatonin levels, sleep, body temperature, heart rate, and circadian rhythms, regardless of any displacement of the sleep-wake cycle (Dijk *et al.,* 1995). Research findings confirmed an interaction between delirium tremens and a drop in melatonin

concentration in alcoholics (Mukai *et al.,* 1998). Some of them reported that cirrhosis may change serum melatonin levels and finally leads to variations in the function of the suprachiasmatic nucleus (Steindl *et al*., 1997). Ethanol may change pineal melatonin synthesis either directly or indirectly by altered receptor function leads a reduction of nocturnal pineal melatonin content with a concomitant elevation of pineal serotonin in alcohol withdrawal syndrome (Moss *et al.,* 1986).

2. To find Sensitivity of Curcumin treatment on Ethanol induced changes in daily rhythms of serotonin Chronometabolome in aging.

Age induced decrease in serotonin has been reported in brain as well as SCN from our laboratory (Jagota and Kalyani, 2008; 2010). With present study, we report age related decrease in levels of 5-HT as well as its metabolites 5-HIAA, NAS, 5-MIAA, 5-MTOH, MEL, NAT in SCN and 5-HTP, 5-HT, 5-HIAA, NAS, 5-HTOH, 5-MIAA, 5-MTOH, MEL, NAT in pineal. However, age related increase was observed in 5-HTP, TRP, 5-HTOH in SCN and TRP only in pineal. General observation found that most of the compounds in serotonin chronometabolome showed age related decrease except TRP. Elevated levels of TRP which is an essential amino acid may be related to decrease in levels with aging (Jagota and Kalyani, 2008; 2010) as well as reduced protein synthesis. Decrease in other metabolites may be related to decrease in enzymatic activity or neurodegeneration in the brain. Age related decrease in serotonin and its related metabolites may lead to behavioral changes or mood disorders etc. This study confirmed that serotonin chronometabolome levels were decreased upon aging. The reduced density of serotonin receptors observed from birth to the mature brain (Azmitia and Whitaker-Azmitia, 1991). It has also been reported that animals exhibit numerous circadian disruptions with advancing age (Weinert and Waterhouse, 1999; Davidson *et al.,* 2008) and exhibits loss of temporal precision (Benloucif *et al*., 1997; Davidson *et al*., 2008; Li and Satinoff, 1995; Valentinuzzi *et al*., 1997; Weinert and Waterhouse, 1999), contributing to a variety of age-related pathologies.

Ethanol treatment caused elevation of the levels in 5-HT, 5-HIAA, TRP, 5-MTOH, NAT in SCN and 5-HTP, 5-HT, 5-HIAA, 5-HTOH, 5-MTOH, NAT in pineal. However, decreased levels were observed in 5-HTP, NAS, 5-HTOH, MEL, 5-MIAA in SCN and TRP, NAS, MEL, 5-MIAA in pineal. Increase in serotonin levels by ethanol may leads to aggressive behavior as well as sleep disorders. Both NAS and MEL levels

were decreased by alcohol. This may be due to decrease in synthesis of regulatory enzymes. Ethanol may act differently in older people than in younger people. Many medical and other problems are associated with both aging and alcohol misuse, the extent to which these two factors may interact to contribute to disease is unclear. Some of them reported that alcoholism may accelerate normal aging or cause premature aging of the brain (Pfefferbaum *et al*., 1997). Aging and alcoholism produce similar deficits in intellectual (i.e., cognitive) and behavioral functioning. Older persons with alcoholism are less likely to recover from cognitive deficits during abstinence than younger persons with alcoholism (Pfefferbaum *et al*., 1997). Elevated levels caused by ET were not restored in ethanol withdrawal. There are no reports available in how alcohol plays a role in elevation of serotonin levels in aging. Few drugs are available to treat alcohol elderly subjects. However, one study has suggested that naltrexone may help prevent relapse to alcoholism in subjects of ages 50 to 70 (Oslin *et al.,* 1997). Results of research in animals suggest that age-related alterations in specific chemical messenger systems in the brain may alter the effectiveness of medications used to treat alcoholism and mental disorders (Druse *et al*., 1997).

Antioxidants (curcumin, turmeric and melatonin) were administered upon alcohol treatment. Curcumin was effective in restoration of alcohol induced levels in 5-HTP, 5-HT, 5- HIAA in SCN and all ten metabolites in pineal in middle age group (1yr). In old age (2yr), Curcumin was effective in restoration of alcohol induced altered levels in 5-HT and 5-MTOH in SCN and 5-HTP, 5-HT, 5-HIAA, 5-MTOH and NAT in pineal. Turmeric was not effective in restoration of alcohol induced altered levels in 1yr as well as 2yr in SCN but in pineal, it was effective in restoration of levels of 5-HTP, 5-MIAA, 5-MTOH, MEL, NAT in 1yr and 5-HTOH, 5-MTOH in 2yr. Melatonin was also sensitive in restoration of alcohol induced levels in 5- HT of 1yr in SCN whereas in pineal, it was effective in restoration of metabolites - 5-MIAA, 5-MTOH, in 1yr and 5-HTP, 5-HT, NAS, 5-HTOH, NAT in 2yr. Curcumin appeared more sensitive than turmeric and melatonin in age related sensitivity to alcoholism. Our results are in agreement with previous studies and found that antioxidants could have beneficial effects in reducing the incidence of ethanol-induced as well as age related changes in cellular as well as molecular level by reducing free radical production, trapping free radicals themselves or reinforcing the natural antioxidant defence (Nordmann, 1994; Polidori, 2003). Middle age group (1yr) showed more sensitivity in restoration of levels

caused by ET than 90 day and 2yr. Curcumin was more sensitive in age related alcohol induced changes in SCN and pineal than turmeric and melatonin.

3. Effect of Curcumin on ethanol induced changes on daily rhythms of *per1* and *per2* expression in SCN and pineal.

Period genes (*per1, per2* and *per3*) have been shown to play a role in the molecular mechanism underlying circadian rhythmicity in mammals (Sherman *et al*., 2000; Bae *et al*., 2001; Cermakian *et al*., 2001; Shiino *et al.,* 2003). Among them, *per1* and *per2* are very important in regulating the circadian clock by negative feedback mechanism. Here, we have studied *per1* and *per2* expression in SCN and pineal. The daily rhythmicity was observed in both *per1* and *per2* expression in SCN and pineal. Upon ET, mean levels were decreased by ~1.5 fold in *per1* and *per2* in SCN whereas in pineal, *per1* gets more affected than *per2* and 2.5 fold decrease in *per1* was observed as compared with control. Though levels were decreased in *per2* of pineal but it was not significant. Rhythmicity was also abolished in *per1* of SCN and *per2* of pineal by ET. Decrease in the levels as well as abolition in the rhythm of *per* genes leads to disturbance in the clock. Finally, this leads to change in execution of circadian output and cause phase shift in body physiological functions. Most of clock genes are also expressed in peripheral structures (Abe *et al*., 2002; Balsalobre, 2002; Balsalobre *et al*. 1998; Yamazaki *et al*., 2000). Disturbance in the SCN may lead to change in the function of peripheral clocks. One of these peripheral clocks is the pineal gland which expresses *per1, per2, clock* and *bmal1* genes (Fukuhara *et al*., 2000; Namihira *et al*., 1999; Takekida *et al*., 2000). In our study, we got similar results in *per* gene expression in SCN. This strongly confirmed that ethanol can change the circadian function of SCN and this resulted to change in pineal function. Such changes in circadian function may be responsible for circadian clock disorders and such clock disorders may be lead to neurological disorders. After ET, we found EW was not helpful to restore rhythmicity as well as levels of gene expression. This is in agreement with previous reports that chronic ethanol administration altered circadian rhythms of *per2* and *per3* mRNA levels in the suprachiasmatic nucleus (Chen *et al*., 2004). The same researchers showed that adult rats displayed an altered circadian expression of *per* genes in the arcuate nucleus after prenatal exposure to alcohol (Chen *et al.,* 2006). Rosenwasser *et al.,* (2005) showed that both chronic ethanol intake and withdrawal affect the period and amplitude of the circadian rhythm of activity in rats. A few studies found that circadian genes are

important regulators of the behavioral responses to drugs of abuse (Rosenwasser, 2010). Mutations in the PAS domain of *per2* have been reported to increase in alcohol intake and it was linked to changes in glutamatergic transmission (Spanagel *et al*., 2005). Drugs of abuse can also serve as powerful Zeitgebers for some of these clocks outside of the SCN (Shibata *et al.*, 2010).

As curcumin have many beneficial properties, we administered to know the effect on *per* gene expression upon ET. Curcumin was sensitive in restoration of levels as well as rhythmicity in SCN only but not in pineal. The effector follower system may not be sensitive. Curcumin dose may needs to be changed to get the promising results. Turmeric, crude form curcumin was not sensitive in restoration of levels as well as rhythm. Little is known about drug studies with *per* gene expression in SCN. Some of them reported that drug consumption has a negative effect on the expression of circadian rhythmicity, since it produces a flattening of the functions and causes a state of desynchronization of endogenous control. Moreover, it has been shown that *clock* and *per2* are associated with vulnerability to addiction (Adan, 2010). External administration of melatonin showed better results than curcumin in our study and it was sensitive in restoration of *per1* and *per2* levels in both SCN as well as pineal. The mechanism of melatonin action on per genes was not yet known.

4. Effect of Curcumin on ethanol induced changes on daily locomotor activity rhythms.

Circadian rhythms modulate several behavioral and physiological responses to alcohol in both humans and experimental animals. These effects are widespread, altering the circadian rhythms of numerous physiological, endocrine and behavioral functions (Brick *et al.*, 1984). In the present study, ethanol treatment caused robust change in circadian activity and resulted in phase shift (advance). Activity was shifted from night to day. Our results similar with previous studies, reported that alcohol administration alter the phase, amplitude, or abolish the expression of circadian rhythms in a variety of behavioral and physiological functions, including locomotor activity, body temperature (Baird *et al.*, 1998), sleep (Ehlers and Slawecki, 2000; Rouhani *et al*., 1990). Hamster studies showed lengthening of the free-running period was observed with ethanol (Joy and Turek, 1989; Mistlberger and Nadeau, 1992; Zucker *et al*., 1976). Ethanol also alters the temporal structure of nighttime locomotor activity and photic entrainment (Ruby *et al*., 2009; Brager *et al*., 2010). Neonatal alcohol exposure

produces permanent changes in the circadian regulation of the rat activity rhythm and its entrainment to LD cycles (Allen *et al*., 2005). Chronic ethanol intake reduces the responsiveness of the circadian pacemaker to acute photic stimulation (Rosenwasser *et al*., 2005). Ethanol intake can modulate both photic and non-photic circadian phase responses (Seggio *et al*., 2005). Chronic ethanol intake alters patterns of circadian phase shifting and free-running period in mice (Seggio *et al*., 2009). In EW, mean locomotor activity decreased further and night time activity also decreased. This confirmed that ethanol withdrawal was not helpful to restore its original activity. This is in agreement with previous studies that EW showed varying responses; including change in circadian period (Mistlberger and Nadeau, 1992). Few of them reported that EW can cause hypo-activity (Hammer *et al*. 2010; Logan *et al.,* 2010). Disruption of normal physiological timing by alcohol may lead to many health consequences (Trujillo *et al.*, 2010).

Curcumin and turmeric administration upon ET showed that locomotor activity was not restored to its original activity. May be these drugs have negative effect on locomotor activity. Some of the workers found that drugs like desipramine, moclobemide, clonidine and fluoxetine had shortening the photoperiod and some of the drugs like clorgyline and lithium can lengthening the photoperiod (Duncan, 1996; Rosenwasser 1992; Rosenwasser, 1996; Rosenwasser and Wirz-Justice, 1997; Dwyer and Rosenwasser, 2000). Action of the drug may be depending on person's sensitivity. In our studies, we got differential results with different animals. Some of them reported that antidepressant drugs can affect circadian pacemaker (Honma *et al*., 1991; Wollnik, 1992; Klemfuss and Kripke, 1994)

Melatonin treatment resulted in restoration of mean, amplitude, percentage of nocturnal activity, mean daily activity and partial restoration in phase. Melatonin was more sensitivity in restoration of changes caused by ethanol than curcumin and turmeric. This is in agreement with previous reports that administration of melatonin can entrain free running activity rhythms in rodents (Weaver, 1999; Cassone and Natesan, 1997). Some of them reported that daily subcutaneous injections of melatonin to rats strongly affect the locomotor activity rhythm and helps in entrainment (Redman *et al.,* 1983, Cassone and Natesan, 1997). Behavioral arousal (1-4 h before activity onset) can induce a phase advance of the locomotor activity rhythm in Syrian hamster (Hastings *et al.,* 1992, Cutrera *et al.,* 1996). So it is generally believed that melatonin can mediate chronobiotic effects through the high-affinity Mel receptors located within

the SCN (Gauer *et al.*, 1993; Vanecek *et al.*, 1987; von Gall *et al*., 2002). Melatonin is also being helpful in scavenging the free radicals in some pathology in which high production of free radicals is the primary cause of the disease (Reiter *et al.*, 2001).

Summary and Conclusion

Summary and Conclusion:

As serotonin is an important neurotransmitter involved in the photic as well as nonphotic regulation of circadian rhythms and is a precursor of neurohormone melatonin, we studied the effect of ethanol on daily rhythms of serotonin chronometabolome in SCN as well as pineal by measuring serotonin chronometabolome levels at various time points. Ethanol treatment caused elevation of majority of compounds in serotonin chronometabolome except 5-HTP and 5-HTOH in both SCN as well as pineal. Increased levels of 5-HTOH may be considered as marker for alcoholism. Ethanol withdrawal was studied upon ET to check the serotonin chronometabolome levels and found that it was not helpful to restore levels in any compound. Among three, curcumin was very sensitive in restoration of ethanol induced changes in levels and daily rhythms of serotonin chronometabolome in SCN as well as pineal than turmeric and melatonin.

In order to understand, how alcohol affects serotonin chronometabolome, we have studied alcoholism in aging. Age related decreases in basal levels were observed in majority of the compounds. Ethanol treatment caused robust change in the levels as well as rhythmicity in 5-HT, 5-HIAA, NAS, MEL, 5-HTOH and 5-MTOH in SCN as well as pineal upon aging. So these metabolites could be considered as possible biomarkers in aging of serotonin chronometabolome with alcoholism. Ethanol withdrawal was not helpful to restore levels of serotonin chronometabolome in aging. In order to know sensitivity of antioxidants, we studied serotonin chronometabolome in aging upon alcoholism. In SCN, curcumin is more sensitive in 90 day than 1yr and 2yr and age related decrease in the sensitivity was observed whereas in pineal, curcumin is more sensitive in middle age group (1yr) than 90 day and 2yr. Turmeric was not effective in restoration of alcohol induced levels in SCN upon aging but in pineal, it was partially sensitive in 5-HTOH and 5-MTOH. Melatonin was also not effective in restoration of alcohol induced levels in SCN as well as pineal upon aging. This study confirms that curcumin is more sensitive in restoration of serotonin chronometabolome levels in alcoholism upon aging than turmeric and melatonin.

In gene expression studies, rhythmicity was observed in *per1* and *per2* in SCN as well as pineal in control group. Ethanol treatment caused reduced levels in *per1* as well

as *per2* in both SCN and pineal. Reduced levels were maintained in ethanol withdrawal like ET in both *per1* and *per2* in SCN as well as pineal.

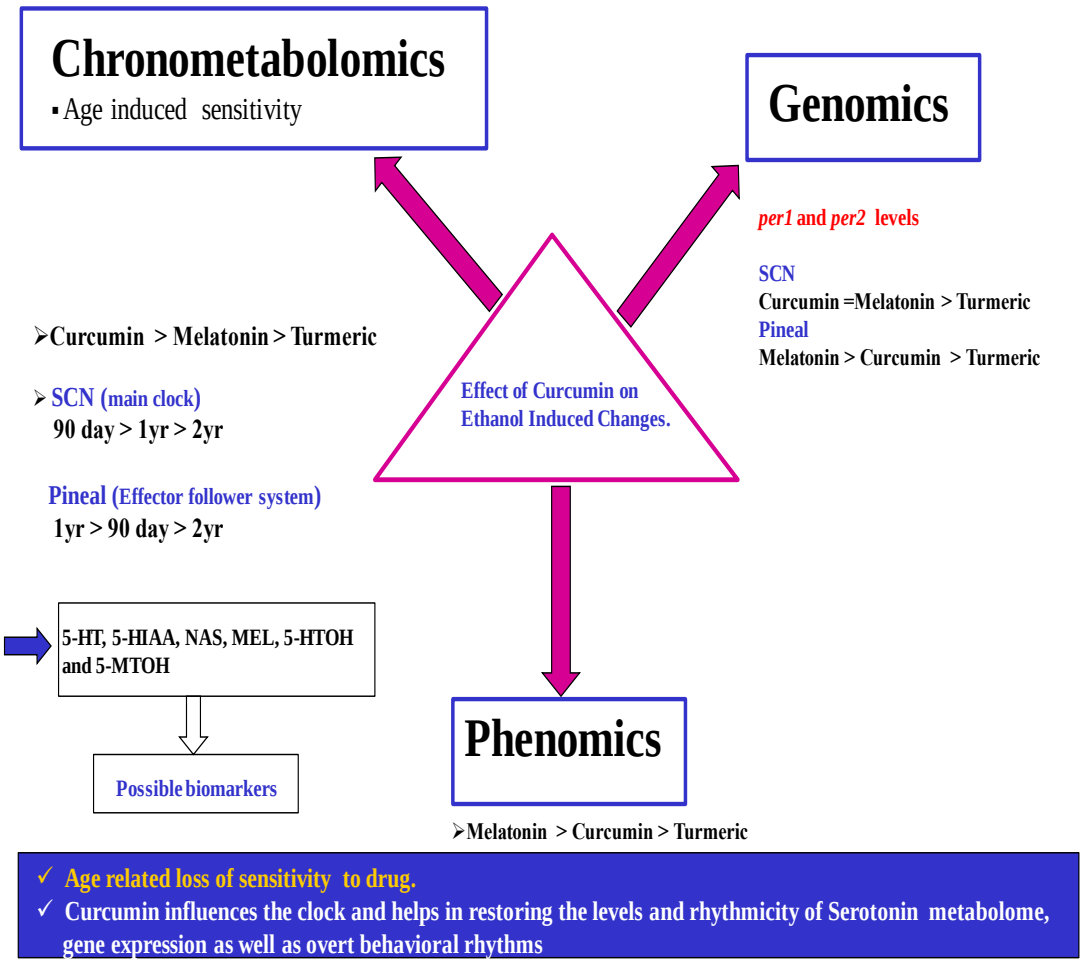

Fig. 78. Flow diagram showing the effects of curcumin on ethanol induced changes in daily serotonin chronometabolomics, genomics and phenomics.

Curcumin was partially sensitive in restoration of levels in *per1* and *per2* in SCN only but not in pineal. Turmeric treatment was not sensitive to restore the levels caused by ET in *per1* and *per2* in SCN as well as pineal. Melatonin treatment partially was helpful to restore the levels caused by ET in *per1* and *per2* in SCN as well as pineal. Studies of *per1* and *per2* demonstrated that curcumin as well as melatonin was sensitive in pineal whereas in SCN, only melatonin is sensitive.

In locomotor studies also, ethanol treatment caused change in phase shift, amplitude, and activity pattern of daily rhythms. The effects caused by ethanol intake could not be reversed by ethanol withdrawal. The results of this experiment indicate that both ethanol intake and ethanol withdrawal could alter the period and amplitude of free-running circadian activity rhythms in the rat. Curcumin was not sensitive to restore its original nocturnal activity. Turmeric was also not sensitive enough in restoration of changes caused by ethanol. Melatonin proved partially sensitive in attaining reversal of changes caused by ethanol. Exogenously administered melatonin has significant effects on circadian functions. Among three antioxidants (curcumin, turmeric, melatonin), only melatonin showed partial restoration on ethanol induced changes of gross locomotor activity of rats than curcumin and turmeric. Further studies are needed to assess the role of melatonin on ethanol induced gross locomotor activity. Duration and dose of melatonin may be changed in order to get promising results in gross locomotor activity.

Final conclusion of our study reported that curcumin influences the clock and sensitive in restoring the levels and rhythmicity of serotonin chronometabolome as well as *per* gene expression but not behavioral rhythms. Melatonin is also effective in restoration of *per* genes as well as locomotor activity.

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ABBREVIATIONS

5-HIAA	:	5-Hydroxy indole acetic acid
5-HT	:	5-Hydroxytryptamine
5-HTOH	:	5-Hydroxy tryptophol
5-http	:	5-Hydroxytryptophan
5-MIAA	:	5-Methoxy indole acetic acid
5-MTOH	:	5-Methoxy indole acetic acid
8-OHDPAT	:	8-hydroxy2 (din propylamino) tetralin
AA-NAT	:	Arylalkylamine N-acetyl transferase
ADD	:	Attention deficit disorder
ADH	:	Alcohol dehydrogenase
ADH	:	Anti-diuretic Hormone
ADHD	:	Attention deficit hyperactivity disorder
ALDH	:	Acetaldehyde dehydrogenase
ALS	:	Amyotrophic lateral sclerosis
ANG II	:	Angiotensin II
ANOVA	:	Analysis of Variance
ASPS	:	Advanced Sleep Phase Syndrome
AVP	:	Arginin vasopressin
AW	:	Alcohol Withdrawal
BAC	:	Blood Alcohol Concentration
BAT	:	Brown adipose tissue
BBS	:	Bombesin
Bmal1	:	Brain-muscle-Arnt-like-protein1
^{0}C	:	degree centigrade/ degree Celsius
Cal B	:	Calbindin
cAMP	:	Cyclic Adenosine Mono Phosphate
CALR	:	Calretinin
CCG	:	Clock controlled genes
CCK	:	Cholecystokinin
cDNA	:	Complentary DNA
CGRP	:	Calcitonin generelated peptide
CkIε	:	Casein kinase Iε
Clock	:	Circadian locomotor output cycles kaput
COAD	:	Chronic Obstructive Airways Disease
Cry	:	*Cryptochrome*
CT	:	Curcumin treatment
Ct	:	Threshold cycle
CYP2E1	:	Cytochrome P450 2E1
DM-SCN	:	Dorsomedial SCN
DSPS	:	Delayed Sleep Phase Syndrome
DT's	:	Delirium tremens
EC	:	Electrochemical detector
EDTA	:	Ethylene di-amine tetra acetic acid
EGF	:	Epidermal growth factor
ENK	:	Enkephalin
ET	:	Ethanol treatment
EW	:	Ethanol withdrawal

GABA	:	Gamma amino butyric acid
GPCRs	:	G protein-coupled receptors
Gal	:	Galanin
GRP	:	Gastrin releasing peptide
IP_3	:	Inositol Phosphate 3
ISAAC	:	Insitu Ag/AgCl electrode
LD	:	Light Dark cycle
LGICs	:	Ligand-gated ion channels
LSD	:	Lysergic Acid Diethylamide
LTP	:	Long-term potentiation
MCV	:	Mean Corpuscular Volume
MEL	:	Melatonin
mg	:	milligram
ml	:	milliliter
mM	:	mill molar
mENK	:	Met-Enkephalin
mRNA	:	messenger ribonucleic acid
MT	:	Melatonin treatment
MT_1	:	Melatonin receptor subtype1
NAD	:	Nicotinamide adenine dinucleotide
NAS	:	N-acetyl serotonin
NAT	:	N- Acetyl tryptamine
NATr	:	*N*-acetyl transeferase
NGF	:	Nerve Growth Factor
NPY	:	Neuropeptide Y
NT	:	Neurotensin
Per	:	*Period*
PHI	:	Peptide histidine isoleucine
PRC	:	Phase response curve
RHT	:	Retinohypothalamic tract
ROS	:	Reactive oxygen species
RP-HPLC	:	Reverse phase high pressure liquid Chromatography
Q-PCR	:	Quantitative- Polymerase chain Reaction
PK2	:	Prokineticin 2
pM	:	Pico mole
PMDD	:	Premenstrual Dysphoric Disorder
PMS	:	Premenstrual Syndrome
PWS	:	Protracted withdrawal syndrome
ror	:	Retinoic acid orphan related
RT	:	Room temperature
SAD	:	Seasonal Affective Disorder
SCG	:	Superior cervical ganglion
SCN	:	Suprachiasmatic nucleus
SS	:	Somatostatin
TGFα	:	Transforming growth factor α
TH	:	Tyrosine hydroxylase
THC	:	Tetrahydrocurcumin
TRP	:	Tryptophan

TT	:	Turmeric treatment
VL-SCN	:	Ventrolateral SCN
VIP	:	Vasoactive intestinal peptide
ZT	:	Zeitgeber time
Ml	:	micro litre
μM	:	micro molar

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ORIGINAL PAPER

The Effect of Curcumin on Ethanol Induced Changes in Suprachiasmatic Nucleus (SCN) and Pineal

Anita Jagota · M. Y. Reddy

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Abstract (1) Circadian clocks have been localized to discrete sites within the nervous system of several organisms and in mammals to the suprachiasmatic nucleus (SCN) in the anterior hypothalamus. The SCN controls and regulates the production and discharge of melatonin (hormonal message of darkness) from the pineal gland via a multisynaptic efferent pathway. The nocturnal rise in melatonin production from serotonin results due to an increased activity of serotonin *N*-acetyl transferase (NAT). (2) The complex interaction between alcohol and biological clock need to be understood as alcoholism results in various clock linked neuronal disorders especially loss of memory and amnesia like state of consciousness, sleep disorders, insomnia, dementia etc. (3) Serotonin, 5-Hydroxy-tryptamine (5-HT) plays an important role in mediating alcohol's effects on the brain. Understanding the impact of alcohol consumption on circadian system is a pre-requisite to help in treatment of alcohol induced neurological disorders. We, therefore, studied the effect of ethanol drinking and ethanol withdrawal on daily rhythms of serotonin and its metabolite, 5-hydroxy-indole acetic acid (5-HIAA) in SCN and Pineal of adult male Wistar rats maintained under light-dark (LD, 12:12) conditions. (4) Curcumin is well known for its protective properties such as antioxidant, anti-carcinogenic, anti-viral and anti-infectious etc. Hence, we studied the effect of curcumin on ethanol induced changes on 5-HT and 5-HIAA levels and rhythms in SCN and Pineal. (5) Ethanol withdrawal could not restore either rhythmicity or phases or levels of 5-HT and 5-HIAA. Curcumin administration resulted in partial restoration of daily 5-HT/5-HIAA ratio, with phase shifts in SCN and in Pineal. Understanding the impact of alcohol consumption on circadian system and the role of herbal medication on alcohol withdrawal will help in treatment of alcohol induced neurological disorders.

Keywords Serotonin · 5-HIAA · SCN · Pineal · Alcohol · Curcumin

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Introduction

Circadian rhythms in mammals are regulated by a master clock located in the suprachiasmatic nucleus (SCN) of the brain (Klein et al. 1991; Jagota et al. 2000; Jagota 2006). Serotoninergic neurotransmission is important in mammalian circadian clock function (Mistleberger et al. 2000). Several serotonin receptor subtypes have been localized in the SCN (Moyer and Kennaway 1999). The SCN controls and regulates the rhythmic production and discharge of serotonin derivative, melatonin (hormonal message for darkness) from pineal gland via multisynaptic efferent pathways. The nocturnal rise in melatonin production from serotonin is a result of increase in the activity of serotonin *N*-acetyl transferase (NAT) (Klein et al. 1997). Disruptions of the biological rhythms can impair the health and result in sleep disorders of elderly, insomnia, dementia, affective illness (mood and depression), hypothalamic tumors, heart problems, and problems associated with congenital blindness, jet lag, shift work, and space etc. (Moore 1991; Jagota 2005).

The complex interaction between alcohol and biological clock (the pacemaker) has become a rapidly expanding area in chronopharmacology (Spanagel et al. 2005). Serotonin plays an important role in mediating alcohol's effects in brain (Lovinger 1999). Alcohol consumption is related with changes in levels of various neurotransmitters such as norepinephrine, GABA, glutamate, dopamine, and noradrenalin etc. (Zarcone 1978; Gewiss et al. 1991; Kawahara et al. 1993; Prospero et al. 1994; Littleton 1998). The specific mechanism underlying the relationships between neurotransmitter function, alcohol and sleep disturbances is obscure. Some drugs such as disulfiram, naltrexone, and acamprostate have been used as anticraving medications and help in overcoming withdrawal symptoms. These drugs have been related with many adverse reactions (Petrakis and Krystal 1997; Oncken et al. 2001; Oscar et al. 2003; Verge et al. 2006). In recent years the development of new medications to treat alcohol dependence has initiated a new era in alcoholism treatment.

The curcumin (1,7-bis (4-hydroxy-3-methoxy phenyl)-1, 6-hetadiene-3, 5-dione), a major yellow phenolic active curcuminoid present in turmeric used in the diet, is non-toxic and protective pharmaceutical, neutraceutical, and phytoceutical agent. It has a plethora of beneficial effects such as antioxidant, anti-inflammatory, anti-carcinogenic, anti-viral, and anti-infectious effects etc. (Arajuo and Leon 2001; Aggarwal et al. 2003; Joe et al. 2004). We report here, the effect of ethanol drinking and its withdrawal on daily rhythms of neurotransmitter serotonin and its metabolite, 5-HIAA in SCN and Pineal and the effect of curcumin on ethanol induced changes in 5-HT and 5-HIAA daily rhythms.

Materials and Methods

Ninety-day-adult male Wistar rats were maintained at 23 ± 1°C with LD, 12:12 (lights on: 06:30 A.M. (Zeitgeber time (ZT)-0) and lights off: 6:30 P.M. (ZT-12)) for 2 weeks prior to experiment. Food and water were provided ad libitum. All experiments were performed as per Institutional Animal Ethics. The rats were separated into four groups—(1) control; (2) ethanol drinking; (3) ethanol withdrawal; (4) curcumin treated.

Group 1 animals were supplied food and water ad libitum. Group 2 were offered for 15 days under the two bottle-free choice regimen with unlimited access of ethanol (10% v/v in tap water) and water. Food pellets were always available. Bottles refilled everyday with a fresh solution and their positions interchanged at random to avoid development of position preference. In Group 3, after ethanol drinking for 15 days as in Group 2, ethanol withdrawal was

followed for 15 days, i.e., only food and water were provided ad libitum. In Group 4 also, after ethanol drinking as in Group 2 for 15 days was given 0.002% curcumin (Sigma) in diet for 15 days ad libitum.

Brains were dissected from all the experimental rats (Group 1–4), following anesthesia at various time points such as ZT-0, 6, 12, 18, and 24. Pineal gland was separated and SCN was carefully punched out with the help of scalpel from 500-μ brain slices which were made using tissue chopper (Prosser and Gillete 1989).

Serotonin and 5-HIAA levels were assayed by using HPLC-EC method (Mefford et al. 1980; Grady et al. 1984). The tissue sample was homogenized with 100 µl of 0.1 N perchloric acid containing sodium bisulfate (1 mM). After homogenization the tissue samples were sonicated for approximately 5 s. The centrifugation was done at 12,800*g* for 10 min to remove tissue debris. The supernatant was filtered through 0.22-µ syringe filters and then clear supernatant was applied to the chromatography system (Waters, USA) by using eluant: 10% methanol; 0.1 M citric acid; 0.1 M sodium acetate, 50 mg/l EDTA (pH 4.1). The protein estimation was done by using Bradford's method (Bradford 1976).

Statistical Analysis

Data was analyzed using Jandel Scientific Sigma stat software by the analysis of variance (ANOVA) and student's *t*-test.

Results

Effect of Curcumin on Ethanol Induced Changes in 5-HT and 5-HIAA Daily Rhythms in SCN

Group 1 (control) showed daily rhythms in 5-HT and 5-HIAA levels. 5-HT levels measured at various time points such as ZT-0, 6, 12, 18, and 24 were 19.37 ± 1.05, 39.29 ± 2.58, 24.26 ± 5.26, 8.65 ± 0.90, and 20.01 ± 1.86 µmol/g protein, respectively (Fig. 1A) and 5-HIAA levels were 2.84 ± 0.67, 8.76 ± 1.66, 12.05 ± 4.32, 2.79 ± 0.46, and 2.93 ± 0.89 µmol/g protein, respectively (Fig. 1B). The 5-HT levels were maximum at subjective mid-day (ZT-6) and minimum at subjective mid-night (ZT-18) whereas 5-HIAA levels were maximum at ZT-12 and minimum at ZT-0. The maximum:minimum ratio (daily pulses) for 5-HT and 5-HIAA were similar (Fig. 1C). The 5-HT/5-HIAA ratio was maximum at ZT-0/24, i.e., at onset of light and minimum at ZT-12, i.e., at onset of darkness (Table 1).

5-HT levels at various time points ZT-0, 6, 12, 18, and 24 in Group 2 (ethanol drinking) were 20.33 ± 3.09, 116.10 ± 7.44, 45.73 ± 6.02, 10.25 ± 0.42, and 18.93 ± 1.412 µmol/g protein, respectively (Fig. 1A) whereas 5-HIAA levels were 34.37 ± 1.48, 37.53 ± 1.27, 37.85 ± 1.64, 100.52 ± 2.30, and 26.68 ± 1.09 µmol/g protein, respectively (Fig. 1B). 5-HT levels showed significant increase at ZT-6 and 12 ($p_a \leq 0.05$) though there was no significant difference at ZT-0, 18, 24. Interestingly 5-HIAA levels showed significant elevation as compared to Group 1 at all time points with about 50 times increase at subjective mid-night (ZT-18) ($p_a \leq 0.05$). The daily pulses of 5-HT were significantly high as compared to control ($p_a \leq 0.05$) (Fig. 1C). The 5-HT/5-HIAA ratio was maximum at subjective mid-day (ZT-6) and minimum at subjective mid-night (ZT-18), i.e., there was a phase delay by about 6 h as compared to control. In addition, the 5-HT/5-HIAA ratio was significantly different at all time points except ZT-12 from controls ($p_a \leq 0.05$) (Table 1).

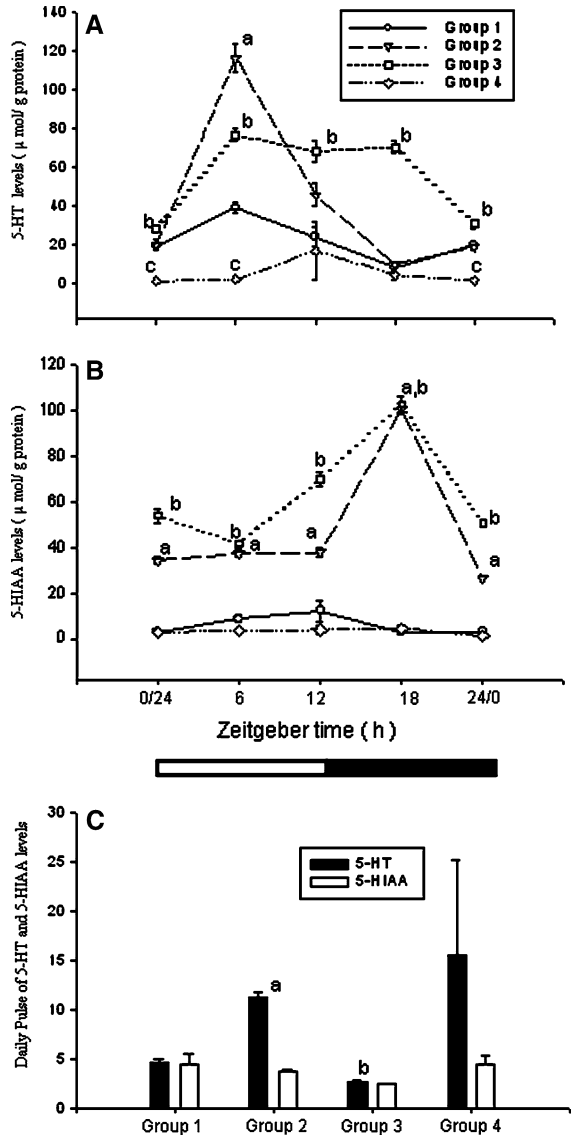

Fig. 1 Effect of curcumin treatment in SCN of ethanol treated 90 day rats. Group 1: control, Group 2: ethanol drinking for 15 days, Group 3: ethanol withdrawal for 15 days after ethanol drinking for 15 days, Group 4: curcumin treatment for 15 days after ethanol drinking for 15 days. (**A**) 5-HT rhythms: rhythmicity persists with a significant increase in 5-HT levels upon ethanol drinking for 15 days, ethanol withdrawal does not result in restoration of 5-HT levels and curcumin treatment results in restoration though with a phase delay of 6 hours for maximum levels. (**B**) 5-HIAA rhythms: ethanol drinking resulted in increase in 5-HIAA levels with a phase shift by 6 h for maximum levels, ethanol withdrawal did not result in restoration of phase or levels and curcumin treatment resulted in restoration of 5-HIAA levels as well as phase. (**C**) Daily pulses of 5-HT and 5-HIAA levels: ethanol drinking resulted in significant increase in daily 5-HT pulse, ethanol withdrawal resulted in significant decrease whereas curcumin treatment resulted in restoration though with fluctuation. $p_a \leq 0.05$, $p_b \leq 0.05$, and $p_c \leq 0.05$ (whereas a, b, c refers to comparison between Groups 1 and 2, 1 and 3, and 1 and 4, respectively). Vertical bars indicate mean ± S.E., $n = 6$

Table 1 Effect of curcumin on 5-HT/5-HIAA ratio in SCN in alcohol treated rat

S.No.	Experimental group	Zeitgeber time (h)				
		ZT-0/24	ZT-6	ZT-12	ZT-18	ZT-24/0
1	Control	7.232 ± 1.01	4.65 ± 0.54	2.308 ± 0.56	3.18 ± 0.36	7.51 ± 1.38
2	Ethanol treated	0.592 ± 0.05[a]	3.09 ± 0.129[a]	1.21 ± 0.09	0.103 ± 0.001[a]	0.948 ± 0.213[a]
3	Ethanol withdrawal	0.526 ± 0.02[b]	1.84 ± 0.05[b]	0.97 ± 0.05	0.68 ± 0.02[b]	0.61 ± 0.02[b]
4	Curcumin treated	0.605 ± 0.219[c]	0.74 ± (0.142[c]	5.36 ± 3.16	1.11 ± 0.38[c]	1.86 ± 0.35[c]

Each value is mean ± S.E., (n = 6); Zeitgeber time (ZT): ZT-0 = 6.30 A.M (Lights on); ZT-12 = 18.30 P.M (Lights off); $p_a \leq 0.05$, $p_b \leq 0.05$ and $p_c \leq 0.05$ (a, b, c same as in Fig. 1A)

In Group 3 (ethanol withdrawal), the 5-HT levels at various time points such as ZT-0, 6, 12, 18, and 24 were 28.36 ± 1.049, 76.51 ± 3.18, 68.25 ± 5.55, 70.30 ± 2.89, and 31.00 ± 2.37 μmol/g protein, respectively which were significantly high as compared to controls at all time points ($p_b \leq 0.05$) (Fig. 1A). The levels remained significantly high not only at subjective mid-day (ZT-6) but also after onset of darkness (ZT-12) as well as subjective mid-night (ZT-18). The 5-HIAA levels on withdrawal were also not restored to normal and were significantly high ($p_b \leq 0.05$) as compared to control. These were 53.85 ± 3.11, 41.58 ± 1.56, 69.86 ± 3.36, 102.76 ± 3.31, and 50.65 ± 1.192 μmol/g protein at ZT-0, 6, 12, 18, and 24, respectively (Fig. 1B). The daily pulses for 5-HT were significantly different as compared to control ($p_b \leq 0.05$) (Fig. 1C). The 5-HT/5-HIAA ratio was maximum at ZT-6 and minimum at ZT-0 and the ratio was reduced in amplitude significantly at ZT-0, 6, 18, 24 ($p_b \leq 0.05$) and phase delayed by 12 h (Table 1).

Group 4 (curcumin treated) animals showed decrease in 5-HT and 5-HIAA as compared to Group 2 and Group 3. The 5-HT levels at ZT-0, 6, 12, 18, and 24 were 1.45 ± 0.71, 2.52 ± 0.54, 17.11 ± 14.69, 4.64 ± 2.47, and 1.85 ± 0.43 μmol/g protein (Fig. 1A) and 5-HIAA were 2.73 ± 0.96, 3.58 ± 0.88, 3.88 ± 1.64, 4.45 ± 1.13, and 1.044 ± 0.230 μmol/g protein (Fig. 1B) respectively. The 5-HT levels though appeared reduced but the maximum levels were at ZT-12. The levels were significantly different at ZT-0, 6, and 24 ($p_c \leq 0.05$). Interestingly, there was no significant difference in the daily pulses of 5-HT and 5-HIAA as compared to control therefore daily pulses were restored though Standard Error was high for serotonin pulses. The 5-HT/5-HIAA ratio was maximum at ZT-12 i.e. onset of darkness and minimum at ZT-0 i.e. onset of light (Fig. 1C). The maximum 5-HT/5-HIAA ratio was not significantly different from that of control. Rhythmicity appeared to be restored, though with a phase reversal (Table 1).

Effect of curcumin on ethanol induced changes in 5-HT and 5-HIAA daily rhythms in Pineal

5-HT and 5-HIAA levels were measured in Pineal similarly as in SCN. In Group 1 (control), at various time points ZT-0, 6, 12, 18, and 24, 5-HT levels were 206.03 ± 39.79, 291.61 ± 58.38, 179.44 ± 28.13, 28.87 ± 12.50, and 203.28 ± 13.25 μmol/g protein, respectively (Fig. 2A) whereas 5HIAA levels were 11.98 ± 1.54, 18.00 ± 1.81, 10.76 ± 1.12, 2.79 ± 0.72, and 12.08 ± 1.35 μmol/g protein, respectively (Fig. 2B). The 5-HT and 5-HIAA levels showed daily rhythms and were maximum at ZT-6 and minimum at ZT-18 respectively as in SCN. The 5-HT and 5-HIAA daily pulses were significantly different (Fig. 2C). The 5-HT/5-HIAA ratio was maximum at ZT-0/24, i.e., onset of light and minimum at ZT-18, i.e., mid subjective night (Table 2).

The 5-HT levels at various time points ZT-0, 6, 12, 18, and 24 in Group 2 (ethanol drinking) were 149.13 ± 5.43, 398.43 ± 30.35, 442.19 ± 26.14, 453.06 ± 22.71, and 141.08 ± 10.15 μmol/g protein respectively (Fig. 2A) and 5-HIAA were 60.12 ± 1.85, 63.40 ± 2.64,

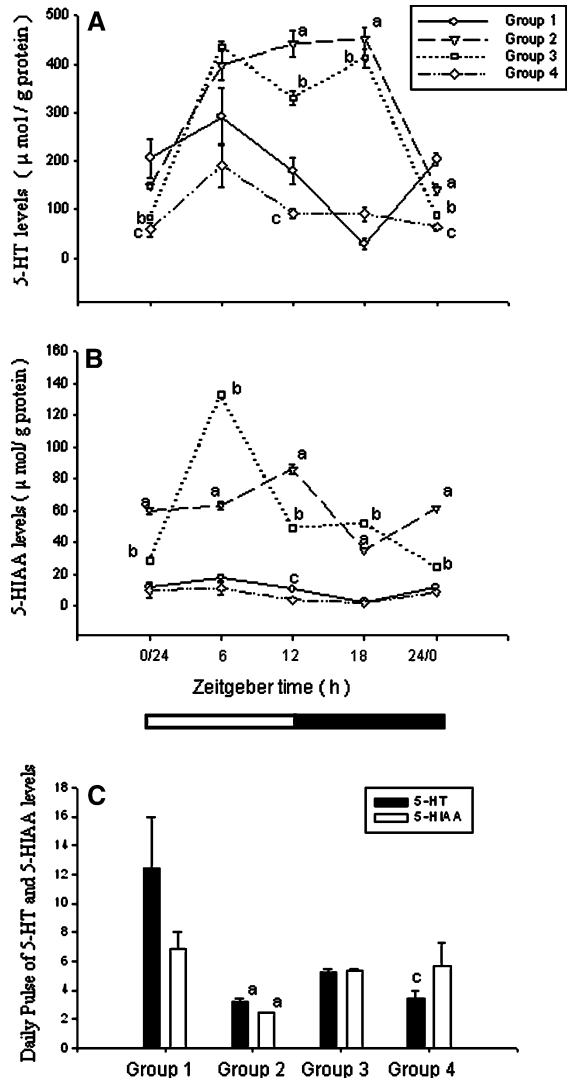

Fig. 2 Effect of curcumin treatment in Pineal of ethanol treated 90 day rats. Group 1–4 same as in Fig. 1. (**A**) 5-HT rhythms: ethanol drinking resulted in increase in 5-HT levels, ethanol withdrawal did not result in restoration and curcumin treatment resulted in restoration of maximum 5-HT levels. (**B**) 5-HIAA rhythms: ethanol drinking resulted in increase in 5-HIAA levels with phase delay of 6 h, ethanol withdrawal restored the phase but 5-HIAA levels were as high as 15 times compared to control and curcumin treatment resulted in restoration of phase as well as 5-HIAA levels. (**C**) Daily pulses of 5-HT and 5-HIAA levels: ethanol drinking resulted in significant decrease in daily 5-HT and 5-HIAA pulses though levels were high in ethanol withdrawal, daily 5-HT, and 5-HIAA pulse was restored and curcumin treatment resulted in restoration of 5-HIAA daily pulse though 5-HT pulse decreased significantly. $p_a \leq 0.05$, $p_b \leq 0.05$, and $p_c \leq 0.05$ (whereas a, b, c same as in Fig. 1). Vertical bars indicate mean ± S.E., $n = 6$

85.94 ± 2.70, 35.35 ± 1.32, and 61.59 ± 0.81 μmol/g protein, respectively (Fig. 2B). The basal levels of 5-HIAA were significantly high as compared to control at all time points though rhythmicity persisted ($p_a \leq 0.05$). 5-HT and 5-HIAA daily pulses were significantly different as compared to controls ($p_a \leq 0.05$) (Fig. 2C). The 5-HT/5-HIAA ratio was maximum at mid night (ZT-18) phase advanced by 12 h or phases were reversed and minimum at ZT-0/24. In addition the amplitude in 5-HT/5-HIAA ratio was significantly reduced at ZT-0, 6, 12, and 24 ($p_a \leq 0.05$) (Table 2).

In Group 3 (ethanol withdrawal), 5-HT levels were 83.28 ± 5.45, 436.44 ± 10.01, 331.23 ± 14.49, 412.72 ± 19.40, and 88.27 ± 4.448 μmol/g protein, respectively (Fig. 2A) and 5-HIAA levels were 28.56 ± 1.07, 132.95 ± 2.13, 49.26 ± 0.69, 52.39 ± 1.85, and 24.93 ± 0.93 μmol/g protein, respectively (Fig. 2B). Neither rhythmicity nor amplitude of 5-HT and 5-HIAA was restored. The 5-HT as well as 5-HIAA values were significantly high as compared to control at all time points ($p_a \leq 0.05$) though daily pulses were not significantly different from control. The maximum levels were 15 times higher as compared to control ($p_b \leq 0.05$) (Fig. 2C). The 5-HT/5-HIAA ratio was maximum at ZT-18 and minimum at ZT-0 and significantly different at all time points from control group ($p_b \leq 0.05$) (Table 2).

Group 4 (curcumin treated) animals showed 5-HT levels at ZT-0, 6, 12, 18, and 24 were 59.35 ± 14.31, 191.40 ± 44.21, 92.08 ± 10.49, 91.25 ± 14.36, and 64.15 ± 6.218 μmol/g protein, respectively (Fig. 2A) and 5-HIAA as 10.08 ± 4.63, 11.394 ± 4.02, 4.04 ± 1.10, 2.18 ± 0.63, and 8.77 ± 0.61 μmol/g protein, respectively (Fig. 2B). The 5-HIAA levels appeared similar to control and were different only at ZT-12 ($p_c \leq 0.05$) with restoration of rhythmicity. The 5-HT levels also showed decreased levels at ZT-0, 12, and 24 ($p_c \leq 0.05$), with restoration of rhythmicity. Daily pulse of 5-HT were significantly different ($p_c \leq 0.05$) but 5-HIAA daily pulses were not significantly different from control (Fig. 2C). The 5-HT/5-HIAA ratio was maximum at ZT-18 and minimum at ZT-0/24 with restoration of rhythmicity, though advanced by 6 h. The maximum ratio was 2.6 times more as compared to control ($p_c \leq 0.05$). Also at ZT-0, 6 and 24, 5-HT/5-HIAA ratio was significantly different as compared to control ($p_c \leq 0.05$) (Table 2).

Discussion

Serotonin has been shown to play a major role in the regulation of circadian pacemaker (Morin 1999; Mistleberger et al. 2000; Rea and Pickard 2000). This is in agreement with our work as there is a significant increase in serotonin and its metabolite 5-HIAA levels upon ethanol drinking in SCN and in Pineal. The ethanol induced shifts in 5-HT and 5-HIAA rhythms in SCN and in Pineal observed in present study is in agreement with earlier workers who have

Table 2 Effect of curcumin on 5-HT/5-HIAA ratio in Pineal in alcohol treated rat

S.No.	Experimental group	Zeitgeber time (h)				
		ZT-0/24	ZT-6	ZT-12	ZT-18	ZT-24/0
1	Control	17.48 ± 2.35	16.36 ± 2.127	16.86 ± 1.84	11.08 ± 3.30	17.03 ± 1.27
2	Ethanol treated	2.48 ± 0.068[a]	6.29 ± 0.310[a]	5.14 ± 0.199[a]	7.38 ± 3.52	2.29 ± 0.09[a]
3	Ethanol withdrawal	2.92 ± 0.126[b]	3.283 ± 0.052[b]	6.72 ± 0.178[b]	7.88 ± 0.26	3.54 ± 0.128[b]
4	Curcumin treated	7.45 ± 2.28[c]	19.18 ± 4.75[c]	24.59 ± 4.21	45.63 ± 8.79[c]	7.34 ± 0.50[c]

Each value is mean ± S.E., ($n = 6$); Zeitgeber time (ZT): ZT-0 = 6.30 A.M (Lights on); ZT-12 = 18.30 P.M (Lights off); $p_a \leq 0.05$, $p_b \leq 0.05$ and $p_c \leq 0.05$ (a, b, c same as in Fig. 1A)

reported ethanol induced shifts in *per* 1 and *per* 2 (important molecular components of the clock) in various brain regions including SCN (Chen et al. 2004; Spanagel et al. 2005). It is also in agreement with reports that alcohol ingestion alters the phase, amplitude or abolish the expression of circadian rhythms in a variety of physiological and behavioral functions, including locomotor activity, body temperature (Baird et al. 1998), sleep (Ehlers and Slawecki 2000), food intake (Barr 1988), secretion of the stress related hormone, corticosterone and other functions (Rajakrishnan et al. 1999; El-Mas and Abdel-Rahman 2000). Alcohol consumption has also been earlier related with reduction in synthesis of several hypothalamic neuropeptides within the SCN (Madeira and Paul-Barbosa 1999) and alteration in the free running circadian period (Mistleberger and Nadeau 1992).

The long-lasting alcohol tolerance has been related to multifunctional neurotransmitters like serotonin, norepinephrine and dopamine (Tabakoff and Hoffman 1996; Valenuzuela and Harris 1997). We report here alcohol induced phase shifts and increase in 5-HT and 5-HIAA levels. In addition, acute alcohol consumption is also related with the release of serotonin, GABA, and taurine, and result in increased chloride flux and decreased neuronal excitability in rat brain (Yoshimoto et al. 1992; Dahchour et al. 1994; LeMarquand et al. 1994). However, some workers have reported decreased serotonin level upon chronic ethanol drinking (Carmichael and Israel 1975; Michaelis et al. 1978).

The ethanol withdrawal resulted in the alteration in 5-HT and 5-HIAA levels as well as rhythms in Pineal and SCN. This is in agreement with earlier reports where ethanol withdrawal has been associated with phase advances of circadian rhythms in body temperature (Kodama et al. 1988), rapid eye movement (REM) sleep (Imatoh et al. 1986) and the levels of 5-HIAA (Sano et al. 1993, 1994) and phase delays in blood cortisol, a key stress hormone (Iranmanesh et al. 1989). It has been reported that both cortisol and melatonin rhythms might severely get abolished upon ethanol withdrawal (Fonzi et al. 1994; Mukai et al. 1998; Danel and Touiton 2006). The cerebral hyperactivity during ethanol withdrawal in some studies (Glue and Nutt 1990; Grant et al. 1990) can be related to altered 5-HT, 5-HIAA levels and rhythms upon ethanol withdrawal in the present study.

As curcumin is known to have antioxidant, anti-inflammatory, anti-carcinogenic properties (Arajuo and Leon 2001; Aggarwal et al. 2003; Joe et al. 2004), we looked into its protective effects on alcohol induced alterations in 5-HT and 5-HIAA levels and rhythms in SCN and Pineal. We report, here, that curcumin influences the clock and helps in restoring the levels of neurotransmitter 5-HT and its metabolite 5-HIAA. Alcohol induced changes in 5-HT and 5-HIAA rhythms in SCN and Pineal are sensitive to curcumin treatment. More experiments are in progress in our laboratory to study the influence of this compound, but certainly the results in this study indicate curcumin provides a viable food based as well as chrono-pharmacologic approach to ethanol induced alteration in 5-HT, 5-HIAA levels and daily rhythms.

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