Methanol leaf extract of Leptadenia hastata ameliorates chronic, unpredictable, mild stress-induced depression

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Abstract

Leptadenia hastata (Pers) Decne is a tropical herb widely used in the phytotherapy of neuropsychiatric disorders, including depression. Despite the availability of synthetic antidepressant drugs, depression remains a major medical problem. The study aimed to evaluate the effect of methanol leaf extract of *Leptadenia hastata* (LHME) on chronic unpredictable mild stress-induced depression. Acute toxicity of *Leptadenia hastata* was determined using Organization for Economic Co-operation and Development (OECD) guidelines. The chronic unpredictable mild stress (CUMS) model-induced depression involves the evaluation of baseline behavioural changes in the sucrose preference test, open field test, and tail suspension test. The brain-derived neurotrophic factor (BDNF) concentrations and serum cortisol were assessed using an enzyme-linked immunosorbent assay (ELISA). The levels of superoxide dismutase (SOD) and malondialdehyde (MDA) were also assessed using standard procedure. The median lethal dose (LD_{50}) value was > 5000 mg/kg. LHME at doses of 250 – 1000 mg significantly ($p<0.01$) decreased CUMS-induced depression in a dose-dependent manner. LHME significantly ($p<0.01$) reversed depression associated weight loss from 14.00±0.62 g in week two to 22.57±1.13 g in week five of the experiment. It also increased sucrose consumption in the sucrose preference test (SPT) from 4.29±0.52 ml in week two to 9.71±0.68 ml in week five. The LHME also significantly ($p<0.01$) decreased the duration of immobility from 190.00±4.55 sec in week two to 158.00±3.83 sec in week five in the tail suspension test (TST). Furthermore, LHME at the tested dose significantly ($p<0.01$) increased the locomotor activity from 36.29±1.25 sec in week two to 62.43±1.73 sec in week five in the open field test (OFT). LHME significantly ($p<0.01$) and dose-dependently increased the levels of BDNF (204.74±22.97 pg/ml) and decreased the levels of plasma cortisol (0.98±0.06 ng/ml). However, treatment with LHME and the standard drug imipramine did

not significantly change SOD activity and the MDA level in CUMS-induced mice.
The findings demonstrated that LHME ameliorates CUMS-induced depressive-like behaviours, and its effect is possibly mediated via the neuroendocrine (cortisol) and neurotrophic (BDNF).

Keywords: *Leptadenia hastata,* Stress, Cortisol, Depression, Behaviour.

Introduction

The Diagnostic and Statistical Manual of Mental Disorders (DSM-V) characterized depression as depressed mood, diminished interest or pleasure (Anhedonia), a significant decrease in weight or appetite, insomnia, psychomotor agitation, fatigue, feelings of worthlessness or excessive feeling of guilt, inability to concentrate or indecisiveness, and suicidal thoughts (APA, 2016). Depression is a significant contributor to the global burden of diseases and a life-threatening disorder that affects hundreds of millions of people in all communities across the world (WHO, 2017). The estimated number of people living with depression in the world is 350 million, which accounts for about 4.4% of the total global population (WHO, 2017). Depression is known to have a higher prevalence among people with chronic medical conditions compared to the general population (Nguyen *et al*. 2016). According to Mello *et al*. (2010), the one-year prevalence of major depression is 7.1%. The lifetime prevalence of 20% and 10% were observed among women and men, respectively. At any given time, 5% of adults are suffering from major depression. Hypercortisolaemia is a common abnormality detected in many depressed patients (Connor and Leonard, 2008). An animal model is an essential tool for studying the pathophysiology of depression and the development of useful therapeutic agents (Willner, 1997). The chronic unpredictable mild stress (CUMS) induced depression is a valid animal model for depression linked to a stressful life. Pizarro *et al.* (2004) has shown that stress regulates brain-derived neurotrophic factor (BDNF) expression. The stressors, including immobilisation, foot shocks, social deprivation, and forced

swimming, significantly decreased BDNF expression in the hippocampus. Another study reported that exogenous cortisol treatment reduces hippocampal BDNF expression (Dwivedi *et al.* 2006). Therefore, a decrease in cortisol by antidepressant treatment increases the level of BDNF in the hippocampus (Chao *et al.* 1998).

Significant numbers of medicinal plant parts such as the leaf, roots, bark, stems, seeds, flowers, and fruits are used for treating different ailments (Dhamija *et al*. 2011). *Leptadenia hastata* (Pers.) belongs to the family *Asclepiadaceae,* locally called *Yadiya* in Hausa, *Iran-aji* in Yoruba, and *Soɓotorooji* in Fulfulde. It is widely used in Tropical Africa as a vegetable (Burkill, 1985). This plant is traditionally used to treat evil spirits, psychiatric disorders, loss of consciousness, hallucination, and depression (Burkill, 1985; Hussain and Karatela, 1989; Kinda *et al.*, 2017). This study aimed to evaluate the effect of methanol leaf extract of *Leptadenia hastata* on chronic unpredictable mild stress-induced depression.

Material and methods

Drugs

The standard drugs used for the experiment were Imipramine (Tofranil® GSK, Britain), Methanol (Sigma Chemical Co. St Louis USA), and Distilled water.

Experimental Animals

Sixty-five Swiss albino mice (18 – 22 g) were obtained from the animal house of the Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Bayero University Kano (BUK). According to the National Academy of Science, mice were maintained under standard laboratory conditions and guidelines for laboratory animals. The College Ethics Committee approved all experimental protocols of Health Sciences, with reference number BUK/CHS/REC/VII/53

Study Site

The study was performed at Neuropharmacology research laboratory of the Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Bayero University Kano, located at Aminu Kano Teaching Hospital, Kano (AKTH)

Plant Collection and Preparation

Fresh leaves of *L. hastata* were obtained from farms in Kumbotso Local Government Area, Kano State. The plant was authenticated in the Department of Plant Biology, Bayero University, Kano. The specimen voucher number (BUKHAN 0248) was given and documented. The leaves were dried and ground using mortar and pestle. The powdered plant materials, 1000 g each, were macerated with 5 Litre 70 % v/v methanol and filtered using Whatman filter paper No: 10. The filtrate was concentrated *in* vacuo 40°C.

Acute Toxicity Study

The median lethal dose (LD_{50}) was determined using the Organization for Economic Co-operation and Development guidelines (OECD, 2001). Three mice fasted 3 hours before the experiment, and the calculated doses given were according to their body weight. Food was withheld for 1-2 hours, after which the LHME was administered orally. The limit test was conducted in two stages. The first stage, 5000 mg/kg, was administered to one mouse and observed for 48 h. The second stage was carried out on survivors by administering 5000 mg/kg to two additional mice. Animals were observed during the first 30 minutes of treatment, occasionally within 24 h, and finally daily for 14 days.

Study Design:

Depressive-like behaviour was induced by the Chronic Unpredictable Mild Stress (CUMS) model with slight modifications (Forbes *et al.* 1996). Sixty mice were randomly divided into six groups. Group1 consisted of normal

control [no stress and no treatment (NoCUMS)]; group 2: the stressed group [(stress + distilled water 10 ml/kg (CUMS)], group 3: CUMS + imipramine (10 mg/kg); group 4: CUMS + LHME (250 mg/kg); group 5: CUMS + LHME (500 mg/kg); group 6: CUMS + LHME (1000 mg/kg). The mice were subjected to chronic unpredictable mild stress daily for five weeks.

The LHME and imipramine were administered for three weeks, starting two weeks after CUMS exposure up to the end of the procedure. The animals were treated orally and assessed an hour after the drug administration. The stressors used include 2 hours' immobilisation, 24 hours of wet bedding, 24 hours' cage tilted at 45º, 24 hours' food deprivation, 24 hours' empty cages, 24 hours' water deprivation, 1 min tail pinch, and 5 min cold swim at 10 ºC. The whole experiment lasted for 42 days. The OPT, TST, and SPT were carried out on three occasions [i.e., week 0 (before stress), week 2 (before drug administration), and week 5 (at the end of drug administration)] in the following chronological sequence: (i) animal adaptation: 1–3 days; (ii) OFT: 4, 17, 39 days; (iii) TST: 5, 18, 40 days; (iv) sucrose consumption test: 6, 21, 41 days; (vi) stress procedure: 6–41 days; (vii) drug administration: 20–41 days.

Sucrose preference test

Mice were trained to consume 2% (w/v) sucrose solution 24 hours before the experiment. The test was initially carried out 24 hours before stress induction, two weeks after stress, and five weeks after stress. The mice were deprived of food and water for 21 hours and then exposed to both test solutions (2% sucrose and drinking water) within the same period. Sucrose consumption was measured by measuring the volume of sucrose solution remaining in the test bottle (Willner *et al.* 1987; Forbes *et al.* 1996). During the stress induction period, drugs were administered from week three to week five.

Tail suspension test

After administering the stressors, this test was carried out at week zero, week two, and week five. For the test, the mice were suspended on the edge of a shelf 58 cm above a tabletop by an adhesive tape placed approximately 1 cm from the tip of the tail. The duration of immobility was recorded within 6 min period (Steru *et al.* 1985).

Open field test

After applying stressors, the test was carried out at week zero, week two, and week five. The mice were placed in a white wooden open-field apparatus. The mouse exploratory behaviour was recorded in the apparatus for 5 minutes. Then, the arena was cleaned with 10% ethanol between tests. The parameters recorded include line crossing, rearing, and central square entry (Prut and Belzung, 2003).

Biochemical Markers of Depression

Blood sample collection and brain harvesting

On day 42 of CUMS test. Seven mice were randomly selected from each group and sacrificed. Blood samples were collected in plain bottles and centrifuged at 1000 *g* within 20 min. Serum was aspirated and used for the cortisol competitive ELISA detection method. The brain samples were harvested and stored in 0.1 M sodium phosphate buffer, pH 7.4, at –20 °C until needed. The brain was minced and homogenised in phosphate-buffered saline (PBS; 0.1M, pH 7.4). The homogenates were centrifuged at 2000 *g,* and the supernatants were obtained. The brain homogenates were used for sandwich ELISA for (BDNF), superoxide dismutase activity assay (SOD), and malondialdehyde assayed (MDA) using a spectrophotometer.

Brain-derived neurotrophic factor level

According to the manufacturer's instructions, the BDNF level in the brain homogenates was measured using a commercially available ELISA kit (Wuhan Fine Biotech Co., Ltd., Cat. no. EM0020). Briefly, the plate was

washed twice before adding the standard, sample, and zero wells. Then, 100 µL of the standard samples were added to each well and incubated for 90 min at 37 °C, after which the plates were aspirated and washed three times. Then 100 µL of the biotin-labelled antibody working solution was added to each well and incubated for 60 min at 37 °C, after which the plate was removed, aspirated, and washed three times. Then, 100 µL of HRP-Streptavidin conjugate (SABC) working solution was added to each well and incubated for 30 min at 37 °C. The plates were removed, aspirated, and washed five times. 90 µL 3,3',5,5'-Tetramethyl benzidine (TMB) substrate was added and finally incubated for 15 min at 37 °C. The plates were removed, and 50 µL of stop solution was added to each well. The absorbance was measured immediately at 450 nm using a microplate reader (Rayto-RT-2100C).

Serum cortisol level

Blood samples were collected in plain bottles and centrifuged at 1000 *g* within 20 min. The serum was aspirated. According to the manufacturer's protocol, the serum cortisol level was measured using a commercially available ELISA kit (Wuhan Fine Biotech Co. Ltd, Cat. no. EM1721). Briefly, the plates were washed twice before adding the samples, then 50 µL of the sample and standard solutions were added to the already pre-coated antibody plate provided with the kit and incubated for 45 min at 37 °C. The plates were removed, aspirated, and then washed three times, followed by adding 100 µL of SABC working solution into each well and incubated for another 30 min at 37 °C. The plates were removed, aspirated, and washed five times. 90 µL of TMB substrate was added. The plate was further incubated for 15 min at 37°C. The reaction was stopped by adding 50 µL of stop solution, and the absorbance was read at 450 nm using a microplate reader (Rayto-RT-2100C).

Superoxide dismutase assay

The SOD activity in the brain homogenate was measured using a colourimetry assay (Arthur and Boyne, 1985). The xanthine and xanthine oxidase reaction system produces the superoxide anion (O2-). The O_2- reduces nitroblue tetrazolium salt to water-soluble blue formazan, which absorbs light at 560 nm. SOD can remove O_2^-, thereby inhibiting formazan formation. The deeper the blue colouration of the reaction mixture, the lower the SOD activity. Briefly, 90 µL of samples and or distilled water was added to test tubes containing 240 µL, 510 µL, 6 µL, and 180 µL of reagents one, two, three, and four, respectively. They were mixed and allowed to stand for 30 min at room temperature. A 1 ml glass cuvette was used, and absorbance was measured spectrophotometrically at 560 nm. The results were expressed as U/mg protein.

Malondialdehyde determination

The level of MDA in the brain homogenate was measured using the colourimetry method. Briefly, 0.6 µL of reagent one was pipetted into a 1.5 ml centrifuge tube, and a mixture of the solution was made by adding 0.2 ml of homogenate. The solution was heated in a boiling water bath for 30 min. After cooling, the sample was centrifuged at 1,000 g for 10 min. The supernatant was transferred into a 1 ml cuvette, and absorbance was measured using a spectrophotometer at 532 nm and 600 nm wavelengths (Ohkawa *et al*. 1979). The level of MDA was expressed as nmol/mg protein.

Statistical analysis

All results were expressed as Mean ± SEM. Data was analyzed using one-way ANOVA followed by Bonferroni's Post Hoc Test. Repeated measurements were analyzed using Repeated Measure Analysis of variance (ANOVA) followed by Bonferroni Post Hoc Tests. Values of $p < 0.05$ were considered statistically significant. The whole analysis was done using SPSS, version 23. (IBM SPSS, Chicago, IL, USA).

RESULTS

Acute toxicity (LD_{50}) of methanol leaf extract of LHME

The oral median lethal dose (LD_{50}) of LHME was estimated to be >5000 mg/kg body weight in mice according to OECD 420 guidelines.

Effect of LHME on CUMS-induced depression

There was no significant difference ($p>0.05$) in the body weight across the groups at week 0. However, two weeks after chronic unpredictable mild stress (CUMS) exposure, the body weight decreased significantly ($p<0.01$) in all the groups except the group with no chronic unpredictable mild stress (noCUMS) group. Also, a significant increase ($p<0.01$) in body weight was observed three weeks after treatment with LHME at doses of 250, 500, and 1000 mg/kg and the standard drug imipramine (10 mg/kg) when compared to the untreated CUMS group (Figure 1).

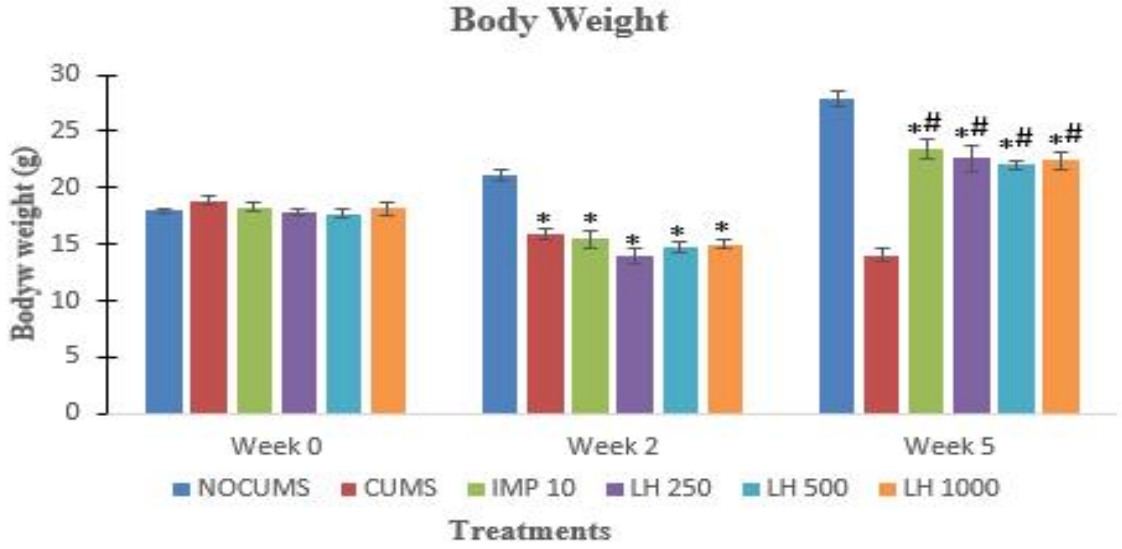

Figure 1: Effect of LHME on body weight in CUMS-induced depression. The body weights at weeks 0 (before stress), weeks 2 (2 weeks after stress) & weeks 5 (3 weeks after stress and treatments). Data presented as mean ± SEM (n = 7). *$p<0.01$ compared to the NoCUMS group; #$p<0.05$ significant difference compared to the CUMS group. CUMS, chronic unpredictable mild stress; NoCUMS, non-chronic unpredictable mild stress; LH=*Leptadenia hastata* methanol extract, and IMP= Imipramine. Treatments = Treatment (mg/kg).

Effect of LHME on Locomotor Activity in Open Field Test

At week 0, no significant difference was recorded in locomotor activity before subjecting the mice to CUMS in OFT. With the exposure to stress for two consecutive weeks, a significant decrease (p<0.01) in the number of lines crossed was observed in CUMS relative NoCUMS group. However, after three weeks of treatment with LHME at doses of 250, 500 and 1000 mg/kg and imipramine (10 mg/kg), the mice significantly (p<0.01) exhibited more crossings as compared to the CUMS group (Figure 2).

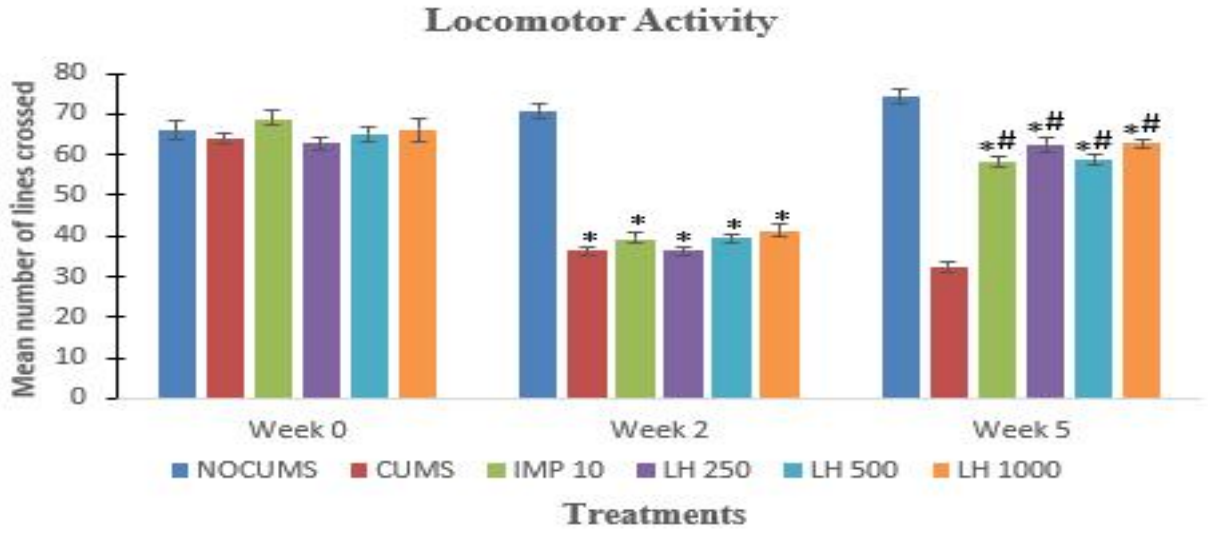

Figure 2: LHME on Locomotor Activity in OFT following CUMS-induced depression. The data were presented as mean ± SEM (n = 7). *p<0.05 significant difference compared to the NoCUMS group; #p<0.01 significant difference compared to the CUMS group. CUMS, chronic unpredictable mild stress, IMP= Imipramine, Treatments = Treatment (mg/kg).

Effect of LHME in Tail Suspension Test

There was no significant difference (p>0.05) in the duration of immobility at week 0 before subjecting the mice to CUMS in TST. However, two weeks after subjecting the mice to stressors, the duration of immobility significantly (p<0.01) increased in all the groups compared to the noCUMS group. Treatment with extract and imipramine for three weeks significantly (p<0.01) decreased the duration of immobility when compared to the untreated CUMS group (Figure 3)

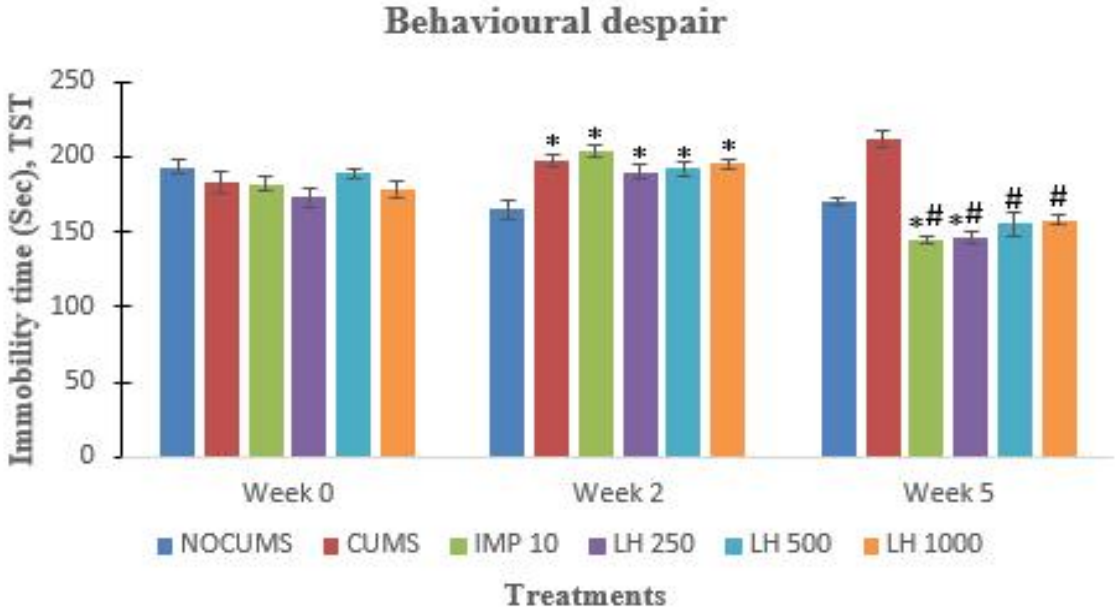

Figure 3: LHME on immobility time in TST following CUMS-induced depression. The results are presented as mean ± SEM (n = 7). *p<0.05 significant difference compared to the NoCUMS group; #p<0.01 significant difference compared to the CUMS group. CUMS, chronic unpredictable mild stress; LH=*Leptadenia hastata* methanol extract and IMP= Imipramine. Treatments = Treatment (mg/kg).

Effect of LHME on Sucrose Consumption

There was no significant decrease (p>0.05) in sucrose consumption across week 0. However, two weeks after CUMS exposure, the sucrose consumption decreased significantly (p<0.01) in all the groups except the NoCUMS group. Three weeks after treatment with extracts LHME at doses of 250, 500 and 1000mg/kg and imipramine (10 mg/kg), there was a significant (p<0.01) increase in sucrose consumption among mice in all the groups compared to the untreated CUMS mice (Figures 4).

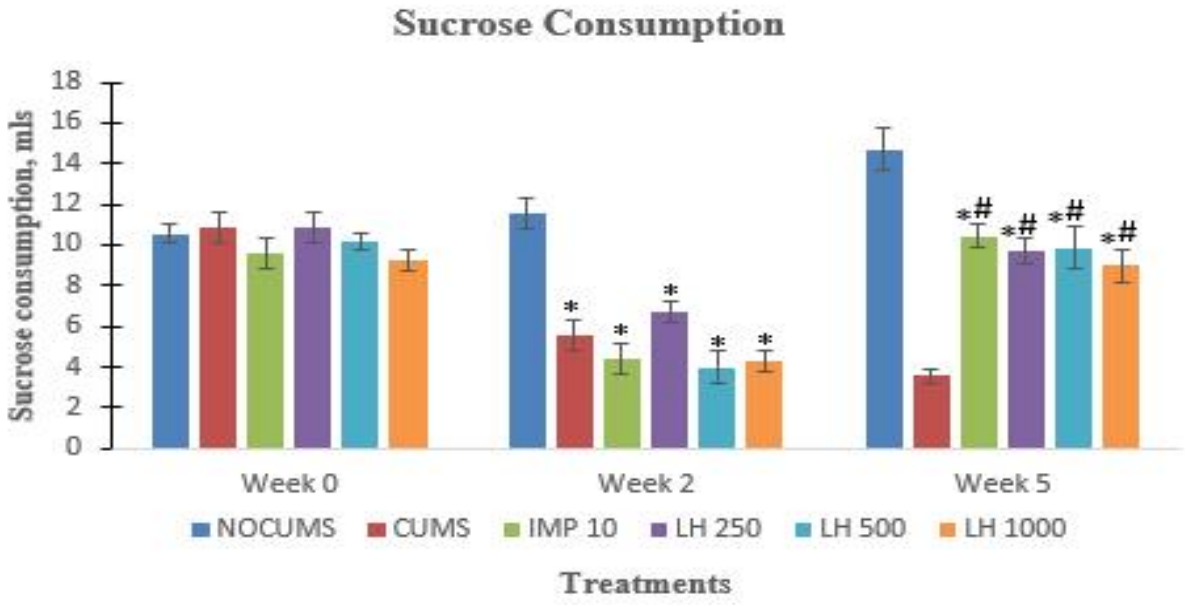

Figure 4: LHME on Sucrose Consumption in mice following exposure to CUMS-induced depression. Results are presented as mean ± SEM (n = 7). **p*<0.01 significant difference compared to the NoCUMS group; #*p*<0.01 significant difference compared to the untreated CUMS group. CUMS, chronic unpredictable mild stress; LH=*Leptadenia hastata* methanol extract, IMP= Imipramine, Treatments = Treatment (mg/kg).

Effect of LHME on Serum Cortisol Level

The serum cortisol levels in the CUMS group were significantly ($p<0.01$) increased compared to the NoCUMS mice. However, treatment with LHME at doses of 250, 500 and 1000 mg/kg and imipramine (10 mg/kg) significantly ($p<0.01$) and dose-dependently decreased the levels of serum cortisol when compared to the untreated CUMS mice (Figure 5).

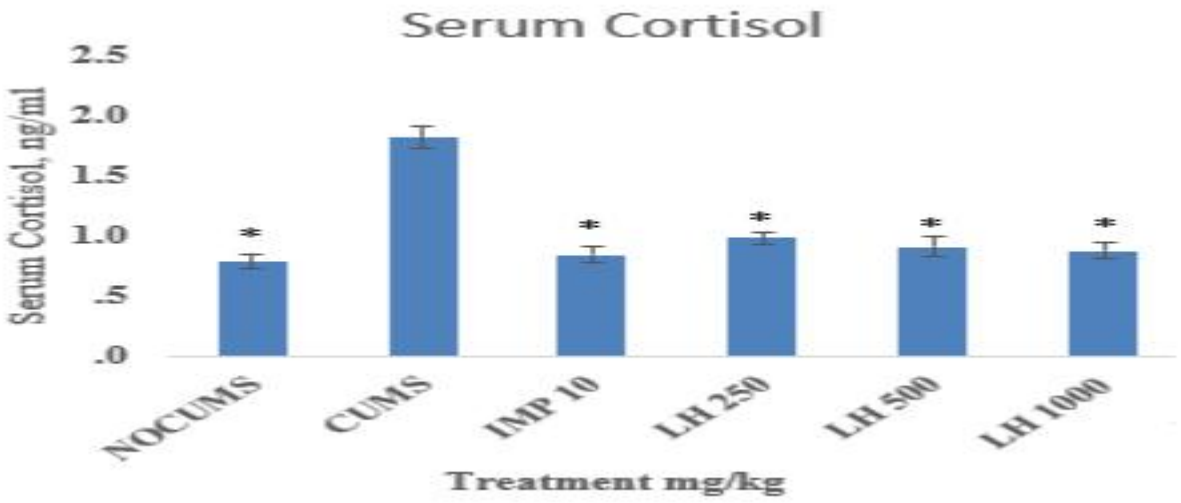

Figure 5: LHME on Serum Cortisol Levels after CUMS-induced depression in mice. The results are expressed as mean ± SEM (n = 7). *p<0.01 significant difference compared to the stressed group; CUMS, chronic unpredictable mild stress; LH=*Leptadenia hastata* methanol extract and IMP= Imipramine.

Effect of LHME on Brain-Derived Neurotrophic Factor (BDNF)

The concentration of BDNF in the brain was decreased significantly (p<0.01) in mice subjected to five weeks of CUMS procedure compared with the NoCUMS mice. However, treatment with LHME (250-1000 mg/kg) and imipramine significantly (p<0.01) increased the concentration of BDNF in the brain and dose-dependent for LHME tested doses when compared to the untreated CUMS mice.

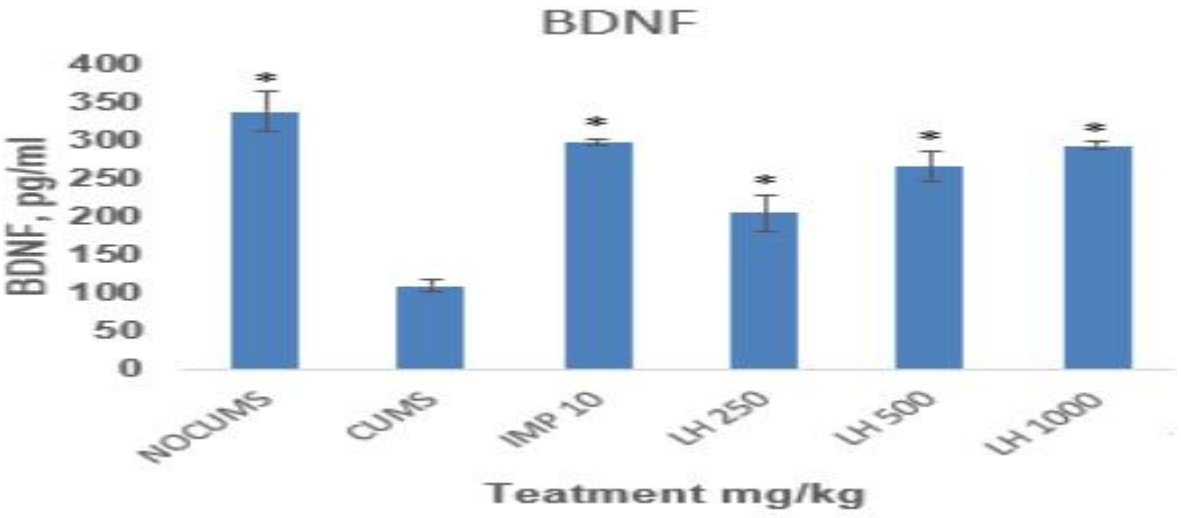

Figure 6: LHME on BDNF concentration after CUMS-induced depression in mice. Results are expressed as mean ± SEM (n = 7). *p<0.01 significant difference compared to the stressed group; CUMS, chronic unpredictable mild stress; LH=*Leptadenia hastata* methanol extract and IMP= Imipramine.

Effect of LHME on malondialdehyde (MDA) levels after CUMS-induced depression in Mice

The MDA levels in mice subjected to CUMS significantly (p<0.05) increased compared to noCUMS mice. However, treatment with LHME at doses of 250, 500 and 1000 mg/kg and imipramine (10 mg/kg) had no significant effect on MDA levels compared to the untreated CUMS group (Figure 7).

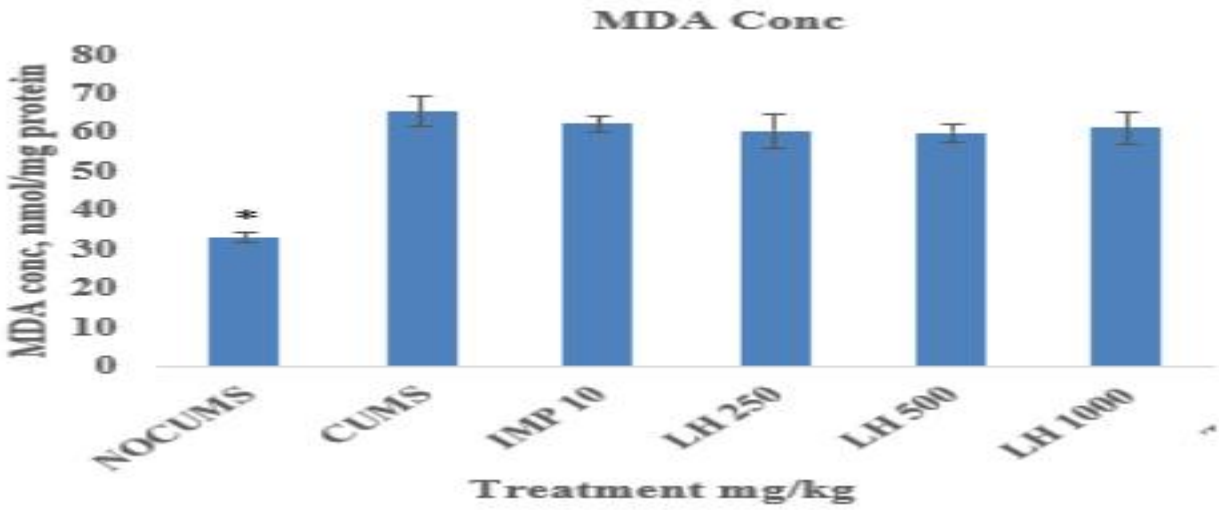

Figure 7: LHME on MDA levels after CUMS-induced depression in mice. Results are expressed as mean ± SEM (n = 7). *p<0.01 significant difference compared to the stressed group; CUMS, chronic unpredictable mild stress; LH=*Leptadenia hastata* methanol extract and IMP= Imipramine. MDA = malondialdehyde;

Effect of LHME on superoxide dismutase (SOD) activity after CUMS-induced depression in Mice

Exposure to stressors for five weeks produced a significant (p<0.01) decrease in the concentration of SOD in CUMS mice when compared with the noCUMS mice. However, treatment with LHME at doses of 250, 500 and 1000 mg/kg and imipramine (10 mg/kg) did not significantly change the level of SOD activity as compared to the untreated CUMS group (Figure 8).

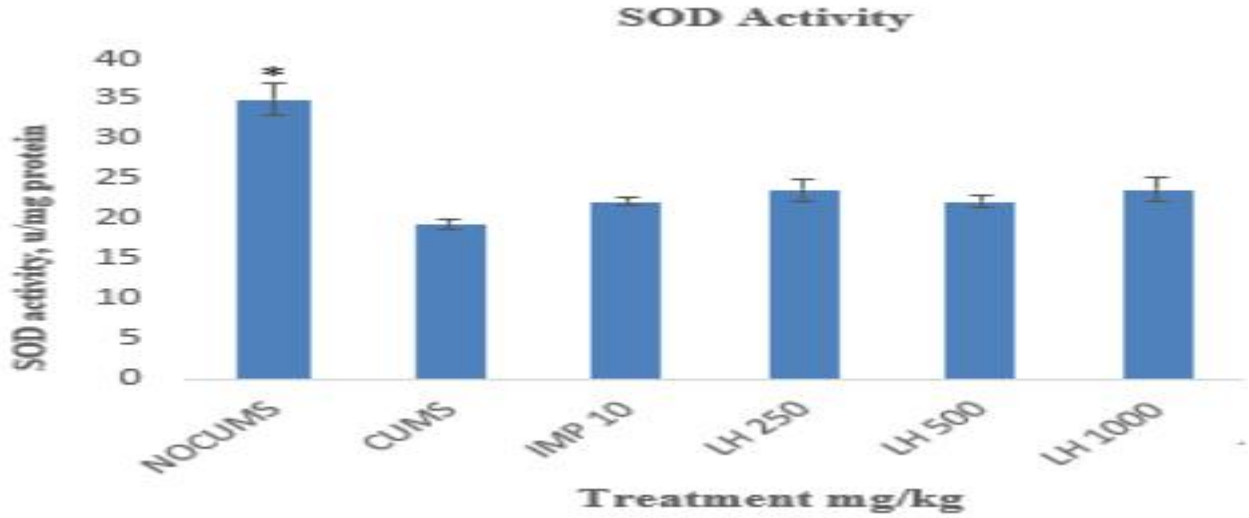

Figure 8: LHME on SOD activity after CUMS-induced depression in mice. Results are expressed as mean ± SEM (n = 7). *p<0.01 significant difference compared to the stressed group; CUMS, chronic unpredictable mild stress; LH=*Leptadenia hastata* methanol extract and IMP= Imipramine. SOD=superoxide dismutase.

Discussion

The acute toxicity profile (LD_{50}) of LHME was found to be non-toxic. Hence, LHME is practically safe, which justifies using the plant as food and treating chronic diseases.

The LHME ameliorated weight loss, poor locomotor activity, behavioural despair and poor sucrose consumption, which were major symptoms of depression in experimental animals (Forbes *et al.* 1996; Qiu *et al*. 2014; Remus *et al.* 2015; Zhai *et al*. 2015; Wang *et al*. 2010; Wang *et al*. 2015).

The CUMS induced depressive-like behaviour in mice treated with either imipramine or LHME showed increased sucrose consumption than the untreated group. This decrease in sucrose consumption in CUMS group was associated with the state of anhedonia, which is a psychological condition characterised by the inability to experience pleasure from activities that were enjoyed previously. These results conform with the previous studies, where impaired sucrose consumption was reversed by antidepressant therapy (Qiu *et al*. 2014; Remus *et al.* 2015; Zhai *et al*. 2015; Wang *et al*. 2015).

The CUMS group lost body weight, while the noCUMS group gained body weight

throughout the experiment. However, a significant increase in body weights following the subsequent three weeks of treatment with LHME and imipramine. These findings align with some studies that reported that antidepressants abated bodyweight loss in depressed individuals (Willner *et al.* 1997; Hurley *et al.* 2013; Remus *et al.* 2015).

In a novel environment, depressed animals displayed fewer locomotor and exploratory activities when compared to the noCUMS group, indicating a loss of interest in external stimuli. However, treatment with LHME and imipramine for three weeks in CUMS-induced animals reversed the impaired locomotor of the mice without causing stimulatory activity.

The unstressed (noCUMS) group showed less behavioural despair in TST, resulting in shorter immobility durations than the stressed-induced CUMS group, indicating antidepressant-like activity (Evans *et al.* 2012). Our findings showed that treatment with LHME and imipramine reversed the behavioural symptoms indicating antidepressant activity.

Our results showed that BDNF concentrations were reduced in the CUMS-induced group (Qiu *et al.*, 2014; Wang *et al.*, 2015). The BDNF level in the animals was upregulated by treatment with LHME and imipramine. Other studies have shown that long-term treatment of depression with antidepressant drugs elevates BDNF levels (Qiu *et al.*, 2014; Wang *et al.*, 2015; Aldoghachi *et al.*, 2019). Studies have shown that BDNF is directly involved in the pathophysiology and aetiology of depression (Yoshida *et al*., 2012; Sakata and Duke, 2014; Aldoghachi *et al.*, 2019). BDNF plays an important role in neuronal development, differentiation, survival and synaptic plasticity of central nervous systems (Sakata and Duke, 2014). Though a short-term treatment with antidepressant drugs elevates the brain's monoamine levels, it also takes more than two weeks to show a clinical

response (Hindmarch, 2001), with about a 70% response rate in patients with depressive illness (Nemeroff, 2007).

Effective antidepressant treatments are associated with improved hippocampal neurogenesis, increased BDNF levels, and brain monoamine neurotransmitters with a resultant increase in hippocampal function as a neuronal response (Monteggia *et al.* 2007). Hence, the effectiveness of the antidepressant effect of these plants may be partly due to the effect on neuroendocrine and neurotrophic factors.

In this study treatment with LHME significantly reduced serum cortisol levels which manifested as decrease in the hyperactivity of the Hypothalamic-Pituitary-Adrenal (HPA) axis. Hence, the antidepressant activity of LHME on CUMS mice might involve the inhibition of the HPA axis and upregulation of the monoamine levels. HPA axis hyperactivity induced by CUMS, has been found to cause a reduction in monoamine neurotransmitters and an increase in serum levels of cortisol (Yang *et al.*, 2017). Antidepressant drugs relieve HPA axis hyperactivity, reduce excess cortisol levels and improve monoamine neurotransmitters (Keller *et al.*, 2017). Also, the CUMS-induced depression model enhanced oxidative stress expressed as the increase in the product of lipid peroxidation (MDA level), as well as decrease in SOD activity has been reported (Galecki *et al.*, 2009; Liu *et al.*, 2015). Expectedly, the mice subjected to CUMS showed decreased SOD activity and increased MDA levels. However, treatment with LHME and imipramine did not significantly change SOD activity and the MDA level in CUMS-induced mice. It has been reported that short-term treatment with antidepressants has little effect on stress biomarkers while long-term treatment with SSRIs but not TCAs has improved oxidative stress imbalance in depression (Duda *et al.,* 2016). This is also supported by the inability of imipramine to produce a significant

antioxidant effect in this study. However, LHME ameliorated the weight loss, poor locomotor activity, and poor sucrose consumption (anhedonia) in CUMS-induced depression model. Additionally, the LHME also improved the level of neurotrophic (BDNF) and neuroendocrine (serum cortisol) markers of depression in this study.

The limitations and strenghts for this study

The limitations of this study are some symptoms of depression are difficult to observe in animals which include: feelings of sadness, guilt, or suicidal thoughts, symptoms mainly limited to humans. However, animal models that served as an essential tool for studying the aetiology of depression, as well as the development of effective therapeutic targets for an antidepressant agent (Porsolt *et al*. 1977; Steru *et al.* 1985; Willner, 1997). Moreover, this research used the ideal animal models of behavioural, neurotrophic (BDNF) and neuroendocrine (serum cortisol) markers of depression.

Conclusion

The methanol leaf extract of *Leptadenia hastata* possesses antidepressant activity that involves reversed CUMS-induced depression elicited via neurotrophic (BDNF) and neuroendocrine (cortisol) systems in its pathophysiology**.**

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Conflict of Interest

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