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# ANTIDEPRESSANT-LIKE EFFECT OF METHANOL ROOT BARK EXTRACT OF *ACACIA SEYAL* DEL. (FABACEAE) IN MICE

**[1*]Gambo, A.Y., [2]Shehu, A., [2]Magaji, M. G and [2]Danjuma, N. M**

[1]*Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Maiduguri, Borno State, Nigeria*
[2]*Department of Pharmacology and Therapeutics, Ahmadu Bello University, Nigeria*

***Author for correspondence**: +234-803-8056502; amandagambo@gmail.com

## ABSTRACT

Depression is a major cause of disability associated with affective and cognitive impairments and has been estimated to be the single biggest burden of health conditions. *Acacia seyal* Del. (Fabaceae) is reported to be used in Zaria, North western Nigeria for the management of depression. The aim of this study is to investigate the antidepressant activity of methanol root bark extract of *Acacia seyal* in mice. The root bark of *Acacia seyal* was collected, dried, pulverised, powdered and extracted using 70% methanol. The methanol root bark extract was then used for preliminary phytochemical screening, acute toxicity study ($LD_{50}$), beam walking assay (BWA) and antidepressant study. The preliminary phytochemical screening was carried out on the extract based on standard procedures, while the acute toxicity study was conducted using Organization for Economic Cooperation and Development (OECD) 425 guideline. Motor coordination deficit was assed using beam walking assay. Antidepressant activity was evaluated using tail suspension test (TST) and open field test (OFT) all in mice. The possible involvement of the monoaminergic, nitritergic and opiodergic systems in the antidepressant activity of the extract was further evaluated. The phytochemical constituents detected include tannins, saponins, flavonoids and steroids. The oral $LD_{50}$ of the extract was found to be ≥5000 mg/kg in mice. The extract did not significantly affect the number of foot slips in the BWA and the locomotory behaviour of mice in the OFT. The extract at the tested doses produced significant ($p$<0.05) decrease in the duration of immobility in the TST. The anti-immobility effect of the extract at 500 mg/kg in the TST was reversed by the pre-treatment of mice with sulpiride (50 mg/kg), metergoline (1mg/kg), cyproheptadine (4mg/kg), prazosin (1 mg/kg), yohimbine (1 mg/kg), atropine (1 mg/kg), L-arginine (50 mg/kg), Nitro-L-Arginine (50 mg/kg) and Naloxone (2 mg/kg). The methanol root bark extract of *Acacia seyal* possesses antidepressant activity possibly mediated through interaction with dopamine, noradrenergic, serotonergic, cholinergic, opioidergic receptors and nitric oxide pathway.

**Keywords**: *Acacia seyal*, Depression, Tail Suspension Test

## INTRODUCTION

Depression is a common mental disorder and represents a global mental health concern (Hussain *et al.,* 2022). Depressive disorders are characterized by sadness, loss of interest, poor concentration, low self-worth, feelings of guilt and tiredness, disturbed sleep and/or appetite (Zhang *et al.,* 2023). Depression has been reported to be on the increase worldwide by 18.4% between 2005 and 2015 (Evans-Lacko *et al.,* 2018). Prevalence rates vary by age, peaking in adulthood out of which 25% of patients develop chronic depression (Ferari *et al*., 2013; Woody *et al.,* 2017).

Antidepressant drugs remain the main forms of effective treatment for the amelioration of depressive symptoms. Despite the improvement in the available therapeutic agents only around 40% of patients with

depression achieve full remission (Warden *et al*., 2007), and side effects are still a serious problem even with the newer medications. There is still a great need for faster acting, safer, better tolerated and more effective treatments for depression (Agid *et al*., 2007; Marwaha *et al.,* 2023).

Globally, there is increased use of medicinal plants as substitutes for orthodox drugs in the management of diseases some of which have produced good results and fewer side effects (Sarko, 2000; Tiwari and Mehta, 2013). The plant, *Acacia seyal* Del. belongs to the family Fabaceae. It is a small to medium-sized tree that reaches a height of 12-17 m, it is commonly called the fodder tree in English and "Dumshee" in Hausa (Hall and McAllan, 1993). *Acacia seyal* is native to Sudan, Egypt, Eritrea, Ethiopia, Ghana, Iran, Kenya, Malawi, Mali, Niger, Nigeria, Saudi Arabia, Senegal, Syrian Arab Republic, Tanzania, Uganda, Yemen, Republic of Zambia, and Zimbabwe. Moreover, it is exotic to Afghanistan, Bangladesh, Bhutan, India, Nepal, Portugal, Sri Lanka and the US (Orwa *et al.,* 2009). It was reported to be used in north-western Nigeria for the management of depression (Shehu *et al*., 2017). The screening of *Acacia seyal* for antidepressant activity will provide the scientific basis for its folkloric use in the management of depression.

## METHODS:

### Plant Collection and Extraction

The whole plant of AS was collected and taken to Herbarium Section of Department of Botany, Ahmadu Bello University, Zaria where it was identified and authenticated by comparing with an existing specimen (voucher number 900347) previously deposited in the herbarium.

The root bark of *Acacia seyal* was transported to the Department of Pharmacognosy and Drug Development where it was dried and size-reduced using mortar and pestle. The powdered plant material was extracted with 70% methanol using cold maceration technique. The solution was concentrated at a temperature of 45ºC on a water bath. The extract subsequently referred to methanol root bark extract of *Acacia seyal* (AS) was stored in air tight container until needed for the work.

### Animals

Swiss Albino mice of either sexes were obtained from the Animal House Facility of the Department of the Pharmacology and Therapeutics, Ahmadu Bello University Zaria. They were housed in standard propylene cages and kept under natural day and light cycle. The animals were fed on standard laboratory animal diet and water *ad libitum*. All experiments were conducted according to the Ahmadu Bello University Animal Ethics Committee.

### Drugs and Chemicals

The following are the chemical/drugs that were used for the experiment are: Fluoxetine (Mebidos Laboratories Pvt. Ltd, India), Diazepam (Roche, France), Methanol (Fluka-Aldrich)

### Phytochemical Screening

Phytochemical screening was carried out on AS using standard protocols of Trease and Evans (2009).

### Acute Toxicity Study

$LD_{50}$ determination was conducted using Organization for Economic Co-operation and Development (OECD 425) guideline in mice. In this method, two groups each of three mice were fasted 3 hours prior to dosing. The fasted body weight was determined for each mouse and dose calculated according to the body weight. AS was administered in a single oral dose using a cannula. A dose of 5000 mg/kg was used for one mouse and observed for 48 hours. The mouse survived, and two additional

mice were dosed and all three mice were observed individually during the first 30 minutes after dosing, periodically during the first 24 hours, and then daily for 14 days. Mice were observed for tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. Time of onset of toxic symptoms and disappearance were also noted.

**Antidepressant Screening**

**Tail suspension test in mice**

Mice were taken to the neurobehavioural laboratory and adapted for 1 hour. Forty (40) mice were grouped into five with eight mice each. Groups 1, 2 and 3 mice were treated with 250, 500 and 1000 mg/kg of AS orally 1 hour prior to test. Group 4 and 5 mice were treated with distilled water (10 ml/kg) and Fluoxetine (20 mg/kg) respectively. For the test, mice were suspended on the edge of the shelf 58 cm above a table top by adhesive tape placed approximately 1cm from the tip of the tail. The duration of immobility was recorded for a period of 6 minutes. Mice are considered immobile if hung passively and completely motionless (Steru *et al*., 1985).

**Open field test in mice**

Forty mice were grouped into five (5) with eight animals each. Groups 1, 2 and 3 mice were treated with 250, 500 and 1000 mg/kg of AS orally 1hour prior to test. Groups 4 and 5 mice were treated with distilled water (10 ml/kg) and Diazepam (15 mg/kg) respectively. Each mouse was placed in white wooden open field apparatus (70×70×35 cm; length × breadth × height) of which one wall is Plexiglas. A plexiglass floor divided into 16 visible squares (15×15 cm) with a central square. Behaviour of each mouse such as peripheral and central square crossing was recorded for 5 minutes. Arena was cleaned with 10% ethanol between tests (Rex *et al*., 1998).

**Beam walking assay**

Mice were trained to walk from a start platform along a ruler (80 cm long, 3 cm wide) elevated 30 cm above the bench by a wooden support to a goal box. Three trials were performed for each mouse, and trained to know that a goal box could be reached. A ruler was used for the training and forty mice that successfully walked along the ruler were grouped into five of eight mice each. The first, second and third groups received 250, 500 and 1000 mg/kg of AS orally one hour prior to test. The fourth and fifth groups received distilled water (10 ml/kg) and diazepam (10 mg/kg) orally respectively. One hour later, each mouse was placed on the beam (60 cm long, 8 mm in diameter and 30 cm elevated above the bench) at one end and allowed to walk to the goal box. Mice that fell were returned to the position they fell from. The measurements taken were the number of foot slips (one or both hind limbs slipped from the beam) and the number of falls (Stanley *et al*., 2005; Magaji *et al*., 2008).

**Mechanistic Studies**

**Serotonergic system**

To assess the involvement of the serotonergic system in the antidepressant-like effect of Thirty-five mice were divided into 7 groups of 5 mice each. Group I, II and III received distilled water (10 ml/kg), AS (500 mg/kg) and Fluoxetine (20 mg/kg) orally respectively. Group IV and V were pretreated with cyproheptadine (4 mg/kg, i. p., a 5-$HT_2$ receptor antagonist). Fifteen minutes after, the animal received AS (500 mg/ kg, *p.o*.) or fluoxetine (20mg/kg, *p.o*.) (Ulak *et al.,* 2010). Group VI and VII were pretreated with metergoline (1mg/kg, i.p., a non-selective 5-$HT_2$ receptor antagonist) (Stachowicz *et al.,* 2009). Fifteen minutes after, the animal received AS (500 mg/ kg, *p.o*.) and fluoxetine (20mg/kg, *p.o*.). Sixty

minutes post-treatment, they were subjected to TST.

**Noradrenergic system:** To investigate the possible involvement of the noradrenergic system in the antidepressant-like effect of AS. Thirty-five mice were divided into 7 groups of 5 mice each. Group I, II and III received distilled water (10 ml/kg), AS (500 mg/kg) and Fluoxetine (20 mg/kg) orally respectively. Group IV and V were pretreated with prazosin (1 mg/kg, *i. p*., an $\alpha_1$-adrenoceptor antagonist). Fifteen minutes after, the animal received *A. seyal* (500 mg/kg, *p.o*.) and fluoxetine (20mg/kg, *p.o*.) and Group VI and VII were pretreated with yohimbine (1 mg/kg, i. p., and $\alpha_2$ - adrenoceptor antagonist) (Gu *et al.,* 2012). After 15 minutes, they received *A. seyal* (500 mg/kg, *p. o*.) and fluoxetine (20 mg/kg, *p.o*.). Sixty-minutes post-treatment, they were subjected to TST.

**Dopaminergic system:** To test the possible involvement of the dopaminergic system in the antidepressant-like effect of AS. Twenty-five mice were divided into five groups of 5 mice each. Group I, II and III were administered distilled water (10 ml/kg), AS (500 mg/kg) and Fluoxetine (20 mg/kg) orally respectively. Group IV and V mice were pretreated with sulpiride (500 mg/kg, *i. p*., a dopamine $D_2$ receptor antagonist) (Gu *et al*., 2012). After 15 minutes, they received AS (500 mg/kg, *p. o*.) and Fluoxetine (20 mg/kg). An hour post-treatment, they were subjected to TST.

**Cholinergic system:** To investigate the involvement of cholinergic system in the antidepressant- like effect of AS, twenty-five mice were divided into five groups of 5 mice each. Group I, II and III were administered distilled water (10 ml/kg), AS (500 mg/kg) and Fluoxetine (20 mg/kg) orally respectively. Group IV and V mice were pretreated with atropine (1 mg/kg, *i.p*.) (Liebenberg *et al.,* 2010). Fifteen minutes post-treatment, the animal received A. seyal (500 mg/kg, p. o.) and fluoxetine (20mg/kg, *p.o*.). An hour post-treatment, they were subjected to TST.

**Opioid receptor:** To investigate the involvement of opioidergic system in the antidepressant- like effect of *A. seyal.* Twenty-five mice were divided into five groups of 5 mice each. Group I, II and III were administered distilled water (10 ml/kg), AS (500 mg/kg) and Fluoxetine (20 mg/kg) orally respectively. Group IV and V mice were pretreated with naloxone (2 mg/kg, *i. p*.). After 15 minutes post-treatment, the animal received AS (500mg/kg, *p. o.)* and fluoxetine (20mg/kg, *p.o*.). An hour post-treatment, they were subjected to TST.

**Nitric oxide pathway (Substrate and inhibitor):** To investigate the possible involvement of the nitric oxide pathway in the antidepressant-like effect of the extract. Thirty-five mice were divided into 7 groups of 5 mice each. Group I, II and III received distilled water (10 ml/kg), AS (500 mg/kg) and Fluoxetine (20 mg/kg) orally respectively. Group IV and V were pretreated with L- arginine (50mg/kg, *i. p*., a nitric oxide substrate) and Group VI and VII were pretreated with L-NNA (50mg/kg i. *p*., a nitric oxide pathway inhibitor). After 15 minutes, they received AS (500 mg/kg, *p. o*.) and fluoxetine (20 mg/kg, *p.o*.). An hour post-treatment, they were subjected to TST.

## STATISTICAL ANALYSIS

All values were expressed as mean ± SEM. The differences in the mean of immobility from TST mean number of foot slips from BWA and mean number of line crossing activity from OFT among different treated groups were analysed by One-way ANOVA followed by Bonferroni post hoc test using

SPSS version 20.0. A significant level of $p$ <0.05 was considered significant.

## RESULTS

### 3.1 Phytochemical constituents

The phytochemical constituents found present in the Methanol Root Bark extract of include glycosides, carbohydrates, saponins, flavonoids, tannins and alkaloids (Table 1).

**Table 1: Phytochemical Constituents Present in the Methanol Root Bark Extract of *Acacia seyal***

| S/No | Phytoconstituents | Inference |
|---|---|---|
| 1 | Alkaloids | + |
| 2 | Flavonoids | + |
| 3 | Tannins | + |
| 4 | Saponin glycoside | + |
| 5 | Cardiac glycoside | - |
| 6 | Unsaturated Steroids and Triterpenes | + |
| | | + |
| 7 | Anthraquinones | - |
| 8 | Carbohydrates | + |

+= present, -=absent

### 3.2 Median Lethal Dose for Methanol Root Bark Extract of *Acacia seyal*

The $LD_{50}$ was estimated to be ≥5000 mg/kg orally in mice.

### 3.3 Effect of Methanol Extract of *Acacia seyal* on Motor Coordination Deficit

The methanol root bark extract of *Acacia seyal* did not impair motor coordination at all tested doses. The standard drug, diazepam, significantly ($p$<0.001) impaired motor coordination at the dose of 10 mg/kg orally (Figure 3).

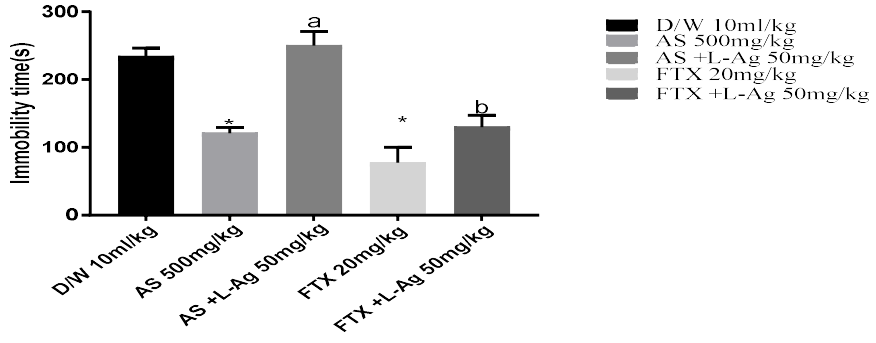


**Figure 1: Effect of Methanol Root Bark Extract of *Acacia seyal* on Motor Coordination in Mice Beam Walking Assay**

Each column represents the mean ± S.E.M. of 6 animals. Data was analysed using One-way ANOVA followed by Bonferroni post hoc test, *$P$≤ 0.001, significantly different from distilled water treated animals. AS= Methanol Root Bark extract of *Acacia seyal*, DW= Distilled water, DZP= Diazepam

### 3.4 Effect of Methanol Root Bark Extract *Acacia seyal* on Tail Suspension Test

The methanol root bark extract of *Acacia seyal* decreased the duration immobility in the treated mice. Significant response was obtained at all the tested doses ($p< 0.05$) as compared to the distilled water (10 ml/kg) group. Similarly, the standard drug, fluoxetine (20 mg/kg), also significantly ($p<0.001$) decreased the duration immobility time (Figure 2).

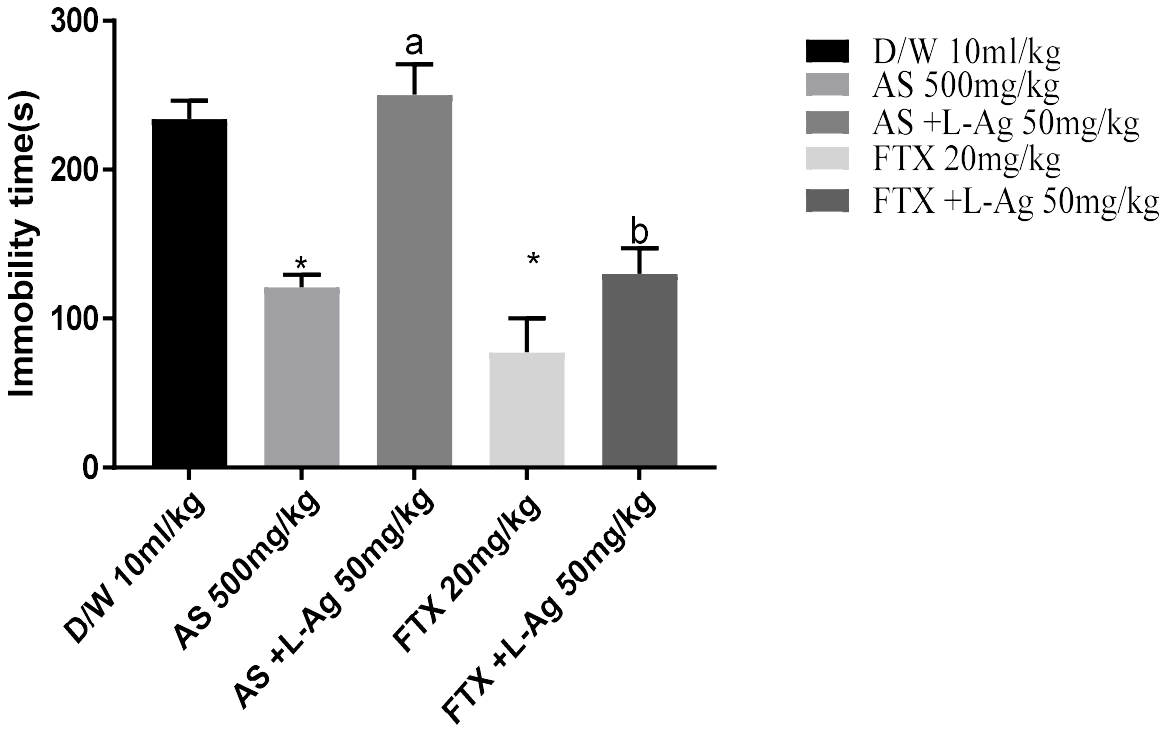


**Figure 2**: **Effect of Methanol Root Bark Extract of *Acacia seyal* on Duration Immobility of Mice in Tail Suspension Test**

Animals were acutely treated with AS (250, 500 or 1000 mg/kg, *po*), distilled water (10 ml/kg, *po*), or Fluoxetine (20 mg/kg, *po*). Each column represents the mean ± SEM of 8 animals. Data was analysed using one-way ANOVA followed by Bonferroni post hoc test, *$p\leq$ 0.05, **$p\leq$ 0.001, ***$p\leq$ 0.0001 significantly different from distilled water treated group. AS= Methanol Root Bark extract of *Acacia seyal*, DW= Distilled water, FTX= Fluoxetine

### 3.5 Effect of Methanol Root Bark Extract of *Acacia seyal* on the Open Field Test

In the open field test, the distilled water treated animals exhibited locomotor activities marked by the number of line crosses (48.83 ± 10.16). The methanol root bark extract of *Acacia seyal* did not significantly increase the number of line crosses activity. But, diazepam significantly ($p<0.01$) increased the number of line crosses activity (Figure 3).

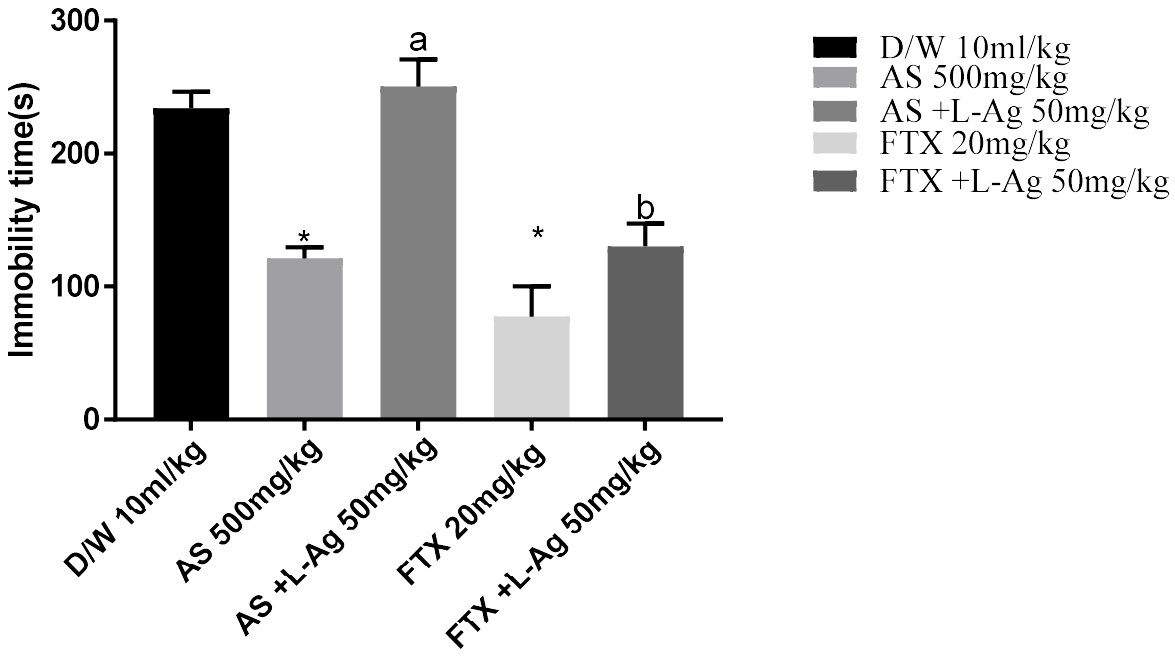


**Figure 3**: **Effect of Methanol Root Bark Extract of *Acacia seyal* on Line Crosses Activity in Mice in the Open Field Test**

Animals were acutely treated with AS (250, 500, or 1000 mg/kg, *po*), distilled water (10 ml/kg, *po*), or diazepam (0.5 mg/kg, *po*). Each column represents the mean ± SEM of 6 animals. Data analysis was performed using One-way ANOVA followed by Bonferroni post hoc test, ***$p \leq 0.001$, significantly different from distilled water treated animals. AS= Methanol Root Bark extract of *Acacia seyal*, DW= Distilled water, DZP= Diazepam

## 3.6 Mechanistic Studies

### 3.6.1 Involvement of dopaminergic system

Pretreatment of mice with sulpiride (50 mg/kg, $D_2$ receptor antagonist, *i.p.*) significantly ($p \leq 0.01$) reversed the anti-immobility effect elicited by the methanol root extract of *Acacia seyal* (500 mg/kg) as compared to treatment alone (Figure 4).

### 3.6.2 Involvement of the serotonergic system

Pretreatment of mice with metergoline (1mg/kg, i.p., a non-selective 5-$HT_2$ receptor antagonist) significantly ($p \leq 0.01$) reversed the reduction in immobility time elicited by methanol root bark extract of *Acacia seyal* extract (500 mg/kg, *p.o.*) as compared to treatment alone (Figure 5).

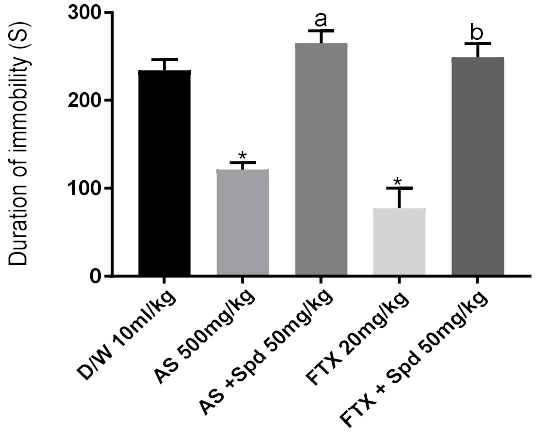


**Figure 4: Effect of Sulpiride on Antidepressant Activity of Methanol Root Bark Extract of *Acacia seyal* in Mice**

Each column represents the mean ± S.E.M. of 5 animals. Data was analysed using One-way ANOVA followed by Bonferroni post hoc test, $^{*}p \leq 0.001$ significantly different from DW treated group; $^{a}$ & $^{b}p \leq 0.01$= significant difference compared to AS or FTX treated groups respectively; AS= Methanol Root Bark extract of *Acacia seyal*; FTX= Fluoxetine; DW= Distilled water, Spd= Sulpiride (50 mg/kg)

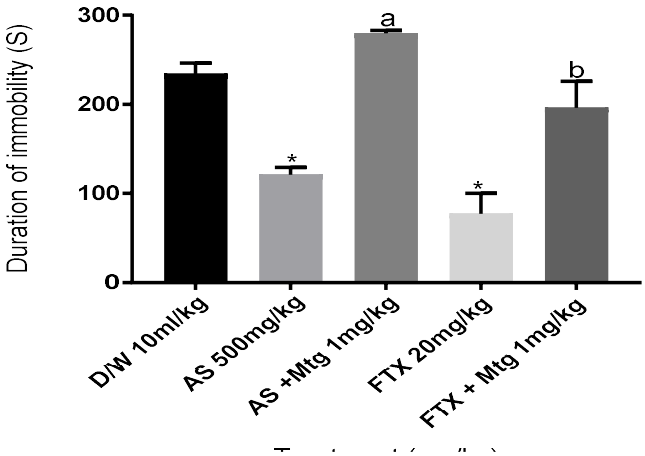


**Figure 5**: **Effect of Metergoline on the Antidepressant Activity of Methanol Root Bark Extract of *Acacia seyal* in Mice**

Each column represents the mean ± S.E.M. of 5 animals. Data was analysed using One-way ANOVA followed by Bonferroni post hoc test, $^{*}p \leq 0.001$ significantly different from DW treated group; a & b=$p \leq 0.01$ significantly different from AS or FTX treated groups respectively; AS= Methanol Root Bark extract of *Acacia seyal*; FTX= Fluoxetine; DW= Distilled water, Mtg=Metergoline (1 mg/kg)

### 3.6.3 Involvement of the serotonergic system

Pretreatment of mice with cyproheptadine (4 mg/kg, *i.p*., a 5-$HT_2$ receptor antagonist) significantly ($p$<0.01) reversed the reduction in immobility time elicited by the methanol root bark extract of *Acacia seyal* (500 mg/kg, *p.o*.) as compared to treatment alone (Figure 4.7).

### 3.6.4 Involvement of the cholinergic system

Pretreatment of mice with atropine (1 mg/kg, *i.p*, muscarinic cholinergic receptor antagonist) significantly ($p$<0.01) reversed the reduction in immobility time elicited by methanol root bark extract of *Acacia seyal* (500 mg/kg, *p.o*.) as compared to treatment alone (Figure 4.8).

### 3.6.5 Involvement of opioidergic system

Pretreatment of mice with naloxone (2 mg/kg, *i.p*., opioid receptor antagonist) significantly ($p$<0.01) reversed the reduction in immobility time elicited by the extract (500 mg/kg, *p.o*.) as compared to treatment alone (Figure 4.9).

### 3.6.6 Involvement of the Nitric Oxide Pathway

Pretreatment of mice with L- Arginine (50 mg/kg, *i.p*., a nitric oxide substrate) reversed the reduction in immobility time elicited by methanol root bark extract of *Acacia seyal* (500 mg/kg) orally (Figure 4.10). Pretreatment of mice with L-NNA (50 mg/kg *i.p*., a nitric oxide synthase enzyme inhibitor) reversed the reduction in immobility time elicited by methanol root bark extract of *Acacia seyal* (500 mg/kg) orally (Figure 9).

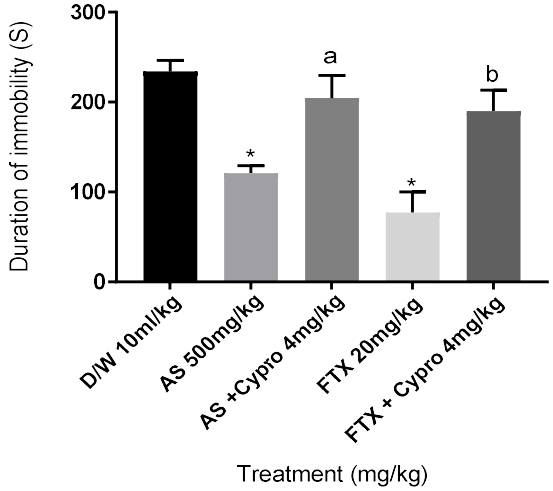


**Figure 6: Effect of Cyproheptadine on Antidepressant activity of Methanol Root Bark Extract of *Acacia seyal* in Mice**

Each column represents the mean ± S.E.M. of 5 animals. Data was analysed using One-way ANOVA followed by Bonferroni post hoc test, $^{*}p\leq$ 0.001 significantly different from DW treated group; $^{a\ \&\ b}p\leq$0.01= significantly different from AS or FTX treated groups respectively; AS= Methanol Root Bark extract of *Acacia seyal*; FTX= Fluoxetine, DW=

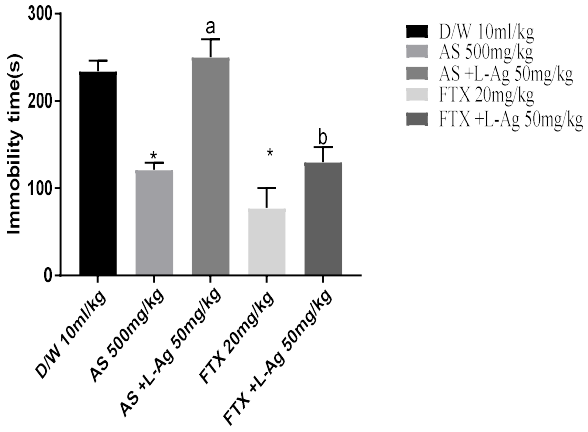


**Figure 7: Effect of Atropine on Antidepressant activity of Methanol Root Bark Extract of *Acacia seyal* in Mice**

Each column represents the mean ± S.E.M. of 5 animals. Data was analysed using One-way ANOVA followed by Bonferroni post hoc test, *$p\leq$ 0.001 significantly different from DW treated group; a & b= $P\leq$0.01significantly different from AS or FTX treated groups respectively; AS= Methanol Root Bark extract of *Acacia seyal*; FTX= Fluoxetine, DW= Distilled water

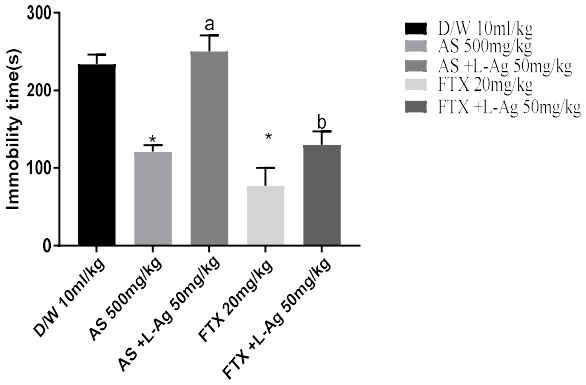


**Figure 8: Effect of Naloxone on Antidepressant Activity of Methanol Root Bark Extract of *Acacia seyal* in Mice**

Each column represents the mean ± S.E.M. of 5 animals. Data was analysed using One-way ANOVA followed by Bonferroni post hoc test, *$p\leq$ 0.001 significantly different from DW treated group; a & b= $p\leq$0.01 significantly different from AS or FTX treated groups respectively; AS= Methanol Root Bark extract of *Acacia seyal*; FTX= Fluoxetine, DW= Distilled water

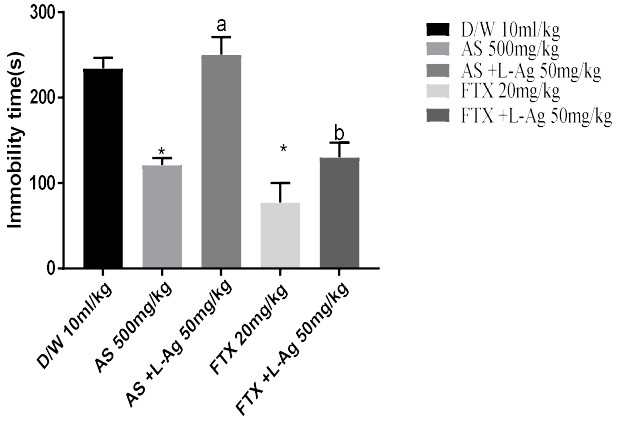


**Figure 9: Effect of L-Arginine on Antidepressant Activity of Methanol Root Bark Extract of *Acacia seyal* in Mice**

Each column represents the mean ± S.E.M. of 5 animals. Data was analysed using One-way ANOVA followed by Bonferroni post hoc test, *$p\leq$ 0.001 significantly different from DW treated group; a & b=$p\leq$0.01 significantly different from AS or FTX treated groups respectively; AS= Methanol Root Bark extract of *Acacia seyal*; FTX= Fluoxetine, DW= Distilled water, L-Ag= L-Arginine (50 mg/kg)

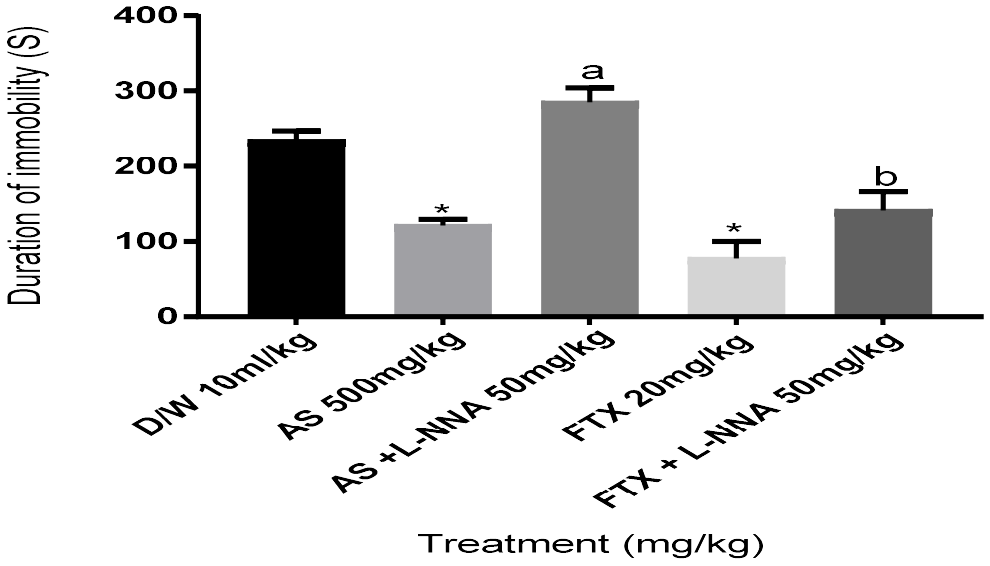


**Figure 10: Effect of L-NNA on the Antidepressant Activity of Methanol Root Bark Extract of *Acacia seyal* in Mice**

Each column represents the mean ± S.E.M. of 5 animals. Data was analysed using One-way ANOVA followed by Bonferroni post hoc test, *$p \leq 0.001$ significantly different from DW treated group; a & b=$p \leq 0.01$ significantly different from AS or FTX treated groups respectively; AS= Methanol Root Bark extract of *Acacia seyal*; FTX= Fluoxetine, DW= Distilled water, L-NNA=N-Arginine (50 mg/kg)

### 3.6.7 Involvement of the noradrenergic system

Pretreatment of mice with yohimbine (1 mg/kg, *i.p*., an $\alpha_2$ - adrenoceptor antagonist) reversed the reduction in immobility time elicited by methanol root bark extract of *Acacia seyal* (500mg/kg) orally (Figure 11). So also, pretreatment of mice with prazosin (1 mg/kg, *i.p*., an $\alpha_1$-adrenoceptor antagonist) reversed the reduction in immobility time elicited by the methanol root bark extract of *Acacia seyal* (500 mg/kg) orally (Figure 12).

## DISCUSSION

Findings in this study provide evidence of antidepressant effects produced by Methanol root bark extract of *Acacia seyal*. The extract at 5000 mg/kg did not induce mortality or any signs of toxicity implying it is safe. It was observed that diazepam at the tested dose increased the number of foot slips in the Beam walking assay but the extract did not show any effect on the number of foot slips. The beam walking assay is used to identify pharmacologically-induced motor coordination deficits (Stanley *et al*., 2005). This finding suggests the extract produces its pharmacological effect without causing significant motor deficits, unlike some clinically used tranquilisers such as benzodiazepines (Stanley *et al*., 2005).

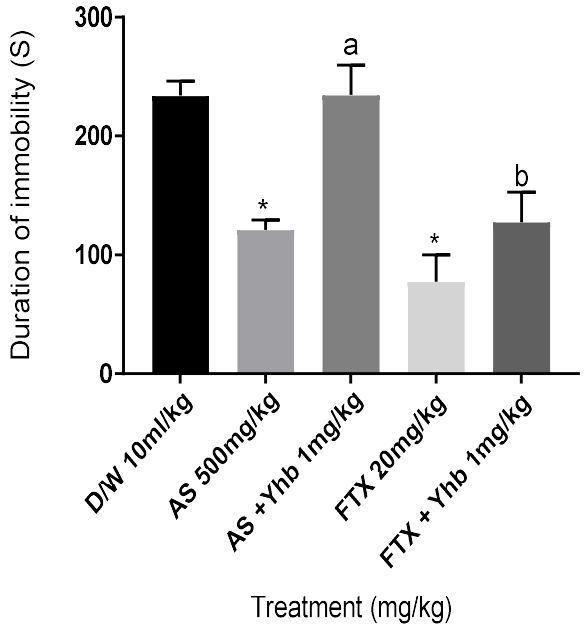


**Figure 11: Effect of Yohimbine on the Antidepressant Activity of the Methanol Root Bark Extract of *Acacia seyal* in Mice**

Each column represents the mean ± S.E.M. of 5 animals. Data was analysed using One-way ANOVA followed by Bonferroni post hoc test, *$p\leq$ 0.001 significantly different from DW treated group; a & b=$p\leq$0.01 significantly different from AS or FTX treated groups respectively; AS= Methanol Root Bark extract of *Acacia seyal*; FTX= Fluoxetine, DW= Distilled water, Yhb= Yohimbine (1mg/kg)

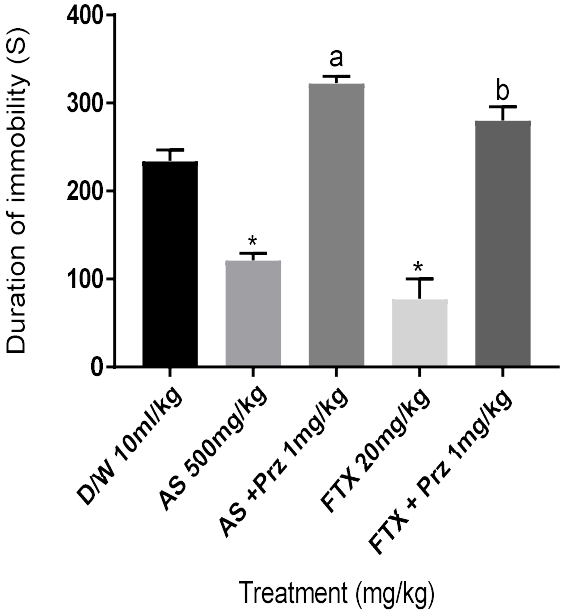


**Figure 12: Effect of Yohimbine on the Antidepressant Activity of Methanol Root Bark Extract of *Acacia seyal* in Mice**

Each column represents the mean ± S.E.M. of 5 animals. Data was analysed using One-way ANOVA followed by Bonferroni post hoc test, *$p\leq$ 0.001 significantly different from DW treated group; a & b= $P\leq$0.01 significantly different from AS or FTX treated groups respectively; AS= Methanol Root Bark extract of *Acacia seyal*; FTX= Fluoxetine, DW= Distilled water, Prz= Prazosin (1mg/kg)

The Tail suspension test (TST) is a predictive behavioural test of antidepressant activity (Chermat *et al*., 1986). Substance under investigation is administered prior to the test, after which the mice will actively pursue escape-directed behaviours over longer periods. The increase in such activity (the decrease in immobility) in the TST is strongly correlated with antidepressant effects in humans (Cryan *et al*. 2005a; Bravo *et al*., 2009). Thus, the ability of the extract to reduce the duration immobility is an indication of its antidepressant properties. The reduction of immobility time elicited by the extract cannot be attributable to any psychostimulant effect because there was no significant increase in locomotor activity when compared with the distilled water group in OFT (Umukoro and Aladeokin, 2010).

The monoamine hypothesis of depression suggests that disturbances in the monoaminergic system, including norepinephrine, 5-hydroxytryptamine, and dopamine, as well as excitatory and inhibitory amino acid receptor families and second messengers, play a crucial role in the

pathogenesis of depression (Marazziti *et al*., 2014; Pytka *et al.,* 2016). Intensive research into the neurobiology of depression suggests that an increase in the monoamine levels at the synapse is believed to be the first step in a complex cascade of events that results in antidepressant activity (Ying *et al.*, 2010).

In this study, the antidepressant-like effect elicited by the extract was reversed by pretreatment of animals with sulpiride (a dopamine $D_2$ receptor antagonist). This indicates that $D_2$ Receptors may play a role in antidepressant-like effect elicited by methanol root bark extract of *Acacia seyal*. Several clinical studies indicate that the noradrenergic system is strongly implicated in the pathophysiology of depression (Frazer, 2000; Nutt, 2006). The $\alpha_1$ -, and $\alpha_2$ -adrenoceptors have been shown to underlie some of the antidepressant-like responses of drugs in behavioural models of depression (Danysz *et al.,* 1986; Masuda *et al.,* 2001). The results obtained from this study show that pretreatment of mice with prazosin ($\alpha_1$ adrenoceptor antagonist) and yohimbine ($\alpha_2$ adrenoceptor antagonist) reversed the antidepressant-like effect of the extract on TST.

Most of the currently used conventional antidepressants directly affect serotonin turnover in the brain (Kress and Lucki,1995), inhibit serotonin reuptake and interact with 5-$HT_1$A and 5-$HT_2$ receptors (Cyran *et al.,* 2005; Moncrieff *et al.,* 2023). Results obtained from the pretreatment of mice with metergoline (a non-selective 5-$HT_2$ receptor antagonist) and cyproheptadine (a 5-$HT_2$ receptor antagonist) reversed the antidepressant-like effect of methanol root bark extract of *Acacia seyal* on TST.

Cholinergic dysfunctions may account for the development of cognitive symptoms associated with depression, especially when the disease is long lasting and treatment resistant. Moreover, changes in hippocampal neurogenesis may be in part mediated by the cholinergic system and may relate to the cognitive disturbances diagnosed in depression (Dugite *et al.,* 2011). Results obtained from the pretreatment of mice with atropine (muscarinic cholinergic receptor antagonist) reversed the antidepressant-like effect of methanol root bark extract of *Acacia seyal* on TST.

There is considerable evidence that suggest a relationship between the opioid system and depression (Hegadoren *et al*. 2009), suggesting that compounds that enhance opioid neurotransmission may exert genuine antidepressant effects (Jutkiewicz, 2006; Berrocoso & Mico, 2009a). Results obtained from the pretreatment of mice with naloxone (opioid receptor antagonist) reversed the antidepressant-like effect of methanol root bark extract of *Acacia seyal*.on TST.

Nitric oxide has been considered an important neurotransmitter substance involved in the pathophysiology of major depression, Nitric oxide is a chemical messenger that possesses an ability to freely diffuse across the cell membranes and unlike other classical neurotransmitters, this molecule is neither stored in the synaptic vesicles nor released by the process of exocytosis (Yun *et al.,* 1997). Results obtained from the pretreatment of mice with L- Arginine (a nitric oxide substrate) and L-NNA (a nitric oxide synthase enzyme inhibitor) reversed the antidepressant-like effect of methanol root bark extract of *Acacia seyal* on TST.

In summary, our results provide evidence of the antidepressant-like effects of methanol root bark extract of *Acacia seyal*. In addition, they suggested that these effects are related to noradrenergic, dopaminergic, serotonergic, opioidergic and the nitric oxide pathway.

## CONCLUSION

The methanol root bark extract of *Acacia seyal* possesses antidepressant-like activity possibly linked to noradrenergic, dopaminergic, serotonergic, opioidergic and the nitric oxide pathways.

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