

Research Article

Antidepressant effects of aqueous extract of *Hibiscus sabdariffa* flower in chlorpromazine induced depression-like phenotype in *Drosophila melanogaster* (Harwich strain)

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ABSTRACT

Background: The research evaluated the Antidepressant Effects of Aqueous Extract of *Hibiscus sabdariffa* Flower in Chlorpromazine Induced Depression-Like Phenotype in *Drosophila Melanogaster* (Harwich Strain).

Method: In the research, a total of 120 flies were utilized and were distributed among six groups labeled as (Group A, B, C, D, E and F), with each group consisting of 20 flies. Group A served as the control and was given standard cornmeal diet for 7 days; group B: Served as positive control and administered 2000 mg/2 ml of chlorpromazine for 7 days, group C was given 2000mg/2ml chlorpromazine for 5 days and 1Mm of imipramine for 2 days, group D was induced with 2000 mg/2 ml chlorpromazine for 2 days and was treated with 100 mg/ml *Hibiscus sabdariffa* for 5 days, group E was administered with 2000 mg/2 ml chlorpromazine for 2 days and were treated with 50 mg/ml *Hibiscus sabdariffa* for 5 days while F was induced with 2000 mg/ml chlorpromazine for 2 days and was treated with 10mg/ml of *Hibiscus sabdariffa*.

Results: The concentration of malondialdehyde, superoxide dismutase and serotonin levels were measured. Chlorpromazine induced significant increase in MDA and SOD activities in flies and decrease in Serotonin levels in depressed groups as compared with other groups (P<0.05).

Conclusion: Results obtained from the study demonstrated that an aqueous extract of *Hibiscus sabdariffa* might have antidepressant activity against chlorpromazine induced depression in *Drosophila melanogaster*, Harwich strain.

Keywords:

Hibiscus sabdariffa,
Drosophila melanogaster,
chlorpromazine, depression

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INTRODUCTION

Depression is a prevalent chronic condition in clinical practice and is expected to become the second leading cause of premature death or disability worldwide (W.H.O, 2017). Studies have shown that depressed individuals have reduced monoamine signalling and metabolite levels in cerebrospinal fluid, and that serotonin, norepinephrine, or dopamine depletion can contribute to a pro-depressive effect. Additionally, depression has been linked to an increase in the monoamine oxidase enzyme in the brain, which leads to reduced levels of monoamines (Leonard, 2020). The flowers of *Hibiscus sabdariffa* have been reported to possess several health-promoting properties, including cardiovascular protection, hypotensive, antidiabetic, anticonvulsant, and antioxidant effects (Ali *et al.*, 2014). These flowers contain flavonoids such as anthocyanin and quercetin, which have been implicated in antidepressant activity (Li *et al.*, 2016).

METHODS

The flies were reared on a standard cornmeal/yeast diet at 25 °C with a photoperiod of 8L: 16D (light: dark) and under non crowded conditions (6 vials of media in 150-ml conical flasks each containing 20 flies).

Equipment

Hand lens, stereo-microscope (fluorescent), forceps, whattman paper, paint brush, hand towel 2 pairs), cooking pot (1), glass wares, spectrometer, refrigerator centrifuge (cold), 1.5mL microtubes (Eppendorf) Micropipette (adjustable), culture vial, treatment vials, foam plug, weighing balance, Fly pi, egg chamber, Courtship chamber.

Chemicals

Corn flour 100 g, baker's yeast 20 g, agar agar 10 g, methyl paraben, water 1700 ml, aqueous extract of *Hibiscus sabdariffa*, 70 % ethanol (1 litre), Phosphate buffered saline (BN: P5493-1L) by Sigma-Aldrich were used.

Plant Extraction and Preparation

Dried extract of the flower of *Hibiscus sabdariffa* was bought from Samaru market Zaria and was freeze dried in the Department of Pharmacognosy Ahmadu Bello University, Zaria, Kaduna State Nigeria. Dried flower of *Hibiscus sabdariffa* was extracted using the cold maceration method. Five hundred grams of powdered plant flower was soaked in 3 liters of distilled water, which was divided into different aliquots using conical flasks. It was shaken for 24 hours. The infused fluid obtained was filtered with No. 1 Whatman's filter paper and the filtrate and was poured onto petri dishes and dried in oven under low temperature (The process was repeated to obtain higher yield of crude extract. Another four hundred and eighty-seven grams of powdered plant leaves was soaked in 4.87 liters of distilled water for 48 hours at room temperature, which was divided into different aliquots using conical flasks. It was also shaken for 24 hours. The infused fluid obtained was filtered with No. 1 Whatman's filter paper and the filtrate was partitioned to obtain two different layers. The filtrate was poured onto petri dishes and dried in oven under low temperature of 0-60°C (104-140°F) to prevent any degradation or loss of active compounds (Olil *et al.*, 2001).

Percentage Yield Determination

Percentage yield was calculated using the formula $W2-W1/W0 \times 100\%$. Where W0 is the weight of the initial dried sample, W1 is the weight of container alone and W2 is the weight of both extract and container (Hsin-Ping, 2012).

Animal Treatment

In order to create traits similar to depression in the flies, Chlorpromazine was administered to them. Flies that were between 3 to 5 days old were given cornmeal diet that had 2000 mg/L of Chlorpromazine mixed in, and they were given a maximum of 48 hours to consume it. After that, the changes in the flies' mating behavior, their ability to resist gravity (known as negative geotaxis assay), and the levels of malondialdehyde and superoxide dismutase

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activities in their bodies was determined. Each group of flies that were treated contained 20 flies (Miang *et al.*, 2017).

After the completion of the experiment, the flies were collected and homogenized together in a 1.5 mL Eppendorf tube. The mixture was then centrifuged at 1500rpm for 5 minutes, and the solid pellet that formed at the bottom was removed. The remaining liquid, also known as the supernatant, was collected and analyzed for biomarkers associated with oxidative stress (SOD, MDA and Serotonin) (Miang *et al.*, 2017).

Mating behavior assay

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 5 inexperienced mates in a courtship chamber and their activities were monitored for 6 hours using a camera to record how many times they attempted and have mated in total. This information comes from a study conducted by Miang *et al.* (2017).

Biochemical assays

MDA level was measured by using thiobarbituric acid MDA assay kit (Jiancheng, Nanjing, China) at 532 nm according to the manufacturer's directions.

SOD activity assays were carried out using water soluble tetrazolium salt assay kit (Jiancheng, Nanjing, China) at 450 nm following the instructions.

Experimental Design

The table (1) below shows the different doses and concentration of drugs used in induction and treatment of depression according to the protocol used by Miang *et al.*, (2017).

Table 1. Different doses and concentrations of drugs used in induction and treatment of depression in the flies

GROUP	IDENTITY	TREATMENT	DURATION (DAYS)
Group A	Normal control	Normal Corn meal Diet	7
Group B	Negative control	2000mg/2ml Chlorpromazine	7
Group C	Standard control	2000mg/2ml Chlorpromazine	2
		1 mM Imipramine	5
Group D	Test 1	2000mg/2ml Chlorpromazine	2
		100mg/ml <i>Hibiscus sabdariffa</i>	5
Group E	Test 2	2000mg/2ml Chlorpromazine	2
		50mg/ml <i>Hibiscus sabdariffa</i>	5
Group F	Test 3	2000mg/2ml Chlorpromazine	2
		10mg/ml <i>Hibiscus sabdariffa</i>	5

Statistical analyses

The results were presented as Mean±SEM (standard error of mean), and the statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test, which was carried out with the help of R statistical software. Additionally, the survival curves were analyzed using the Log-rank (Mentel-cox) test, and this analysis was conducted using Graphpad Prism statistical software version 7.04. The statistical significance was set at a P-value (P<0.05.)

RESULTS

Malonaldehyde Activity

The malondialdehyde content of fruit flies administered *Hibiscus* flower extract ranged from 21863-84842.5 nMol/ml. The highest malondialdehyde content was recorded in Chlorpromazine-treated flies while the lowest malondialdehyde was recorded in 100 mg/ 10 g diet *Hibiscus* flower extract. Chlorpromazine significantly elevated (p<0.05) the malondialdehyde concentration in fruit fly compared to normal control. Imipramine (1 mM) and *Hibiscus* treated flies significantly decreased (p>0.05) malondialdehyde content

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compared to Chlorpromazine as seen in the figure 1 below

Superoxide Dismutase Activity

The superoxide dismutase activities of fruit flies that received Hibiscus sabdariffa flower extract ranged from 3.20 ± 0.30 to 5.80 ± 0.30 U/ml. The highest superoxide dismutase activity was observed in flies treated with Chlorpromazine, while the lowest activity was detected in the control group. Chlorpromazine significantly increased ($p < 0.05$) the superoxide dismutase activities in fruit flies when compared to the normal control. Conversely, flies treated with Imipramine (1 mM) and Hibiscus sabdariffa extract showed a significant reduction ($p > 0.05$) in superoxide dismutase activities when compared to those treated with Chlorpromazine, as illustrated in figure 2 below.

Serotonin Level

The range of serotonin levels in fruit flies given Hibiscus sabdariffa flower extract was between 1.5 ± 0.20 - 7.1 ± 0.10 $\mu\text{g/ml}$. The group of flies induced with Chlorpromazine showed a statistically significant reduction in serotonin levels ($p < 0.05$) when compared to the groups treated with Imipramine (1 mM) and Hibiscus sabdariffa, which showed increased levels of serotonin (figure 3).

Mating Assay

The mean number of attempts and successes during mating varied from 7.3 ± 0.02 - 31.3 ± 0.12 and 2.3 ± 0.01 - 14.6 ± 0.32 , respectively. The greatest number of attempts and successes were observed at 50 mg/10 g diet, while the lowest values were recorded for flies treated with chlorpromazine (2000 mg/2 ml). The difference between attempted and successful matings was statistically significant ($p < 0.05$). Flies that consumed 10 mg/10 g and 50 mg/10 g diets had significantly higher mating attempts and successes compared to the control group, as well as compared to flies treated with chlorpromazine, imipramine (1 Mm), and those

fed a normal cornmeal diet containing 100 mg/10 g, as illustrated in the figure 4 below.

Negative Geotaxis Assay

The climbing activities of male and female flies ranged from 56-58% and 94-97%, respectively. The control group exhibited the highest percentage of climbing activities for both males and females, while the lowest percentage of climbing activities were observed in flies treated with chlorpromazine (2000 mg/2 ml). There was no significant difference ($p > 0.05$) in the percentage of climbing activities between males and females. In contrast, imipramine 1Mm (Standard control) significantly reduced ($p < 0.05$) the percentage of climbing activities in both males and females when compared to the control group, as well as to flies treated with Chlorpromazine at 10 mg/10 g, 50 mg/10 g, and 100 mg/10 g diets, as depicted in the figure below.

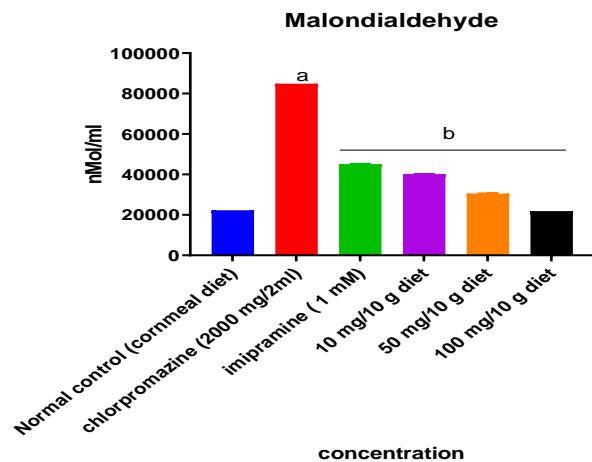


Fig 1: The malondialdehyde content of fruit flies that were given Hibiscus Flower extract was measured. It was observed that Chlorpromazine significantly increased ($p < 0.05$) the malondialdehyde concentration in the fruit flies compared to the normal control. However, the flies that were treated with Imipramine (1 mM) and Hibiscus extract displayed a significant decrease ($p < 0.05$) in malondialdehyde content as compared to the flies treated with Chlorpromazine.

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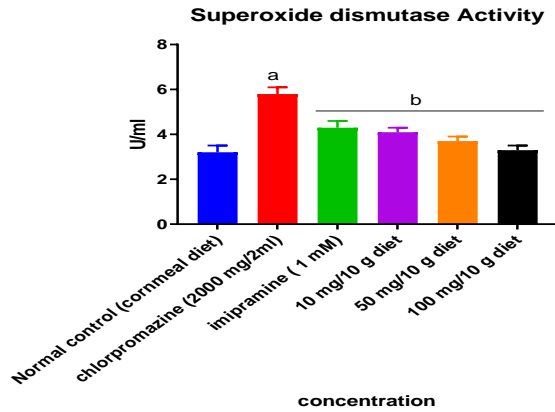


Figure 2: The Superoxide Dismutase activity of *Drosophila melanogaster*. Chlorpromazine significantly elevated ($p < 0.05$) the superoxide dismutase activities in fruitfly compared to normal control^a. Imipramine (1 mM) and Hibiscus treated flies significantly had decreased ($p < 0.05$) superoxide dismutase activities compared to Chlorpromazine^b.

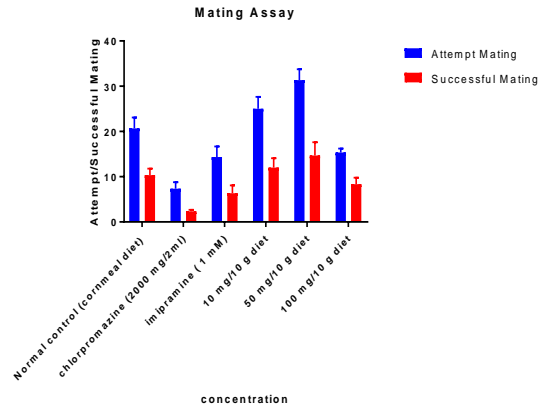


Figure 4: Mating behavior of *Drosophila melanogaster* at different concentrations following treatment with Hibiscus sabdariffa extract. It was observed that there was a notable distinction ($p < 0.05$) between attempted mating and successful mating. The flies fed with diets containing 10 mg/10 g and 50 mg/10 g of the extract displayed a significant increase in both attempted and successful mating compared to the control group, as well as the flies treated with Chlorpromazine, imipramine (1 Mm), and 100 mg/10 g diet.

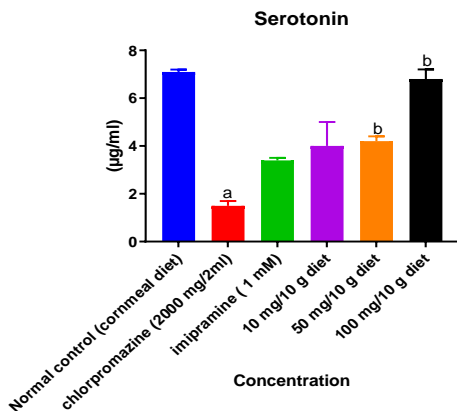


Figure 3: Serotonin levels of *Drosophila melanogaster* (Chlorpromazine significantly decreased ($p < 0.05$) Serotonin in fruitfly compared to normal control^a. Imipramine (1 mM) and 50-100 mg/10 g Hibiscus treated flies significantly had elevated ($p < 0.05$) Serotonin compared to Chlorpromazine^b).

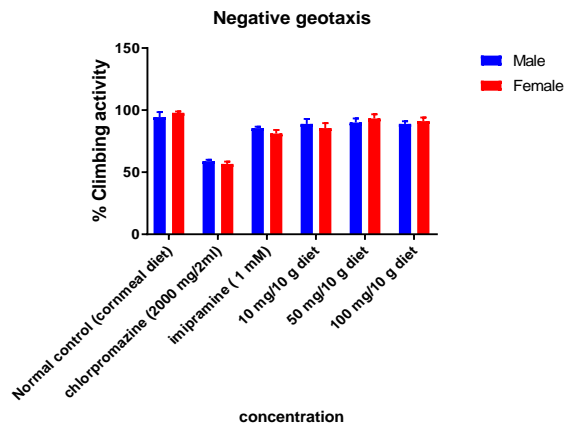


Figure 5: The Negative Geotaxis Assay was conducted on *Drosophila melanogaster*, and it was observed that there was no significant distinction ($p > 0.05$) between the percentage of male and female climbing activities. However, Chlorpromazine significantly reduced ($p < 0.05$) the percentage of male and female climbing activities as compared to Imipramine, Control, and graded doses of Hibiscus sabdariffa (10 mg/10 g, 50 mg/10 g, and 100 mg/10 g diet) in the flies.

Discussion

When the flies were induced with depression and given different doses of positive controls, standard controls, and *Hibiscus sabdariffa* extract, their mating behavior was affected. The groups treated with chlorpromazine showed reduced sexual activity compared to those treated with *Hibiscus sabdariffa* and imipramine, which is a common behavioral abnormality found in depression. Both male and female flies had significantly reduced copulation times compared to the positive and standard control groups and the *Hibiscus sabdariffa* extract group. The decrease in mating frequency was more dramatic in males, suggesting that they may be more sensitive to these drugs. These findings are consistent with previous research conducted by Miang *et al.* in 2017, which found that sexual activity is reduced in groups treated with chlorpromazine compared to other groups.

After inducing depression, the negative geotaxis assay was performed to observe the effects of different doses of negative controls, standard controls, and *Hibiscus sabdariffa* extract. The results showed that groups treated with chlorpromazine had a reduced ability to climb up to 6cm as compared to those treated with *Hibiscus sabdariffa* and imipramine, which is a common behavioral abnormality found in depression. Both male and female flies had significantly reduced climbing ability compared to the control groups (negative and standard) and the *Hibiscus sabdariffa* extract-treated group. This finding supports the work of Ming *et al.*, 2017, which also reported reduced climbing attempts in groups treated with chlorpromazine compared to other groups. This study supports the work of Andreas *et al.*, 2016, which stated that *Drosophila melanogaster* flies are negatively geotactic, and any difficulty in overcoming gravity indicates an abnormality.

Hibiscus sabdariffa has not been previously utilized for the treatment of depression or to uncover the molecular and physiological underpinnings of this condition in *Drosophila melanogaster*, despite similarities in altered behavior between fly mutants and human

patients. In this study, we induced depression-like features in *Drosophila melanogaster* using Chlorpromazine. The induced flies demonstrated significantly reduced climbing abilities and mating frequencies, indicating a loss of interest or sense of pleasure (anhedonia) and decreased energy levels or fatigue. These phenotypes resemble those seen in depressed patients. According to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision, depressed mood and anhedonia are the two primary symptoms of depression, and at least one of these symptoms must be pre

Furthermore, we conducted a comparison of serotonin concentrations in flies treated with Chlorpromazine, standard drug (imipramine), and control flies. Our findings revealed a significant reduction in serotonin levels in flies exposed to Chlorpromazine. This supports the hypothesis that the state of depression may result from a deficiency of serotonin in certain parts of the brain stem (Coppen, 1967). Some models used to identify clinically relevant antidepressants are also characterized by a decrease in serotonin levels (Antkiewicz *et al.*, 2014).

Additionally, we measured the concentration of MDA, an indication of lipid peroxidation, and SOD activity, considering that oxidative stress may be associated with depression (Xu *et al.*, 2014; Black *et al.*, 2015; Ormonde do Carmo *et al.*, 2015). Our results showed that Chlorpromazine significantly increased MDA content and reduced SOD activities in flies, supporting the work of Wiang *et al.* (2017), which reported similar findings.

Several studies have reported the association between depression and oxidative stress, with the lipid peroxidation product MDA being a commonly used biomarker (Bilici *et al.*, 2001; Kotan *et al.*, 2011). In a recent *Drosophila* study, the induction of depression-like behavior using Chlorpromazine was found to increase MDA levels and decrease SOD activity in flies, indicating the presence of oxidative stress (Antkiewicz *et al.*, 2019). This is consistent with previous studies suggesting that oxidative

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stress may play a role in the development of depression (Xu *et al.*, 2014; Black *et al.*, 2015; Ormonde do Carmo *et al.*, 2015). Taken together, these findings suggest that oxidative stress may be a key factor in the development of depression-like phenotypes in flies induced with Chlorpromazine.

despite the significant differences between *Drosophila* and humans in terms of their morphology and physiology, both species share a conserved genetic and molecular basis that regulates many critical biological processes, including those associated with the central nervous system (Hirth and Reichert, 1999).

Recent studies have revealed remarkable similarities between *Drosophila* and human neurobiology, including neuronal signaling, synaptic transmission, and neurotransmitter systems (O’Kane, 2011). For instance, both *Drosophila* and humans use glutamate and GABA as the primary excitatory and inhibitory neurotransmitters, respectively (Grotewiel, 2003). Moreover, both species also utilize many of the same neurotransmitter receptors and second messenger signaling pathways, such as cAMP, calcium, and phospholipase C (Grotewiel, 2003).

This similarity in neurobiological processes between *Drosophila* and humans has made *Drosophila* an ideal model organism to study the molecular and genetic basis of many neurological and psychiatric disorders, including depression (Bodai and Sztanek, 2021). Therefore, the results obtained from this study may have significant implications for understanding the molecular and physiological bases of depression in humans.

Conclusion

This study investigated the potential antidepressant effects of *Hibiscus sabdariffa* in *Drosophila melanogaster* induced with depression using Chlorpromazine. The results suggest that the administration of different concentrations of aqueous extract of *Hibiscus sabdariffa* had antidepressant activity, as evidenced by improved climbing ability and mating frequency in the flies. This highlights

the potential of using *Drosophila* as a model organism for studying depression

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