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Effects of Aqueous Extract of *Garcinia lucida* on Chronic Stress Induced Depression-Like Behaviours in Mice

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ABSTRACT

This study examines anti-depressive effects of *Garcinia lucida (G. lucida)* using the Forced Swimming Test (FST), the Tail Suspension Test (TST) and Sugar Preference Test or anhedonia test after 21 days of unpredictable chronic mild stress (UCMS) in mice. Three doses of aqueous extract of *G. lucida* (200, 400 and 800 mg/kg) were used to evaluate activities of this plant on depression-like behaviours in mice. *G. lucida* showed significant anti-depressive activities in the FST and the TST by specific increase of the delay of immobility occurrence and decrease of immobility time. At dose 200 mg/kg *G. lucida* exhibits significant ($P < 0.001$) increase of the occurrence of immobility for about 90.6% and dose of 400 mg/kg reduced the immobility time from 111 ± 5.57 s (in the control group) to 26.33 ± 1.82 s in the FST. In the TST, the total immobility time of mice treated by *G. lucida* significantly decreased for about 25.89%, 32.63% and 35.92 at doses 200, 400 and 800 mg/kg respectively when compared to the control group. In addition, the delay of immobility occurrence has significantly ($P < 0.01$) increase for about 77.79% in the group treated by dose 800 mg/kg of *G. lucida*. *G. lucida* showed in the anhedonia test a preference to sweaty water as it consumption consumed significantly ($P < 0.001$) increased from 84.7 mL to 155.29 mL at dose 800 mg/kg. The results revealed that *G. lucida* possess compounds probably responsible of the anti-depressive effects observed in the FST, the TST and anhedonia test.

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INTRODUCTION

Stressful experiences have been reported to favor the development of depression in humans [1,2,3]. Therefore, stress-based animal models were developed to explore depressive phenomena. Depression is thought to result from interactions between the effects of environmental stress and genetic predisposition. Depression is one of the top five most prevalent diseases worldwide. By 2020, it is expected to be the second-leading cause of disability globally. Depression is typically presented as lowered mood, difficulty in thinking, loss of interest and physical complaints such as headache, disturbed sleep, loss of energy and change in sex drive [4,5]. According to the estimations of the WHO, depression will be the second leading cause of disability in 2020 [6]. Recent epidemiological studies indicate that severe forms of depression affect 2-5% of the population worldwide, and up to 20% are affected by milder forms of the disease [7]. Moreover, depressive patients have increased risk to develop cardiovascular diseases and 10-15% of individuals with major depression commit suicide [8]. The need for better-tolerated and more efficient treatments is remaining high. It is becoming very useful considering and looking for alternative and low cost effective herbal therapy, especially in low income countries, where much of the population relies on herbs remedies [9]. The broad use of medicinal plants is often attributable to its efficiency, accessibility and affordability. Moreover, traditional medicine is sometimes the only affordable source of health care in developing countries. *G. lucida* is a plant found in Cameroon, Gabon and Equatorial Guinea [10]. Earlier pharmacological studies have shown that bark and seeds extract of *G. lucida* possesses an inhibitory effect upon the actions of curare [11]. Chemical studies revealed the presence of alkaloids, flavonoids, phenols and polyphenols, tannins, anthraquinones, steroids, saponins and glycosides [12,13,14]. The target of the present work is to assess effects of aqueous extract of *G. lucida* upon depression-like behaviors after 21 days of unpredictable chronic mild stress in mice.

MATERIALS AND METHODS

Plant material

The barks of *G. lucida* were collected at Mfou (vicinity of Yaoundé), Cameroon (March 2018). A Voucher specimen was deposited at the National Herbarium in Yaoundé and identify under the reference number 9269.

Preparation of the extract of aqueous extract

Fresh barks of *G. lucida* were dried in the shade and ground. 800 g of bark powder were boiled for 20 min with distilled water. After cooling, the supernatant was collected and filtered with Whatman paper N° 1. The filtered solution was freeze-dried at the Medical and Research Institute of Medicinal Plants of Cameroun (IMPM). 90.56 g of freeze-dried powder of barks of *G. lucida* were obtained and served to prepare the stock solution. The yield of the extraction was about 11.32 %. Three concentrations of aqueous extract of *G. lucida* (80; 40 and 20 mg/mL) were prepared by dissolving the freeze-dry powder in distilled water. As solutions were administrated at the volume of 10 mL/kg, the three doses required for the experiments were obtained (800; 400 and 200 mg/kg).

Animals

Adult *Musmusculus* Swiss mice (23 ± 0.6 g) were obtained from the animal room of the higher teacher's training college of Yaoundé (Cameroon). They were housed at a room temperature of about 25°C in a 12h light/12h dark cycle. Food and water were available ad libitum. For experiments, animals were randomly assigned to control or treatment groups. The study was done in accordance with the national (reg. N° FWA-IRB00001954) ethical committee guidelines for the care and used of laboratory animals.

Chemicals and treatments

Hydro-chlorate of imipramine (Amdipharm limited, Ireland) (Tofranil 25 mg) and sugar (Cameroonian society of sugar) were used. Chemicals were diluted in distilled water and were administered in a volume of 10 ml/kg of mouse body weight. Mice were randomly allocated to one of the following five groups (n = 6/group): (CP) Imipramine (25 mg/Kg), (D 800) *G. lucida* (80 mg/ml), (D 400) *G. lucida* (40 mg/ml), (D 200) *G. lucida* (20 mg/ml), and (CN) vehicle (distilled water, 10 ml/kg).

Unpredictable Chronic mild stress

The UCMS model procedure involves relatively continuous exposure of mice to a variety of mild stressors, such as periods of food and water deprivation, small temperature reductions, changes of cage mates, and other similar individually innocuous, but unpredictable, manipulations [15]. For 3 weeks (21 days) animals of each group received the corresponding

treatments listed above and were later subject to a stress factors which could be forced swimming in cold water, electric choc, pinching of a rear paw, inclination of the cage or a litter anchorage. The stress factors were changed from day to day.

Pharmacological tests

Forced swimming test

The FST, also known as forced swim test, behavioral despair test or the Porsolt test, was developed in 1977 by Porsolt and colleagues in the rat and subsequently in the mouse. The FST is a representative behavioral test for depression which is used for the screening of anti depressant effects of drugs [16]. The apparatus was a cylinder with 20 cm in diameter and 46 cm in height. It was filled with water at the depth of 20 cm. The possible antidepressant effects of our plant extract were studied by the FST. At day 22, 60 minutes after different treatments, mice were individually placed into the FST apparatus at room temperature and left to swim for 6 min. Test parameters including latency to immobility and duration of immobility [17], were evaluated for the last 4 min.

Tail suspension test

The TST, which was first introduced in 1985 to measure the potential effectiveness of anti-depressant drugs, shares a common theoretical basis and behavioral measure with the FST [15]. In this procedure tails of mice were suspended using adhesive tape to a horizontal bar for 6 min. Test parameters including latency to immobility and duration of immobility, were evaluated for the last 4 min. This procedure was performed at day 22, 60 minutes after administration of different treatments.

Anhedonia test

One of the major symptoms of depression in humans is anhedonia, a reduction in interest or pleasure in daily activities [18]. For 3 weeks of UCMS, mice were individually housed in cages with two bottles of drinkable solution, one containing 100 ml of sucrose solution (1%) and the other 100 ml of tap water. Consumed volumes of sucrose solution and tap water were daily recorded. At the end of every week, body weight of each mouse was evaluated with scales JACO.

Data analysis

The animal's behavioral activities in the TST and FST were statistically analyzed with one-way analysis of variance (ANOVA). All results were expressed as mean ± SEM. According to the degrees of freedom (F), values of $p<0.05$ were regarded as statistically significant. Significant differences between individual groups were determined by Fisher post hoc test. The statistical package used for the analysis was STATISTICA 6.0.

RESULTS AND DISCUSSION

Forced swimming test

Effects of *G. lucida* on the latency of immobility

The latency to the onset of the first episode of immobility (swimming time before the first immobilization) in the CN group was 13.66 ± 2.43 s. It was noted a significant increase of this latency in the group of animals that received Imipramine (CP) to 60.33 ± 1.4 s. All doses of *G. lucida* (800, 400and 200 mg/kg) induced an increase of the latency to the onset of the first episode of immobility. The highest latency time was 54.66 ± 3.74 s observed in the group of animals treated with *G. lucida* at dose 200 mg/Kg (D 200) as shown in Figure 1.

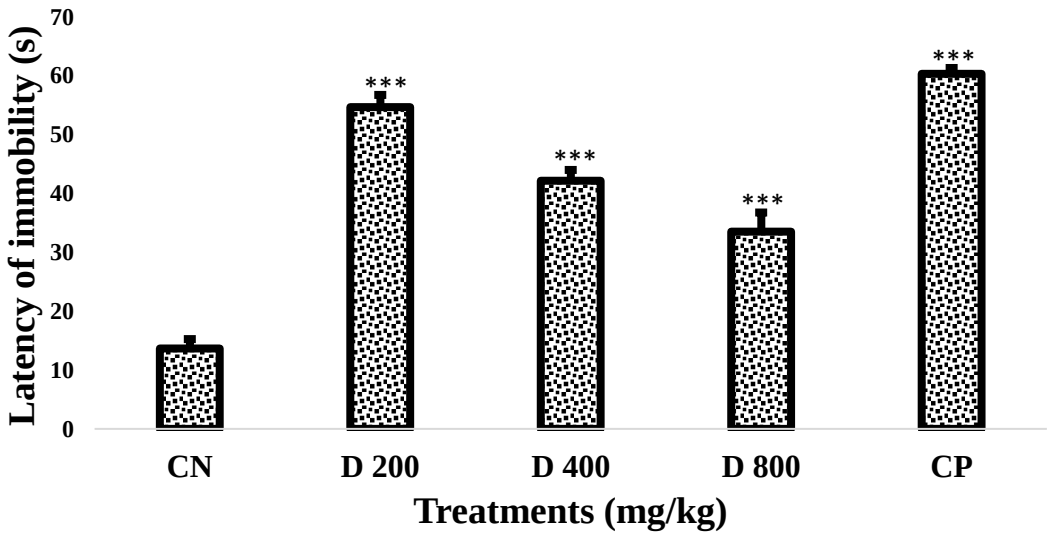

Figure No. 1: Effects of *G. lucida* on the latency of immobility in the FST

Each bar represents the latency of immobility ± SEM. Treatments were administered 1 hour before the test. CN=distilled water; CP=Imipramine (25mg/Kg). N = 6, ***$p<0.001$, when compared to CN (ANOVA, followed by Fisher test).

Effects of *G. lucida* on the immobility time

Figure 2 shows that *G. lucida* significantly decreased the immobility time in the FST. The immobility time of 111.67 ± 5.57 s in the CN group, was reduced to 26.33 ± 1.82 s in the group of animals treated with *G. lucida* at dose 400 mg/Kg (D 400). Imipramine (25 mg/Kg) produced similar outcomes showing a reduction of about 42 % in comparison with the CN group.

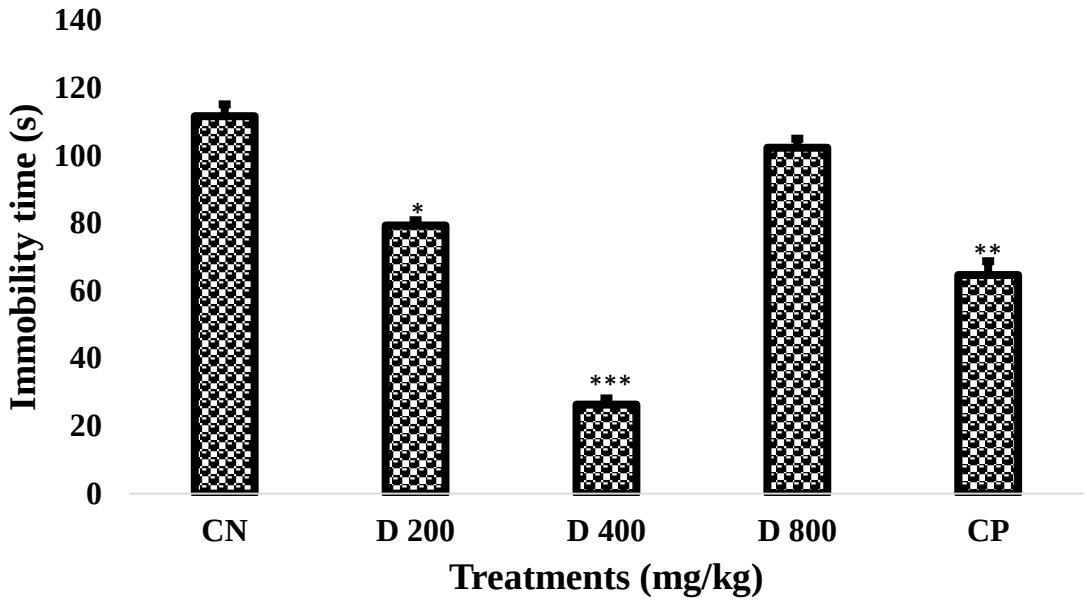

Figure No. 2: Effects of *G. lucida* on immobility time in the FST

Each bar represents the immobility time ± SEM. Treatments were administered 1 hour before the test. CN=distilled water; CP=Imipramine (25mg/Kg). N = 6 per dose, *p<0.05, **p<0.01, ***p<0.001, when compared to CN (ANOVA, followed by Fisher test).

Tail suspension test

Effects of *G. lucida* on the latency of immobility

As expected in the TST, Imipramine (25 mg/Kg) induced a significant delay (p<0.001) of the latency to the onset of the first episode of immobility from 34.5 s ± 3.56 s in the CN group to 81.83 ± 1.44 s in the CP group. The effect of *G. lucida* was dose-dependent, and dose D 800 group (*G. lucida* 800 mg/Kg) produced similar outcome, a significant increase (p<0.01) of the latency of immobility to 63.66 ± 6.90 s as shown in Figure 3.

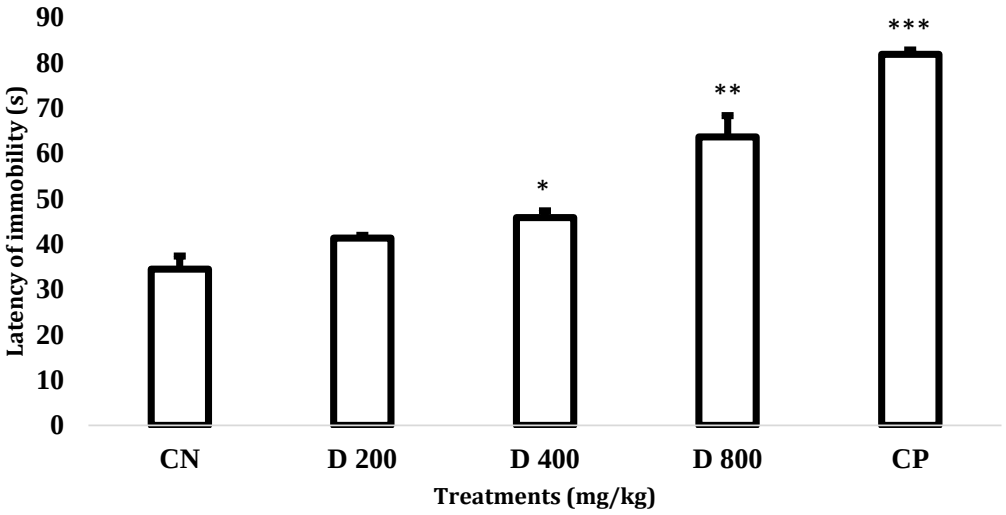

Figure No. 3: Effects of *G. lucida* on the latency of immobility in the TST

Each bar represents the latency of immobility ± SEM. Treatments were administered 1 hour before the test. CN=distilled water; CP=Imipramine (25mg/Kg). N = 6 per dose, *p<0.05, **p<0.01, ***p<0.001, when compared to CN (ANOVA, followed by Fisher test).

Effects of *G. lucida* on the immobility time

The effect of *G. lucida* on the immobility time was dose-dependent. *G. lucida* induced a significant decrease (p<0.001) of the immobility time for about 25.89%, 32.63% and 35.92% respectively at doses 200, 400 and 800 mg/kg. As predicted, imipramine (25 mg/Kg) induced a significant decrease (p<0.001) of the immobility time from 111.33 ± 1.33 s in the CN group to 62.60 ± 5.20 s in the CP group as shown in Figure 4.

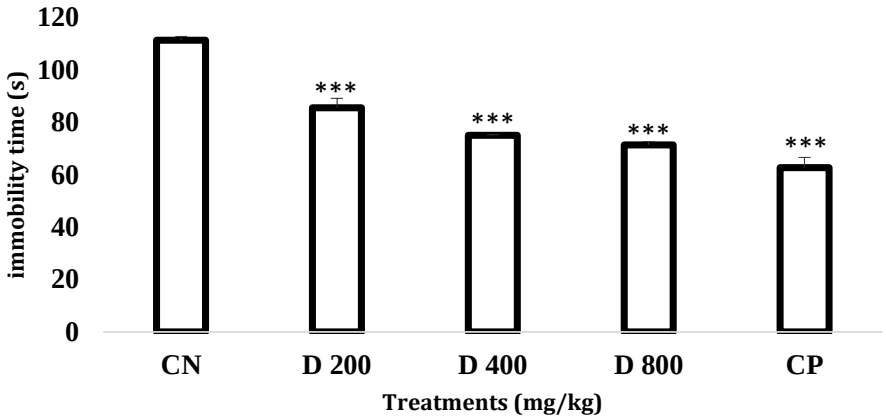

Figure No. 4: Effects of *G. lucida* on immobility time in the TST

Each bar represents the immobility time ± SEM. Treatments were administered 1 hour before the test. CN=distilled water; CP=Imipramine (25mg/Kg). N = 6 per dose, ***p<0.001, when compared to CN (ANOVA, followed by Fisher test).

Anhedonia test

Effects of *G. lucida* on the weekly tap water consumption

Figure 5 shows that *G. lucida* (200, 400 and 800 mg/kg) like Imipramine (25 mg/Kg) induced a weekly decrease of tap water consumption in comparison with the CN group. This observation demonstrates similar outcomes between *G. lucida* and Imipramine as shown in figure 5.

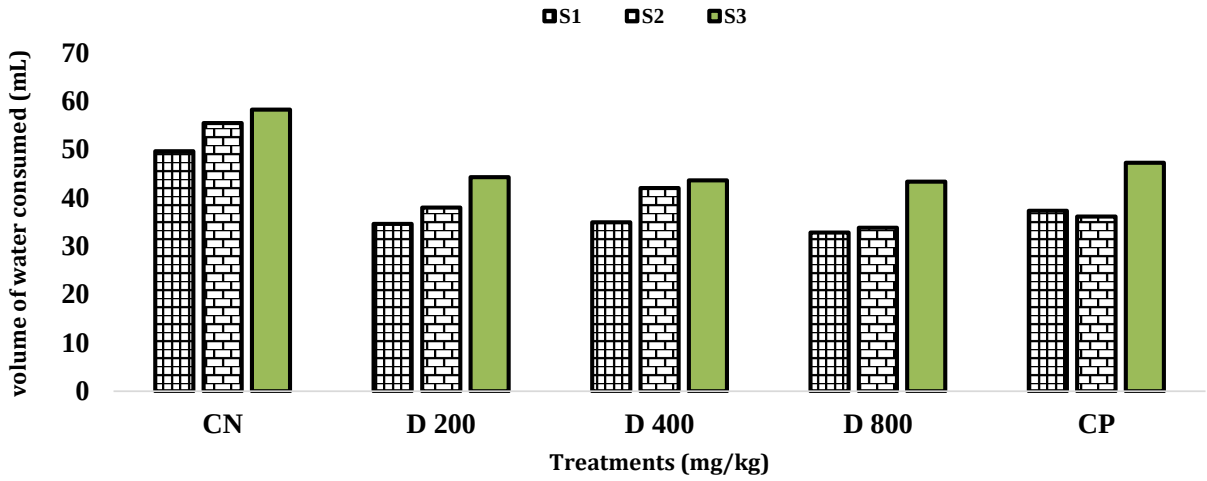

Figure No. 5: Effects of *G. lucida* on weekly consumption of tap water in anhedonia test

Each bar represents the amount of water consumed ± SEM. Treatments were administered 1 hour before the test. CN=distilled water; CP=Imipramine (25mg/Kg). N = 6 per dose. S1: week 1, S2: week 2, S3: week 3.

Effects of *G. lucida* on the weekly consumption of sweet water (sucrose 1%)

The CN group demonstrated a decrease of consumption of sucrose (1%) from week 1 to week 3. Nevertheless, *G. lucida* induced an increase of consumption of sucrose (1%) at all doses. Amongst weeks, *G. lucida* induced significant increase (p<0.05) for the first and second weeks; (p<0.01) for the third week) of consumption of sucrose (1%). Similar effects were observed in the CP group as shown in figure 6.

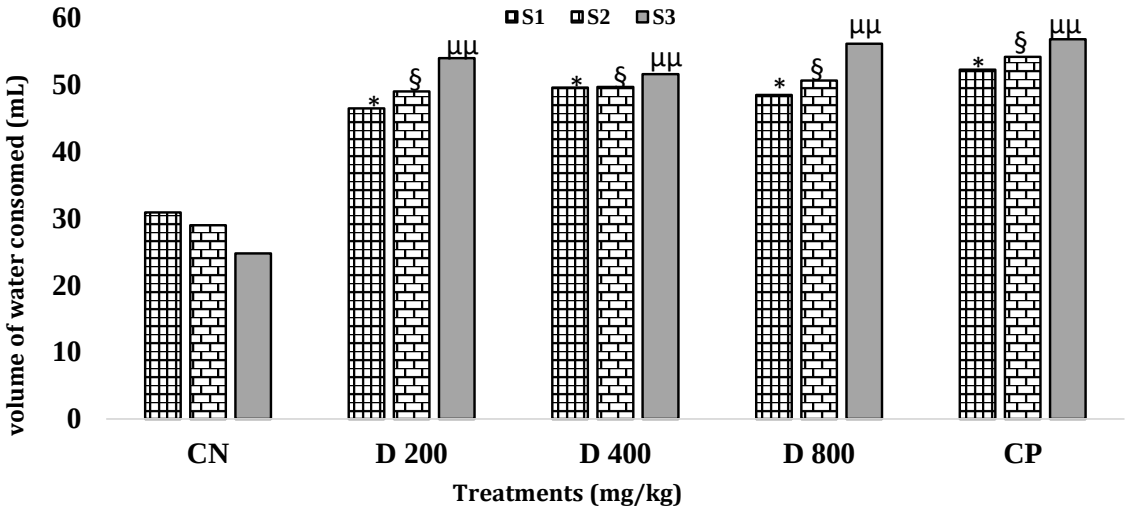

Figure No. 6: Effects of *G. lucida* on weekly consumption of sweat water in anhedonia test

Each bar represents the amount of sucrose consumed ± ESM. Treatments were administered 1 hour before the test. CN=distilled water; CP=Imipramine (25mg/Kg).S1: week 1, S2: week 2, S3: week 3. N = 6 per dose, *P<0.05, §P<0.05, µµP<0.01 when compared to CN (ANOVA, followed by Fisher test).

Effects of *G. lucida* on sucrose (1%) preference

Figure 7 is a comparison between total tap water consumed and total sweat water (sucrose 1%) consumed during the three weeks of experiments. This Figure shows that in the CN group, the amount of tap water consumption is greater than the amount of sweat water consumption. *G. lucida* at all doses induced opposite effects. Our plant induced a significant dose-dependent increase (p<0.001) of sucrose consumption at all doses.

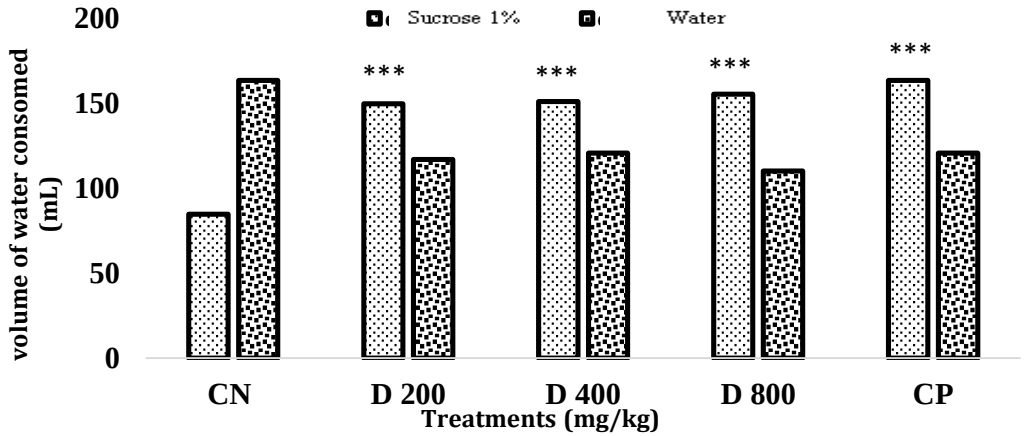

Figure No. 7: Effects of *G. lucida* on sucrose (1%) preference in anhedonia test

Each bar represents the amount of sucrose (1%) or water consumed. Treatments were administered 1 hour before the test. CN=distilled water; CP=Imipramine (25mg/Kg). N = 6 per dose, ***p<0.001, when compared to CN (ANOVA, followed by Fisher test).

Swing of body weight

The mean body weight of animals of CN group decreased from 25.18 g at week 1 to 21 g at week 3. Contrary to the CN group, *G. lucida* (200, 400 and 800 mg/kg) and Imipramine (25 mg/Kg) induced a not significant increase of body weight for about 1.64 g; 3.50 g; 4.0 g and 4.77 g at D 200, D 400, D 800 and CP group respectively.

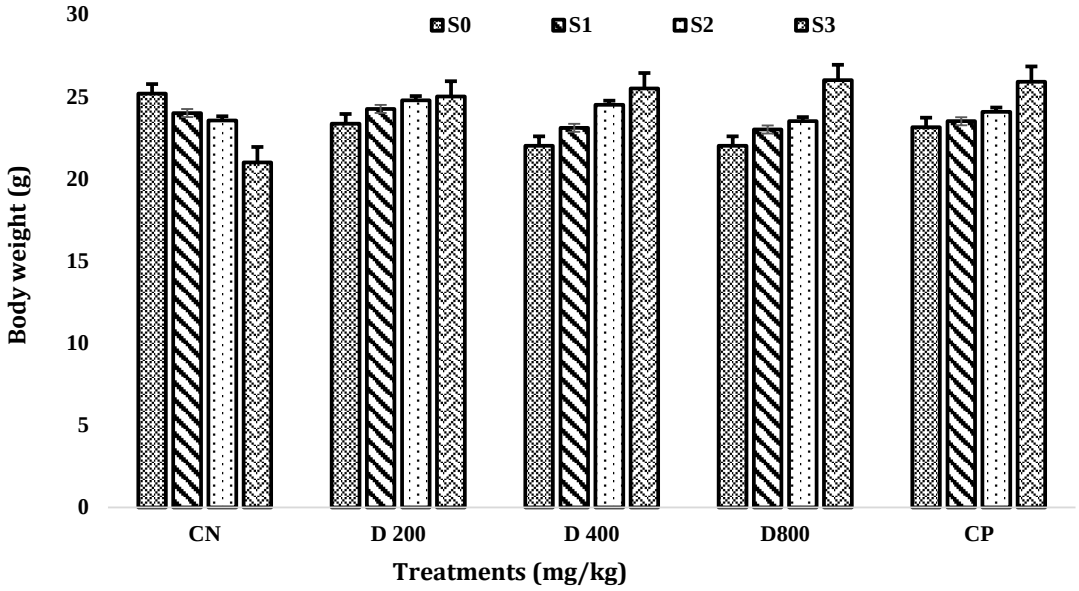

Figure No. 8: Effects of *G. lucida* on the body weight

Each bar represents the body weight ± SEM. Treatments were administered 1 hour before the test. CN=distilled water; CP=Imipramine (25mg/Kg).S0: week 0, S1: week 1, S2: week 2, S3: week 3. N = 6 per dose.

DISCUSSION

The pharmacological properties of *G. lucida* uses in traditional medicine have been assessed. *In-vivo*, three classical experimental models of depression (FST, TST, and sugar consumption test) were used to evaluate the antidepressant properties of *G. lucida*. The UCMS model has been widely used to induce persisting stress-related behavioral changes in rodents [19]. This scheme prevents the stress adaptation process observed in other models of chronic stress [20].

The unpredictable chronic stress-induced animal model of depression is widely used in behavioral assays such as the sucrose preference and FST for evaluating the efficacy of chronic antidepressant treatments [21,22].

FST, a representative behavioral test for depression, is frequently used to evaluate the activities of potential antidepressant drugs in rodent models. This test was developed as an animal model of depression that aimed to measure the effects of antidepressant compounds. The antidepressant treatments decrease the immobility behavior accompanying with an increase in the escape responses such as climbing and swimming. In this test, an increase in immobility time is interpreted as an indicator of depression [23]. Our results show that aqueous extract of *G. lucida* influenced immobility scores (immobility occurrence and total immobility time) when compared to mice treated with distilled water. *G. lucida* demonstrated a significant increase of the latency of immobility and a significant decrease of the immobility time. According to Abelaira and colleagues, various classes of antidepressants reduce immobility time during the FST by increasing the swimming and/or climbing time [18]. It is known that drugs affecting noradrenergic neurotransmission (imipramine) increase climbing behavior, whereas drugs affecting serotonergic neurotransmission (fluoxetine, sertraline, paroxetine, citalopram) increase swimming time [24]. A decrease of immobility time in the FST involves anti-depressive effects of the tested compound [25, 26].

The TST is one of the most widely used models for assessing antidepressant-like activity in mice. *G. lucida* demonstrated a significant increase of the latency of immobility and a significant decrease of the immobility time. The time spent immobile by the animal during a period of 4 minutes is interpreted as a measure of depressive-like behavior. Various antidepressant medications reverse this immobility and promote the occurrence of escape-related behavior [18]. The ability to inhibit immobility involves antidepressant activities of the tested compound [27]. The sucrose preference test serves as an index of anhedonia-like behavior. Anhedonia referred as reduced ability to experience pleasure is a fundamental symptom of human depression [28, 29, 30]. Specifically, reduction in sucrose solution consumption is associated with depression, while the restoration of this response is observed in response to chronic antidepressant treatments [23]. In this test the parameters recorded were the sweat water consumption, tap water consumption and the changes of body mass. It was observed that *G. lucida* induced a significant increase of sweat water (sucrose 1%) consumption. This consumption of sweat water was higher than tap water consumption in all

groups treated with *G. lucida* extract. In an animal, the loss of reactivity for a reward, a pleasant stimulus as observed in the test of preference to sucrose expresses depressive behavior [31]. *G. lucida* antagonized these decrease of sweat water consumption observed in CN group as shown in Figure 6, this suggests that the extract of *G. lucida* could have antidepressant properties, probably responsible of the protection of treated mice against anhedonia symptoms. Regarding mean body mass, mice of CN were characterized by a gradual weight loss over the weeks. It was initially stated that a stressed mouse is characterized by depressive behavior [32]. The loss of body weight and the decrease of exploration are the consequences of the prolonged stress therefore the development of anhedonia. In contrast our results showed that the long term administration of extract of *G. lucida* resulted in a non-significant increase of the body mass in mice, as observed in CP (mice treated with imipramine) over the weeks. In fact, body weight was evaluated weekly for 3 consecutive weeks to identify whether the repeated administration of *G. lucida* would reverse the stress induced body weight loss as previously established by Mozhui and colleagues in 2010 [33]. Mice treated with increasing doses of *G. lucida* as well as a single dose of imipramine demonstrated a gradual non-significant increase of body weight within the testing period. This observation suggests that *G. lucida* could have an inhibitor effect on the chronic unpredictable stress -induced body weight loss. *G. lucida* demonstrated inhibitor effects on depression-like behaviors in FST, in the TST, in anhedonia test and body weight loss induced by chronic unpredictable stress. These findings lead to understand that *G. lucida* possesses anti-depressant effects. Besides, previous phytochemical studies revealed that *G. lucida* contains saponins, alkaloids, bioflavonoids [12,13,14,34]. The anti-depressant effects of *G. lucida* could be related to the presence of some of these chemical compounds. Saponins are responsible of some biological effects [35]; these chemical compounds exert an anti-depressant activity through an inhibition of morphine receptors hypersensitivity [36]. Flavonoids are known to have anxiolytic effects on the central nervous system [37], and neuroprotective effects through the enhancement of the blood-brain barrier [38]. Alkaloids have some direct effects on the nervous system [39]. Caffeine found in seeds of *G. lucida* and probably in bark has a competitive antagonist effect of adenosine receptors leading to the stimulation of the central nervous system [40]. These mechanisms could be some of different ways that underlie the antidepressant effects of *G. lucida*.

CONCLUSION

At the end of this study, which focused on the evaluation of the antidepressant activity of the

aqueous extract of *G. lucida* in white mice, it appears that, the aqueous extract of *G. lucida* increases the time of immobility occurrence and decreases the total immobility duration in the FST and the TST; The aqueous extract of *G. lucida* also increases the total consumption of sweat water, probably responsible for a (not significant) increase of body mass in mice. All these behavioral changes induced by extract of *G. lucida* observed in the FST, the TST and sucrose consumption test indicate the effectiveness against depressive-like behavior, probably due to the neuroactives components present in the extract of barks of this plant.

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