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Antidepressant effect of Doum palm (*Hypaene thebaica*) Aqueous Extract on Chlorpromazine -induced Depression in *Drosophila melanogaster* (Harwich strain)

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ABSTRACT

Depression is a major neuropsychiatric disorder, and limitations of current antidepressants have stimulated interest in plant-derived therapies. *Hyphaene thebaica* (doum palm) contains bioactive compounds with reported neuroprotective and antioxidant properties. This study evaluated the antidepressant-like effects of aqueous doum palm extract in a chlorpromazine-induced depression model of *Drosophila melanogaster*. One hundred and eighty flies were allocated into six groups comprising a control, chlorpromazine-treated group, standard antidepressant-treated group, and three extract-treated groups (500, 750, and 1000 mg/mL). Antidepressant-like activity was assessed using climbing, social spacing, mating, and eclosion assays. The data were analyzed through a one-way ANOVA, establishing a significance threshold at $p<0.05$. Doum palm extract produced dose-dependent improvements in behavioral performance, with the 1000 mg/mL group showing significant improvements in climbing activity, social spacing behavior, mating performance, and eclosion rate compared to the chlorpromazine-treated group ($p<0.05$). Aqueous doum palm extract exhibited significant antidepressant-like activity in *D. melanogaster*, supporting its potential as a candidate for further investigation in mammalian models and mechanistic studies.

Keywords: *Hyphaene thebaica*, depression, chlorpromazine, *Drosophila melanogaster*, antidepressant

INTRODUCTION

Depression is the most prevalent and crippling mental illness. Its occurrence and impact have consistently grown over the last few decades. For instance, in 1990, the World Health Organization (WHO) anticipated that depression would rise from the 4th to the 2nd most common cause of global disability by 2020. By 2008, it was classified as the 3rd and was expected to become the leading cause of disability by 2030.[1]

Depression poses a major concern for public health in Africa, affecting millions of people across the continent. As per the World Health Organization (WHO), mental health conditions, including depression, are prevalent in Africa, with an estimated 80% of individuals with mental health conditions not receiving adequate treatment.[2] The prevalence of depression in Africa is shaped by multiple factors, such as socio-economic circumstances and cultural customs, and limited access to mental health care services.[3] A study by the African Development Bank [4] highlights that depression is a major contributor to the burden of disease in the region, with an increasing number of cases reported annually.

Nigeria, being one of the most populous countries in Africa, faces a substantial burden of depression; a systematic review by Gureje *et al.* [5] indicates that the prevalence of depression in Nigeria ranges from 7% to 12% in the general population. The study emphasizes that despite the high prevalence, mental health services remain underdeveloped, with many Nigerians lacking access to appropriate care.

Although conventional antidepressants, including selective serotonin reuptake inhibitors and tricyclic antidepressants, remain important in the management of depression, their clinical usefulness may be limited by delayed onset of action, incomplete therapeutic response, and adverse effects such as weight gain, sexual dysfunction, and withdrawal symptoms following abrupt discontinuation.[6] These limitations, together with poor access to mental health services and the cost of treatment in many low-resource settings, have encouraged interest in plant-derived

compounds as possible adjuncts or alternative therapeutic candidates.[7]

D. melanogaster has become a useful model organism for neurobehavioral research and preliminary screening of candidate antidepressant compounds. Its short life cycle, low maintenance cost, genetic tractability, and conservation of major neurotransmitter systems, including serotonergic and dopaminergic pathways, make it suitable for studying depression-like phenotypes and treatment responses.[8] These advantages allow behavioral and developmental outcomes to be assessed in a simple, reproducible, and ethically acceptable experimental system.

Chlorpromazine, a typical antipsychotic drug, has been used to induce depression-like phenotypes in *D. melanogaster*. Previous studies have reported that chlorpromazine exposure produces behavioral changes such as reduced locomotor activity, decreased mating frequency, and altered social behavior, together with biochemical changes including increased malondialdehyde, reduced superoxide dismutase activity, and decreased serotonin levels. [6,7] This makes the chlorpromazine-induced model useful for evaluating the antidepressant-like potential of novel compounds, including medicinal plant extracts.

Doum palm (*Hyphaene thebaica*) is widely consumed in parts of Africa and the Middle East and is also used in traditional medicine. Its fruit contains phenolic compounds, flavonoids, and other bioactive constituents with reported antioxidant and antimicrobial activities. [1,3] Since oxidative stress has been linked with chlorpromazine-induced depression-like phenotypes, the antioxidant properties of doum palm suggest that it may possess antidepressant-like activity.

Therefore, the current research examined the effects similar to antidepressants of aqueous doum palm (*Hyphaene thebaica*) extract in a chlorpromazine-induced depression model of *D. melanogaster*, using climbing performance, social spacing, mating behavior, and eclosion as behavioral and developmental endpoints.

MATERIALS AND METHODS

Drugs and chemicals

Agar agar, baker's yeast, corn meal, 1g 5-hydroxy methyl paraben, imipramine, Chlorpromazine, aqueous extract of *Hyphaene thebaica*, 70% ethanol.

Equipment

Treatment Vials, Ice pack, Whatman paper, Sterilized funnel, Towel or Tissue paper, Paintbrush, Tapping pad, Petri dish, Sterilized foam, Pipette, Mortar and Pestle.

Plant extraction, study area and diet preparation

The Doum fruits were rinsed with tap water and subsequently drained. The outer layer (epicarp) and the edible section (mesocarp) were mashed into a fine powder after removing the seeds with a stainless steel knife. The crushed doum fruits were boiled in water at a ratio of 1:5 (w/v) for 10 minutes. The mixture was then strained through a single layer of cheesecloth to yield a clear extract, which was subsequently filtered through cotton to eliminate any fine particles.[3]

The research was conducted in the Biomedical Research, Science and Training Centre (BioRTC), at Yobe State University, where controlled environmental conditions (temperature of 27%, humidity of 25% and light/dark cycles) were maintained to ensure consistent experimental conditions. The study was performed in accordance with institutional guidelines for ethical research involving model organisms.

850 ml of water was split into 700 ml and 150 ml using a measuring cylinder. One gram of methyl paraben was dissolved in 10 ml of ethanol in a Falcon tube.

Vials were cleaned with 70% ethanol. The heat source was then turned on, and a clean pot was placed on it. The 700 ml of water was poured into the pot and left to boil. A small amount of the boiling water was fetched to dissolve the yeast, and this was stirred periodically for 10 minutes.

Eight grams of agar-agar were added to the boiling water and stirred periodically for 10 minutes. Fifty grams of corn flour were mixed with 125 ml of water and then added to the mixture on the heat. Stirring continued for another 10 minutes.

The dissolved yeast and the remaining 25 ml of water were then added to the mixture, which was stirred periodically for another 15 minutes. The mixture was left to cool slightly until the pot could be held without burning.

The dissolved methyl paraben was then added and stirred in. The meal was transferred from the pot into sterile vials, which were covered with mesh cloth to cool to room temperature.

Once cooled, the vials were cleaned with sterile paper towels (tissue) to remove moisture. Flies were then transferred into the sterile vials, which were covered with foam plugs.

Inducing depression in Drosophila melanogaster

Flies in the negative control group were exposed to chlorpromazine-supplemented food medium for seven days to induce depression-like behavior. Flies in the standard control and treatment groups were similarly exposed to chlorpromazine-supplemented food medium for two days to induce depression-like behavior, after which they received the standard antidepressant (imipramine) or doum palm extract, respectively, for seven days.

Previous research has demonstrated that D. melanogaster fed with a medium containing Levodopa or Chlorpromazine could exhibit depression-like traits in both behavioral and biochemical markers, which include a significant reduction in food consumption,

mating frequency, and serotonin (5-HT) levels, along with an increase in malondialdehyde (MDA) levels and a decrease in the activity of superoxide dismutase (SOD). Additionally, the offspring of flies treated with Chlorpromazine also displayed these characteristics associated with depression.[9,10]

Social space assay

The test chamber, shaped like a vertical triangle, was built using a pair of square glass plates (18 × 18 cm), kept apart by 0.47 cm with acrylic spacers to limit the flies to a two-dimensional area. Four spacers were utilized: two right triangles for each side and two rectangular ones at the bottom, creating an internal chamber in the form of an isosceles triangle (base: 15.2 cm, height: 15.2 cm). The two bottom spacers were temporarily separated to enable the introduction of flies into the chamber.[11]

Mating assay

The mating assay is employed to observe the influence of depression and subsequent treatment with doum palm extract on the reproductive behavior of *D. melanogaster*. Mating behavior is a well-established indicator of overall health and well-being in flies. Depressed flies, due to exposure to chlorpromazine, typically exhibit reduced sexual activity and mating success, which can be restored upon effective treatment.[12]

After the flies received treatment, they were placed back into their usual living environment in order to study their mating behavior. To test their mating behavior, the flies that were given drugs were paired with inexperienced mates in a courtship chamber, and their activities were monitored for 6 hours using a camera to record how many times they attempted to mate and have mated in total. This information comes from a study conducted by Miang *et al*.[13]

Eclosion assessment during acute toxicity

Eclosion is a critical stage in the *D. melanogaster* life cycle and, during acute toxicity testing, serves as an important endpoint for assessing the effects of doum palm extract on fly development and survival.

Fly eggs from all experimental groups were collected and placed in separate vials containing standard food medium. The developmental process was monitored, particularly focusing on the time taken for larvae to pupate and for adults to emerge (eclose) from the pupal case. Eclosion rates (percentage of larvae that successfully emerge as adults) were recorded daily until all flies in the control group had eclosed.[14,15]

Startled induced negative geotaxis (SING)

This was carried out as outlined earlier by Coulom and Birman.[16] Each group contained 10 to 20 flies and was placed in a vertical column (25 cm long, 1.5 cm diameter) with a conic bottom end. They were suddenly startled by gently tapping them down, to which *D. melanogaster* responded by climbing up. After 1 min, flies having reached the top of the column (above 22 cm) and flies remaining at the bottom end (below 4 cm) were separately counted. Three rounds per column were performed at 1 min intervals.[17]

Experimental design

The study employed an experimental design to assess the antidepressant effects of doum palm (*Hyphaene thebaica*) aqueous extract on chlorpromazine-induced depression in *D. melanogaster*. The experimental design consisted of six groups: a normal control group, a negative control group, a standard control group, and three treatment groups (Test 1, Test 2, and Test 3) receiving low, medium, and high doses of doum palm extract, respectively.

Table 1. Experimental groups

Group	Identity	Treatment	Duration (Days)
I	Normal control	Normal cornmeal Diet	7
II	Negative control	2000 mg/2 ml chlorpromazine	7
III	Standard control	2000 mg/2 ml chlorpromazine 1 Mm Imipramine	27
IV	Test 1	2000 mg/2 ml chlorpromazine 500 mg/ml doum palm extract	27
V	Test 2	2000 mg/2 ml chlorpromazine	27
VI	Test 3	750mg/2 ml doum palm extract 2000 mg/2 ml chlorpromazine 1000 mg/2 ml doum palm extract	27

Number of flies per group = 30

Statistical analysis

The data obtained were analyzed using GraphPad Prism (9.3.1, NY, USA). The results were expressed as Mean ± SEM (standard error of the mean). Comparison among groups was determined using analysis of variance (ANOVA) and the Kruskal-Wallis test for non-parametric data. The identification of notable differences was conducted using Tukey's Honestly Significant Difference test for parametric data and Dunn's post hoc test for non-parametric data. A threshold of $p<0.05$ was established to indicate statistical significance.

RESULTS

Startled-induced negative geotaxis assay

The graph showed that the Normal Control group performed best, indicating typical motor function, while the Negative Control group displayed the poorest performance, potentially due to a toxic or inhibitory factor. Test 1 appeared to decrease climbing performance over time, while Test 2 and Test 3 showed moderate to slight improvements, possibly indicating dose-dependent effects on motor ability.

The results imply that different concentrations or treatment conditions impact climbing ability variably, with the highest doses potentially impairing function, while lower concentrations may have more tolerable or even slightly beneficial effects.

Mating assay

This graph represents the results of the mating assay for *D. melanogaster*, measuring the Success rate (%) across various groups. The high success rate in the NC group suggests that in a normal, untreated environment, *D. melanogaster* has a higher mating success. The low success rate in the Negative Control and STD Control suggests that the treatments or conditions applied in these groups negatively affect mating success. Test groups (1, 2, and 3) do not show significant improvement in mating success compared to the Negative Control, indicating that the doum palm aqueous extract does not significantly enhance mating success in chlorpromazine-induced conditions.

Social spacing assay

The social space assay revealed significant alterations in social behavior among different treatment groups: Control flies maintained the closest social spacing (approximately 0.75 cm). CPZ-only-exposed flies showed increased social distance (approximately 1.3 cm). CPZ + 500mg Doum, Chlorpromazine + 750mg Doum, and Chlorpromazine + 1mM Imipramine groups demonstrated further increased social spacing (1.3 cm and 1.8 cm, respectively). CPZ + 1000mg Doum treatment showed a significant reduction in social distance (approximately 0.6 cm), suggesting a potential protective effect at this concentration.

Statistical analysis using GraphPad and ImageJ software indicated significant differences between CPZ + 500mg Doum and CPZ + 750 mg Doum groups ($p < 0.05$), indicating significant differences ($p<0.05$ and $p<0.01$) when compared with the Control Only, as compared with two-way ANOVA and Tukey's multiple comparison post-hoc test.

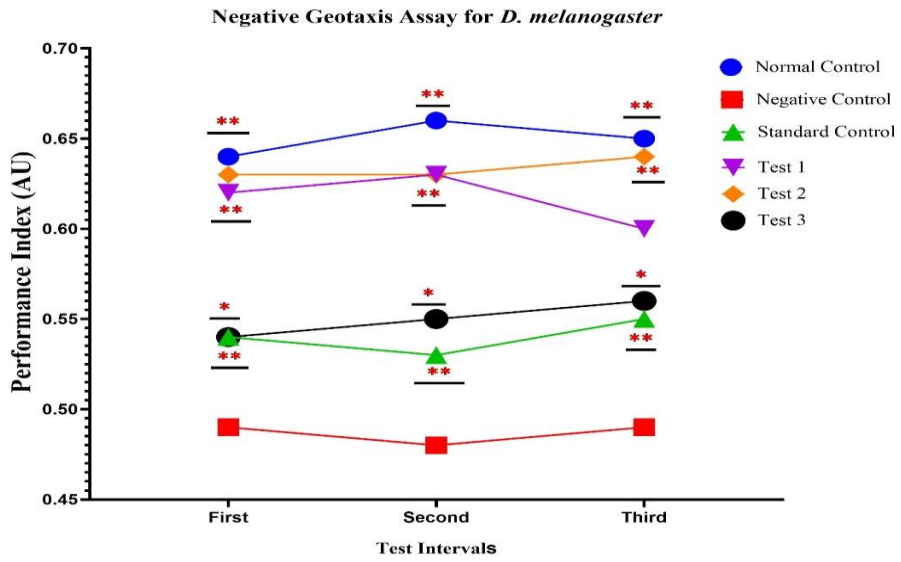

Figure 1. Effect of aqueous doum palm extract on climbing performance (SING assay) in chlorpromazine-induced depression-like phenotype in *Drosophila melanogaster*. Climbing performance was assessed using the negative geotaxis assay across three test intervals. Normal control = flies fed a normal cornmeal diet; Negative control = flies exposed to chlorpromazine only; Standard control = chlorpromazine-exposed flies treated with imipramine; Test 1 = chlorpromazine-exposed flies treated with 500 mg/mL doum palm extract; Test 2 = chlorpromazine-exposed flies treated with 750 mg/mL doum palm extract; Test 3 = chlorpromazine-exposed flies treated with 1000 mg/mL doum palm extract. AU = arbitrary units. *p<0.05 and **p<0.01 denote significant statistical variations between the groups being compared.

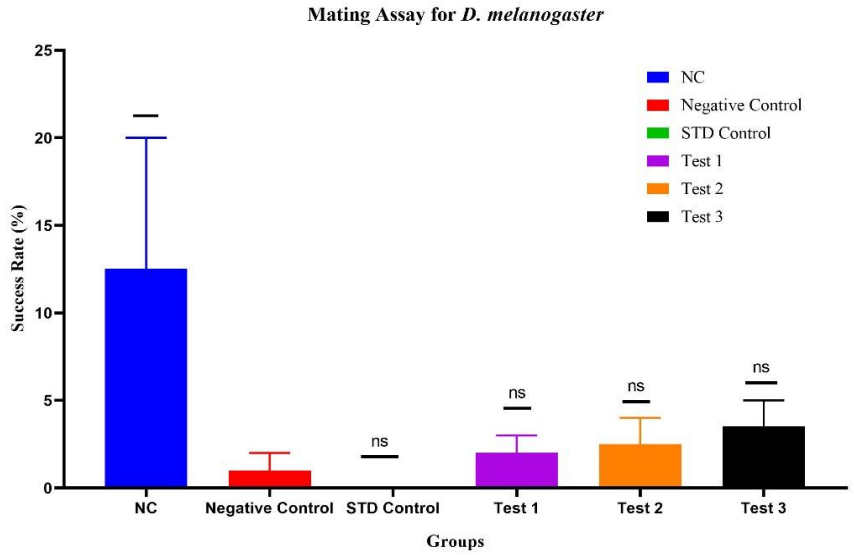

Figure 2. Effect of aqueous doum palm extract on mating success in chlorpromazine-induced depression-like phenotype in *Drosophila melanogaster*. Mating behavior was assessed as percentage mating success across the experimental groups. Normal control (NC) = flies fed normal cornmeal diet; Negative control = flies exposed to chlorpromazine only; Standard control (STD) = chlorpromazine-exposed flies treated with imipramine; Test 1 = chlorpromazine-exposed flies treated with 500 mg/mL doum palm extract; Test 2 = chlorpromazine-exposed flies treated with 750 mg/mL doum palm extract; Test 3 = chlorpromazine-exposed flies treated with 1000 mg/mL doum palm extract. Values are presented as mean ± SEM. ns = not significant.

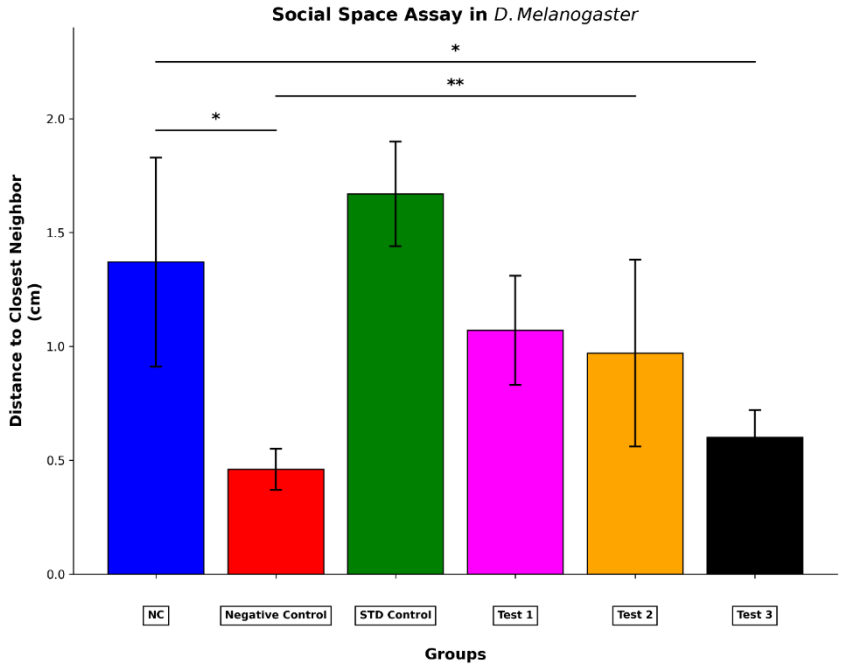

Figure 3. Effect of aqueous doum palm extract on social spacing in chlorpromazine-induced depression-like phenotype in *Drosophila melanogaster*. Social spacing was assessed as the proximity to the closest neighboring fly and expressed in centimeters. Normal control (NC) = flies fed normal cornmeal diet; Negative control = flies exposed to chlorpromazine only; Standard control (STD) = chlorpromazine-exposed flies treated with imipramine; Test 1 = chlorpromazine-exposed flies treated with 500 mg/mL doum palm extract; Test 2 = chlorpromazine-exposed flies treated with 750 mg/mL doum palm extract; Test 3 = chlorpromazine-exposed flies treated with 1000 mg/mL doum palm extract. Values are presented as mean ± SEM. *p<0.05 and **p<0.01 indicate statistically significant differences between compared groups.

DISCUSSION

The current study assessed the antidepressant-like properties of the aqueous extract of *Hyphaene thebaica* on chlorpromazine-induced depression-like behaviors in *D. melanogaster*. The behavioral endpoints assessed were startled-induced negative geotaxis, mating behavior, social spacing, and eclosion. Overall, the findings suggest that doum palm extract produced assay-dependent effects, with clearer improvement in locomotor and social behavior than in mating performance.

In the startled-induced negative geotaxis assay, chlorpromazine-treated flies showed reduced climbing performance compared with the normal control group, indicating impaired locomotor activity. This agrees with previous reports that chlorpromazine exposure can induce depression-like phenotypes in *Drosophila*, including reduced activity and altered behavioral performance.[10] Treatment with doum palm extract improved climbing performance, particularly at 750 and 1000 mg/mL, suggesting partial reversal of chlorpromazine-induced locomotor impairment. This finding is also consistent with Coulom and Birman's use of negative geotaxis as a sensitive assay for detecting motor dysfunction in *Drosophila*.[13] The enhancement was inconsistent across various testing intervals, indicating that the extract might not lead to a straightforward linear dose-response relationship. The enhancement was inconsistent across various testing intervals, indicating that the extract might not lead to a straightforward linear dose-response relationship.

The mating assay demonstrated a lower success rate in mating among flies exposed to chlorpromazine in comparison to individuals in the normal control group. This agrees with previous evidence that chlorpromazine-induced depression-like states in *Drosophila* may reduce mating frequency and reproductive behavior.[6,7] However, unlike the climbing assay, doum palm extract did not significantly improve mating success in comparison with the negative control group. This disagrees with the general expectation that successful antidepressant-like treatment should improve multiple behavioral outcomes. The finding may indicate that mating behavior is less sensitive to doum palm extract within the treatment period used in this study, or that reproductive behavior requires a longer recovery period than locomotor performance.

In the social spacing assay, chlorpromazine exposure altered social interaction patterns, suggesting disruption of normal social behavior. This supports the use of social spacing as a behavioral endpoint for assessing neurobehavioral changes in *Drosophila*.8 Treatment with 1000 mg/mL doum palm extract produced the clearest improvement in social spacing, suggesting that the highest concentration may have a protective effect on social behavior. This agrees with previous plant-extract studies in chlorpromazine-induced depression-like models, where antioxidant-rich extracts improved behavioral outcomes.[7] However, the lower extract concentrations showed weaker or more variable effects, suggesting that the behavioral effect of doum palm may be concentration-dependent.

The possible antidepressant-like effect of doum palm extract may be related to its reported phytochemical constituents, including phenolic and flavonoid compounds. These compounds may contribute to antioxidant and neuroprotective activity. This interpretation agrees with previous reports linking chlorpromazine-induced depression-like behavior in *Drosophila* to oxidative stress, increased malondialdehyde, reduced antioxidant enzyme activity, and altered serotonin levels.[6,7] However, the present study did not measure serotonin, dopamine, malondialdehyde, superoxide dismutase, catalase, or glutathione. Therefore, the mechanism remains only a possible explanation and should not be presented as a confirmed finding.

Taken together, the findings suggest that aqueous extract of *Hyphaene thebaica* has antidepressant-like potential in chlorpromazine-induced depression-like phenotypes in *Drosophila melanogaster*, particularly in locomotor and social behavior assays. The strongest effect was observed at 1000 mg/mL, although the lack of significant improvement in mating behavior suggests that the extract's effect may be selective rather than generalized across all behavioral outcomes. Future studies should include biochemical markers, clearer dose-response analysis, and validation in mammalian models.

CONCLUSION

This research explored the possible antidepressant effects of *Hyphaene thebaica* in D. melanogaster subjected to depression through Chlorpromazine. The findings indicate that various concentrations of aqueous extract of Hyphaene thebaica exhibited antidepressant properties, as demonstrated by enhanced climbing performance and increased mating frequency in the flies. This underscores the potential of utilizing D. *melanogaster* as a model organism for investigating depression.

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