DISCUSSION

This study was conducted to determine the possible beneficial role of melatonin in ethanol dependence, which is a complex disorder with an urge to drink and loss of control over the quantity of ethanol consumed. The study was conducted on rodent models of acute and chronic high ethanol consumption. Several types of rodent models were employed to assess the effect of melatonin on ethanol consumption, based on previous reports [27-29] stressing the need for the same to allow for higher pharmacological differentiation in the efficacy of the test compound.

Ethanol-induced reinforcement can be assessed directly in laboratory animals by either operant or non-operant self-administration methods. Operant procedures require sophisticated equipment. In this study, non-operant procedures were used. Oral self-administration procedures have clear face and construct validity as models of human ethanol consumption. Both in animal models as well as in humans, subjects can choose whether to consume ethanol or not, as well as choose its quantity and timing. These procedures have been proven to be useful in identifying the drugs efficacious in preventing excessive ethanol consumption and confirming their predictive validity. In addition, these procedures are technically simple and yield reproducible results. [324]

The rodent models employed in the present study included voluntary as well as forced ethanol consumption models. Voluntary ethanol consumption models included ethanol consumption in ethanol naïve rats by using drinking in dark (DID) model, two bottle choice paradigm and ethanol deprivation model. Two bottle choice paradigm was further subdivided into two models: continuous access and intermittent access models. We have used male Wistar rats in all the models. It was assumed that making standard laboratory rats voluntarily consume large quantity of ethanol is difficult, without the use of some initiation techniques. [15,308,324] However, other studies have also proved that standard laboratory rats, such as Wistar rats will voluntarily consume high levels of ethanol and are used as rodent models of voluntary ethanol consumption. [15,324,328]

Ethanol consumption in ethanol naïve rats was assessed using drinking in dark (DID) model. The DID model has shown predictive validity with opioid and dopaminergic mechanism, as described by Kamdar NM et al. [8] The DID model is useful to
evaluate the process of rapid initiation of the high level of ethanol drinking which ultimately progresses to ethanol dependence. In this model, melatonin has significantly reduced ethanol consumption in ethanol naïve rats (Table 13, Figure 13), the effect being in a dose dependent manner. This effect of melatonin seems to be specific to ethanol as it did not have any effect on intake of plain tap water (Table 11, Figure 11) and sugar solution (Table 12, Figure 12).

Naltrexone was used as a standard drug in the present study. It significantly reduced ethanol consumption in the ethanol naïve model and the effect was specific to ethanol (Table 13, Figure 13), as it did not affect plain water (Table 11, Figure 11) or sugar solution consumption (Table 12, Figure 12). Naltrexone, an opioid antagonist, has been proven to reduce ethanol consumption clinically as well as in several preclinical studies. [5,572,573,574]

Both melatonin and naltrexone did not have any effect on normal reinforcement for drinking sweet fluids or consumption of plain water. Ethanol, plain water and sugar water consumption are influenced by different motivational pathways, i.e. motivation for ethanol is different from motivation for natural rewards. [8] The fact that naltrexone did not affect consumption of plain water or sugar water confirms that opioid signaling is involved in ethanol consumption, but not in natural motivation to drink water or palatable fluids like sugar water. Our observation has shown that, similar to naltrexone, melatonin also selectively decreases ethanol drinking without having a significant effect on plain water or sugar water consumption. Thus, the findings of this model suggest that melatonin may affect the process of rapid initiation of ethanol drinking, which may be the preliminary step in the development of ethanol dependence.

Alternatively, it can be argued that melatonin reduced ethanol consumption by causing sedation or affecting locomotor activity. But the fact that melatonin selectively affected the ethanol drinking, but not plain water or sugar solution, rules out this possibility.

The major challenges in preclinical studies of ethanol dependence is the development of animal models showing high ethanol consumption and resembling the progressive transition from low levels to high level of ethanol consumption, eventually becoming
DISCUSSION

The voluntary ethanol consumption models using two bottle choice paradigm procedure effectively show a gradual increase in voluntary ethanol consumption as well as preference in rats, ultimately reaching pharmacologically relevant blood ethanol concentrations. [331] Standard laboratory rats like Wistar rats voluntarily consume ethanol using the intermittent-access ethanol drinking paradigm without the need for any initiation procedures like sucrose fading technique. [15] This procedure is simple, has high validity, and reliability, making it a useful and relevant procedure for preclinical evaluation of potential therapeutic agents against alcohol dependence. [331] Two bottle choice paradigm is one of the best known models for voluntary ethanol consumption. In this model, rats were presented with access to two bottles, one containing an ethanol solution and the other containing drinking water. [324, 273] This ethanol drinking paradigm has been a successful preclinical tool to evaluate the therapeutic drugs used in ethanol abuse disorders. [331] The limitations of this model include relative coarseness as a measure of ethanol dependence and the inability to measure the motivational component of behavior.

In the continuous-access two-bottle choice drinking paradigm model, animals received a single dose of test drug (different dose levels) and the standard drug (Figure 4). The findings of this model showed that melatonin significantly reduced ethanol consumption in rats habituated to long term high ethanol consumption (Table 15; Figure 14). This effect of melatonin can be considered as dose dependent, since there was a statistically significant difference in ethanol consumption between melatonin 50mg/kg and 100mg/kg (Table 15; Figure 14) (p=0.02). Naltrexone also produced similar reductions in ethanol consumption. As there was no significant difference between the effect of naltrexone and melatonin on ethanol consumption, the effect of these two drugs can be considered similar. The single dose of melatonin as well as that of naltrexone did not have an effect on ethanol consumption on the second day after the drug administration in continuous-access two-bottle choice drinking paradigm model (Table 16; Figure 15). This finding suggests that the duration of effect of a single dose of these drugs is less than 24 hours. None of these drugs had any influence on water consumption as the volume of water intake remained similar throughout the study (Table 17, 18; Figure 16, 17). In our study, the total fluid intake by rats significantly reduced with higher dose of melatonin and
naltrexone when compared with baseline consumption (Table 19; Figure 18) \((p=0.01)\) and the effect lasted only for one day after the drug administration (Table 20; Figure 19).

The intermittent-access two-bottle choice drinking paradigm involved in study included, acute as well as chronic experiment. In the acute experiment, all drugs were given to the animals as a single dose (Figure 5) whereas in the chronic experiment, drugs were given for six days continuously (Table 5). The animals were given voluntary access to ethanol solution by using intermittent access two-bottle-choice 20% ethanol drinking paradigm without any initiation procedures like sucrose fading technique.

As observed in the previous model, this model also demonstrated a significant reduction in the ethanol consumption in the melatonin and naltrexone groups of male Wistar rats. Acute administration of melatonin in higher dose (100 mg/kg) as well as naltrexone effectively reduced ethanol intake in rats voluntarily consuming high ethanol levels (Table 21; Figure 20). The volume of water consumed remained same irrespective of increase or decrease of ethanol consumption after the drug treatment in all the drug groups (Table 22; Figure 21). A statistically significant reduction in ethanol consumption was not observed at a lower dose of melatonin i.e., 50 mg/kg. Findings of chronic experiment in this model showed that long term administration of melatonin in higher dose (100 mg/kg) as well as naltrexone effectively reduced ethanol intake in high ethanol consuming rats (Table 24; Figure 22). As in the acute experiment, the volume of water consumed remained same irrespective of increase or decrease of ethanol consumption after the drug treatment in all the groups (Table 25; Figure 23); an appreciable reduction in ethanol consumption was not observed at lower dose of melatonin i.e., 50 mg/kg. The ratio of ethanol to total fluid consumed decreased significantly after the administration of melatonin 100 mg/kg, signifying that rats have consumed less ethanol after the drug administration. The same ratio was found to increase in control group (distilled water), showing a gradual increase in ethanol consumption, resembling transition from low to medium to high level of ethanol drinking (Table 26; Figure 24).
In the next model, we investigated the possible beneficial effect of melatonin on relapse to ethanol seeking, induced by re-exposure to ethanol in ethanol deprivation models. Relapse is one of the pathognomonic features of ethanol dependence and the prevention of the same is of paramount importance in the successful treatment of ethanol dependence. Relapse behaviour can be studied in animals by giving long-term access to voluntary consumption of ethanol and then depriving the access to ethanol for several days to months. Following re-exposure to ethanol, animals usually show a transient increase in their ethanol consumption over baseline drinking, which is termed as alcohol deprivation effect (ADE). The ADE has been suggested to be an animal model of human relapse. An increase in the reinforcing property of ethanol or ethanol itself acting as a cue with smell/taste or as a priming stimulus may be responsible for this behaviour. Naltrexone and acamprosate, both have significantly reduced an ADE in rodents. The ADE model used widely to screen the new agents for the possible anti-relapse property. [13,575]

The findings of the ethanol deprivation model in our study revealed a significant increase in total daily ethanol intake after a deprivation phase, as compared to baseline ethanol intake in the control group. As has been observed in previous studies [564], such a relapse-like ethanol consuming behavior or ADE was transient in our study, lasting only for the first day after ethanol was reinstated after the first deprivation phase (Table 30; Figure 27) and the relapse-like behavior could not be appreciated following the second deprivation phase (Table 31; Figure 28). Analysis of our study findings indicate that treatment with both doses of melatonin tested not only checked relapse-like ethanol intake, but also significantly reduced ethanol consumption compared to the baseline ethanol intake values (Table 30; Figure 27).

The effect of melatonin was similar to naltrexone, the standard drug approved for the treatment of relapse in alcohol dependence. An earlier study has shown that agomelatine (melatonin receptor agonist) by activation of melatonin receptors significantly reduces the ADE, but the ethanol consumption was more than the baseline intake before deprivation. [564] Our study is unique in this finding of decline in ethanol consumption over and above the prevention of relapse-like ethanol intake.

Melatonin after a single oral dose of 50 mg/kg has shown reduction in ethanol consumption only for day-1 after the first deprivation phase compared to its baseline
(Table 30; Figure 27), but in the second post-deprivation phase the suppressive effect of same dose of melatonin on ethanol consumption could not be appreciated (Table 31; Figure 28). Similarly, in the first deprivation phase, the reduction in ethanol consumption was seen in melatonin 100mg/kg treated group (for 5-days) and in naltrexone 20mg/kg treated group (for 3-days) when compared with their baseline values and the suppression in the ethanol consumption was also significant in melatonin 100mg/kg as well as naltrexone treated groups on day-1 when compared with the vehicle treated control group, without any effect on day-3 and day-5 consumption (Table 30; Figure 27). In the post-second deprivation phase, melatonin 100mg/kg and naltrexone 20mg/kg treated groups significantly reduced ethanol consumption compared to their respective baseline values only for day-1 ($P = 0.006$; $P = 0.021$ respectively). However, in comparison to vehicle treated control group, ethanol consumption was significantly reduced in melatonin 100mg/kg and naltrexone 20mg/kg treated groups till 3rd day of the second post-deprivation phase (Table 31; Figure 28). This shows that melatonin 100mg/kg and naltrexone 20mg/kg administered in a single dose significantly reduced voluntary ethanol consumption following second deprivation phase and the effect persisted for three days. Unlike in the first post-deprivation phase, there was no effect seen with melatonin 50 mg/kg. The second deprivation phase, however, did not show any ADE in the control group, unlike the first deprivation phase. Hence the effect seen with both melatonin and naltrexone seems to be just an effect on ethanol consumption and not on ADE. Due to this, the 3rd deprivation phase which was earlier planned in our study was abandoned. None of these drugs had any influence on water consumption after any deprivation phases throughout the study (Table 32 and 33; Figure 29 and 30) and the gradual increase in body weight observed throughout study did not show any statistical significant difference among any drug treated groups (Table 34; Figure 31).

The repeated use of deprivation phases has proven to be a useful method in inducing features of dependence in long-term ethanol drinking rats. The animals undergoing repeated deprivation phases develop behavioral changes resembling the core features of dependence. It was confirmed by using operant procedure that after repeated deprivation phases, rodents exhibit a higher motivation for ethanol. [324]
Peres et al showed that rats receiving 10% ethanol in drinking water for 35 days display an altered daily profile of melatonin production, with a phase delay and a reduction in the nocturnal peak. [542] Lower levels of melatonin during the early part of the night along with a delay in the onset of the nocturnal plateau or peak value was observed in alcoholic patients. The prolonged sleep latency observed in these individuals correlated with the nocturnal delay of melatonin secretion. [563] Patients with ethanol dependence show sleep disturbances, such as difficulty in the onset as well as maintenance of sleep. [576] Alteration in the melatonin secretion during ethanol consumption may be involved in the relapse-like drinking in rats. Consistent with these conclusions, in our study too administration of melatonin abolished post abstinence drinking. Vengeliene et. al., demonstrated that the administration of melatonin at the end of the light phase advanced circadian phase and completely abolished ADE. [564]

However, in contrast to these reports, Crespi has reported higher levels of melatonin in the pineal gland of ethanol drinking rats and there was a significant reduction of spontaneous consumption of ethanol in rats receiving melatonin antagonist. [561]

The ratio of ethanol to total fluid intake (preference to ethanol over water) was calculated. The ratio of ethanol/total fluid intake was decreased in melatonin 100 mg/kg and naltrexone treated groups after first and second deprivation phases.

In the forced ethanol consumption model, animals had access to ethanol solution as their sole fluid for a restricted period i.e., they were forced to drink ethanol solution during this period, which helps to attain a pharmacologically relevant quantity of ethanol in rodents. [324] As observed in the previous models, this model also showed similar results, indicating that both melatonin and naltrexone significantly reduced ethanol consumption in high ethanol consuming rats. Ethanol consumption was not affected when animals received melatonin receptor antagonists (like luzindole and prazosin) before receiving melatonin (Table 35; Figure 32). These findings suggest that the effect of melatonin on ethanol consumption may have been blocked by luzindole (MT1 and MT2 receptor blocker) and prazosin (MT3 receptor blocker). With these results, we can deduce that all three melatonergic receptors may be involved in the effect of melatonin in reducing the ethanol consumption. Our results in this model
also showed that luzindole had a much higher effect on blocking the effect of melatonin on ethanol consumption than prazosin because the significant difference in ethanol consumption was seen only between luzindole+melatonin and melatonin 50mg/kg groups. Prazosin treated group did not display any difference in ethanol consumption compared to the melatonin 50mg/kg treated group (Table 36; Figure 32), and there was no significant difference when compared to its baseline.

The ratio of ethanol/total fluid intake also decreased in melatonin 25mg/kg, melatonin 50mg/kg and naltrexone treated groups.

In the forced ethanol consumption model, we included a group of animals with access to water as the sole drinking solution (i.e., water fed group) during the restricted period instead of ethanol. These animals received melatonin (at higher dose at the same time point at which different drugs were administered in ethanol exposed rats depending on their allocated groups) and the water consumption was recorded for this group at the same time points as was done in ethanol exposed groups. The results showed that the water fed group did not show any significant difference in water consumption when compared with pretreatment baseline water consumption (Table 38). These findings suggest that melatonin did not have any effect on normal drinking behavior of animals and thus rules out the possibility of sedative effect of melatonin affecting the ethanol consumption.

In this model, at the end of the study, rats were sacrificed and the nucleus accumbens was dissected out and processed for the estimation of dopamine levels by using ELISA kit. Dopamine levels in the nucleus accumbens were significantly elevated in the rats receiving distilled water which had high ethanol consumption whereas the levels of dopamine were significantly decreased in melatonin and naltrexone treated groups (Table 40; Figure 34). Thus, the groups which showed an increase in ethanol consumption also had an elevated dopamine levels in their nucleus accumbens (NAc) and vice versa. This shows that there is a direct correlation between ethanol consumption and dopamine levels in the NAc.

We assume that the effect of melatonin on ethanol consumption and dopamine level could be through the involvement of melatonin receptors (MT₁, MT₂ and MT₃) present in the nucleus accumbens, which was confirmed by administering melatonin
receptor antagonists like luzindole (MT\textsubscript{1} and MT\textsubscript{2} receptor blocker) and prazosin (MT\textsubscript{3} receptor blocker). The suppressive effect of melatonin on ethanol consumption was not seen in animals treated with luzindole + melatonin i.e., there was a statistically significant increase in ethanol consumption which augmented the release of dopamine in luzindole treated group (Table 35, 36, 40; Figure 32 and 34). However, prazosin treated group did not show any statistically significant difference in ethanol consumption and dopamine level when compared with melatonin 50mg/kg or naltrexone treated groups (Table 35, 36, 40; Figure 32 and 34). This shows that melatonin acts through MT\textsubscript{1} and MT\textsubscript{2} receptors (blocked by luzindole) to reduce ethanol consumption and thereby dopamine levels, whereas MT\textsubscript{3} receptor (blocked by prazosin) may not be involved in such an effect.

Melatonin through its action in the brain is known to regulate various physiological and neuroendocrine functions. It has been confirmed that chronic alcohol consumption not only reduces melatonin levels, but also delays the pineal melatonin production. [577] Heavy alcohol use is associated with a decrease in melatonin level and may contribute to the often-reported alcohol induced sleep disturbance. [578]

The potential efficacy of melatonin has been demonstrated in several types of drug dependence in the preclinical studies. A large body of research suggests that melatonin has some reversal effects on different drugs of abuse and is being useful in some aspects of drug dependence. Administration of melatonin was able not only to reverse the development of morphine tolerance and dependence but also to inhibit the morphine withdrawal syndromes in mice. [551,552,553,579,580] Reversal of the expression of morphine-induced rewarding effect by melatonin may be mediated by the activation of melatonin MT\textsubscript{2} receptor subtype within the central nervous system. [26]

It was also established that physiological doses of melatonin reduce craving in heavy smokers during acute withdrawal from nicotine. [581] Melatonin is involved in cocaine induced reward and has reduced chronic use of benzodiazepines in patients with insomnia. [582,583]

Targeting the melatonergic system with melatonin in patients suffering from alcoholism, especially those with comorbid depression, may help to restore normal
sleep architecture of an addicted patient, reduce alcohol wanting and thereby relapse behavior. [564]

Dopamine signaling is known to be involved in reinforcing properties of dependence producing agents, including ethanol dependence. [2] The dopaminergic mesolimbic system plays a significant part in the motivational and reinforcement mechanisms related to behavior. Ethanol increases dopaminergic transmission in the mesolimbic pathway and increases the firing rate of dopaminergic neurons enhancing dopamine release. The transient surge in dopamine level in these areas occurring with administration of dependence producing agents contributes to reinforcement properties. A transient surge in dopamine also occurs when rodents taste sweet fluids, which may underlie reinforcement of sugar water drinking in rodents. [584]

Some of the neurotransmitter genes like D2 dopamine receptor genes are associated with the increased risk of developing ethanol dependence. [167,43] Dopamine release at VTA has a clear role in the initiation of ethanol consumption in the nucleus accumbens. [110,111]

Direct evidence of a role for dopamine in ethanol reward comes from the finding that the rats that operantly self-administer ethanol will stimulate its release in the nucleus accumbens. During chronic ethanol abuse, larger amounts of ethanol may need to be consumed to evoke dopamine release, in order to obtain the pleasurable effects of ethanol intake. During ethanol withdrawal, dopamine release will be reduced, thereby reducing the firing of related neurons leading to dysphoria, malaise and depression. [44] Ethanol, acting through opioid receptors in the ventral tegmental area and nucleus accumbens, modulates the activity of the mesolimbic dopamine system. [2] Evidences indicate that ethanol influences the actions of the mesolimbic dopaminergic pathway in brain, mediating through opioid receptors. Its interaction with opioid receptors is either directly or indirectly. [7]

The increased extracellular dopamine levels during ethanol deprivation periods suggests the role of dopaminergic system in enhancing the ethanol consumption during ADE. [115]
Repeated deprivation phases in alcohol-preferring rats leads to upregulation as well as increased binding sites of dopamine receptors in nigrostriatal dopaminergic pathway, indicating the role of this pathway in mediating ethanol like relapse behaviour. [114,116,117]

Melatonin receptors are found in several locations that receive dopaminergic innervation, such as prefrontal cortex, striatum, nucleus accumbens and amygdala in the mammalian brain. [585] Inhibition of dopamine release by melatonin has been demonstrated in sites such as hypothalamus, hippocampus, medulla-pons, and retina. [586,587] Studies have proven the anti-dopaminergic activities of melatonin in the striatum. From various studies, it has been demonstrated that melatonin in physiological doses generally produces an antidopaminergic effect on the brain, [586] while high doses of melatonin has no effect on dopaminergic neurons. [588] The present study showed that the melatonin at the dose of 50mg/kg and 25mg/kg had anti-dopaminergic effect. This effect could be due to the indirect effect by decreasing the ethanol consumption.

Several studies have demonstrated the efficacy of naltrexone in ethanol dependence with progress in maintaining abstinence and suppression of heavy drinking. [7,194,195,196] It produces its effects through interaction with dopamine and the endogenous opioid systems. [50] Inhibitory effect of naltrexone on GABAergic neurons in the ventral midbrain may affect the dopamine reward pathway in ethanol dependence. [8]

It is well established that melatonin has antioxidant properties and functions as an endogenous free-radical scavenger. [589,590] Recent studies have shown that ethanol dependence is associated with the induction of oxidative stress. [23] Nitric oxide has a role in oxidative stress by forming peroxynitrite and superoxide radical. Studies have shown that brain nitric oxide levels increase following ethanol administration. [591] Melatonin has the ability to suppress nitric oxide synthase (NOS) activity. [552] Studies have shown that chronic melatonin treatment has reversed cognitive deficits in ethanol-intoxicated mice, which is associated with its antioxidant property. [557] Administration of melatonin has reversed the increase in lipid peroxidation and a decline in glutathione induced by the chronic administration of ethanol in rats. This
protective activity of melatonin is by scavenging free radicals and stabilizing glial activity against ethanol induced injury to the nervous system. [592] These evidences may suggest that antioxidant property of melatonin may be a possible basis for its efficacy in reducing the ethanol consumption in rodent models of high ethanol consumption.

Melatonin also increases the release of β-endorphin from the periaqueductal gray matter in rats. [593] Therefore, one may surmise that melatonin also may show some effects on the ethanol rewarding properties as well, since ethanol is also considered as a substance of abuse.

Melatonin is found to be useful in mental diseases like anxiety disorders, depression, schizophrenia, phobias and autism, attention deficit hyperactivity disorder and has a quick and lasting effect. [594,595,596] Melatonin has been successfully used to ease the anxiety in patients with insomnia. [597] Melatonin levels have also been used to differentiate clinical subtypes of schizophrenia. The paranoid subtype has been reported to have lower melatonin levels than healthy subjects. [542] The findings of our study suggest that melatonin is a promising drug in the management ethanol dependence and relapse. Due to its effect on sleep architecture, melatonin may help to restore normal sleep rhythm in patients with ethanol dependence. Although three drugs, disulfiram, acamprosate and naltrexone have been approved for the ethanol dependence, none of them is devoid of limitations. While naltrexone is considered most efficacious among these drugs it is said to bring about abstinence in only about 25-35% patients. [598] It also carries a black box warning of possible hepatotoxicity which might already be a problem in patients with alcohol dependence. Acamprosate and disulfiram have been found wanting in their efficacy to sustain abstinence. Melatonin is known to have a favorable safety profile and is approved for the treatment of insomnia and jet lag. The findings of the present study showed promising results with melatonin in rodent models of high ethanol consumption. Based on these findings further clinical studies can be planned in individuals with ethanol dependence to explore whether the same beneficial effect seen in rodent models is translated in patients with ethanol dependence also. If this discovery is positive, melatonin or its congeners may become safer alternatives in the treatment of ethanol dependence.
The limitations of our study should be considered. We could not procure high ethanol consuming strains of rodents (genetically selected strains with an inborn preference for ethanol) for the present study. However, the studies have shown that standard laboratory rats like Wistar rats will voluntarily consume high levels of ethanol and are used as rodent models of voluntary ethanol consumption. [328,329,575] Also, use of genetically selected rodent strains may result in reduced generalization potential. [324] Animal models used in the present study exhibit only high ethanol consumption, without the evidence of dependence or depicting the motivational component of ethanol dependence. Motivational component of dependence can be studied using operant procedures, which requires sophisticated equipment. We did not measure the blood ethanol concentration in animals after exposure to ethanol solution and hence, we could not assess whether the animals have achieved pharmacologically relevant blood ethanol concentration. We studied the role of dopamine in relation to the mechanism of action of melatonin. However, the effect of melatonin on other neurotransmitters or opioidergic system as well as possible antioxidant like effect of melatonin was not been evaluated in the present study.

Selective MT₁ receptor antagonists are not available. Though selective MT₂ receptor antagonists were available, we could not procure it. Hence we could not specify whether MT₁, MT₂ or both receptors are involved in the beneficial effect of melatonin in reducing the ethanol consumption in rodents. The earlier studies of melatonin in morphine dependence has shown that MT₂ receptors were involved in reversing the expression of morphine-induced rewarding effect. [26]

Further scope for research in this area includes confirmation of the findings of this study in other species of animals. Effect of melatonin on motivational component of ethanol dependence can be studied using suitable operant models like reinstatement model which uses operant chamber. Here, the animal works to get ethanol, which is paired concurrently with some conditioning cue, such as light, sound, etc. After the prior cue, the animal’s reinstatement of lever pressing for ethanol is measured. Lever pressing acts as a dependent measure of motivation to seek ethanol. Role of other neurotransmitters like GABA, glutamate or opioid system in the effect of melatonin on ethanol drinking can be explored.