RESEARCH PAPER

Genistein Influences Behavioral and Neuroendocrine Responses in an Antibiotic-Induced Physiological Stress Model

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ABSTRACT

Background and aim: The global prevalence of mental health issues, predominantly depression and anxiety, affects over a billion people and has socioeconomic implications. Research indicates the microbiota-gut-brain (MGB) axis as a key communication pathway that can be disrupted by antibiotics, leading to physiological stress and behavioural changes. This study examined the therapeutic potential of Genistein, a compound with antioxidant and neuroprotective effects, in reducing stress responses caused by antibiotic treatment.

Materials and Methods: Using juvenile female Wistar rats, gut dysbiosis was induced through a 14-day course of Amoxicillin. Behavioural outcomes were assessed through the sucrose preference test (SPT), forced swim test (FST), and tail suspension test (TST), while neuroendocrine activation was measured by serum cortisol levels. Amoxicillin administration increased behavioural despair, demonstrated by longer immobility in both the FST and TST.

Results: The antibiotic treatment caused a rise in serum cortisol (79.19 ng/ml compared to 28.44 ng/ml in controls), indicating hyperactivity of the primary stress response system. The subject showed no significant decrease in sugar water preference. Genistein treatment effectively mitigated these responses, reducing immobility times and lowering cortisol levels to 54.2 ng/ml. These protective effects were similar to those of the standard antidepressant Fluoxetine. The findings indicate that Genistein functions as an effective agent for stress mitigation, effectively stabilising the MGB axis.

Conclusion: This research paves the way for interventions to reduce neuropsychiatric risks associated with early-life exposure to antibiotics. Furthermore, it supports the incorporation of both behavioural and biochemical markers in studies exploring the gut-brain axis.

Keywords: Microbiota-gut-brain axis, Gut dysbiosis, Antibiotic – induced stress, Amoxicillin, Genistein, Neuroprotective effects, Behavioural despair.

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1. INTRODUCTION

Worldwide, depression and anxiety are the most common and disturbing psychiatric disorders contributing to the overall burden of disease [1]. Estimates suggest that more than one billion individuals are affected by mental health conditions, particularly anxiety and depressive disorders [2]. This disease imposes substantial socio-economic costs, with productivity losses from depression and anxiety estimated expenditure of $1 trillion annually [3]. Furthermore, studies demonstrate that females are excessively affected, being more susceptible to developing depression and anxiety, particularly in stressful situations. Although the pathophysiology of depression is complex, it underscores the serious role of the microbiota-gut-brain

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(MGB) axis in its development and manifestation [4]. This bidirectional signalling system connects the gut microbiota, the digestive tract, and the central nervous system, affecting both physical and mental health. Gut dysbiosis, an imbalance in the gut microbiota, is recognized as a key factor that may exacerbate symptoms of anxiety and depression [5].

The widespread use and misuse of broad-spectrum antibiotics, such as beta-lactams, have been shown to alter the diversity and structure of the intestinal flora [6]. This antibiotic-induced dysbiosis can interrupt MGB axis communication by changing the levels of neuroactive bacterial metabolites, such as short-chain fatty acids (SCFAs), and by interfering with hypothalamic-pituitary-adrenal (HPA) axis regulation [7,8]. The preclinical studies in rodent models have connected antibiotic-induced gut dysbiosis to the development of depressive and anxiety-like behaviours [9]. Despite the availability of multiple pharmacologic treatments, a generous proportion of patients fail to achieve a complete response, leading to the designation of Treatment-Resistant Depression [10]. Moreover, the slow onset of action and poor tolerability profile, as well as side effects such as weight gain and sleep disorders, severely limit therapeutic compliance and efficacy. This requires an urgent search for novel therapeutic agents with a favourable efficacy and tolerability profile, potentially by targeting emerging pathways such as the MGB axis.

Genistein, a primary isoflavone derived from soybeans, has diverse pharmacological effects, including antioxidant and neuroprotective properties. Recent research has shown the behavioural effects and anxiolytic potential of genistein in various rodent preclinical models, with mechanisms suggesting it influences gut microbiota composition [11]. Moreover, genistein has been found to modify central nervous system signalling pathways, particularly by increasing brain-derived neurotrophic factor expression [12]. The increased vulnerability of females to depression points to the possibility of a neuroprotective agent like genistein. Therefore, this study aims to investigate whether gut dysbiosis induced by Amoxicillin leads to measurable physiological stress responses in juvenile female Wistar rats. Additionally, it will evaluate the potential of Genistein as a neuroendocrine modulator that may reduce hyperactivity of the HPA axis. This research examines the relationship between physiological stress activation and behavioural outcomes within a stress model associated with gut dysbiosis.

2. MATERIALS AND METHODS

This study was designed as an experimental interventional study to investigate the neuroendocrine modulation effects of Genistein against antibiotic-induced physiological stress in juvenile female Wistar rats. The study was conducted at the Central Animal House, Aarupadai Veedu Medical College and Hospital, Puducherry. All experimental procedures were reviewed and approved by the Institutional Research Committee (IRC) and the Institutional Animal Ethical Committee (IAEC) with IAEC no. AVMC/IAEC/2024/06/01/01.

2.1 Experimental Animal Models

A total of 24 juvenile female Wistar rats (4 – 6 weeks old), weighing between 100g and 120g, were procured from CCSEA-approved breeders. The animals were housed in a controlled laboratory environment with a regulated temperature of 22 ± 2°C. They experienced a 12-hour light/dark cycle, with lights on at 7:00 AM, and humidity levels were carefully monitored to ensure their well-being. They had ad libitum access to standard pellet feed and distilled drinking water. The animals underwent a 7-day quarantine period, followed by a 7-day acclimation period, before the initiation of the experimental protocol.

2.2 Drugs and Chemicals

The study used Amoxicillin to induce gut dysbiosis and test the effects of Genistein on neuroprotection, with Fluoxetine as a control. All compounds were administered orally, and animals received a standard diet and water for health maintenance.

2.3 Experimental Design and Grouping

The study was conducted over 14 days. The 24 juvenile female Wistar rats were randomly allocated into four groups (n = 6 rats per group) after acclimatisation. All drugs were administered through the oral route daily for 14 consecutive days (Day 1 to Day 14). The experimental grouping was summarized in Table 1.

Table 1: Experimental Grouping and treatment strategy for evaluating behavioral and neuroendocrine effects in a dysbiosis model

Group	Treatment	Rationale
Group I (Control)	Vehicle (DMSO 0.5% in 99.5% Distilled Water)	To establish baseline behaviour and physiologic parameters.
Group II (Disease Model)	Amoxicillin (50mg/kg/day)	To induce gut dysbiosis-associated physiological stress
Group III (Positive Control)	Amoxicillin (50mg/kg/day) + Fluoxetine (12mg/kg/day)	To validate the sensitivity of the pharmacological reference compound (Standard antidepressant).
Group IV (Test Group)	Amoxicillin (50mg/kg/day) + Genistein (10mg/kg/day)	To investigate the behavioural and neuroendocrine effects of Genistein.

2.4 Behavioural and Biochemical Parameters
All behavioural and biochemical parameters were assessed at two time points: Day 0 (Baseline) and Day 14 (Post-treatment). The observers conducting the behavioural tests were blinded to the treatment groups to minimise bias.

2.4.1. Sucrose Preference Test (SPT)
The SPT evaluates anhedonia, which is a fundamental symptom of depression. Rats were offered a choice between two bottles: one filled with a 1% sucrose solution and the other with distilled water, for a duration of 24 hours. The amount consumed (in grams) of both the water and sucrose solution was determined by weighing the bottles before and after 24 hours. Sucrose Preference (%) was calculated using the following formula:

$$\text{Sucrose Preference (\%)} = \frac{\text{Sucrose Intake}}{\text{Sucrose Intake + Water Intake}} \times 100$$

A decrease in sucrose preference indicates anhedonia.

2.4.2 Forced Swim Test (FST)
The Forced Swim Test (FST) evaluates behaviours associated with immobility or passive coping strategies in rodents. In this assessment, rats are individually placed in a glass cylinder measuring 40 cm in height and 18 cm in diameter, filled with 15 cm of water maintained at a stable temperature of 25°C. The total duration of immobility is recorded over a five-minute testing period. Immobility is defined as remaining afloat without active struggle, engaging only in minimal movements necessary to keep the head above the water surface. A decrease in immobility duration is interpreted as an indicator of enhanced active behaviour or escape-oriented responses.

2.4.3 Tail Suspension Test (TST)
Rats were suspended 50cm above the ground on a horizontal rod by fixing their tail with adhesive tape approximately 2cm from the tail tip. The entire experimental period was 6 minutes, and the activity for the final 4 minutes was recorded. Immobility time (seconds) was defined as the time the rat remained completely motionless, having given up struggling. A reduction in immobility time suggests a decrease in immobility behaviour.

2.4.4 Physiological and Biochemical Parameters
Individual rat body weight was measured using a standard laboratory balance on Day 0 and Day 14. Blood samples were collected from each rat on Day 0 and Day 14 under mild anaesthesia. The serum was separated, and serum cortisol levels were measured using a chemiluminescent Immunoassay (CLIA) kit. Elevated cortisol levels are indicative of chronic stress and activation of the HPA axis.

2.5. Statistical Analysis

Data were compiled and examined using SPSS software (Version 29). A One-Way Analysis of Variance (ANOVA) was performed to compare means among the four experimental groups, followed by a post-hoc Dunnett's test for comparisons between the Amoxicillin-induced model group (Group II) and the treatment groups (Group III and Group IV). A p-value of less than 0.05 ($p < 0.05$) was set as the threshold for statistical significance.

3. RESULTS

3.1 Sucrose Preference Test (SPT)
In behavioural assessment using the Sucrose Preference Test (SPT), there was no significant difference in consumption percentage among the groups, indicating that no anhedonia was induced in this model. The control group exhibited a high baseline preference for sucrose at 86.95 ± 3.39%, while the Amoxicillin-treated disease model group showed a nearly identical preference of 84.52 ± 0.89%. Although the Amoxicillin + Genistein group recorded a numerically lower mean preference of 67.75 ± 5.55%, statistical analysis confirmed that these differences were not significant compared with the control or disease groups ($p \geq 0.05$) (Table 2). This numerical shift was driven by greater variability among animals within the group, reflecting idiosyncratic responses to the treatment vehicle or individual taste sensitivity, rather than a consistent depression-related behaviour. These findings suggest that the juvenile female Wistar rats maintained their reward-seeking behaviour despite the antibiotic intervention.

Table 2: Effect of Amoxicillin and in combination with Fluoxetine or Genistein on Sucrose Preference Test (SPT)

Groups	Sucrose preference test (%)
Control	86.95 ± 3.39
Amoxicillin	84.52 ± 0.89
Amoxicillin +Fluoxetine	74.48 ± 11.73
Amoxicillin+Genistein	67.75 ± 5.55

3.2 Forced Swim Test (FST)
The Forced Swim Test was conducted to assess the potential of Genistein and Fluoxetine to reduce physiological stress and to induce gut dysbiosis-associated stress that could lead to behavioural effects caused by Amoxicillin in juvenile female Wistar rats. One-way ANOVA revealed a statistically significant difference in immobility times across the experimental groups ($F = 8.29$, $p = 0.0013$). Post-hoc analysis using Dunnett's test

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confirmed that the administration of Amoxicillin alone significantly increased immobility time (Mean ~144.4) compared to the control group (Mean~49.7, $p < 0.01$). This indicates that the Amoxicillin-induced dysbiosis protocol successfully established a state of behavioural despair in the juvenile rats. Treatment with the standard antidepressant Fluoxetine and the test compound Genistein both resulted in a reduction of immobility compared to the Amoxicillin-only group. Fluoxetine attenuated the behavioural impact of Amoxicillin, reducing immobility time to a mean of approximately ~84 ($p \leq 0.05$ compared to Amoxicillin alone). Genistein demonstrated a significant reduction in immobility time (Mean ~ 101.5) compared to the Amoxicillin-treated disease model group ($p \leq 0.05$). Unlike the previous assessment of "high behavioural resilience," these results demonstrate that Amoxicillin administration successfully induced immobility behaviour (Fig.1). Furthermore, both Fluoxetine and Genistein interventions improved swimming behaviour, suggesting a protective or restorative effect against the stressors induced by the dysbiosis model.

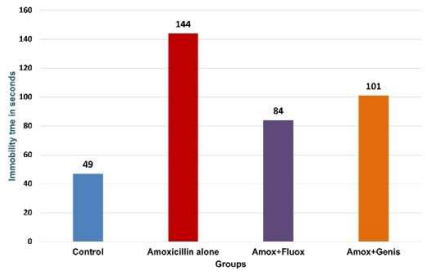

Figure 1: Effect of Amoxicillin – induced gut dysbiosis and treatments with Fluoxetine and Genistein on immobility time in the Forced Swim Test in Juvenile female Wistar rats

3.3 Tail Suspension Test (TST)

The Tail Suspension Test was utilised to further validate the antidepressant-like potential of Genistein in juvenile female Wistar rats following Amoxicillin-induced stress. Statistical analysis through One-way ANOVA followed by Dunnett's post-hoc test revealed that Amoxicillin administration considerably increased the duration of immobility (142.84 ± 32.17 s) compared to the control group (90.13±3.006s) ($p \leq 0.01$). This confirms that the dysbiosis-induced stress model effectively produced a state of behavioural despair, as the rats spent more time in a passive, immobile posture. The standard antidepressant group showed a substantial reduction in immobility time (107.50±9.87s) compared to the Amoxicillin-treated group ($p \leq 0.05$). Fluoxetine demonstrated the maximum level of efficacy in restoring active escape-related behaviour. Similarly, the group treated with Genistein showed a decrease in immobility duration (134.75±30.78s) when compared to the Amoxicillin-only group ($p \leq 0.05$) (Fig.2). While Genistein effectively reduced the behavioural symptoms of stress, its effect was less pronounced than that of the reference drug, Fluoxetine. However, the significant reduction ($p \leq 0.05$) compared to the disease model (Amoxicillin) suggests that Genistein possesses notable antidepressant-like properties in this juvenile rat model.

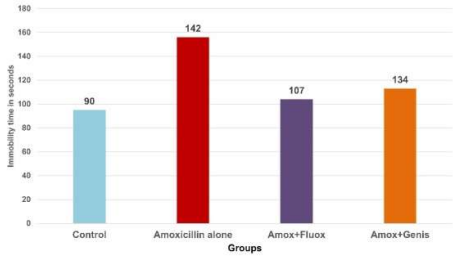

Figure 2: Effect of Amoxicillin – induced gut dysbiosis and treatments with Fluoxetine and Genistein on immobility time in the Tail Suspension Test in Juvenile female Wistar rats

3.4 Assessment of Body Weight

Analysis of physical parameters showed no significant difference in body weight among all experimental groups of rats (One-way ANOVA, $p = 0.646$). The mean body weight remained comparable across all groups, with the control group at 202.00 ± 18.19 g, the Amoxicillin group at 197.50 ± 14.86 g, and the treatment groups (Amoxicillin + Fluoxetine and Amoxicillin + Genistein) showing values of 188.50 ± 23.81 g and 196.17 ± 15.01 g, respectively. Post - hoc Dunnett's test (with the Amoxicillin group as reference) further confirmed the absence of significant differences between the treatment groups and the disease model group. These findings indicate that neither Amoxicillin – induced gut dysbiosis nor the administration of Genistein or Fluoxetine caused metabolic distress in the juvenile rats.

3.5 Biochemical Parameter: Serum Cortisol

The biochemical assessment focused on serum cortisol levels as a biomarker for hypothalamic-pituitary-adrenal (HPA) axis activation and stress response. The results showed that the administration of Amoxicillin led to a marked increase in serum cortisol levels (79.19 ng/ml) compared to the control group (28.44 ng/ml). This rise in cortisol indicates a physiological stress state induced by the antibiotic treatment. The administration of Genistein in the test group appeared to attenuate this response, with cortisol levels recorded at 54.2 ng/ml, which was lower than the levels seen in the Amoxicillin-induced physiological stress model group. One-way ANOVA followed by Dunnett's post-hoc test revealed that this reduction was statistically significant compared to the Amoxicillin-induced physiological stress model group ($p \leq 0.05$). Similarly, the positive control group treated with Fluoxetine showed a reduction in cortisol levels to 41.1 ng/ml. Notably, all observed values remained within the established normal physiological range for the species (25.2 to 208.2 ng/ml), and the modulation of these levels by Genistein suggests a potential protective role for the phytoestrogen in regulating the stress response associated with gut-brain axis disturbances (Fig.3).

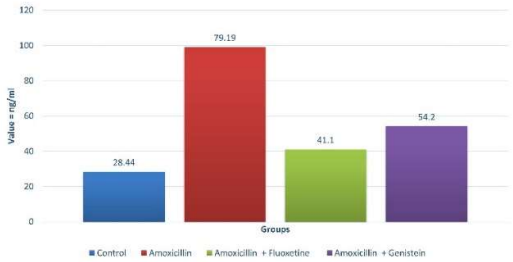

Figure 3: Effect of Amoxicillin – induced gut dysbiosis and treatments with Fluoxetine and Genistein on Serum Cortisol Test in Juvenile female Wistar rats

4. DISCUSSION

4.1 Neuroendocrine Dysregulation and HPA Activation

In this study, the serum cortisol levels revealed that Amoxicillin administration triggered an endocrine stress response (79.19 ng/ml vs. 28.44 ng/ml in controls). This activation of the Hypothalamic-Pituitary-Adrenal (HPA) axis was a consequence of gut microbiota disruption. According to [13], gut dysbiosis increases intestinal permeability, allowing microbial antigens to stimulate systemic inflammation and HPA hyperactivity.

Interestingly, the administration of Genistein attenuated this cortisol rise (54.2 ng/ml, $p \leq 0.05$). This statistical significance was a crucial finding, as it demonstrates that while the subjects showed certain behavioural resilience in reward processing, Genistein was active at a neuro-endocrine level. This supports recent evidence by [11], who found that Genistein exerts neuro-endocrine protective effects by regulating the expression of stress-related genes and reducing corticosterone levels in rodent models. Despite these biochemical shifts, body weight remained stable across all groups ($p \geq 0.05$), indicating that the antibiotic dose did not exceed a systemic toxicity threshold that would impair growth or metabolic health [14].

4.2 Selective Behavioural Despair and Hedonic Resilience

Amoxicillin administration prolonged immobility in both the FST (mean ~144.4 s vs. ~ 49.7 s in controls; $p < 0.01$) and TST ($p < 0.01$), establishing a state of "behavioural despair" reflective of passive coping under acute stress [15]. However, a striking finding was the lack of depressive-like behaviour in the Sucrose Preference Test (SPT), where preference remained above 84% in the Amoxicillin group. The lack of anhedonia suggests the model shows partial depressive-like behaviour instead of a full depressive phenotype. This behavioural resilience in the SPT contrasts with the findings of [9,16], which suggest that more aggressive or multi-antibiotic treatments were effective in causing broader affective disturbances,

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including anhedonia. This indicates that the microbial depletion was a critical factor; while our model was sufficient to disrupt stress-coping circuits, the reward-seeking pathways remained healthy.

4.3 Divergent Responses to Targeted Microbial Depletion

Not all microbiota disruptions cause depression. Recent research has found that drug class, treatment duration, and the host's developmental window modulate behavioural expression following antibiotic exposure. Our observations of hedonic stability align with contemporary rodent models where acute microbiota disruption, in the absence of sustained systemic inflammation or concurrent psychosocial stress, fails to elicit anhedonia [17,18]. As noted by Faysal et al., 2025 [19], the emergence of behavioural pathology may depend on reinforcing environmental factors rather than microbiota disruption alone.

4.4 Developmental and Sex-Dependent Plasticity

The observed resilience was further attributed to the ontogenetic and sex-dependent plasticity of the subjects. The neuroplasticity and compensatory remodelling capabilities of juvenile and young adult brains are superior, which may mitigate the behavioural effects of disruptions to the MGB axis [20,21]. The utilisation of female Wistar rats presents a protective variable, as estrogens have been established as modulators of gut barrier integrity and suppressors of neuroinflammation. This phenomenon has the potential to obscure the behavioural outcomes that are typically observed in male cohorts [22,23].

4.5 Methodological Sensitivity and Subclinical States

The SPT, FST, and TST are standard methods for evaluating depressive disorders, but their sensitivity is often limited to severe conditions [24,25]. In this study, the group treated with Genistein demonstrated a numerically lower sucrose preference (67.75%); however, this was not statistically significant and was driven by individual variability rather than a consistent anhedonic shift. Given that the Amoxicillin-only group displayed no significant anhedonic deficit (84.52%), these variations likely reflect idiosyncratic taste sensitivity or neophobia.

4.6 Neuroprotective Potential of Genistein

Genistein efficaciously reduced FST and TST immobility and mitigated the elevation of cortisol induced by Amoxicillin. This result indicates that Genistein acts as a stress-buffering agent within the MGB axis. These findings align with Diksha et al., 2023[26], who demonstrated that Genistein upregulates brain-derived neurotrophic factor (BDNF) expression and stabilises monoaminergic signaling. By suppressing HPA hyperactivity, Genistein enhances the physiological stability of the MGB axis, preserving homeostatic signalling despite microbial disruption [27,28].

This study demonstrates that juvenile Amoxicillin-induced gut dysbiosis in female Wistar rats leads to a depressive-like state with increased despair and cortisol levels, but no anhedonia or weight change. Treatment with Genistein and Fluoxetine reduced despair and normalized cortisol, suggesting early-life antibiotics may raise neuropsychiatric risk via dysbiosis. Evidence that early-life antibiotic exposure may elevate neuropsychiatric risk through dysbiotic mechanisms [29,30].

5. CONCLUSION

In conclusion, our study shows that Amoxicillin alters gut bacteria in young female Wistar rats, causing physiological stress and depressive-like behaviours. This is evidenced by increased immobility in the forced swim and tail suspension tests, as well as higher cortisol levels. However, the rats maintained normal interest in sweet solutions in the sucrose preference test and steady body weight. Both Genistein and Fluoxetine reduced immobility and normalized cortisol levels, indicating they can mitigate stress from gut bacteria changes. These findings reflect the complex gut-brain relationship during early antibiotic use and suggest that Genistein, a plant compound, may help reduce mental health risks. They also support using behavioral tests and blood markers together in animal studies of gut-brain issues.

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