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PROTECTIVE EFFECTS OF AGMATINE ON PRENATAL ETHANOL-RELATED BEHAVIORAL DEFICITS

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Abstract:
Prenatal exposure to ethanol is known to cause significant behavioral deficits, often leading to long-term neurodevelopmental disorders collectively referred to as fetal alcohol spectrum disorders (FASD). This study investigates the therapeutic potential of agmatine, a polyamine derived from the amino acid arginine, on prenatal ethanol-induced behavioral deficits in offspring. Pregnant rats were administered 0 or 6 g/kg ethanol [20% (wt/vol)] in two divided doses and on weekends a single daily dose of 0 or 4 g/kg to induce developmental alteration similar to FASD, and agmatine was subsequently administered to offspring from PND 21 to PND 35 to evaluate its neuroprotective properties. Behavioral assessments, including tests for anxiety, and motor coordination, were conducted on the offspring. The results showed that prenatal ethanol exposure led to marked impairments in motor function as well as increased anxiety-like behaviors in offspring. However, offspring treated with agmatine exhibited significant improvements in these behavioral domains compared to the untreated ethanol-exposed group. Specifically, agmatine treatment was associated with reduced anxiety levels OFT, and better motor coordination on beam walking and rota rod apparatus. In conclusion, agmatine shows promise as a protective agent against prenatal ethanol-induced neurodevelopmental deficits. These findings highlight the therapeutic potential of agmatine for mitigating the adverse effects of prenatal alcohol exposure and improving behavioral outcomes in affected offspring.
Key words: Fetal alcohol spectrum disorder (FASD), Attention-deficit/hyperactivity disorder (ADHD), Agmatine, Anxiety, Motor coordination.

Introduction

Ethanol exposure during pregnancy is a well-documented risk factor for the development of a range of neurobehavioral abnormalities, collectively known as fetal alcohol spectrum disorders (FASD) [1,2]. These disorders are characterised by a variety of behavioral, cognitive and physical impairments. The neurotoxic impact of ethanol disrupts normal brain development, leading to long-term behavioral abnormalities that can persist into adulthood [3–5].

Prenatal ethanol exposure impairs neurogenesis and disrupts neural plasticity, leading to cognitive and behavioral deficits. Ethanol exposure during development period has been associated with excitotoxicity, involving excessive glutamate release, which overstimulates NMDA receptors, resulting in neuronal damage and death [6,7]. Additionally ethanol exposure induces oxidative stress by generating excessive reactive oxygen species (ROS). These ROS damage cellular components, leading to neuronal apoptosis [8,9]. Chronic neuro-inflammation is a hallmark of ethanol-induced neurotoxicity. Ethanol activates microglia and astrocytes, leading to the release of pro-inflammatory cytokines, which exacerbate neuronal injury [10,11].

Agmatine, a biogenic amine derived from the decarboxylation of arginine, has emerged as a promising neuroprotective agent [12]. It is well known for its capacity to modulate cell survival pathways, and a variety of neurotransmitter systems. Agmatine has been shown in earlier research to be effective in reducing the consequences of a range of neurological insults, such as neurodegenerative illnesses, ischemia, and neuro-trauma [13–15]. It reduces the production of pro-inflammatory cytokines and inhibits microglial activation, thereby decreasing neuro-inflammation and protecting neurons [14,16–18]. Agmatine can inhibit NMDA receptor activity, thereby reducing glutamate-induced excitotoxicity and protecting neurons from damage [19,20]. Agmatine exhibits antioxidant properties that help neutralize ROS, thus mitigating oxidative damage and enhancing neuronal survival [14,21–23]. It promotes neurogenesis by stimulating the proliferation and differentiation of neural progenitor cells. Its capacity to mitigate the neurobehavioral consequences of ethanol consumption during pregnancy is still not fully understood [13,24,25].

The potential benefits of agmatine in controlling alcohol addiction and withdrawal have long been acknowledged. According to research, agmatine can regulate how the central nervous system reacts to alcohol, which can lessen the cravings and the symptoms of withdrawal [26–32]. In our previous study we found beneficial effect of agmatine in gestational ethanol exposure. Administration of agmatine during early postnatal period effectively mitigates the neurobehavioral and cognitive deficits associated with ethanol exposure during pregnancy [33]. The study also highlighted that agmatine administration found to reduce neuro-inflammation and improved BDNF immunocontent in hippocampus. This study aims to investigate the protective effects of agmatine on prenatal ethanol-related behavioural deficits specifically focusing on the anxiety-like behaviour and motor impairment. In this study, we examined the behavioural abnormalities which are commonly disrupted by prenatal ethanol exposure, including anxiety and, motor coordination. Through these analyses, we aim to determine whether agmatine administration can normalize these behavioural parameters. Understanding the role of agmatine in this context could pave the way for novel therapeutic strategies to mitigate the long-term consequences of prenatal ethanol exposure.

2. Methodology:

2.1 Animals

The adult male and female Sprague-Dawley rats were employed and housed in pairs with animals of the same sex in a facility with regulated lighting (12/12 h light/dark cycle) and temperature (25 °C). Food and water were freely available to all animals. Institutional Animal Care (IAEC) of Smt. Kishoritai Bhoyar College of Pharmacy, Kamptee, Nagpur, India (853/1AEC/20-21/23) reviewed and approved all animal experiments, ensuring to the guidelines by the Committee for the Purpose of Control and Supervision of Experiments on Animals, Govt. of India (CPCSEA).

2.2 Drugs

Ethanol (Merck Chemicals; Mumbai, India) was administered through intragastric intubation. Agmatine Sulphate, L-arginine (Agmatine precursor), Aminoguanidine (diamine oxidase inhibitor), Arcaine (Agmatinase Inhibitor) (Sigma-Aldrich Co.; USA) were dissolved in saline (0.9%) and administered by intraperitoneal (i.p.) route. The period of time frame for conducting behavioral

studies was 9:00 AM–1:00 PM. Based on earlier studies, the dosages and timings of injections were used for behavioral testing

2.3. Prenatal treatment

Animals were housed under standard facility and female were paired and mated with male SD rats in cages overnight. The conformation of copulation was carried out through vaginal plug examination and presence of anestrus phase were regarded as gestation day (GD) 0. In order to mimic behavioural and cognitive deficits associated with ADHD, the ethanol was administered via intragastric intubation between GD9-20. Pregnant rats were administered with 0 or 6 g/kg ethanol (20% [wt/vol]) diluted in saline daily in two divided doses (6 h apart), except on weekends a single daily dose of 0 or 4 g/kg ethanol was administered [34]. The dams in control group were administered with the equal amount of sucrose solution (30% [wt/vol]) diluted in saline as isocaloric substitute for ethanol. After the delivery, the pups were weaned with mother for 3 weeks. In present experiment, the animal model differs from Aglawe et al., 2021, in which they administered ethanol in liquid modified diet [33]. As the unit for analysis was litter, thus single male litter from pre-treated damps were considered for further analysis so as to avoid litter effect. The offspring were separated from mother on post-natal day 22 and the male offspring were assigned to different treatment (n = 6 per group). Agmatine and modulators were administered intraperitoneally from PND 21 to PND 35 and different paradigm were performed using adult offspring.

2.4 Open field test

The open field test (OFT) was conducted following established protocols. Each rat was placed individually in a rectangular container (60×60×30 cm). The floor of the apparatus was divided into 16 squares (15×15 cm). Each rat was allowed to explore the arena freely for 5 minutes. The number of central zone crossings, the time spent in central and peripheral zones, and the frequency of rearing events to evaluate anxiety like behavior [35].

2.5 Beam walking test

Rats were assayed for motor coordination on PND 64 on beam walking test, which involved evaluating their ability to navigate a horizontal, narrow beam (2.3 cm–120 cm) at 50 cm above a foam-padded cushion. The rats had two minutes to move across the beam during the test. If they did not complete the task or if they fell off the beam, the trial was ended. The latency to cross the beam and the number of foot slips were recorded [36].

2.6 Rota rod

In the rota rod test, rats were placed on a rotating rod apparatus to assess motor coordination and balance (PND 66). The test was performed in two trials. On first day, training phase animals were placed on the apparatus and allow to maintain their balance on rotating rod at 16 rpm. On test day, rat were positioned on the rod, and the time they remained on it without falling was recorded 24 rpm. The apparatus was cleaned between trials to prevent scent marking [37].

2.7. Statistical Analysis

All values are stated as mean ± SEM. One-way and Two-way analysis of variance (ANOVA) with post hoc Sidak's comparison was used to define statistical significance. Values of $p < 0.05$ were considered statistically significant for all the tests.

3. Results

3.1. Effect of agmatine on anxiety like behaviour in OFT

As shown in fig. 1, the anxiolytic effects of agmatine was analysed using the OFT. One-way ANOVA revealed that preanatal ethanol exposure significantly affects the behavior of the animal in OFT as the EtOH exposed offspring spent significant less time in the central zone [$F(4, 25) = 60.64$, $p < 0.001$], while spent more time in periphery [$F(4, 25) = 9.205$, $p < 0.001$] and the number of

crossing were significantly reduced indicating anxiety like behavior [F(4, 25) = 20.59, p < 0.001]. Specifically, the ethanol-treated offspring rats spent a mean of 23.67±1.229 sec in the central zone, whereas control rats averaged 48±1.789 sec (p < 0.01). Rats treated with agmatine 40 and 80 mg/kg demonstrated a notable increase in time spent (t = 3.961 and t = 8.291) and number of entries (t = 3.398 and t = 5.227) in the central zone of the compared to the control group, indicating reduced anxiety levels. However, there was no significant differences in time spent in peripheral area and rearing count between the agmatine-treated and control groups.

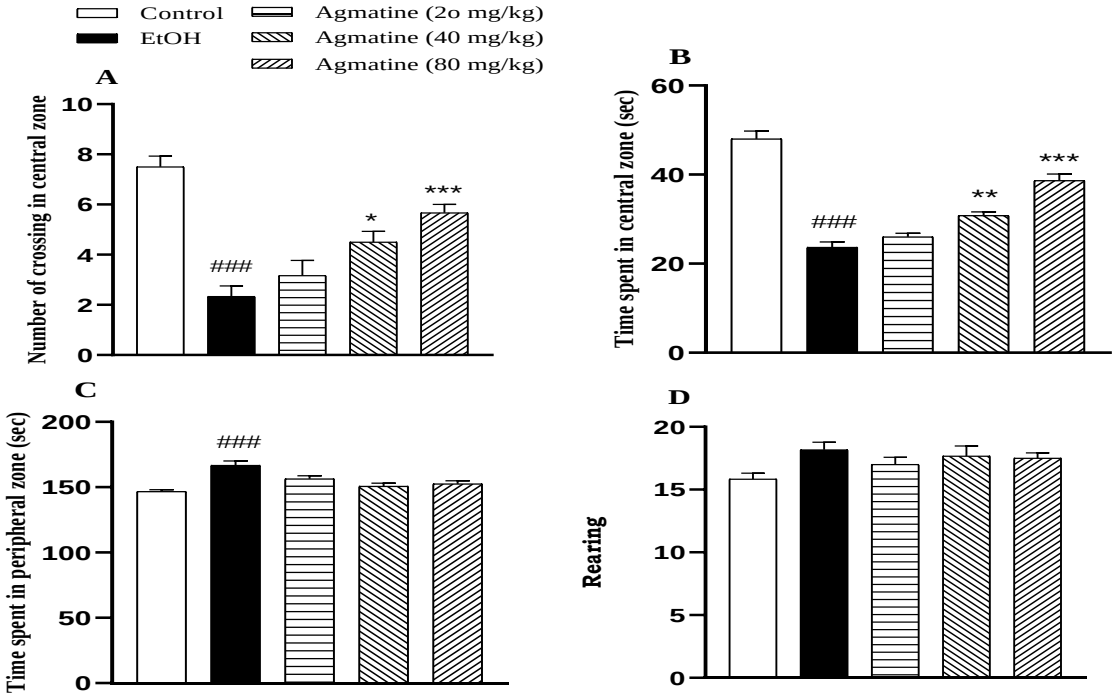

Fig. 1. Effect of prenatal ethanol exposure on anxiety-like behaviour in OFT. The results are expressed as mean ± SEM (n = 6). ###P < 0.001 compared to the control group; *P < 0.05, **P < 0.01, ***P < 0.001 compared to the EtOH (One-way ANOVA followed by *post hoc* Sidak's multiple comparison test).

3.2. Effect of agmatine on motor balance on beam walking test

The motor coordination in ethanol exposed offspring was assayed using the beam walk test. Prenatal ethanol exposure significantly alters motor coordination in beam walk tests indicated with significant increase in the number of foot slips (t = 7.474) and higher transfer latency (t = 4.056) compared to control offspring (p < 0.001). As shown in the fig.2, post hoc Sidak's multiple comparison tests revealed that administration of agmatine 40 and 80 mg/kg showed significant decreases in the number of foot slips [t = 4.130, t = 4.196, F (4, 25) = 14.41]and transfer latency [t = 3.244, t = 4.056, F (4, 25) = 5.592] in the ethanol exposed offspring.

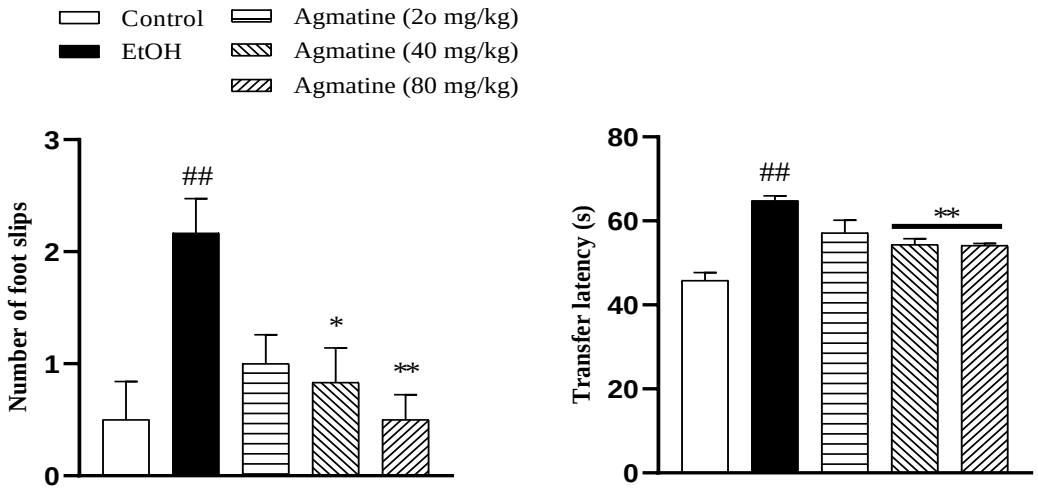

Fig. 2. Effect of prenatal ethanol exposure on balance in beam walking test. The results are expressed as mean ± SEM (n = 6). $^{\#\#}P < 0.01$ compared to the control group; $^{*}P < 0.05$, $^{**}P < 0.01$, compared to the EtOH (One-way ANOVA followed by *post hoc* Sidak's multiple comparison test).

3.3. Effect of agmatine on motor coordination on rota rod apparatus

As shown in fig 3, in addition to beam walking motor coordination on EtOH offspring were also assayed using rotarod apparatus. Two-way ANOVA revealed that prenatal ethanol exposure has significant altered fall latency in as compared with control rats (t = 8.229). In addition, *Post hoc* analysis showed that agmatine administration (80 mg/kg) significantly improved motor coordination by increasing the latency time in 2nd and 3rd trial, whereas treatment with 40 mg/kg agmatine was found to be effective in 3rd trial [$F_{Interaction} = 3.419$, $F_{Treatment} = 22.93$, $F_{Trials} = 13.02$] as compared to ethanol offspring.

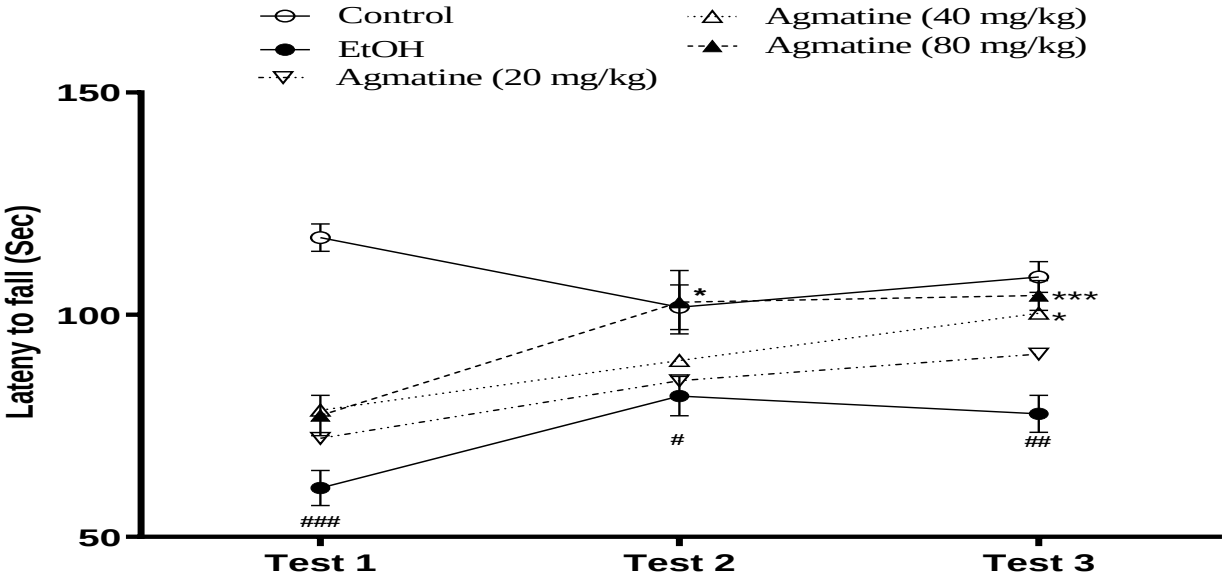

Fig. 3. Effect of prenatal ethanol exposure on motor coordination in rotarod apparatus. The results are expressed as mean ± SEM (n = 6). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ compared to the control group; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared to the EtOH (Two-way ANOVA followed by *post hoc* Sidak's multiple comparison test).

4. Discussion

The present study aimed to investigate the effects of agmatine on anxiety-like behaviors and motor coordination in offspring exposed to prenatal ethanol. Our findings demonstrate that agmatine significantly ameliorates the behavioral deficits induced by prenatal ethanol exposure, indicating its potential as a therapeutic agent for addressing neurodevelopmental disorders associated with prenatal ethanol exposure.

In our previous finding we revealed that prenatal ethanol exposure significantly alters the development in offspring and the behavioural alterations were persisted in adulthood. In the study treatment with agmatine was found to improve the behavioural outcomes in adult offspring through reducing the oxidative stress in brain [38]. The open field test results revealed that prenatal ethanol exposure significantly increased anxiety-like behaviors in offspring, as evidenced by a substantial reduction in the time spent in the central zone and a corresponding increase in the time spent in the periphery. These findings are consistent with existing literature that associates prenatal ethanol exposure with increased anxiety and altered exploratory behavior [39–42]. Administration of agmatine resulted in a notable increase in time spent and entries into the central zone, suggesting a significant reduction in anxiety levels. This anxiolytic effect of agmatine is likely due to its ability to modulate neurotransmitter systems and its neuroprotective properties [43]. This results are align with our previous findings indicating anxiolytic action of agmatine in ethanol addition and withdrawal condition [31,33,44].

Motor coordination deficits are another hallmark of prenatal ethanol exposure, as evidenced by increased foot slips and higher transfer latency in the beam walk test [45]. Our study found that ethanol-exposed offspring showed significant impairments in motor coordination, which were effectively mitigated by agmatine treatment. Specifically, agmatine administration at 40 and 80 mg/kg significantly reduced the number of foot slips and transfer latency. The rotarod test further supported these findings, demonstrating significant improvements in motor coordination and balance with agmatine treatment. Ethanol-exposed rats had significantly lower fall latency times, which were substantially increased by agmatine administration.

The neuroprotective effects of agmatine observed in this study can be attributed to its multifaceted mechanisms of action. Agmatine modulates various neurotransmitter systems, inhibits nitric oxide synthase, and interacts with imidazoline and α2-adrenergic receptors, all of which play crucial roles in maintaining neural integrity and function [12,27,28,46]. By mitigating excitotoxicity through NMDA receptor inhibition and reducing neuro-inflammation, agmatine helps preserve neural circuitry and promote neurogenesis, which are often disrupted by prenatal ethanol exposure [19,47].

In conclusion, our study provides compelling evidence that agmatine significantly reduces anxiety-like behaviors and improves motor coordination in offspring exposed to ethanol. These findings highlight agmatine's potential as a therapeutic agent for behavioral deficits associated with prenatal ethanol exposure. Future research should explore the long-term effects of agmatine treatment and its efficacy in clinical settings to further establish its role in mitigating the adverse outcomes of prenatal ethanol exposure.

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