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Neuroprotective role of agmatine in prenatal acute ethanol exposure induce alterations in rats

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Abstract

The present study investigates the impact of agmatine, a neuromodulator with neuroprotective and anxiolytic properties, on behavioral changes associated with prenatal ethanol exposure in rats. The research focuses on the vulnerability of adolescents to alcohol-related problems and explores the potential link between prenatal alcohol exposure, anxiety, and adolescent alcohol use. The study also delves into the neurotoxic effects of ethanol on social behavior, cognitive function, and emotional regulation. The pregnant (GD12) Sprague Dawley rats were exposed to ethanol 2.5 g/kg, 20% v/v followed by a second i.p. injection of 1.25 g/kg ethanol and administered agmatine, along with its modulators, during adolescence. The results reveal that prenatal ethanol exposure induces behavioral changes such as increased locomotor activity, anxiety, social interaction deficits, and depression-like behavior. Agmatine administration, particularly at doses of 40 and 80 mg/kg, mitigates these effects, indicating its potential therapeutic role. Moreover, agmatine treatment improves recognition memory impaired by ethanol exposure and reduces oxidative stress, emphasizing its neuroprotective properties. In conclusion, the study suggests that agmatine holds promise in addressing the behavioural and neurochemical alterations induced by prenatal ethanol exposure during adolescence. The findings contribute to understanding the potential therapeutic capabilities of agmatine in mitigating the adverse consequences of early alcohol exposure on brain function and behaviour.

Keywords: Prenatal ethanol, Agmatine, Depression, Anxiety, Oxidative stress

Introduction

The initiation of alcohol use commonly commences during early adolescence, and this early onset significantly contributes to later alcohol-related problems. Early engagement in episodic heavy drinking is associated with an increased risk of alcohol dependence and enduring adverse psychosocial consequences (Grant et al., 2001;

Hingson et al., 2006). Notably, adolescent dependent on alcohol often exhibits heightened negative emotionality, characterized by elevated levels of anxiety, depression, and increased stress reactivity (Martin et al., 2000). Recent research suggests a potential link between prenatal alcohol exposure, anxiety, and adolescent alcohol use. While affective disorders, particularly anxiety, are prevalent in children with fetal alcohol spectrum disorder (FASD), studies indicate that anxiety is associated with alcohol use, abuse, and dependence during adolescence (Schmidt et al., 2007). Although not directly investigated in human studies, it is hypothesized that anxiety disorders, including social anxiety, in individuals with fetal alcohol exposure may contribute to early initiation of drinking during adolescence. Alcohol may become appealing to these individuals due to its perceived anxiolytic, calming, and stress-relieving effects. Moreover, prenatal alcohol exposure not only correlates with anxiety disorders but also induces alterations in various aspects of social behavior. Children and adolescents with fetal alcohol exposure struggle with considering consequences, understanding social cues, and communicating in social contexts (Thomas et al., 1998). Similarly, older individuals with FASD encounter difficulties in peer interactions. Consequently, alcohol may become attractive to these individuals due to its perceived ability to facilitate social interactions with peers (Lugo et al., 2003). Laboratory rodent studies also reveal sensitivity in the social behaviour of animals subjected to prenatal ethanol exposure, manifesting alterations in both adolescent-typical and adult-typical forms of social interactions (Hamilton et al., 2014). These modifications, when viewed collectively, have the potential to induce neurotoxic effects, leading to structural and functional adjustments in the brain. These transformations are often linked to prenatal exposure to ethanol during the crucial developmental phase, influencing cognitive function and emotional regulation.

Agmatine, an endogenous aminoguanidine compound synthesized from L-arginine by arginine decarboxylase has been projected as a neuromodulator in the brain (Halaris & Plietz, 2007; Laube & Bernstein, 2017). It has a multi-receptorial affinity and binds to α2-adrenoreceptors (Li & Mei, 1994), imidazoline binding sites (Raasch et al., 2001; Reis & Regunathan, 2000a), blocks N-methyl-D-aspartate (NMDA) receptors and inhibits nitric oxide synthase (NOS) (Auguet et al., 1995; Galea et al., 1996). Agmatine exhibits neuroprotective (M. Dixit et al., 2018; Kotagale et al., 2019), anxiolytic (Taksande et al., 2014), anti-depressant (Taksande et al., 2009), anti-compulsive (M. P. Dixit et al., 2014) and also inhibits the process of addiction (Kotagale et al., 2018a). Agmatine, a polyamine precursor, is known to inhibit the NMDAR via binding at the polyamine site (Gibson et al., 2002). Exogenous administration of agmatine attenuates glutamate induced neurotoxicity in cell cultures of rat cerebellum (Olmos et al., 1999), hippocampus (Wang et al., 2006), and cortex. Based on numerous studies, it is evident that Agmatine may have a potential role in addressing ethanol exposure and withdrawal induced complications. But, the exact mechanism involved in this treatment is yet to be investigated. The possible mechanism behind this can be its neuroprotective action, interaction with NMDA and imidazoline receptors. The present study seeks to investigate the therapeutic capabilities of agmatine, a powerful antioxidant, in addressing behavioural changes linked to prenatal ethanol intoxication in rats.

2. MATERIALS AND METHODS

2.1. Subjects:

Male and Female *Sprague Dawley* Rats were used. After the weaning period pups were grouped housed under a controlled temperature (22 ± 1°C), relative humidity (50 ± 5%) and maintained under a controlled 12/12 h light-dark cycle with free access to Food and water. All experimental procedures were approved by the Institutional Animal Ethical Committee and executed in strict accordance with the guidelines of Committee for the Control and Supervision of Experiments on Animals (CCSEA), Govt. of India.

2.2. Drugs:

Ethanol (95% w/v; Merck chemicals: Mumbai, India), Agmatine sulphate, L-arginine monohydrochloride and amino-guanidine hemisulphate (Sigma-Aldrich Co.; USA) were dissolved in saline (0.9%) and administered by intraperitoneal (i.p.) route. Behavioural experiments were carried out during the light hours between 9:00 AM and 1:00 PM. Doses and timings of injections were selected on the basis of previous and pilot experiments carried out in our laboratory (Aglawe et al., 2014; Taksande et al., 2010).

2.3. Experimental procedure

In the present study, Female Sprague Dawley Rats were mated during proestrus phase in a large plastic cage. Vaginal smears were collected every morning, with the first day of detectable sperm designated as gestational day (GD) 1. On GD12, the damps were divided in two groups, Ethanol (EtOH) and control group. Dams in ethanol group were injected intraperitoneally (i.p.) with 2.5 g/kg ethanol (20% v/v ethanol in physiological saline) followed by a second i.p. injection of 1.25 g/kg ethanol (2 h later). Control Dams were given two i.p.

injections of equivalent volumes of saline. The females in both the group were left undisturbed and allowed to have normal delivery. The pups were separated after weaning period of 21 days and pups having weight 16 to 35gm were used for further experiment (Diaz et al., 2016). After the weaning period pups from ethanol and saline treated dams were divided in following groups-

Control- Pups from saline treated dams were administered with saline (1 ml/kg, i.p.) on postnatal day 21 to 30.

EtOH- Pups from EtOH treated dams were administered with saline (1 ml/kg, i.p.) on postnatal day 21 to 30.

EtOH + Agmatine – pups from EtOH treated dams were administered with Agmatine (20, 40 and 80 mg/kg, i.p.) on postnatal day 21 to 30.

EtOH + L-arginine – pups from EtOH treated dams were administered with with L-arginine (60 mg/kg, i.p.) on postnatal day 21 to 30.

EtOH + Aminoguanidine – pups from EtOH treated dams were administered with with Aminoguanidine (50 mg/kg, i.p.) on postnatal day 21 to 30.

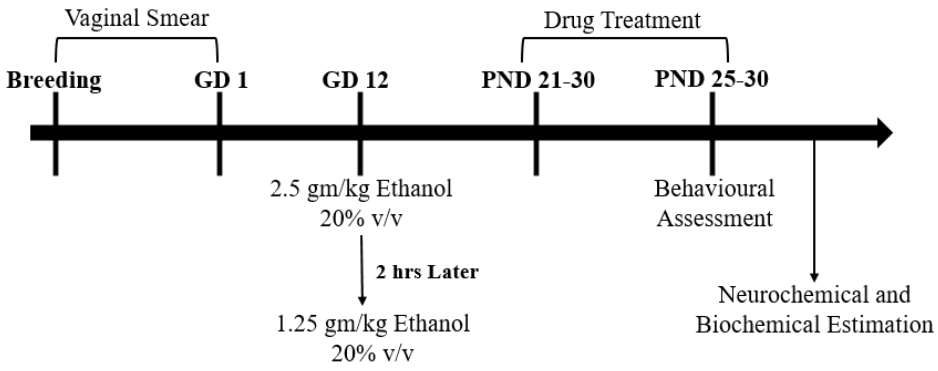

Figure 1: Schematic representation of experimental design

2.4. Behavioural Assessment

2.4.1. Open field test (OFT):

The open field test was used to assess the effect of acute administration of ethanol on locomotor activity of animals during adolescence period. Briefly, the animals were placed in an open field area (100 X 100 X 40 cm) with a square shape made of acrylic material illuminated with bright ligh (200 lux). For the assessment each rats were place in the centre of the apparatus and allowed to explore freely for 5 minutes. Following the experiment, a video camera mounted was used to assess squares crossed by the animal during the 5 min. The area was cleaned with 50% ethanol in between tests (Kim et al., 2011).

2.4.2. Elevated plus maze (EPM):

The plus-maze was used to assess the effect of acute administration of ethanol on anxiety-like behaviour of animals during adolescence period. The apparatus was consisted of a black Plexiglas with two open arms measuring 50 cm × 10 cm and two closed arms measuring 50 cm × 10 cm × 50 cm with an open roof, arranged so that the two open arms were across from one another (Pellow et al., 1985). At the beginning, each rat was placed in the centre facing towards one of the open arms. The total number of entries to the closed and open arms, the cumulative time spent were then automatically calculated by a video camera system for 5 minute (Saitoh et al., 2005).

2.4.3. Social interaction test:

The social interaction test was used to assess the effect of acute administration of ethanol on social behaviour of animals during adolescence period. The animals were allowed to interact in open box made up of white acrylic (120 x 120 x 60 cm). The social interaction test was conducted for 10 min under 40 W red lamp. Briefly, dummy partner receiving no medication same age, sex and weight were placed with the subject animals in the open space. For the period of 10 minutes the animals were assessed for (a) Following/chasing, (b) Anogenital interactions, for instance genital investigation, (c) Adjacent interactions: play-fighting, climbing over/under, adjacent laying, following, boxing, biting, grooming, sniffing, (d) Head-to-head interactions and touching or almost touching their faces with the untreated dummy partner were visually assessed and (c) Total social interaction: the total amount of time spent engaging in all of the aforementioned behaviors (Dandekar et al., 2008; Kokare et al., 2010; Seo et al., 2013).

2.4.4. Novel object recognition (NOR)

The Novel object recognition test was used to assess the effect of acute administration of ethanol on learning and memory of animals during adolescence period. The apparatus for the test consisted of closed plexiglass chamber (40 × 20 × 20 cm3). On first day of experiment, the animals were habituated with the open field for 5 minutes. On day 2, a training session of 10 min was conducted by two identical wooden objects (Size- 4 cm × 4 cm × 4 cm; Shape - building blocks resistance to biting demolition) in the open field positioned diagonally 6 cm away from the walls. On test day 6 (24 h later), animals were subjected to the recognition memory test by replacing one familiar object from training session with novel object of same size but having slight different shape, the individual mouse was allowed to explore one familiar and one novel object placed on the same diagonal positions for 10 min. Sniffing the object from a distance of 3 cm, touching the object with the nose and/or forepaws was considered as exploration. Discrimination index was calculated as exploration time with novel object/exploration time with familiar object + novel object.

2.4.5 Forced swim test (FST):

The forced swim test was used to assess the effect of acute administration of ethanol on depression-like behaviour of animals during adolescence period (Porsolt el al., 1977). Rats are exposed to the cylindrical tank (30 cm height X 20 cm diameter) for the first time during pre-test, the training phase for 15 minutes, followed by a second exposure for 6 minutes, 24 hours later. The duration of immobility were recorded during the test trial on day 2.

2.5. Biochemical Assessments

Following behavioral assessment, animals were euthanized by an overdose of pentobarbital sodium and brains were removed surgically. Brains were rinsed in isotonic saline and 10% (w/v) tissue homogenates were prepared in 0.1 M phosphate buffer (pH 7.4). The homogenates were centrifuged at 10,000 ×g at 4 °C for 15 min and the supernatant was used for oxidative stress parameter determination.

2.5.1. Lipid peroxidation

The thiobarbituric acid (TBA) reaction was used to estimate lipid peroxidation after acute prenatal exposure. Briefly, the brain supernatant was mixed with 2 volumes of trichloroacetic acid (TCA) and was reacted with 0.67% w/v TBA in a boiling water bath. The pink color pigment obtained after MDA formation was measured at 532 nm using a spectrophotometer (Wills, 1966).

2.5.2. Reduced glutathione (GSH)

The reduced GSH concentration in the sample was determined by method described by Ellman and colleagues. Briefly, 1 ml of tissue supernatant was digested and precipitated using 4% sulfosalicylic acid. After centrifugation at 1200 rpm for 15min the supernatant was reacted with 5,5-dithiobis 2-nitrobenzoic acid (DTNB) in 0.1 M phosphate buffer (pH 8). The yellow product so formed was read at 412nm using a UV-VIS spectrophotometer. The GSH was calculated using a molar extinction coefficient (1.36 l04 M cm-1) (Ellman, 1959; Luck et al., 1997).

2.5.4. Nitrate/nitrite

The nitrite levels in the supernatant was used as an indicator of the nitric oxide generation. An equal volume of brain supernatant and Griess reagent were mixed and incubated at 37°C for 15 minutes. The absorbance was recorded at 540 nm using a spectrophotometer and the nitrite concentration was calculated using the standard curve of sodium nitrite (Green et al., 1982).

2.8. Statistical Analysis

All values are stated as mean ± SEM (n = 6). One-way or Two-way analysis of variance (ANOVA) was used to define statistical significance with post hoc Tukey's comparison test. Two-way ANOVA followed by post hoc Tukey's comparison test were used to analyses the results of NOR. Values of $p < 0.05$ were considered statistically significant for all the tests.

3. Results

3.1. Effect of Agmatine and its modulators on locomotion activity in acute prenatal ethanol exposure

As shown in fig 2, One-way ANOVA followed by post hoc Tukey's comparison test revealed a significant increase in ambulations count in ethanol + saline treated animals as compared to control group ($p<0.0$). Treatment of Agmatine (20, 40 & 80 mg/kg i.p) group showed significant decrease in ambulations [F (6, 35)

= 8.804, P<0.0001], and rearing [F (6, 35) = 4.823, P=0.0011], as compared to ethanol + saline animals. Furthermore, significant reduction in ambulations and rearing was observed with L-arginine (P< 0.0001) and aminoguanidine (P=0.0171) treatment as compared with ethanol + saline treated group. Treatment with agmatien as well as modulators does not show any significant alterations in the grooming [F (6, 35) = 1.540, P=0.1943].

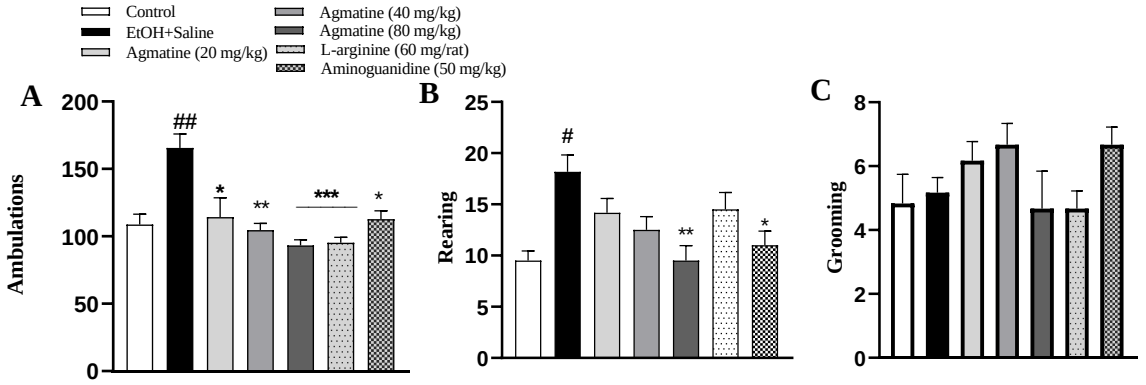

Figure. 2: Effect of Agmatine (20, 40 and 80 mg/kg) and it's Modulators (L-Arginine and Aminoguanidine) on Locomotor activity in rats. Each data point represent the mean of number of A) Ambulations, B) Rearing and C) Grooming ± SEM (n=6), #P<0.01, ##P<0.001 vs Control, *P< 0.01 vs Ethanol + saline, **P <0.01 vs Ethanol+ Saline, ***P <0.001 vs Ethanol+ Saline (one-Way ANOVA followed by post hoc Bonferroni's multiple comparison test).

3.2. Effect of agmatine and its modulators on anxiety like behaviour in acute prenatal ethanol exposure

As shown in figure 3, One-way ANOVA followed by post hoc Tukey's comparison test demonstrated that prenatal ethanol exposed animals showed a significant increase in number of entries in open arm as compared with control group (p<0.05). Treatment of Agmatine (80 mg/kg) group showed significant decrease in number of entries [F (6, 35)= 6.319,P<0.0001], and time spend in open arm [F (6, 35)= 8.467,P<0.0001], as compared to ethanol + saline treated group. Additionally, post hoc analysis also revealed treatment with Aminoguanidine (50 mg/kg) reduces number of entries (P=0.0197) and time spent (P= 0.0171) in open arm as compared to ethanol + saline treated group. No significant changes were observed in the number of entries (P=0.1033) in open arm with L-arginine treatment whereas its treatment effectively reduced the time spent in open arm [p<0.01] in ethanol + saline treated group. No significant changes were observed in the number of entries in close arm [p>0.9999] in ethanol + saline and Agmatine treated group.

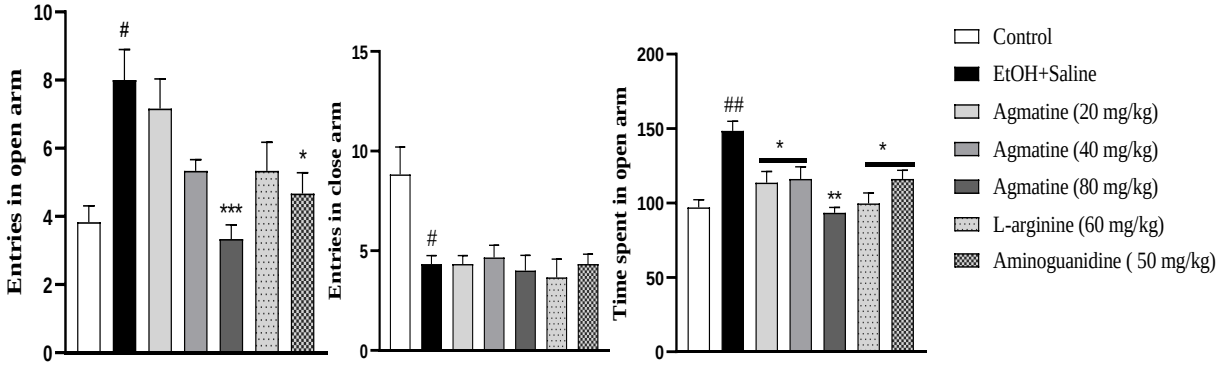

Figure. 3: Effect of Agmatine (20, 40 and 80 mg/kg) and it's Modulators (L-Arginine and Aminoguanidine) on Anxiolytic activity in rats. Each data point represent the mean of number of A) Entries in open arm B) Entries in close arm and C) Time spent in open arm ± SEM (n=6), #P<0.01 vs Control, ##P <0.001vs Control, *P <0.01, **P<0.001, ***P<0.0001 vs Ethanol + Saline. (one-Way ANOVA followed by post hoc Tukey's comparison test).

3.3. Effect of Agmatine and its modulators on Anti-social behaviour in acute prenatal ethanol exposure

As shown in figure 4, One-way ANOVA followed by post hoc Tukey's comparison test revealed that prenatal ethanol exposure showed a significant decrease in social interaction time during adolescent period as compared to saline control group ($p<0.001$). Treatment of Agmatine (40 and 80 mg/kg) and its modulators group showed significant increase in social interaction time in ethanol treated animals [$F(6, 35)= 9.371$, $P<0.0001$]. Treatment with low dose of agmatine (20 mg/kg) did not showed any improvement in social interaction as compared with ethanol treated adolescent rats ($P=0.8616$).

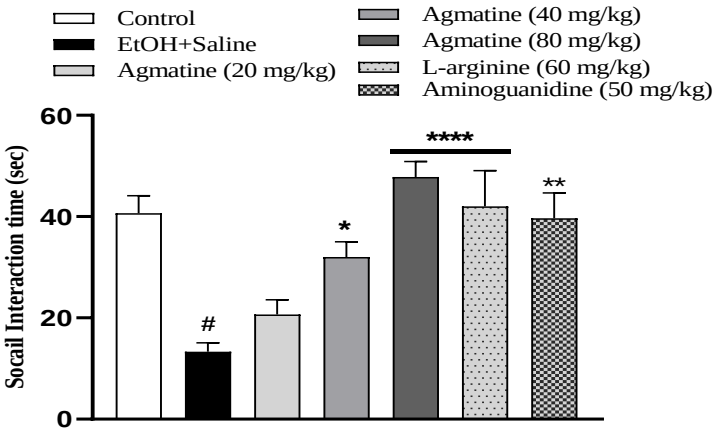

Figure 4: Effect of Agmatine (20, 40 and 80 mg/kg) and it's Modulators (L-Arginine and Aminoguanidine) on duration(sec) to assess Social interaction time in rats. Each data point represent the mean of Social interaction time ± SEM (n=6), #P<0.001 vs Control, *P<0.01, **P<0.001, ***P<0.0001 vs Ethanol+ saline (one-Way ANOVA followed by post hoc Tukey's comparison test).

3.4. Effect of Agmatine and it's modulators on recognition memory in acute prenatal ethanol exposure

As shown in Figure 5, Two-way ANOVA followed by *post hoc* Tukey's Comparison test demonstrates that no significant changes were observed in the Exploration time (sec) with familiar object with the animals prenatally exposed to ethanol as compared with saline treated animals which spent more time with the novel object compared with familiar object indicating novelty seeking behavior in control group ($p<0.01$). Thus results showed that the ethanol exposure during pregnancy alters the recognition memory and novelty behavior in adolescence period. The Exploration time (sec) with novel object was significantly increased by agmatine (20, 40 and 80 mg/kg), L-Arginine (60 mg/kg) and Aminoguanidine (50 mg/kg) [F_{Group} (1, 5) =58.23, p=0.0006, $F_{Treatment}$ (6, 30) =8.645, P<0.0001, $F_{Interaction}$ (6, 30) = 11.07, P<0.0001] as compared to Ethanol + Saline group. Additionally, One-way ANOVA followed by *post hoc* Tukey's Comparison test demonstrates a significant decrease in Discrimination index in Ethanol +Saline treated group as compared to control group ($p<0.0001$), which was increased by treatment of Agmatine (40 & 80 mg/kg), L-Arginine (60 mg/kg) [$F(6, 35) = 4.939$, $p<0.0001$] as compared to Ethanol + saline group.

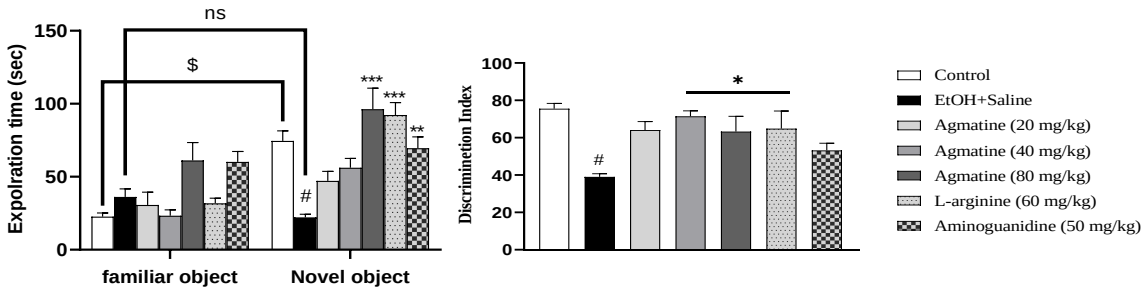

Figure 5: Effect of Agmatine (20, 40 and 80 mg/kg) and it's Modulators (L-Arginine and Aminoguanidine) on A) Exploration time (sec) and B) Discrimination index to assess inattention in rats. Each data point represents mean of Exploration time (sec) or Discrimination index + SEM (n=6). #P<0.001 vs control, *P< 0.01, **P<0.001, ***P<0.0001 vs Ethanol + Saline, $P<0.01vs control group for Familiar object (One way and Two way ANOVA followed by *post hoc* Tukey's comparison test).

3.5 Effect of agmatine and its modulators on depression-like behaviour in acute prenatal ethanol exposure

As shown in figure 6, One-way ANOVA followed by post hoc Tukey's Comparison test revealed that a significant increase in Immobility time in animals exposed to ethanol prenatally as compared to saline treated group indicating depressive like behaviour (P<0.001). Treatment of Agmatine and modulators showed significant decrease in Immobility time [F (6,35)= 6.038,P=0.0002] as compared to ethanol + saline treated group.

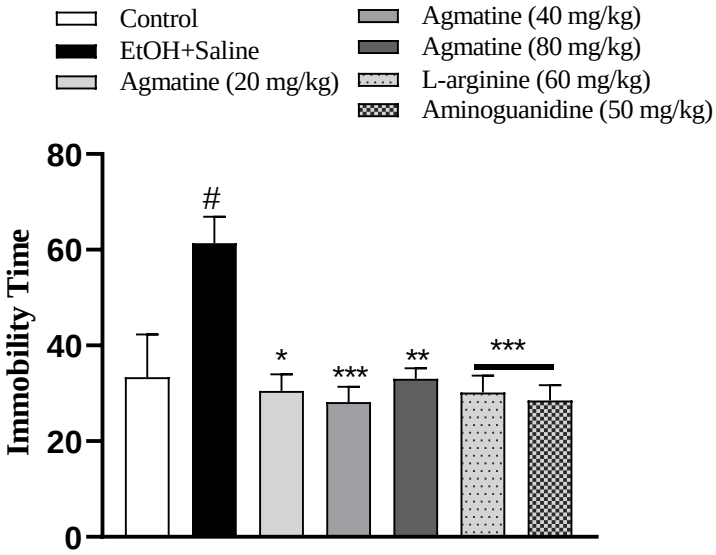

Figure 6: Effect of Agmatine (20, 40 and 80 mg/kg) and it's Modulators (L-Arginine and Aminoguanidine) on Immobility time in rats. Each data point represent the mean of Immobility time ± SEM (n=6), #P<0.001 vs Control, **P<0.001, ***P<0.0001 vs Ethanol+ saline (one-Way ANOVA followed by post hoc Tukey's comparison test).

3.6. Effect of Agmatine on Oxidative stress parameters

As shown in Table 1, One-way ANOVA followed by *post hoc* Tukey's comparison test demonstrates a significant increase in brain lipid peroxidation and nitrite level in Ethanol + Saline group as compared to control group (p<0.001) indicating increased oxidative stress due to exposure of ethanol during prenatal period, which was significantly reduced by treatment of agmatine (40 and 80 mg/kg) as compared to Ethanol + Saline group. Additional, agmatine improved antioxidant status in ethanol treated animals evident with increased GSH levsl in ethanol treated animals. However, treatment with low dose of agmatine (20 mg/kg) did not produced any significant alterations in oxidative stress and antioxidant levels.

Table 1: Effect of Agmatine on Oxidative stress parameters

Parameters	Control	EtOH	Agmatine		
			20 mg/kg	40 mg/kg	80 mg/kg
Lipid peroxidation (nM/mg of brain tissue)	0.01136 ± 0.0001769	0.02446 ± 0.0003577$^{\#}$	0.02410 ± 0.0006803	0.02267 ± 0.0005161**	0.02077 ± 0.0003461***
Reduced glutathione (GSH) (mM/mg of brain tissue)	0.08665 ± 0.001314	0.04903 ± 0.001364$^{\#}$	0.04968 ± 0.002365	0.07336 ± 0.002672*	0.07116 ± 0.002747***
Nitrate/nitrite	99.40 ± 2.225	134.4 ± 1.820$^{\#}$	120.9 ± 0.7959*	112.9 ± 1.032**	101.4 ± 2.989***

Discussion

This study aimed to investigate the impact of agmatine administration on prenatal ethanol exposed rats. Our findings indicate that acute ethanol exposure led to a significant increase in oxidative stress and alterations in basal GABA and glutamate levels. This suggests a potential connection between compromised oxidative defense and behavioral changes following ethanol administration. Existing literature underscores the vulnerability of the developing brain to ethanol-induced toxic effects, especially exposed to mother, which is characterized by heightened susceptibility to dependence and addiction. The study delves into the extensively

explored realm of prenatal acute ethanol exposure in rats, aiming to comprehend both immediate effects and potential long-term consequences of alcohol consumption during this critical developmental period. Our observations revealed that acute ethanol administration increased motor activity, as evidenced by heightened ambulation and rearing counts. Notably, treatment with agmatine (at doses of 40 and 80 mg/kg) normalized these effects. Furthermore, our study unveiled an anxiolytic effect of acute ethanol exposure, as indicated by an increase in the number of entries and time spent in the open arm. This aligns with previous findings that highlight the anxiolytic effects of ethanol. Encouragingly, administration of agmatine demonstrated a promising role in alleviating anxiety. This effect is attributed to agmatine's modulation of neurotransmitters, reduction of oxidative stress parameters, and exertion of neuroprotective effects. These outcomes resonate with our earlier research, which emphasized the anxiolytic effects of agmatine (Kale et al., 2020; Taksande et al., 2010, 2015).

In this study, we explored the potential of agmatine treatment during adolescence in mitigating ethanol-induced memory impairment in rats. Notably, agmatine administration significantly enhanced the discrimination index in ethanol-treated animals. The hippocampus, a region crucial for learning and memory, has been identified as the site for endogenous agmatine activity, as evidenced by previous research (Liu & Bergin, 2009; Reis & Regunathan, 1998, 2000b). Other studies have reported increased agmatine levels in the hippocampus during object recognition and water maze training (Aglawe et al., 2021; Kotagale et al., 2018b; Liu & Bergin, 2009), suggesting the potential involvement of hippocampal agmatine in mitigating behavioral and cognitive deficits associated with adolescent ethanol exposure. The impact of ethanol exposure on social behavior was also assessed, revealing impairments in sociability among animals exposed to ethanol prenatally. Intriguingly, agmatine treatment demonstrated an improvement in the social behavior of these animals. Furthermore, our results indicated that adolescent alcohol exposure was linked to depression-like behavior, as evidenced by increased immobility time during a forced swim task. Agmatine treatment significantly reduced immobility in ethanol-treated animals, aligning with previous studies highlighting agmatine's antidepressant effects (Neis et al., 2016; Taksande et al., 2009). Ethanol exposure's association with neurotoxicity in the hippocampus, a region critical for spatial working memory, was also explored. Studies have linked ethanol-induced neurotoxicity to NMDA receptor hyperexcitability, contributing to cognitive deficits associated with early postnatal ethanol exposure (Koretz et al., 1994; Littleton et al., 2001; Vetreno et al., 2011; Wilson et al., 2016). The impact of acute alcohol exposure prenatally and during adolescence on the generation of reactive oxygen species (ROS) is well-documented, with numerous studies demonstrating a decrease in antioxidant levels and an increase in free radical production in both animals and humans following ethanol exposure (Bondy, 1992; Haorah et al., 2005; Hernández et al., 2016; Sun et al., 2001). In our current study, acute ethanol exposure significantly raised brain MDA levels, indicating heightened lipid peroxidation. Additionally, a decrease in GSH levels in the ethanol-exposed group suggested ethanol-induced oxidative stress (Pinto et al., 2014). Agmatine treatment emerged as a protective strategy against oxidative stress, evidenced by the improvement in antioxidant defense, as indicated by increased GSH levels in the brains of ethanol-treated animals. Moreover, agmatine treatment demonstrated a protective effect against nitrosative stress, a consequence significantly elevated due to acute ethanol exposure. In conclusion, our findings suggest that acute ethanol administration during adolescence induces alterations in glutamatergic and GABAergic neurotransmissions, coupled with the generation of oxido-nitrative stress. Notably, agmatine treatment alleviates these changes, highlighting its potential as a therapeutic approach to counter the vulnerability of adolescents to ethanol abuse.

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