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"A Systematic Review On Effect Of Agmatine In GBA For The Treatment Of Anxiety In Rats".

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ABSTRACT:

Accumulative experimental evidence has suggested an important involvement of gut microbiota in the modulation of host's immunological and neurological functions. The present study investigated the role of agmatine in dysregulation of gut brain axis with special reference to anxiety in rats. Experimental intestinal dysbiosis in rats was induced by daily intragastric introduction of 500 mg/kg of body weight Ampicillin for fourteen days. Male wistar rats (weight 200–250 g) were randomly divided into seven groups with 6 animals in each group. After fourteen days of antibiotic consumption, rats were administered with different doses of Agmatine (5, 10, 20, mg/kg) and lactobacillus clausii (0.25, 0.5, 1 ml/kg) intragastrically for consecutive three days. Group of rats received Agmatine along with lactobacillus clausii in sub effective doses for consecutive three days. combination of Agmatine (10mg) and lactobacillus (0.5ml) administered orally also shows Anxiolytic effect. If Agmatine and lactobacillus given orally the Agmatine level in brain was seen to be increase due to which dysbiosis induced anxiety was been prevented. It was also assume that the Agmatine level increase in the brain due to lactobacillus.

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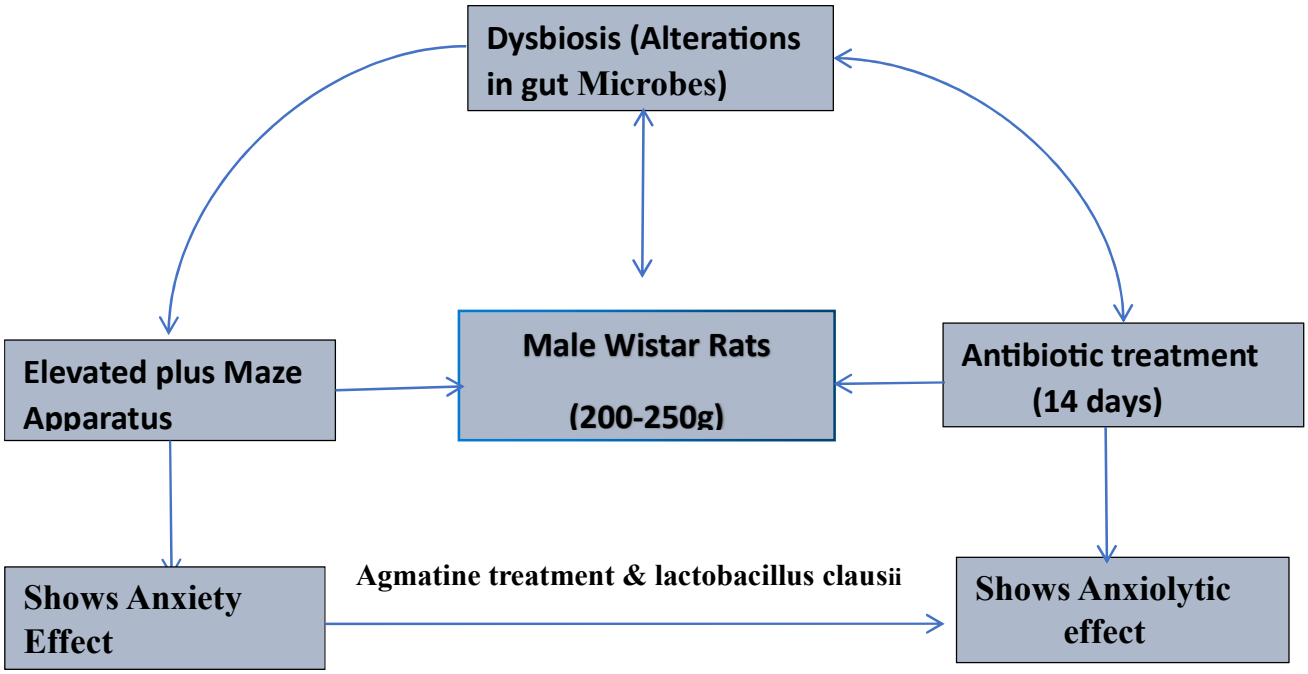
GRAPHICAL ABSTRACT:



KEYWORDS: Gut Brain Axis, Agmatine, Dysbiosis, Anxiety, Probiotics.

INTRODUCTION:

The GBA represents the bidirectional communication system between the central nervous system (CNS) and the enteric nervous system (ENS), linking the emotional and cognitive centers of the brain with peripheral intestinal functions. This connection is mediated through various channels, including neural pathways, endocrine signaling, immune responses, and biochemical messengers.

At the heart of this interaction lies the gut microbiota, the diverse community of microorganisms inhabiting the gastrointestinal tract. These commensal microbes play a pivotal role in maintaining the delicate balance of body homeostasis and are implicated in a wide array of physiological processes, ranging from metabolism to immune function.

The influence of the gut microbiota extends beyond the confines of the gastrointestinal tract, exerting profound effects on mental health as well. Emerging evidence suggests that alterations in the composition and diversity of gut microbiota, known as dysbiosis, may contribute to the

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development of various central nervous system disorders, such as autism spectrum disorders, anxiety, and depression. Moreover, dysbiosis has been closely linked to functional gastrointestinal disorders like irritable bowel syndrome (IBS), further underscoring the intricate interplay between gut microbiota and host physiology. Understanding the complex interrelationship between the gut microbiota and the brain holds great promise for novel therapeutic interventions aimed at restoring microbial balance and promoting overall health and well-being.

Gut is controlled by the autonomic nervous system (ANS) consisting of parasympathetic and sympathetic systems, and also by the local enteric nervous system consisting of the myenteric (Auerbach plexus) and Meissner's (submucosal plexus). Parasympathetic control of the CNS is through vagal nerve with efferent cholinergics acting on the myenteric plexus (motor movements) and Meissner plexus (submucosal glands secretions).

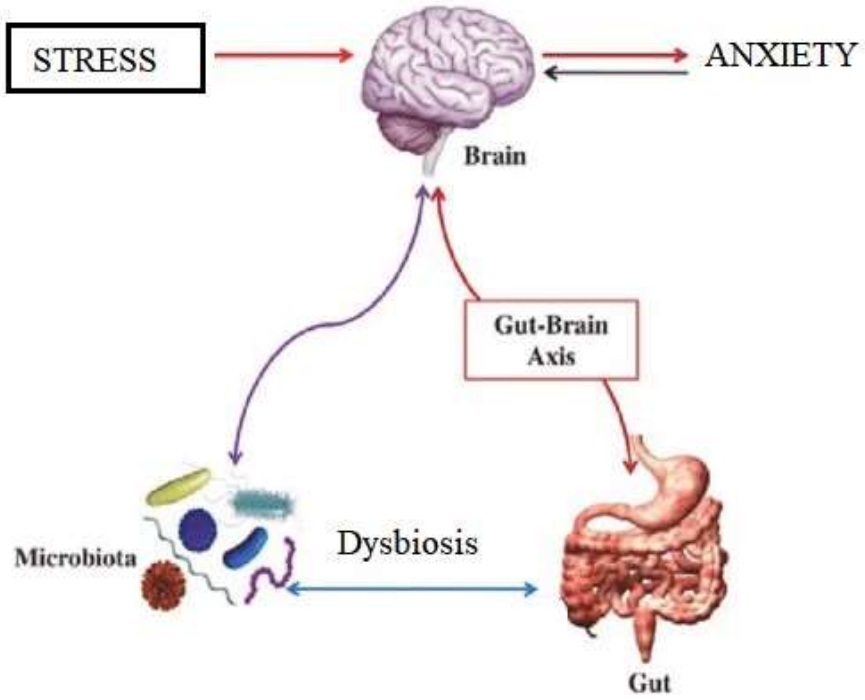

Fig.1:Bidirectional communication between gut and brain

MATERIAL AND METHODS:

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Animal

Adult male and female Wistar Rats (220-250 g) were used for protocol. They were housed in a temperature (22+1°C) and humidity- controlled (50+5%) environment with free access to food and water. All experimental procedures were carried out under strict compliance with Institutional Animal Ethical Committee (IAEC) according to guideline of the Committee for the Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India, New Delhi. Every possible effort was made to reduce the suffering of animal in all the experimental designs.

Drug and administration

Agmatine, Lactobacillus clausii (sporogenes), Ampicillin, Agmatine were purchased from Sigma chemicals, Agmatine (5, 10, 20, mg/kg), were dissolved in saline (0.9% w/v) and administered orally and Lactobacillus clausii (sporogenes) (0.25, 0.5, 1 ml/kg). Ampicillin (500mg/kg) was administered orally.

Rat model of antibiotic-associated dysbiosis

Experimental intestinal dysbiosis in rats was induced by daily intragastric introduction of 500 mg/kg of body weight Ampicillin for fourteen days.

Design Of The Study:

Male wistar rats (weight 200–250 g) were randomly divided into
seven groups with 6 animals in each group.
After fourteen days of antibiotic consumption, rats were administered with different doses of agmatine and lactobacillus clausii intragastrically for consecutive three days.
Group of rats received agmatine along with lactobacillus clausii in sub effective doses for consecutive three days.

Microbiological studies

Qualitative contents of the intestinal microbiota were determined in different periods of the experiment (before and after exposure to antibiotics and at the end of the experiment).
Changes in the gut microbiota were tested by bacteriological analysis of the fecal samples.
At the end of the experiment, fecal samples were taken from the different experimental group.

These samples were diluted in phosphate buffer and further diluted in $10–10^{-9}$ times employing method of serial dilutions using distilled water.

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Preparation of serial dilution:

1ml of bacterial suspension was added in 9ml of distilled water (Stock solution).

Like that prepared another 9 test tube with 9ml of distilled water and autoclaved at 121psi for 15 min.

Then repeated by bacterial suspension 1ml of newly prepared solution was added into 2^{nd} test tube.

Bacterial suspension and resuspension continues in this manner until the final tube was reached, diluting the stock solution by a factor (10^{-1} to 10^{-9}).

Ten agar media plates were prepared for each serial dilution then with the help of spreader spread overall bacterial suspension on agar media and incubate up to 24 hrs. At 37ºC.

After 24hrs. Three types of colonies were found i.e. regular shape, Irregular shape, and tiny colony (small).

For colony isolation regular, irregular, and tiny colonies were selected.

After that one way streaking method was performed with loop full of culture was taken on inoculating needle and streak on agar media then incubates up to 24 hrs. At 37ºc. After 24hrs. Results were observed.

After observation four ways streaking was performed with taking one loop full of culture from one way streaking plate and streak on another agar media plate and again incubate for 24hrs at 37ºC.

After that biochemical method (IMVIC) was performed for the confirmation of microorganism.

Level of micro-organism in gut was estimated by using colony forming unit (CFU) procedure. And all the procedure wasperformed aseptically.

Colony Forming Unit

Five ml of Bacterial Culture was added to 45 ml of sterile diluents. From this suspension, two serial, 1/100 dilutions were made, and 0.1 ml was plated onto Plate Count Agar from the last dilution. After incubation, 137 colonies are counted on the plate. Calculate CFU/mL of the original Sample.

Formula: cfu/ml = (no. of colonies x dilution factor) / volume of culture plate

We monitored for the presence and quantity of bacteria that have been identified in our earlier studies as marker bacteria undergoing significant changes under the influence of Metronidazole and Ampicillin.

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It was determined that bacteria belonging to the genera Lactobacillus, Enterococcus, Escherichia, and Klebsiella under the following selective and differential diagnostic culture media were used for the bacteriological studies: MacConkey's agar, and Blaurock medium (Nutrient medium, Russia).

After enumeration of the colonies on the agar plates, three to four colonies presenting different microscopic appearances were analyzed.

These different morphotypes were isolated and submitted to microscopic examination.

Microscopic examinations were done by way of the gram stain procedure of pure cultures of bacteria.

Elevated Plus Maze:

The elevated plus maze were used for assessing anti-anxiety effects of drugs. Rats were placed at the junction of the four arms of the maze, facing an open arm, and entries/duration in each arm were recorded for 5 min. (Walf, A. A. et.al., 2007) The EPM apparatus was shaped like a plus sign in wood, with two unwalled (open) arms. The maze was elevated to a height of 70 cm. Maze area was provided by room light illumination and the rat's behavior was recorded on video-tape as to avoid the presence of investigators inside the room. During test, each rat was placed at the center of maze facing one enclosed arm. All entries on open or closed arms were scored for 5 min and total time spent on each arm was recorded. An entry was defined by placing all four paws into an arm and no time was recorded when the animal was in the center of maze. Rats falling from the maze were excluded from the study.

Statistical analysis

The time spent into open and enclosed arms of EPM, the number of entries into each arm and total number of crossings, rearing responses and fecal bolli on open field were analyzed by one-way analysis of variance (ANOVA), followed by a post hoc Bonferroni Multiple Comparison test. For analysis of latency to leave the first square of open field we used the Kruskall/Wallis test. Significant differences were considered when PB/0.05; data are presented as mean 9/S.E.M. or median and interquartile range.

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DISCUSSION:

The aim of this study was to investigate the role of agmatine in Gut- Brain-Axis (GBA) signaling in context of anxiety disorder. The present study demonstrated the effect of agmatine and probiotic on dysbiosis induced anxiety in rats using EPM. We found that acute treatment of agmatine and probiotic by oral route increased open arm entries and time spent into open arm and indicate of an anti-anxiety like property.

Based on available research and clinical data, it is believed there are several causes of intestinal dysbiosis: Putrefaction (the result of changes in diet); Fermentation dysbiosis resulting from inefficient host digestion; Deficiency dysbiosis, which is often caused by antibiotic exposure; sensitization dysbiosis which is the result of abnormal immune responses caused by an alteration of the normal intestinal flora(Elena et al. 2013). In this research study we found that consumption of Ampicillin caused significant changes in intestinal microbiota (dysbiosis) and Microbiologically, an increase in the numbers of putative opportunistic bacteria and decrease in concentrations *Lactobacillus spp.*, and *Enterococcus spp.* in the intestines were determined.

The microbiome plays an essential role in maintaining gastrointestinal health, but also broadly impacts brain function and behavior via bidirectional communication between the brain and gut microbe communities (Sylvia and Demas, 2018; Winter et al., 2018). changes in intestinal microbial communities have been linked to a variety of neurological and psychiatric disorders including Alzheimer's disease, Parkinson's disease, Autism, Anxiety disorders, and Depression (Dinan and Cryan, 2012; Valles-Colomer et al., 2019).

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In present study we found that dysbiosis induced anxiety and this was confirmed by using animal model (Elevated Plus Maze). The EPM test was conducted as described (Cohen et al., 2015) in a black Plexiglas EPM consisting of four elevated arms (70 cm from the floor, 45 cm long, 12 cm wide) arranged in a cross. Two opposite arms are enclosed by 45 cm high walls (lighting 3–5 lux), and the remaining arms are open (lighting 30 lux). To start the test, rats were individually placed in a central square platform facing the same closed arm at the intersection of the open and closed arms provided access to all arms. The latency to enter the open arms, the amount of time spent in the open arms, closed arms, and center square, and the total distance traveled over the course of the 5 min test were recorded. An animal was considered to be in the open arm when the rat's body fully crossed out of the center square onto an open arm platform maze). Antibiotic administration cause significant decrease into no. of open arm entries and % time spent into open arm in elevated plus maze.

In the present study agmatine was administered orally in dose dependence manner (5, 10 and 20mg/kg) in dose 10mg/kg and 20mg/kg oral significantly reduced anxiety like behavior in dysbiosis rats. Likewise lactobacillus and their combination with agmatine were administered orally these also show reduced anxiety like behavior in rats. On the basis of microbial estimation of gut by fecal matter we also analyzed the effects on the possible changes of gut microbiota and shows reduce abundance of lactobacillus. The GIT is inhabited by an intensive population of organized and highly specialized microbial flora that is a key determinant of health and disease (Petrof and Khoruts; 2014).

Compositional and functional changes in commensal microbiota are thought to be involved in the pathogenesis of many diseases. In this study, our observations revealed that dysbiosis reduced the relative abundance of Lactobacillus, (Hui-wen Xiao; 2018). It has been shown that microbiota play a pivotal role in this process and affect central neurochemistry and behavior. We also observed that in dysbiosis animal it decreases the concentration of agmatine in brain as compared to control group with the help of HPLC method. After treatment with agmatine, lactobacillus and their combination shows significantly increases the agmatine concentration in brain. In conclusion, Anxiety associated with reduction of lactobacillus level in GI tract and brain agmatine level in dysbiosis induced anxiety. Oral agmatine and lactobacillus administration increases brain agmatine level which might be responsible for reduction in anxiety

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and shows agmatine and probiotics play an important role in restoration of dysregulated gut-brain-axis in dysbiosis induced anxiety.

SUMMARY AND CONCLUSION

In view of this background, the present study investigated the role of Agmatine in dysregulation of gut brain axis with special reference to Anxiety in rats using EPM paradigm and microbial estimation. Adult wistar rats (weight 200–250 g) were randomly divided into seven groups with 6 animals in each group. After fourteen days of antibiotic consumption, rats were administered with different doses of Agmatine and lactobacillus clausii intragastrically for consecutive three days. Group of rats received Agmatine along with lactobacillus clausii in sub effective doses for consecutive three days. Agmatine was administrated in dose dependent manner (5, 10 and 20 mg/kg, oral), administration sessions at 9 am and 4 pm. The administration of Agmatine (10 mg/kg and 20 mg/kg, oral) significantly Agmatine exerted anxiolytic action in rats. Lactoctobacillus 0.5ml/rat and their combination with Agmatine also shows anxiolytic effect and increase level of lactobacillus in gut and agmatine concentration in brain and exerted anti- anxiety effect .In dysregulation of gut brain axis lactobacillus level reduce (Hui-wen Xiao 2018) and it is identified microbial estimation by fecal matter. Dysbiosis associates reduce lactobacillus level in GI tract and reduction of brain Agmatine in dysbiosis induced anxiety in rats. If Agmatine and lactobacillus given orally the Agmatine level in brain was seen to be increase due to which dysbiosis induced anxiety was been prevented. It was also assume that the Agmatine level increase in the brain due to lactobacillus. Thus, Agmatine and Probiotics play an important role in restoration of dysregulated gut-brain-axis in dysbiosis induced- anxiety.

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