# AGMATINE MODULATES BEHAVIOURAL AND OXIDATIVE EFFECT OF ACUTE ETHANOL EXPOSURE IN ADOLESCENT RATS

**Mayur B. Kale[1]*, Nitu L. Wankhede[2], Chandrashekhar D. Upasani[3], Aman B. Upaganlawar[4]***

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**Abstract**

This study is design to examine the potential of agmatine on acute ethanol-induced behavioral alterations during adolescence. Acute ethanol exposure has been associated with range of disturbances, including, anxiety, depression, social impairment and cognitive deficits. Ethanol's impact on neurotransmitter systems, oxidative stress, and excitotoxicity can contribute to these alterations. Agmatine, a novel neurotransmitter and neuromodulator in brain, has shown promising results in preclinical studies for its multifaceted neuroprotective properties. It has been investigated for its potential to modulate neurotransmitter systems, and reduce oxidative stress. In this experiment, rats were subjected to acute ethanol administration from PND 34 - 41 and administered with agmatine (20, 40 and 80 mg/kg) to evaluate its effect on ethanol-induced behavioral alterations. The results from different behavioral paradigms showed that agmatine in doses 40 mg/kg and 80 mg/kg showed prominent anxiolytic and antidepressant effect. Additionally, it improved the recognition memory in object recognition task and also improved altered social behavior associated with acute ethanol exposure. Furthermore, biochemical analysis revealed involvement of neurotransmitter modulation antioxidant potential of agmatine by reducing enhanced glutamate levels, NO and lipid peroxidation and improving antioxidant defense and GABA levels in brain. The results of this study provide valuable insights into the potential benefits of agmatine in mitigating the acute behavioural consequences of ethanol exposure.

**Key words-** Alcohol, Agmatine, Adolescence, Oxidative stress, anxiety

[1*, 2, 3, 4]*SNJB's Shriman Sureshdada Jain College of Pharmacy, Neminagar, Chandwad, Nasik, India-423101
E-mail:- mayur.kale28@gmail.com; amanrxy@gmail.com
[1*, 2, 4]*Smt. Kishoritai Bhoyar College of Pharmacy, Kamptee, Nagpur, Maharashtra, India-423101

***Corresponding Author: -** Mayur B. Kale, Aman B. Upaganlawar
*SNJB's Shriman Sureshdada Jain College of Pharmacy, Neminagar, Chandwad, Nasik, India-423101
E-mail:- mayur.kale28@gmail.com; amanrxy@gmail.com

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## 1. Introduction

Ethanol exposure during adolescence can have profound effects on physical, mental, and emotional development (Dow-Edwards et al., 2019; Konrad et al., 2013). As the adolescent brain is still maturing, alcohol use can disrupt development, potentially leading to behavioural alterations, cognitive impairments, and increased susceptibility to mental health issues. Alcohol exposure during adolescence is associated with a higher risk of developing substance use disorders in adulthood (Grant & Dawson, 1997; Room, 2005). Additionally, alcohol use can contribute to negative social consequences. Adolescence is a critical period for brain development characterized by synaptic pruning, myelination, and maturation of neural pathways. Exposure to ethanol during this time can disrupt these processes leading to long-term neurobehavioural consequences. Oxidative stress plays a crucial role in brain alterations resulting from alcohol exposure during adolescence (Crews & Nixon, 2009; James Haorah et al., 2008). Alcohol consumption leads to production of reactive oxygen species that can overwhelm the antioxidant defenses in brain, triggering lipid peroxidation and mitochondrial dysfunction. Mitochondrial dysfunction due to oxidative stress is closely associated with several neurological diseases (Banarase et al., 2023; Chandurkar et al., 2023; Mangrulkar et al., 2023; Sammeta et al., 2023; Tiwari et al., 2021; Umare et al., 2022). Furthermore ethanol exposure during adolescence leads to altered neurotransmission of glutamate and GABA. Use of ethanol disrupts the balance between these neurotransmitters, resulting in increased glutamate activity and decreased GABA activity, which can triggers excitotoxicity, impair synaptic plasticity, and contribute to neuronal damage (Chefer et al., 2011; Valenzuela, 1997). These changes can collectively contribute to neurotoxicity and ultimately lead to the brain's structural and functional alterations associated with early ethanol exposure during this critical developmental period, affecting cognitive function and emotional regulation.

Agmatine is a polyamine synthesized from L-arginine through the enzyme arginine decarboxylase (Chandurkar et al., 2023; Dhokne et al., 2023; Donald J. Reis & Regunathan, 2000). It functions as a potential neurotransmitter and neuromodulator in various neurological disorders. Agmatine has demonstrated antidepressant and anxiolytic effects, anticonvulsive, anti-inflammatory as well as antinociceptive activity (Aglawe et al., 2014; Kale et al., 2020; Neis et al., 2015; Satriano et al., 2001; Taksande et al., 2009, 2014; Taksande, Gawande, et al., 2017). It also exhibits neuroprotective qualities and found to enhance memory (Aglawe et al., 2021b; Dixit et al., 2018; Lee et al., 2009). Aditionally, agmatine has been involved in drug addiction and associated complications (Donald J. Reis & Regunathan, 2000).

Agmatine is predominantly localized in the ventral tegmental area (VTA), nucleus accumbens and amygdala, which are known to play central roles in the reinforcement, reward, and withdrawal to psychoactive substances (Otake et al., 1998; Donald J. Reis & Regunathan, 2000).. Notably, agmatine exerts its antioxidant effects by reducing the generation of free radicals and augmenting the brain's antioxidant defense mechanisms (Piletz et al., 2013). Agmatine modulates diverse receptor activity, mainly through interacting with α2-adrenoreceptors and imidazoline receptors, while also inhibiting the glutamate NMDA receptor and nitric oxide synthase (NOS) (Raasch et al., 2001; Donald J. Reis & Regunathan, 2000; Taksande, Sharma, et al., 2017; Yang & Reis, 1999). Number of studies have reported that agmatine can normalize levels of key molecules like hippocampal BDNF in adult rats exposed to prenatal ethanol, suggesting its neurotrophic effects associated with ethanol exposure (Aglawe et al., 2021b).

Thus current study aims to discover the therapeutic potential of agmatine, a potent antioxidant, on behavioural alterations associated with acute ethanol intoxication during adolescence in rats. The study seeks to investigate whether acute exposure to ethanol during this critical developmental period exacerbates cognitive and social deficits and whether agmatine treatment can ameliorate these impairments. Additionally, the research aims to delve into the mechanisms underlying agmatine's actions, specifically examining how it may counteract ethanol-induced activation of oxidative stress and neurotrans-mission alteration during adolescence.

## 2. Material and method

### 2.1. Subject

Male and female Sprague Dawley Rats were bred in a plastic cage during a 4-day period. Following the conformation of pregnancy, Female rats were separated and allowed to have normal delivery. All the experimental animals were housed in controlled environment with 12:12 hour light-dark cycle and were provided with food and water ad

libitum. Pups were weaned on PND 21 and were placed in separate plastic cages with same-sex littermates. The maintenance of all the animals were in accordance with the guidelines of Institutional Animal Ethics Committee and the experiment were approved by the Committee for the Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India, New Delhi (Moosavi et al., 2012).

### 2.2. Drug Preparation

Ethanol was procured from Merck Chemicals; Mumbai, India. Agmatine sulphate, and agmatine modulators including L-arginine monohydro-chloride, arcaine sulphate and amino-guanidine hemisulphate, were procured from Sigmae Aldrich Co.; United States, and were prepared in sterile saline. In the present study, the doses and administration protocol were employed based on previous experiments carried out in our laboratory (Aglawe et al., 2014; Taksande et al., 2010a).

### 2.3. Experimental design

Animals were randomly assigned to different treatment groups and administered with either ethanol (1g/kg, 16% v/v in sterile saline) or isovolumetric sterile saline via i.p. injection from PND 34 – 41. This post-natal age was selected as an animal model of adolescence based on scientific literatures representing that rats undergo physiological development during this period similar to adolescent humans (Spear, 2000; Vincent-Fiquet et al., 1984). Blood ethanol concentrations were measured 60 min after the first dose of ethanol. The Agmatine (20, 40 and 80 mg/kg) was administered 30 min prior administration of ethanol daily. All the behavioural experiments were carried out between 9:00 AM and 1:00 PM.

### 2.4. Behavioural Measures

#### 2.4.1. Open field test

The exploratory behavior and locomotor activity were evaluated using open-field apparatus. The apparatus consisted of a square box of opaque material divided into a grid (100 x 100 x 40 cm). Before the test session, animals were acclimated to the experimental room and handled to minimize stress. The animals were individually placed in the center of an open field arena illuminated with bright light (200 lux) and left to explore the environment for 1 min. The behavioural parameters including distance travelled, ambula-tion, rearing, and grooming were recorded for the next 5 min. The arena was cleaned between sessions with 75% ethanol to prevent odor cues, and video recordings were analyzed using video tracking software (VJ instrument, India) for quantifying behavioural parameters.

#### 2.4.2. Elevated Plus Maze

The anxiety-like behavior was assessed using an elevated plus maze (EPM). The apparatus consisted of two open arms and two enclosed arms (50 x 10 x 50 cm) arranged in a plus-shaped configuration, elevated above the floor at a height of 70 cm. Rats were individually placed at the center of the maze facing towards the open arm, and time spent in open and closed arms, and the number of entries into each arm type were recorded as indicators of anxiety-like responses for 5 min. The maze was cleaned between sessions with 75 % ethanol to eliminate olfactory cues.

#### 2.4.3. Social interaction Test

The social interaction test was performed to assess sociability and communication behaviors in adolescent rats. The test was performed by placing the animal in an arena with a same-sex unfamiliar rat and allowing them to freely interact for 10 minutes. The rats were individually placed in an opaque plastic cage (30 × 20 × 20 cm) before testing. After a brief period, each animal was placed into the testing arena with a same-age and sex test partner. Partners were unfamiliar rats that had been socially active before the test. During the 10-min session, the parameters including sniffing, grooming, following, and physical contact, were recorded by a video camera to evaluate social preferences, and communication skills. After each test, the apparatus was cleaned between sessions with 75 % ethanol, and the bedding was replaced with fresh to eliminate olfactory cues.

#### 2.4.4. Novel Object Recognition test

The novel object recognition (NOR) test was used to assess the memory and recognition abilities of adolescent rats. The apparatus consisted of a closed plexiglass arena (40 × 20 × 20 cm) with three identical objects (a ping-pong ball). The test was performed in three different trials. On day 4, rats were habituated individually with the apparatus for 5 min without any object in an open field. On day 5, an acquisition trial was performed, rats were exposed to two identical objects placed equidistant from the corners of the apparatus for 10 min. During test trials on day 6, one of the familiar objects was replaced with a novel object and animals were reintroduced to the arena, and their interaction times with the familiar

and novel objects were recorded for 10 min. Sniffing the object from a distance of 3 cm, and touching the object with the nose and/or forepaws was considered exploration whereas sitting on the object or turning around the object was not considered as an exploratory behavior. A preference for exploring the novel object over the familiar object indicated successful recognition memory. The discrimination index was calculated as exploration time with novel object/ familiar object + novel object.

### 2.4.5. Forced swim test

The depressive-like behaviors were assessed using a forced swim test (FST). The FST was performed in two sessions, training, and testing. In the training session, rats were placed individually into a glass tank (30 x 20 cm) filled with water for 15 minutes. The post-training, test session was performed and immobility duration was recorded for 5 min. Immobility time, is characterized by minimal movement and floating necessary for keeping their head above water.

## 2.5. Blood Ethanol Determination

For analysis, blood was collected from a lateral saphenous vein, 3 min post-ethanol administration. For the determination of BEC, ~50 µL of blood was mixed with 160 µL 3% $HClO_4$, and ethanol concentration was quantified using an alcohol dehydrogenase assay (Zapata et al., 2006). For estimation of BEC, 20 µL of sample aliquot was reacted with alcohol dehydrogenase and 1.5 mM β- nicotinamide adenine dinucleotide (β-NAD) in 0.5M Tris-HCl buffer (pH 8.8). The absorbance was recorded at 340 nm and ethanol concentration was expressed as mg/dL.

## 2.6. Oxidative stress parameters

For the analysis of oxidative stress parameters, animals were euthanized with an overdose of pentobarbital sodium and brains were removed. For biochemical estimation, the brain was homogenized with ice-cold Tris-HCl buffer (20 mM, pH 7.4) and centrifuged at 10,000 ×g at 4°C for 15 min. These tissue homogenates were used for the estimation of lipid peroxidation, SOD, GSH, nitrate/nitrite level, and protein.

### 2.6.1. Lipid peroxidation

The amount of MDA was evaluated for the determination of lipid peroxidation. In the thiobarbituric acid (TBA) reaction, 1 molecule of MDA reacts with 2 molecules of TBA forming a pink pigment. The reaction was performed at 90-100° in pH 2-3 for 10-15 min. Briefly, the supernatant was mixed with 2 volumes of trichloroacetic acid (TCA) (10% w/v) and centrifuged. The Aliquot of supernatant was reacted with 0.67 % w/v TBA (1:1) in a boiling water bath for 10 min and cool it. At 532 nm absorbance was measured using a spectrophotometer (Wills, 1966).

### 2.6.2. Reduced glutathione (GSH)

The reduced GSH concentration in the sample was determined using the method previously described by Ellman and colleagues. Briefly, 1 ml of supernatant was digested at 4 °C for 1 hour and precipitated using 4% sulfosalicylic acid. The mixture was centrifuged at 1200 rpm for 15min. Following 1 ml of supernatant was reacted with 0.1 M phosphate buffer (pH 8) and 5,5-dithiobis 2-nitrobenzoic acid (DTNB). The yellow product so formed was read at 412 nm using a UV-VIS spectrophotometer. Results were expressed as µM/mg of protein using a molar extinction coefficient (1.36 l04 M $cm^{-1}$) (Luck et al., 1997; Ellman, 1959).

### 2.6.3. Superoxide dismutase

The total SOD was determined by measuring the inhibition of epinephrine oxidation by the enzyme in the sample (Misra & Fridovich, 1972). The radicle sequestering activity of SOD directly inhibits the increase in oxidation in epinephrin. For estimation, the reaction was initiated by the addition of 0.3 mmol/L epinephrine to the reaction mixture of supernatant and 0.05 mol/L carbonate buffer (pH 10.2). The change in absorbance in the sample was recorded at 480 nm for 2 min. The results were expressed as units of SOD/mg of protein, one unit of enzyme activity is defined by a 50% inhibition rate of reaction by the enzyme.

### 2.6.4. Estimation of nitrate/nitrite

Accumulation nitrite n the supernatant was used as an indicator of the nitric oxide generation and was estimated using the Griess reagent. An equal volume of brain sample and Griess reagent (0.1% N-naphthyl ethylenediamine dihydrochloride, 1% sulfanilamide, and 2.5% phosphoric acid) were mixed and incubated at 37°C for 15 minutes. The absorbance was recorded at 540 nm using a spectrophotometer. The nitrite concentration was calculated using the standard curve of sodium nitrite(Green et al., 1982).

### 2.6.5. Protein Estimation

Protein content was used for the correction of MDA and nitrite levels. Tissue homogenate was mixed with Bradford reagent for 5 min and

absorbance was recorded at 570 nm. The protein estimation was done by comparing it with the standard bovine serum albumin solution (MM, 1976).

### 2.7. Data Analysis

Data from the behavioural experiment were analyzed independently using a one-way analysis of variance (ANOVA) followed by post-hoc test to assess potential differences in the measured variables across various experimental groups. All analyses were performed with Graphpad prism. Each value represents mean ± SEM.

## Results:

### 3.1. Agmatine attenuate the Hyperactivity induced by acute ethanol administration in Adolescent rats

One-way ANOVAs was performed to evaluate significance betweent different groups. A significant difference in ambulation and rearing count was found when animals were administered with alcohol during adolescence (PND 34 - 41). The administration of agmatine 40 mg/kg and 80 mg/kg significanly attenuated increased motor activity i. e. ambulation [F(4, 25) = 8.218] along with rearing [F(4, 25) = 11.16 ] associated with acute ethanol administration. However, low dose of agmatine failed to alter the locomotor behavior in ethanol treatded rats. Administration of ethanol in adolescent animals did not alter the grooming behavior when observed in OFT.

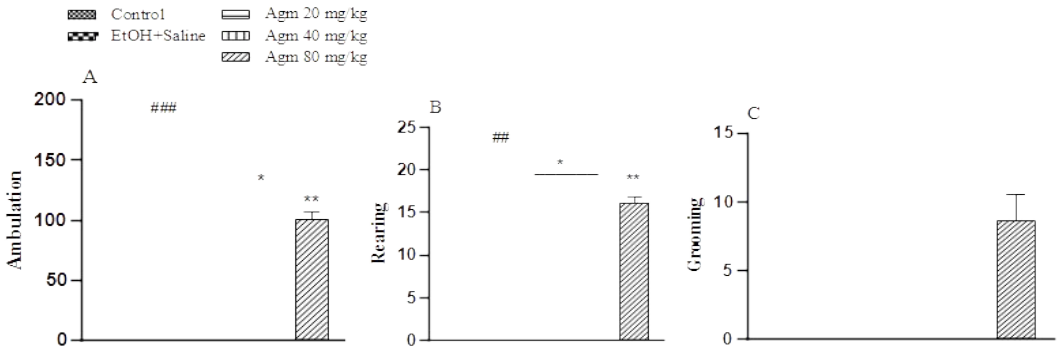


**Figure 1.** Effect of Agmatine (20, 40 and 80 mg/kg) on Ambulation (A), Rearing (B) and grooming (C) in ethanol treated rats. Each values represents mean ± SEM (n = 6). $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs. saline control animals, *p < 0.05, **p < 0.01 vs. ethanol withdrawal animals (One-way ANOVA followed by *post hoc* Sidak's multiple comparison test).

### 3.2. Agmatine modulate anxity like behavior induced by acute ethanol administration in Adolescent rats

As shown in figure, One-way ANOVA followed by post hoc Sidak's Multiple Comparison test revealed that a significant increase in number of entries in open arm in animals administred with ethanol from PND 34- 41 ($p<0.05$). Treatment of agmatine 40 and 80 mg/kg showed significant decrease in number of entries [F(4,25) = 8.674], and time spend in open arm [F(4,25) = 7.806],as compared to ethanol treated adolescent animals. No significance alterations were observed in the entries in close arm. Administration low dose of agmatine significantly reduced the number of entries in open arm ($p= 0.0128$) whereas fails to show any alteration in time spent ($p=0.7382$).

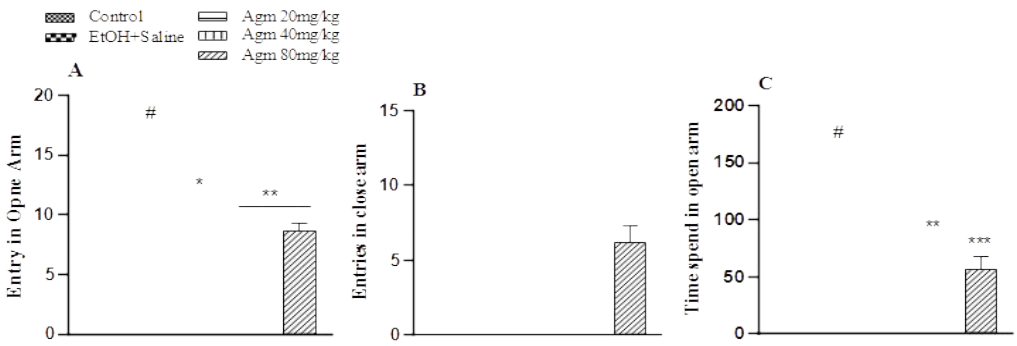


**Figure 2.** Effect of Agmatine (20, 40 and 80 mg/kg) on entries in Open (A) and closed arm (B) and time spent

in open arm (C) in ethanol treated rats. Each values represents mean ± SEM (n = 6). #p < 0.001 vs. saline control animals, *p < 0.05, **p < 0.01, ***p < 0.001 vs. ethanol withdrawal animals (One-way ANOVA followed by *post hoc* Sidak's multiple comparison test).

**3.3. Agmatine modulate social behavior in acute ethanol administration in Adolescent rats**

As shown in figure 3, One-way ANOVA followed by post hoc Sidak's multiple comparison test revealed that a significant decrease in social behavior in animals administered with ethanol during adolescence age (t = 10.18, p<0.001). Treatment of Agmatine 40 mg/kg (p < 0.01) and 80 mg/kg (p < 0.001) showed significant increase in social interaction time [F(4, 25) = 30.49] as compared with ethanol treated animals. Administration of agmatine in low doses fails to show any alteration in impaired social behavior associated with acute ethanol administration (p = 0.7321).

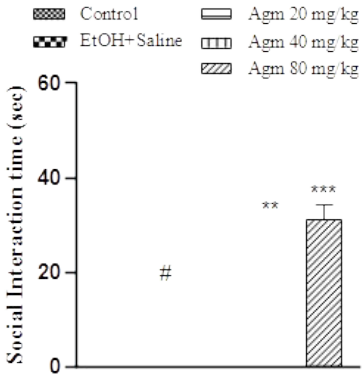


**Figure 3.** Effect of Agmatine (20, 40 and 80 mg/kg) on exploration time with social animal. Each values represents mean ± SEM (n = 6). #p < 0.001 vs. saline control animals, **p < 0.01, ***p < 0.001 vs. ethanol withdrawal animals (One-way ANOVA followed by *post hoc* Sidak's multiple comparison test).

**3.4. Agmatine protect against memory impairment induced by acute ethanol administration in Adolescent rats**

As shown in Figure 4, Two-way ANOVA followed by *post hoc* Sidak's multiple comparison test demonstrates memory impairment with administration that no significant changes were observed in exploration time (sec) with familiar object of Ethanol + Saline treated group as compared to control group and also a significantly decrease in Exploration time (sec) with novel object of Ethanol + saline treated group as compared to control group (p<0.05). The Exploration time (sec) with novel object was significantly increased by agmatine (80 mg/kg) [F (4, 16) = 3.704, p=0.0427]; Arcaine (30 mg/kg) [F (4, 16) =7.699, p=0.0501] as compared to Ethanol + Saline group.

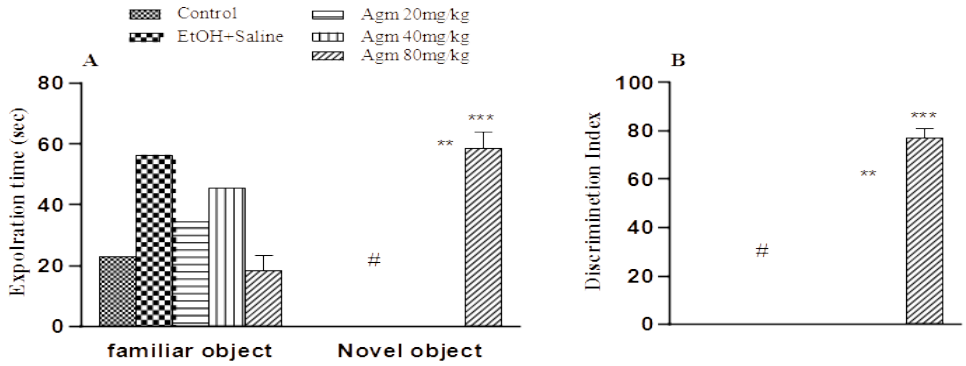


**Figure 4.** Effect of Agmatine (20, 40 and 80 mg/kg) on object exploration time (A) and Discrimination index (B) in NOR. Each values represents mean ± SEM (n = 6). #p < 0.001 vs. saline control animals, **p < 0.01, ***p < 0.001 vs. ethanol withdrawal animals (One-way ANOVA and Two-way ANOVA followed by *post hoc* Sidak's multiple comparison test).

**3.5. Agmatine protect against depressive behavior induced by acute ethanol adminis-**

**tration in Adolescent rats**

Acute exposure of ethanol during adolescence produced marked depression like behavior in animals. Post hoc Sidak's mean comparison showed that the immobility duration of ethanol treated animals were significantly increased as compared to control animals ($t = 3.652$, $p < 0.001$). The One-way ANOVA also revealed that, treatment with agmatine showed antidepressant effect when administered for 7 days. The administration of agmatine 20, 40 and 80 mg/kg showed significant reduction in immobility time as compared to ethanol treated animals [$F (4, 25) = 5.436$].

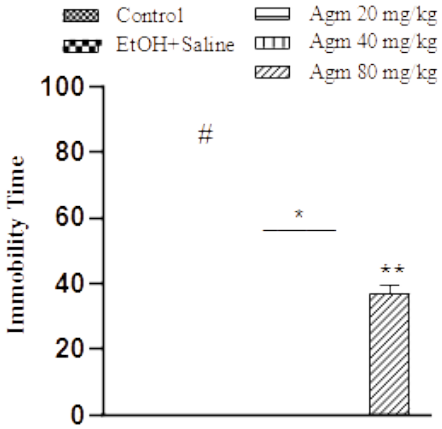


**Figure 5.** Effect of Agmatine (20, 40 and 80 mg/kg) on immobility time in ethanol treated rats. Each values represents mean ± SEM (n = 6). $^{\#}p < 0.001$ vs. saline control animals, $^{*}p < 0.05$, $^{**}p < 0.01$ vs. ethanol withdrawal animals (One-way ANOVA followed by *post hoc* Sidak's multiple comparison test).

### 3.6. Agmatine protect against oxidative stress induced by acute ethanol administration in Adolescent rats

Table 1. Illustrate the effect ethanol on oxidative stress parameters in brain of adolescent animals. Ethanol exposure significantly increased the lipid peroxidation ($t = 17.63$) and reduced the levels of total GSH ($t = 10.80$) in brain of adolescent rats. Administration of agmatine in doses 40 mg/kg and 80 mg/kg reduced the lipid peroxidation [$F (4, 25) = 97.60$, $p < 0.001$] and improved the antioxidant defense revealed by increased activity of GSH [$F (4, 25) = 39.19$, $p < 0.001$] in ethanol treated rats. Additionally, the levels of NO were significantly increased after acute ethanol exposure which was subsequently reduced after treatment with agmatine in dose dependent manner [$F (4, 25) = 56.92$, $p < 0.001$].

| Parameters | Control | EtOH | Agmatine | | |
|---|---|---|---|---|---|
| | | | 20 mg/kg | 40 mg/kg | 80 mg/kg |
| **Lipid peroxidation (nM/mg of brain tissue)** | 0.01249 ± 0.0002819 | 0.02534 ± 0.0005237$^{\#}$ | 0.02410 ± 0.0008603 | 0.02252 ± 0.0004161$^{**}$ | 0.02197 ± 0.0002461$^{*}$$^{**}$ |
| **Reduced glutathione (GSH) (mM/mg of brain tissue)** | 0.09565 ± 0.001614 | 0.05930 ± 0.002326$^{\#}$ | 0.05968 ± 0.003265 | 0.07113 ± 0.002572$^{*}$ | 0.07600 ± 0.001747$^{***}$ |
| **Nitrate/nitrite** | 92.60 ± 1.225 | 125.4 ± 1.720$^{\#}$ | 116.9 ± 0.7959$^{*}$ | 114.9 ± 1.032$^{**}$ | 101.4 ± 2.989$^{***}$ |

### 3.7. Agmatine modulate GABA/Glutamate levels in Adolescent rats administered with ethanol

Acute ethanol administration during the adolescent (PND 34- 41) reduced the GABA levels as compared with animals administered with saline [$t = 6.876$, $p < 0.001$]. The administration of agmatine in dose of 40 mg/kg ($p < 0.05$) and 80 mg/kg ($p < 0.01$) significantly increases the brain GABA levels in animals administred with ethanol [$F(4, 25) = 12.76$]. Acute ethanol administration during the adolescent period (PND 34- 41) increases the glutamate levels as compared with animals administered with saline [$t = 15.46$, $p < 0.001$]. The glutamate levels were decreased with agmatine treatemt. The administration of agmatine 40 and 80 mg/kg siginificantlly reduces the enhance glutamate levels as compared with ethanol treated adolescent rat [$F (4, 25) = 75.96$].

A one-way ANOVA followed by post hoc analysis using Sidak's multiple comparison test revealed there was no effect on low dose of agmatine 20 mg/kg on levels on GABA and glutamate in ethanol treated animals.

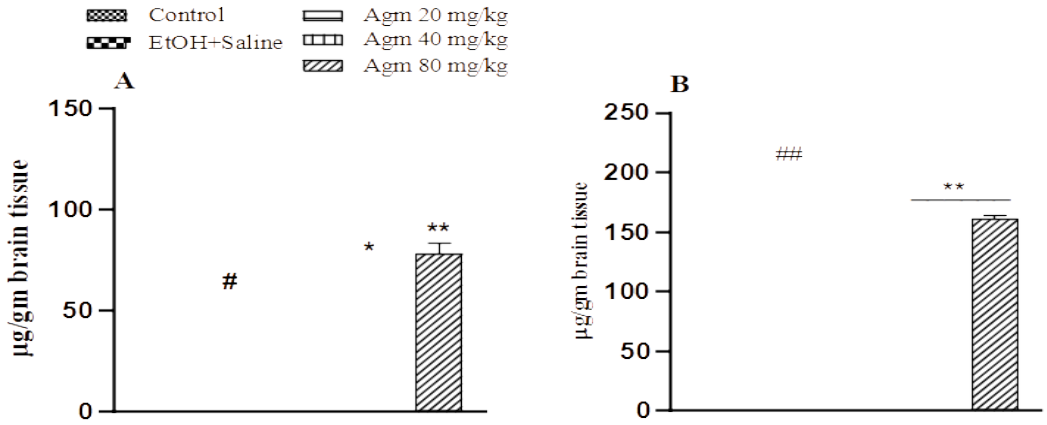


**Figure 6.** Effect of Agmatine (20, 40 and 80 mg/kg) on Brain GABA (A) /Glutamate (B) levels (µg/gm of brain tissue) in ethanol treated rats. Each values represents mean ± SEM (n = 6). $^{\#}p < 0.001$ vs. saline control animals, **p < 0.01 vs. ethanol withdrawal animals (One-way ANOVA followed by *post hoc* Sidak's multiple comparison test).

**Discussion**

In present study we examined the impact agmatine administration during ethanol exposure in adolescent animals. Our results reveal that acute ethanol administration resulted in significantly increased oxidative stress along with alteration in basal GABA and glutamate levels. Suggesting involvement of blunted oxidative defense after ethanol administration during behavioural alteration.

Number of literature demonstrated vulnerability of developing brain to ethanol induce toxic effect. Ethanol exposure during adolescence has higher susceptibility of dependence and addition. Acute ethanol exposure during adolescence in rats has been extensively studied to understand the immediate effects and potential long-term consequences of alcohol consumption during this critical developmental period. In our study we found that, acute ethanol administration has been associated with increased in motor activity evident by increased ambulation and rearing count which was normalized by treatment with agmatine (40 and 80 mg/kg). The present finding also suggested that ethanol exposure during adolescence has anxiolytic effect indicated by increased in number of entries and time spent in open arm. These results are in agreement with the previous findings indicating anxiolytic effect of ethanol (Varlinskaya & Spear, 2002, 2006a). In addition administration of agmatine also showed promising action in alleviating anxiety by modulating neuro-transmitters, reducing oxidative stress parameters, and exerting neuroprotective effects, which is consistant with our previous results highlighting anxiolytic effect of agmatine (Kale et al., 2020; Taksande et al., 2010b, 2010b, 2015).

In the present study, treatment with agmatine during the adolescent period attenuated ethanol induced memory impairment in rats. Agmatine treatment significantly improved the discrimination index in ethanol treated animals. Endogenous agmatine has been localized in hippocampus and involved in the process of learning and memory (Leitch et al., 2011; P. Liu & Bergin, 2009; Ping Liu et al., 2008; D. J. Reis & Regunathan, 1998; Donald J. Reis & Regunathan, 2000). Numerous research has also suggested increased agmatine levels in object recognition and water maze training in the hippocampus of animals (Aglawe et al., 2021a; Kotagale et al., 2018; P. Liu & Bergin, 2009). These results suggest involvement of hippocampal agmatine in inhibition of behavioural and cognitive deficits associated with adolescent ethanol exposure. Ethanol administration has been also associated with social inhibition (Varlinskaya & Spear, 2006b). In our study, we assessed impact of ethanol exposure on social behavior of animals. The results from social interaction study revealed that ethanol exposure during adolescence impairs sociability of animals and treatment with agmatine has been found to improve the social behavior in these animals.

The results above also suggest that alcohol exposure during adolescence has been also

associated with the depression-like behavior revealed with increased immobility time during forced swim task. Additionally, treatment with agmatine found to reduce the immobility in ethanol treated animals. These results are in agreement with our previous studies suggesting that agmatine possess antidepressant effect (Neis et al., 2015; Taksande et al., 2009; Zomkowski et al., 2002).

Studies showed that ethanol exposure has been associated with neurotoxicity in the hippocampus a brain region critically involved in spatial working memory. Recent research also suggests ethanol induced neurotoxicity is due to NMDA receptor hyperexcitability which might account for the cognitive deficits associated with ethanol exposure during early postnatal life (Littleton et al., 2001; Vetreno et al., 2011; von B. Ahern et al., 1994; Wilson et al., 2016). Such damage make adolescent animals particularly vulnerable to future insults to hippocampal function, including acute exposure to ethanol. In the present study, we estimated the levels of GABA and glutamate in rat brains, and we found significantly increased in glutamate concentration and reduced GABA levels in brain. Agmatine is known to inhibit the NMDAR via binding at the polyamine site (Gibson et al., 2002; Wang et al., 2006; Zhu et al., 2003). In present study we found that treatment with agmatine (40 and 80 mg/kg) increases the GABA level and reduces the elevated glutamate level in ethanol exposure rats. These results are in agreement with previous finding suggesting exogenous adminis-tration of agmatine attenuates glutamate induced neurotoxicity in cell cultures of rat cerebellum, hippocampus, and cortex (Gibson et al., 2002; Olmos et al., 1999; Wang et al., 2006; Zhu et al., 2003). These evidence attract more attention to the possibility of using agmatine, a glutamate blockers as therapeutic approach to treat ethanol associated behavioural and neurochemical alterations.

Acute alcohol exposures are well documented to increase the generation of reactive oxygen species (ROS). Many investigations have revealed a decreased level of antioxidants and increased production of free radicals in animals and humans following ethanol exposure (Bondy, 1992; J Haorah et al., 2005; Hernández et al., 2016; Sun et al., 2001). In present study, acute ethanol exposure significantly elevated the MDA level in the brain indicating enhanced lipid peroxidation. In addition we also found decrease in the GSH in the ethanol group that may be associated with ethanol induced oxidative stress (Pinto et al., 2014; Ramezani et al., 2012). Treatment with agmatine found to reduce oxidative stress via improving the antioxidant defense revealed by increased GSH levels in brain sample of ethanol treated animals. Additionally, treatment with agmatine protected the brain against nitrosative stress which was significantly elevated due to acute exposure of agmatine.

In conclusion, the results presented here suggests that acute ethanol administration during adolescence induces alteration in glutamatergic and GABAnergic neurotransmissions along with generation of oxido-nitrative stress. These changes could be alleviated by treatment with agmatine which might contribute to potential treatment against vulnerability of adolescents to ethanol abuse.

## 5. References


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