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Anxiolytic and antidepressant effects of ethanol extract of *Ocimum gratissimum* L. (Lamiaceae)

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Abstract
Ocimum gratissimum, commonly known as clove basil or aromatic basil, has been traditionally utilized for its potential medicinal benefits, including anxiolytic and antidepressant effects. This study aimed to evaluate the anxiolytic and antidepressant properties of methanol extract of *Ocimum gratissimum* at doses of 100, 200, and 400 mg/kg po. Using a Chronic Unpredictable Mild Stress (CUMS) model, mice were divided into 5 groups (n = 6/group). The animals were weighed during the CUMS exposure to monitor stress-induced changes. The negative control group received normal saline, while the positive control group was administered Imipramine (30 mg/kg po) and three treatment groups received the extract. Behavioural responses were assessed using the elevated plus maze, open field test, and forced swim test, with body weight changes monitored as a stress indicator. Results showed that animals treated with *O. gratissimum* extract at all doses tested showed significant reductions in anxiety-like and depressive-like behaviours compared to the negative control. Weight loss, a common indicator of stress, was significantly mitigated in the *O. gratissimum* treated groups. These findings suggest that *O. gratissimum* possesses substantial anxiolytic and antidepressant effects, with higher doses showing greater efficacy and improved weight maintenance, supporting its traditional use in managing anxiety and depression.

Keywords: *Ocimum gratissimum*; Chronic Unpredictable Mild Stress (CUMS); Anxiety-like behaviour; Depressive-like behaviour

INTRODUCTION

Depression is a prevalent and debilitating mental health disorder characterized by persistent sadness, loss of interest or pleasure in daily activities, and a range of emotional and physical problems. It is a major contributor to the global disease burden, affecting over 264 million people worldwide [1]. This pervasive condition has far-reaching implications, not only for the individuals affected but also for public health systems, economic productivity, and societal well-being [2-4]. Commonly prescribed medications include selective serotonin reuptake inhibitors (SSRIs) and serotonin-norepinephrine reuptake inhibitors (SNRIs), which primarily target the serotonergic and noradrenergic systems. Despite their

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widespread use, these medications often have limited effectiveness, with response rates varying significantly among individuals. Furthermore, issues such as delayed onset of therapeutic effects, side effects, and emerging resistance to traditional antidepressants highlight the need for alternative or adjunctive therapies (Hamon and Blier, 2013; Keller et al., 2017). [5,6]. This has driven ongoing research into alternative therapeutic approaches, particularly those based on natural products, which may offer safer and more effective options for managing depression.

Chronic stress is one of the primary risk factors for depression, and it is known to induce a range of biochemical and physiological changes that contribute to the development and persistence of depressive symptoms. Among these changes, alterations in monoamine oxidase A (MAO_A) activity and oxidative stress have been highlighted as significant contributors to the pathophysiology of depression [7]. MAO_A is an enzyme responsible for the catabolism of monoamine neurotransmitters such as serotonin, norepinephrine, and dopamine, which are crucial in regulating mood and emotional responses [8]. Elevated MAO_A activity has been observed in individuals with depression, leading to reduced levels of these neurotransmitters and contributing to depressive symptoms [7].

Ocimum gratissimum, commonly known as African basil or clove basil, is a medicinal plant widely used in traditional medicine across Africa and Asia for its various therapeutic properties, including antimicrobial, anti-inflammatory, and antioxidant activities [9,10]. Recent studies have shown that *Ocimum gratissimum* also possesses neuroprotective and antidepressant-like effects, making it a promising candidate for the treatment of stress-induced depression [11,12]. The potential of *Ocimum gratissimum* to modulate MAO_A activity and mitigate oxidative stress in the brain suggests that it could play a role in alleviating depressive symptoms by restoring neurotransmitter balance and protecting against oxidative damage.

This study aims to investigate the antidepressant-like effects of *Ocimum gratissimum* in a mouse model of chronic unpredictable mild stress (CUMS), a well-established model for inducing depressive-like behaviours that mimic the symptoms observed in human depression [13]. This research seeks to contribute to the development of novel, plant-based therapies for anxiety and depression that may offer a safer and more effective alternative to current treatments.

EXPERIMENTAL METHODS

Drugs and treatment. All drugs and chemicals used were analytical grade. Diazepam injection from Roche Product Ltd. Abuja regional office, Nigeria and imipramine hydrochloride (Sigma-Aldrich, St. Louis, USA) were used as reference standards for antidepressant activity. Physiological saline solution was used as solvent for dissolving drugs and extract. All drugs were administered orally and the extract was given orally at a volume of 5 mL/kg body weight.

Animals. Thirty male Swiss mice, aged 10–12 weeks and weighing 20–25 g, were used in the study, with six animals allocated per group. The animals were sourced from the Animal House, Pharmacology Department, University of Jos, Jos, Nigeria. The animals were housed in plastic cages at room temperature under standard hygienic conditions. They had unrestricted access to rodent pellets and potable water, except as dictated by the experimental design. All experimental procedures were conducted in compliance with the National Research Council's Guide for the Care and Use of Laboratory Animals (2011) and adhered to the ARRIVE 2.0 guidelines [14]. All protocols were also approved by the

University of Jos Animal Care and Use in collaboration with the Office of Laboratory Animal Welfare (OLAW). With Reference: Assurance Approval-UJ/FPS/F17 -00379.

Preparation of plant extract. Fresh leaves of *Ocimum gratissimum* were washed and dried at room temperature (23-25°C). The dried leaves were powdered using a mortar and pestle and stored in a cool, dry place until needed. Three hundred grams of the powdered plant material were weighed using a precision balance and transferred into a glass container. To perform the extraction, 1000 ml of 80% methanol was measured and added to the glass container. The mixture was left to macerate for 72 hours with intermittent shaking. Following the extraction period, the mixture was filtered to separate the residue from the filtrate. The filtrate was then concentrated by evaporation using an oven set at 40°C to obtain a dried extract. The resulting plant extract was sealed and stored in a refrigerator until further use.

Chronic unpredictable mild stress (CUMS). The CUMS procedure was conducted as previously described by Wilner [13] with minor modifications. Mice were subjected to CUMS for two weeks, with fourteen different stressors randomly administered at designated times. The animals were exposed to two different stressors each day, which were unpredictable in nature and timing [15]. These stressors included water deprivation, food deprivation, cage tilting, light cycle alteration, noise stress, and social isolation. Two different stressors were randomly selected and administered at unpredictable times each day. The sequence and timing of stressors were carefully randomized to ensure no predictable pattern. The stressor schedule was unique each day, with no repetition of the same stressor on consecutive days.

Experimental design. Mice were randomly assigned to five groups (n = 6 animals per group). Group 1 received normal saline5ml/kg po and served as the control group; group 2 received imipramine 30 mg/kg one hour before exposure to CUMS; groups 3, 4, and 5 received *O. gratissimum* 100, 200, and 400 mg/kg/day (per oral) + CUMS. Treatment was administered daily for fourteen days. The doses and treatment schedule for *O. gratissimum* were selected based on previous studies that explored similar herbal interventions in stress models [16].

Weight measurement. Body weights of the mice were measured on Day 1 (before initiating the CUMS procedure) and on Day 5, 10 and 14 (the final day of the CUMS paradigm). Additionally, weights were recorded every three days throughout the CUMS procedure using a precision electronic balance.

Behavioural studies. Behavioural assessments were conducted in a controlled environment with adequate lighting and minimal noise. The tests were conducted to evaluate anxiety, and depressive-like behaviours using the following sequence: elevated plus maze (EPM), forced swim test (FST), and tail suspension test (TST).

Anxiety-like behaviour was assessed using the EPM, following the protocol outlined by Kraeuter et al, [17]. Mice were individually placed in the centre of the EPM with their heads facing an open arm and allowed to explore for 5 minutes. The duration of entries into both the open and closed arms was recorded. An entry was scored when all four paws of the mouse entered an arm. The maze was cleaned with 70% ethanol between tests to remove olfactory cues.

The FST was conducted following the methodology of [18]. Mice were placed in a transparent cylinder (25 cm height, 10 cm diameter) filled with water (25°C) to a depth of 10 cm. Mice were observed for 6 minutes and the immobility time was recorded, indicative of depressive-like behaviour.

The TST was carried out according to the procedure described by Steru et al. [19]. Mice were suspended by the tail using adhesive tape on the edge of a table, 50 cm above the floor. Immobility time was recorded over 6 minutes, with immobility defined as the absence of any limb or body movements except for those required for respiration.

Statistical analysis. Data were analysed using one-way ANOVA followed by Dunnett's post hoc test. Results were expressed as mean ± standard deviation (SD). p-values < 0.05 were considered statistically significant. Statistical analysis was conducted using GraphPad Prism 9.0 (GraphPad Software, USA).

RESULTS

The effect of methanol extract of *O. gratissimum* on body weight in mice subjected to Chronic Unpredictable Mild Stress (CUMS) was assessed and compared with a negative control group (normal saline) and a positive control group (Imipramine, 30 mg/kg). Table 1 presents the mean body weights (± S.E.M.) of the mice across four different time points: Day 1, Day 5, Day 10, and Day 14.

Figure 1 shows the effects of *O. gratissimum* on mice in the Open Field Test, comparing the activity levels of mice treated with various doses of *O. gratissimum* (100 mg/kg, 200 mg/kg, 400 mg/kg), Imipramine (30 mg/kg), and normal saline (control). The results are represented as Mean ± S.E.M. (n=6) with statistical significance tested using One-Way ANOVA followed by Dunnett's test. Significant differences ($p<0.05$) from the normal saline group indicate the anxiolytic effects of *O. gratissimum* and its ability to enhance exploratory behaviour in the Open Field Test.

Figure 2 Depicts the effects of *O. gratissimum* on mice in the Tail Suspension Test (TST) and Forced Swim Test (FST). The results compare the immobility times of mice treated with normal saline, Imipramine (30 mg/kg), and *O. gratissimum* at doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg. Each bar represents the Mean ± S.E.M. (n=6). One-Way ANOVA was employed to assess mean differences, followed by Dunnett's test for multiple comparisons. Significant reductions in immobility time ($p<0.05$) compared to the normal saline group suggest that *O. gratissimum* has antidepressant-like effects.

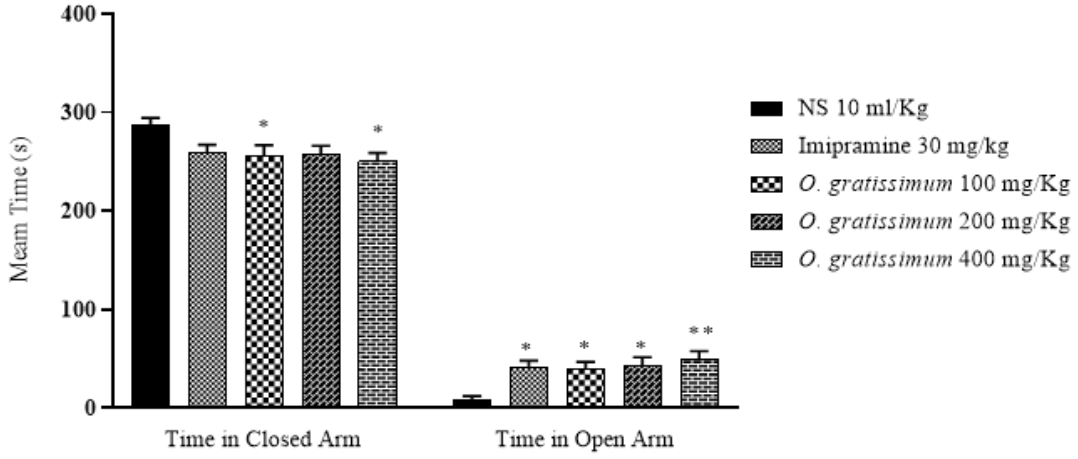

Figure 1: Effect of *O. gratissimum* on Mice in Open Field Test
Values are represented as Mean ± S.E.M. n=6; One-Way ANOVA was used for mean differences followed by Dunnett's test for multiple comparison *p<0.05, significantly different from normal saline (NS).

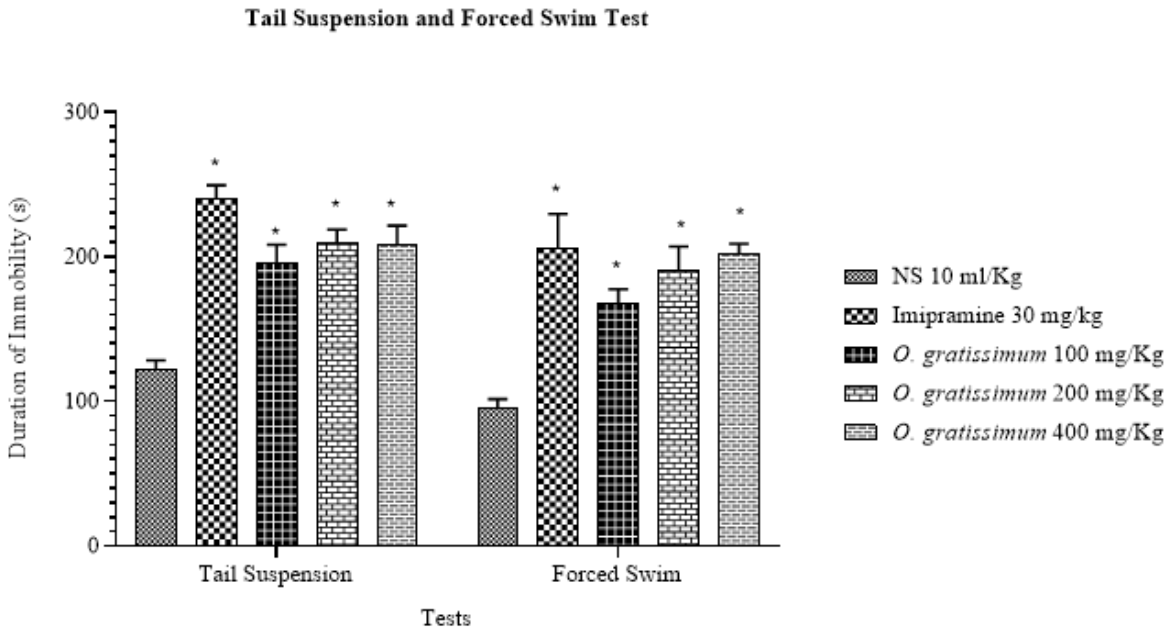

Figure 2: Effect of *O. gratissimum* on Mice in Tail Suspension and Forced Swim Test
Values are represented as Mean ± S.E.M. n=6; One-Way ANOVA was used for differences in mean followed by Dunnett's test for multiple comparison *p<0.05, significantly different from normal saline (NS).

DISCUSSION

Effects of CUMS on weight of mice. The observed weight loss in mice subjected to CUMS and treated with normal saline reflects the physiological impact of chronic stress. Chronic stress alters appetite regulation, metabolism, and energy balance, often leading to significant weight loss in animal models [13]. Weight restoration in groups treated with *Ocimum gratissimum* suggests that the plant extract mitigates stress-induced physiological disruptions. This finding aligns with prior research indicating that natural products with neuroprotective and antioxidant properties can attenuate stress-related metabolic changes [7,12,16].

2. Elevated Plus Maze (EPM): Anxiety behaviour. In the EPM, CUMS-exposed mice exhibited reduced exploration of the open arms, indicative of heightened anxiety. Treatment with *O. gratissimum* significantly increased time spent in the open arms, suggesting anxiolytic effects. These results support earlier studies demonstrating the anxiolytic potential of *O. gratissimum* due to its interaction with neurotransmitter systems and its antioxidant properties [8,17]. This anxiolytic activity could be attributed to the plant's ability to modulate GABAergic and serotonergic pathways, as observed in similar herbal therapies [20].

Forced Swim Test (FST): Depressive-like behaviour. In the FST, CUMS exposure resulted in increased immobility time, a hallmark of depressive-like behaviour. Increased immobility in this test is interpreted as a sign of behavioural despair and reduced responsiveness [18]. *O. gratissimum* treatment significantly reduced the immobility time, indicating an antidepressant-like effect. The reduction in immobility suggests that *O. gratissimum* can counteract depressive-like states induced by chronic stress [19].

4. Tail Suspension Test (TST): Depressive-like behaviour. Similar to the FST, the TST revealed increased immobility in stressed mice, reflecting depressive-like behaviour. The administration of *O. gratissimum* reduced immobility time in the TST, further supporting its potential antidepressant effects. This finding complements the results from the FST and suggests a robust effect of *O. gratissimum* in alleviating stress-induced depressive symptoms [21].

The findings of this study underscore the potential of *O. gratissimum* as a natural therapeutic agent for anxiety and depression. Its ability to counteract stress-induced behavioural and physiological changes highlights its relevance in developing alternative or adjunctive treatments for mood disorders. Given the limitations of current pharmacological treatments, such as side effects, delayed efficacy, and resistance, plant-based therapies like *O. gratissimum* offer promising avenues for safer, more effective interventions [5,6,8].

Conclusion. The study provides evidence supporting the anxiolytic and antidepressant properties of *O. gratissimum*. Significant improvements in anxiety-like and depressive-like behaviours, at all doses, were observed, alongside better weight maintenance compared to the negative control group. The reduction in stress-induced weight loss further supports the efficacy of *O. gratissimum* in alleviating the physiological effects of stress. These results underscore the plant's potential as a natural therapeutic agent for anxiety and depression. Overall, *O. gratissimum* demonstrates promising potential as an alternative treatment for mental health conditions.

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