Neurobehavioural Mechanism of Antidepressant Effect of Methanol Stem Bark Extract of *Adansonia digitata* (Linn) in Tail Suspension Test in Mice

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Abstract Aim: An earlier study has demonstrated the *in-vivo* antidepressant effect of methanol stem bark extract of *Adansonia digitata*, using soxhlet extraction protocol, but there is a lack of scientific data on its neurobehavioural mechanism of action. This study, therefore, investigated its antidepressant potentials, using cold maceration method, and determined the probable neurobehavioural mechanism of its antidepressant-like effect. **Methodology:** The antidepressant-like effect of the extract was evaluated in tail suspension test, at graded doses in mice. Subsequently, the probable neurobehavioural mechanism of the antidepressant-like effect of the extract was investigated by intraperitoneal pretreatment with adrenergic, serotonergic, dopaminergic, and muscarinic cholinergic receptor antagonists; GABA agonist; nitric oxide precursor and inhibitors; and using a putative neuromodulator at NMDA receptors prior to the extract administration. **Results and discussion:** The extract at all the doses used, significantly ($p < 0.05$) and dose-dependently decreased the immobility time in tail suspension test without significant ($p > 0.05$) alteration on locomotor behaviour in mice. However, the anti-immobility potential of the extract was significantly ($p < 0.05$) reversed by prazosin, yohimbine, sulpiride, methylene blue, L-arginine and baclofen, suggesting the involvement of adrenergic, dopaminergic, GABAergic and nitergic pathways. **Conclusion:** This study, therefore, concluded that the extract may possess antidepressant effect and its mechanism may involve multiple pathways.

Keywords *Adansonia digitata*, Cold Maceration, Antidepressant, Neurobehavioural Mechanism, Multiple Receptor Pathways

depression is among the top ten causes of morbidity, and mortality world over [1], and it is envisaged to be the leading cause of morbidity and mortality by the year 2030 [2, 3]. Patients with depression have symptoms that reflect a functional deficit in brain monoamine neurotransmitters, specifically norepinephrine, serotonergic [4] and dopaminergic systems [5]. Other neurotransmitters such as GABA acting via GABA_B receptor [6], β adrenoceptors [7], muscarinic cholinergic receptor [8], glutaminergic via NMDA receptor [9], and nitric oxide signaling pathways [8] have been implicated in depression.

Despite the availability of synthetic antidepressant drugs, depression remains a major medical problem [10]. This is because the currently available antidepressant drugs are associated with a numerous side effect which includes weight gain, hypopiasia, sexual dysfunction, and cardiac toxicity and sleep disorder [11, 12, 13]. Therefore, herbal therapies should be considered alternative or complementary medicines [14]. Among the demonstrated herbal therapies effective for the treatment of depression are *Hypericum perforatum* L. [15], *Cordyceps sinensis* [16], and *Perilla frutescens* [17].

Adansonia digitata L. (Bombacaceae), otherwise called baobab, is a large iconic tree indigenous to Africa, where it is found in many African countries [18]. The African species *Adansonia digitata* is indigenous to, and widely distributed throughout the savannas and savanna woodlands of sub-Saharan Africa [19].

Numerous ethnomedicinal values are attributed to the various plant parts of *Adansonia digitata* [18]. For example, stem bark is used in the treatment of neuropsychiatric disorder [20], depression [21], malaria [19], wound healing [22], among other indications in Africa traditional medicine and have been evaluated as a substitute for imported western drugs [23, 24].

The different parts of *Adansonia digitata* have numerous biological properties including antimicrobial, antiviral [25], antioxidant [26], hepatoprotective [27], anti-inflammatory,

1. Introduction

According to The World Health Organization,

analgesic and antipyretic activities [24].

According to the World Health Organisation, depression will be the second leading cause of disability in the developed countries in 2020 [28], and the continuous use of the currently available synthetic antidepressant drugs acting via multiple neurotransmitters system [29], have been associated with some severe side effects [30]. Therefore, a continuous search for new affordable therapeutic agents with minimal side effects from medicinal plants is imperative. Although the antidepressant effect of *Adansonia digitata* stem bark has been demonstrated using hot extraction protocol on forced swimming (FST) and tail suspension (TST) tests [21], but there is lack of scientific data on its probable neurobehavioural mechanism of action, therefore, the objective of this study was to investigate the antidepressant effect of the stem bark extract using cold extraction protocol, as well as determine its neurobehavioural mechanism of action in TST.

2. Materials and Methods

Adansonia digitata stem bark was collected in Bode Osi in Ola Oluwa Local Govt area of Osun State. It was authenticated by Mr. G. A. Ademoriyo of the Herbarium Unit, Department of Botany, Faculty of Science, OAU, Ile-Ife and herbarium voucher Ife 17705 was obtained and herbarium specimen deposited.

2.1. Preparation of Plant Materials

Adansonia digitata stem barks were air dried at room temperature. The dried stem barks were pulverized, and 300 g of the powdered material, was extracted with 1.5 litres of eighty percent (80%) methanol for 72 h. The filtrate was then concentrated in vacuo at a temperature of 40°C and further freeze-dried to yield 2.3 g (0.77 %) crude extract and coded MSAD.

2.2. Drugs

Prazosin hydrochloride, cyproheptadine hydrochloride, yohimbine hydrochloride, fluoxetine hydrochloride, propranolol hydrochloride, baclofen, ($\pm$) sulpiride, atropine sulphate, L-arginine, L-NG-Nitroarginine, methylene blue and ascorbic acid were all from Sigma Aldrich, St. Louis, MO, USA; and normal saline (Unique Pharmaceutical Limited, Lagos, Nigeria). MSAD and other drugs were dissolved in normal saline and freshly prepared on each day of the experiment.

2.3. Animals

Male adult albino mice weighing 18–25 g were obtained from the Animal House, Igbinedion University, Okada, Edo State, Nigeria. The animals were fed with a standard

commercial diet (Guinea feeds brand, Bendel Feeds Nigeria) and water *ad libitum*. The animals were acclimatized to the laboratory condition for at least one week before the commencement of the behavioural experiments. The experimental procedures employed in this study are in accordance with the approved institutional guidelines and within the confine of internationally accepted principles for Laboratory Animal Use and Care (EEC Directive of 1986; 86/609/EEC) [31]. All efforts were made to reduce animal sufferings and to use the minimum number of animals in this study.

2.4. Acute Toxicity Test

Acute Oral Toxicity Study

The acute oral toxicity test of MSAD was conducted according to the Organization for Economic Cooperation and Development (OECD) guidelines 425 of 2008. The experimental procedures were carried out in two phases. In the first phase, a single female mouse was orally administered 2000 mg/kg of MSAD after 3 h of fasting. The mouse was observed for any signs of acute behavioural toxicity within the first 24 h. Based on the outcome of the first phase; another 4 female mice were orally administered the same dose of MSAD and subsequently observed for any sign of delayed toxicity or death in the next 14 days [32]. Based on the outcome of the experiment, the two phases of the experiments were repeated for 5000 mg/kg.

2.5. Pharmacological Experiments

Effect of MSAD in Tail Suspension Test (TST)

The TST employed for screening the potential antidepressants effect of MSAD was carried out as previously done elsewhere [33]. Mice were randomly divided into 5 groups, containing 5 mice per group ($n=5$). Group I (Vehicle): mice received normal saline p.o (10 mL/kg); Groups II-IV (Treatment groups): mice received 200, 400 and 800 mg/kg, p.o of MSAD, and Group V (received the reference drug): mice received fluoxetine (20 mg/kg, i.p.). One hour after oral ingestion of MSAD and 30 min after intraperitoneal injection of fluoxetine, each mouse was suspended on the edge of a table, 50 cm above the floor with the help of adhesive tape placed approximately 1 cm from the tip of the tail. Immobility duration was recorded for the last 4 minutes during 6 minutes period in different groups. The mouse animal was considered immobile, when it did not show any movement of the body, and hanged passively.

2.6. Mechanism of Action of MSAD in TST

Involvement of Noradrenergic Pathway

Mice were randomly divided into 5 groups containing 5 mice per group ($n=5$). Group I: mice in this group received normal saline p.o (10 mL/kg); Groups II: mice received

200 mg/kg, p.o of MSAD; Group III-V: mice were pretreated with prazosin (62.5 µg/kg, an α_1 adrenoceptor antagonist), yohimbine (1 mg/kg, α_2 adrenoceptor antagonist), propranolol (2 mg/kg, β -receptor antagonist), 30 min before the administration of MSAD (200 mg/kg, p.o.) respectively. One hour after normal saline and extract administration in Group I and II and 30 min after oral ingestion of MSAD in Group III-V, the mice were submitted for TST [7].

Involvement of Serotonergic Pathway

Mice were randomly divided into 3 groups, containing 5 mice per group (n=5). Group I: mice in this group received normal saline p.o (10 mL/kg); Groups II: mice received 200 mg/kg, p.o of MSAD; Group III: mice were pretreated with cyproheptadine (3 mg/kg, i.p., a 5-HT receptor antagonist), and 15 min later they received MSAD (200 mg/kg, p.o.). One hour after oral administration of normal saline or extract in Groups I-III, mice were evaluated on TST as previously described [34].

Involvement of Dopaminergic Pathway

Mice were randomly divided into 3 groups, containing 5 mice per group (n=5). Group I: mice received normal saline p.o (10 mL/kg); Groups II: mice received 200 mg/kg, p.o of MSAD; Group III: mice were pretreated with sulpiride (50 mg/kg, i.p., a selective dopamine D₂ receptor antagonist), 30 min before administration of MSAD (200 mg/kg, p.o.). One hour after oral administration of normal saline or extract in Groups I & II, and 30 min after oral ingestion of MSAD in Group III, mice were subjected to TST [7].

Involvement of Muscarinic Cholinergic Receptor Pathway

Mice were randomly divided into 3 groups, containing 5 mice per group (n=5). Group I: mice received normal saline p.o (10 mL/kg); Groups II: mice received 200 mg/kg, p.o of MSAD; Group III: mice were pretreated with atropine (1 mg/kg, i.p., a muscarinic cholinergic receptor antagonist), 15 minutes before the orogastric ingestion of MSAD (200 mg/kg, p.o.). One hour after oral administration of normal saline or extract in Groups I & II, and 45 min after oral ingestion of MSAD in Group III, mice were subjected to TST [35].

Involvement of N-Methyl-D-aspartate (NMDA) Receptor Pathway

Mice were randomly divided into 3 groups, containing 5 mice per group (n=5). Group I: mice received normal saline p.o (10 mL/kg); Groups II: mice received 200 mg/kg, p.o of MSAD; Group III: mice were pretreated with ascorbic acid (100 mg/kg, i.p., a putative neuromodulator that antagonizes NMDA receptors) 30 min before oral administration of MSAD (200 mg/kg, p.o.). One hour after oral administration of normal saline or extract in Groups I & II, and 30 min after oral ingestion of MSAD in Group III,

mice were subjected to TST [36].

Involvement of Gabaergic Pathway

Mice were randomly divided into 3 groups, containing 5 mice per group (n=5). Group I: mice received normal saline p.o (10 mL/kg); Groups II: mice received 200 mg/kg, p.o of MSAD; Group III: mice were pretreated with baclofen (10 mg/kg, i.p., GABA_B agonist) 15 minutes before the oral administration of MSAD (200 mg/kg, p.o.). One hour after oral administration of normal saline or extract in Groups I & II, and 45 min after oral ingestion of MSAD in Group III, mice were subjected to TST [6].

Involvement of L-Arginine-NO-cGMP Pathway

Mice were randomly divided into 5 groups, containing 5 mice per group (n=5). Group I: mice received normal saline p.o (10 mL/kg); Groups II: mice received 200 mg/kg, p.o of MSAD; Group III & IV mice were pretreated with L-Arginine [750 mg/kg, i.p., a precursor of nitric oxide (NO)] and methylene blue [10 mg/kg, i.p., an inhibitor of nitric oxide synthase and an inhibitor of soluble guanylate cyclase (sGC)] respectively 15 min before MSAD; Group V: mice orally treated with MSAD, 30 min before L-NNA (0.3 mg/kg, i.p., a competitive inhibitor of NO synthase with selectivity for the neuronal and endothelial isoforms of the enzyme). One hour after oral administration of normal saline or extract in Groups I & II, 45 min after MSAD ingestion in Group III & IV [37] and 30 min after MSAD administration in Group V, mice were tested on TST [38].

2.7. Statistical Analysis

Results were expressed as mean $\pm$ S.E.M. The significance of the difference between treated groups and the negative group were analyzed using one-way analysis of variance (ANOVA), followed by Dunnett's post hoc analysis. The level of significance for all tests was set at $p < 0.05$ compared to the negative control group.

3. Results

3.1. Result of LD₅₀ Determination

The LD₅₀ of MSAD was found to be > 5000 mg/kg, p.o. in mice.

3.2. Effect of MSAD on Tail Suspension Test (TST) in Mice

The effect of MSAD (200 - 800 mg/kg, p.o.) on TST is shown in Figure 1. MSAD at 200, 400 and 800 mg/kg showed significant [$F_{(4, 20)} = 20.642$, $p < 0.05$] dose-dependent decrease in anti-immobility time when compared to the vehicle-treated control group. MSAD at all the doses used also shortened the immobility time more than the positive control drug fluoxetine.

3.3. Effect of Various Receptor Antagonists on the Antidepressant Effect of MSAD on TST in Mice

The results obtained for the effects of various antagonists on the antidepressant-like activity of MSAD are presented in Figure 2A-H. Pretreatment with prazosin (62.5 µg/kg), yohimbine, (1 mg/kg), sulpiride (50 mg/kg), L- arginine (750 mg/kg), methylene blue (10 mg/kg,) and

baclofen (10 mg/kg), significantly [$p < 0.05$, $F_{(2, 12)} = 17.714$, 12.721, 18.073, 20.776, 11.056 and 35.798] reversed the antidepressant-like effect of MSAD (200 mg/kg, p.o.) respectively. Propranolol (2 mg/kg), atropine (1 mg/kg), cyproheptadine (3 mg/kg), L- nitroarginine (10 mg/kg), and ascorbic acid (100 mg/kg), did not reverse the antidepressant effect of MSAD in TST.

Figure 1. Antidepressant effect of methanol stem bark extract of *Adansonia digitata* on TST test in mice. VEH; Vehicle (10 mL/kg, normal saline), MSAD; Methanol stem bark extract of *Adansonia digitata*, FLUOX; fluoxetine (20 mg/kg). Each bar represents Mean $\pm$ SEM, $n=5$. * $p < 0.05$ compared to the vehicle [$F_{(4, 20)} = 11.438$, $p < 0.0001$] (ANOVA, Student-Newman-Keuls post hoc test).

Figure 2. A–K: The effects of various antagonists, agonist, inhibitor and precursor: A (prazosin), B (yohimbine), C (propranolol), D (sulpiride), E (atropine), F (cyproheptadine), G (L-nitroarginine), H (L-arginine), I (methylene blue), J (ascorbic acid) and K (baclofen) on the anti-immobility activity of MSAD in TST.

Table 1. Effects of MSAD on locomotor and rearing behaviours in mice in the open field

Group	Dose (mg/kg)	Number of square crossed	Number of rearing	Total activities
		Mean $\pm$ S.E.M		
Vehicle	(10 mL/kg)	68.8 $\pm$ 5.0	27.0 $\pm$ 1.9	95.8 $\pm$ 5.9
MSAD	100	73.2 $\pm$ 2.3	26.8 $\pm$ 1.9	100.0 $\pm$ 2.7
MSAD	200	69.6 $\pm$ 3.6	26.2 $\pm$ 1.9	92.8 $\pm$ 6.0
MSAD	400	74.0 $\pm$ 4.6	26.0 $\pm$ 1.7	100.2 $\pm$ 6.2
DZP	1	85.4 $\pm$ 6.2	13.6 $\pm$ 2.5*	99.0 $\pm$ 7.8

MSAD; methanol stem bark extract of *Adansonia digitata*, DZP; diazepam. Each value represents Mean $\pm$ SEM, n=5. *p<0.05 compared to the vehicle. (ANOVA, Dunnett's post hoc test)

3.4. Effect of MSAD on Locomotor Behaviour in Open Field (OFT) Test in Mice

MSAD at 200, 400 and 800 mg/kg, per oral, did not show any significant effects on locomotor activity in mice. Although, there was a dose-dependent decrease in rearing but not significant from the vehicle-treated control group as assessed from the OFT (Table 1). However, the standard sedative drug, diazepam (1 mg/kg) significantly [$F_{(4, 20)} = 8.516$, $p = 0.004$] reduced rearing in mice compared to the vehicle-treated control group. The result is presented in Table 1.

4. Discussion

This study examined the acute toxicity (LD_{50}) and mechanism of antidepressant-like effect of methanol stem bark extract of *Adansonia digitata* (MSAD) using various receptor antagonists in tail suspension (TST) behavioural test in mice. The results obtained revealed that MSAD may not be toxic and possessed antidepressant-like activity in TST acting via multiple receptor neurotransmission.

The acute toxicity (LD_{50}) study of MSAD was greater or equal to 5000 mg/kg suggesting that MSAD may not be toxic to mice. Since, according to Hayes, no toxicity could be considered above 5 g/kg [39]. Therefore, low oral doses of 200, 400 and 800 mg/kg, per oral of MSAD were chosen for this study, since antidepressant drugs are expected to be used for a long period of time [40].

In addition, the doses of MSAD used in this study, were also selected, such that, they did not significantly, alter the spontaneous locomotor activity of mice using an open field test (Table 1), thereby ruling out the possibility of getting a false positive result in the antidepressant effects of MSAD and as such, the observed antidepressant effect of MSAD may not be due to psychostimulant effect which decreases immobility time by stimulating locomotor activity [41, 42, 43].

The TST presents some advantages over FST in that it allows an objective measure of immobility, by avoiding

hypothermia, induced by immersion in water. In addition, TST is strongly correlated with antidepressant effects in humans, and can be used to distinguish between antidepressants and other psychotropic drugs, such as antipsychotics, and anxiolytics [44], thus, TST was chosen for the delineation of the probable neural mechanism of MSAD [45] in this study.

MSAD shortened immobility time in TST suggesting an antidepressant-like effect. This finding is in line with previous findings of agents that shortened immobility time in TST, which were suggested to have antidepressant-like effect in TST [37, 46]. Of particular interest, is the dose-dependent increase in antidepressant effect of MSAD reported by earlier researchers [47] with highest antidepressant effect exhibited at 1000 mg/kg, per oral in their study, but in our study, the lowest tested dose of 200 mg/kg, per oral of MSAD showed the highest antidepressant effect.

The reason for this pattern of result could be the different methods of the plant extraction protocol employed. For example, Shehu *et al.* [47] used a soxhlet extraction protocol, while we used a cold maceration method using the same solvent. It could probably be suggested, that *Adansonia digitata* stem bark may contain thermolabile constituents, thereby making cold maceration a better alternative to soxhlet extraction, since cold maceration is best used in case of thermolabile drugs [48]. This suggestion may be supported with earlier reports, that prolong heating using soxhlet extraction protocol, may lead to the degradation of compounds, and not suitable for thermolabile compounds [49, 50, 51]. However, cold maceration techniques may be a better technique of preparing such medicinal plant extract [48]. Subsequently, our finding may probably favour the long use of the plant extract at 200 mg/kg, compared to 1000 mg/kg in the previous study, as antidepressant drugs are normally ingested over a long period of time [40].

Of all the tested doses of 200, 400 and 800 mg/kg of MSAD in this study, 200 mg/kg showed to be most effective at reducing immobility in TST, therefore, 200 mg/kg was selected for the delineation of the mechanism of

antidepressant-like effect of MSAD in TST.

Previous reports have indicated that acute administration of α_1 -adrenergic receptor antagonist and α_2 -adrenergic receptor antagonists abolished the antidepressant effect of antidepressant agents in TST [37, 46]. In this study, the anti-immobility effect of MSAD was abolished by prazosin (an α_1 -adrenoceptor antagonist) (Figure 2 Panel A) and yohimbine (an α_2 -adrenoceptor antagonist) (Figure 2 Panel B) suggesting that MSAD might be acting via interaction with α_1 - and α_2 -adrenoceptors. This observation is in line with previous reports of medicinal plants acting via these mechanisms [37, 46].

Antagonism of dopaminergic receptor pathway has been demonstrated to reverse antidepressant effect of medicinal agents, acting via dopaminergic neurotransmission in TST [38, 46]. The reversal of the antidepressant-like effect of MSAD by sulpiride (Figure 2 Panel D) suggests that MSAD may be acting via agonistic action on a dopaminergic neuronal pathway to exert its antidepressant-like effect. This experimental finding is in line with earlier experimental findings of the medicinal agents whose antidepressant-like effects were reversed by sulpiride and suggested to be acting via dopaminergic pathways [38, 46].

Inhibition of 5-HT₂ receptors plays an important role in the antidepressant effect of the antidepressant agent in TST [37]. In this study, pretreatment of mice with cyproheptadine (a 5-HT₂ receptor antagonist), did not reverse the antidepressant-like effect of MSAD, indicating that its antidepressant-like effect, may not be mediated via 5-HT₂ receptor neurotransmissions.

In this study, pretreatment of mice with baclofen reversed the antidepressant-like effect of MSAD (Figure 2 Panel K) suggesting that GABA_B receptor neurotransmission may be involved in the antidepressant effect of MSAD in TST. Previous studies have shown that antidepressant-like effects of medicinal plant extracts were abolished by baclofen (a GABA_B receptor agonist) in TST suggesting the involvement of GABA_B receptor in the antidepressant-like effect of these extracts [52, 53] since GABA_B receptor antagonism may serve as a basis for the generation of novel antidepressants [54]. This study is in conformity with earlier reports of the involvement of GABA_B receptor neurotransmission in the antidepressant effect of medicinal plants [52, 53].

Pretreatment of mice with propranolol (a β adrenergic receptor antagonist), atropine (a muscarinic cholinergic receptor antagonist) and ascorbic acid (a putative neuromodulator at the NMDA receptor) did not reverse the antidepressant effect of MSAD suggesting that the observed antidepressant effect of MSAD may not be mediated via β adrenergic, muscarinic cholinergic and glutaminergic via NMDA receptor pathways.

Numerous studies have ascribed an important role to the L-arginine-NO-cGMP pathway in the pathophysiology of depression [8, 55]. In this study, pretreatment with

L-arginine reversed the antidepressant effect of MSAD (Figure 2 Panel H) which is consistent with an earlier report [56]. However, L-NNA did not reverse or potentiate the antidepressant effect of MSAD rather pretreatment with L-NNA before the administration of MSAD showed antidepressant effect; this observation could be supported with earlier finding that nitric oxide inhibitors exert antidepressant-like effects in animal models predictive of antidepressant activity [57, 58].

5. Conclusions

This study concluded that MSAD may possess antidepressant effect, and its probable neural mechanism of action may involve multiple receptor pathways.

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