

Original Research Article

Antidepressant effect of methanol root bark extract of *Acacia seyal* Del. (Fabaceae): Possible involvement of the inflammatory pathway

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Abstract

Purpose: To study the involvement of inflammatory pathways in the antidepressant activity of *Acacia seyal* in mice.

Methods: The median lethal dose (LD_{50}) of the extract *Acacia seyal* (AS) was determined using OECD guideline 425. The antidepressant activity of AS was assessed against BCG (0.2 mg/kg, ip)-induced depression in mice using Tail suspension test (TST) and open field test (OFT) at 4, 24 and 48 hours post BCG administration.

Results: The median lethal dose (LD_{50}) for the extract was > 5000 mg/kg orally. The extract AS at all tested doses (250 – 1000 mg/kg) significantly ($p \leq 0.001$) decreased the duration of immobility in TST but increased the number of line crossing in OFT post-BCG.

Conclusion: The antidepressant activity of the methanol root bark extract of *Acacia seyal* in mice may involve an inflammatory mechanism. Thus, the extract of *Acacia seyal* may be suitable for the management of depression in humans resistant to other conventional antidepressant agents. However, further studies are required to ascertain this

Keywords: Depression, *Acacia seyal*, Tail suspension test, Open field test, *Bacillus Calmette-Guerin*

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INTRODUCTION

Depression is an incapacitating psychiatric ailment that has been estimated to affect 21% of the world population [1]. Depression can occur at any age from childhood to late life and is a tremendous burden to the society. The disorder causes severe distress and disruption of life linked to all-cause mortality [2]. Depression is a highly complicated disorder with heterogeneity observed in etiology, diagnosis and treatment

response [3]. Heterogeneity in depression hinders progress in treatment and research [4]. Although a number of drugs are available for the treatment of depressive disorders, most of drugs produces poor response, relapse and remission conferring great challenge [5]. Inflammatory pathways play a role in the pathophysiology of depression and this can be highly beneficial in forming basis for new treatment approaches [6]. The release of pro-inflammatory cytokines leads to neuroinflammation, which contributes to

depression [7]. Inflammation reduces the availability of serotonin in the brain [8]. Therefore, antidepressant drugs required activation of the inflammatory pathways to increase the synaptic availability of serotonin. Antidepressants are likely to be less effective in the presence of inflammatory stimulus to reduce the depressive symptoms especially in major depression [9]. Excessive production of inflammatory mediators by macrophages has been linked to suicidal attempts [10]. Thus, an urgent need to target inflammatory pathways as an alternative approach to the management of depression. *Acacia seyal* is reported useful in the management of depression elicited via monoaminergic, neuroendocrine and neurotrophic pathways [11].

However, the involvement of the anti-inflammatory mechanism in the antidepressant activity of the plant has not been reported. This study therefore aimed to determine the antidepressant effect of methanol root bark extract of *Acacia seyal* against *Bacillus Calmette-Guerin* (BCG) induced depression in mice.

EXPERIMENTAL

Plant preparations and extraction

The whole plant *Acacia seyal* was collected in March 2018 from Sabon Gari Local Government Area of Kaduna State. It was taken to Herbarium Section of the Department of Botany, Ahmadu Bello University, Zaria where it was authenticated by Mr Namadi Sanusi. A voucher specimen (no. 900347) was deposited in the Herbarium of Department of Botany, Ahmadu Bello University, Zaria.

The root bark extract of *Acacia seyal* collected was dried and sized reduced using mortar and pestle. Two hundred grams of the dried powdered material was extracted with 1500 milliliters of methanol by cold maceration technique for 2 days with occasional shaking. The resultant extract was evaporated to dryness over a water bath which gave a brown solid mass. All solutions for administrations were freshly prepared using distilled water.

Animals

Male Swiss albino mice (18 - 20 g) were obtained from the Animal House of the Department of Pharmacology and Therapeutics, Ahmadu Bello University Zaria, Nigeria. They were housed in cages and kept under natural day and light cycle. Standard laboratory animal diet was provided and water *ad libitum* and followed experimental

conditions and procedures stipulated in international guidelines [12]. An ethical approval was obtained from the Ahmadu Bello University Animal Ethics Committee (no. ABUCAUC/2017/022).

Acute toxicity study

Median lethal dose (LD₅₀) was determined using Organization for Economic Co-operation and Development (OECD) guideline in mice [13]. Two groups of three mice each were fasted 3 h prior to administration of methanol root bark extract of *Acacia seyal* and 2 h after the administration of the extract. In limit test, 5000 mg/kg was given to one mouse and observed for 48 h. The first mouse survived, thereafter, two more mice were given same dose of extract. In all case, mice were observed during the first 30 min of treatment and then at intervals over 24 h and then daily for 14 days. The animals were monitored for tremor, convulsion, salivation, diarrhoea, sleep, behavioural changes and coma. The time of onset of toxic symptoms and disappearance were also noted. The weights of animals were determined weekly for 14 days individually.

Treatments

On the day of injection, fresh solution of BCG was prepared and administered intraperitoneally to forty mice at the dose of 0.2 mg/kg. Rectal temperatures were taken at 0, 4, 24 and 48 h after BCG administration. The mice were divided into 5 groups of 8 mice each, then monitored for signs of motion sickness for a period of 4 h. Three hours after BCG treatment, Group 1 mice were given distilled water (10 ml/kg) and group 2 with fluoxetine (20 mg/kg) via the oral route. Mice in groups 3, 4 and 5 received methanol root bark extract of *Acacia seyal* at dose of 250, 500 and 1000 mg/kg per orally respectively. Mice in all groups were then subjected to tail suspension and open field test 1 h after treatment, with another hour interval allowed between each test.

Behavioural tests

Tail suspension test

Tail suspension test (TST) was performed according to the method described by [14]. Mice in all the groups were subjected to tail suspension test by individual suspension of the mouse 60 cm above the surface of the table with an adhesive tape placed 1 cm away from the tip of the tail and a cylindrical rubber stopper. Mice were considered immobile only when they hung passively and were completely motionless.

Experiments were video-taped for 6 min. This test was done at 4, 24 and 48 h post BCG injection.

Open field test

Each mouse was placed in a white wooden open field apparatus (70 × 70 × 35 cm, length × breadth × height), one side and floor were made up of transparent plexiglas, with the floor divided into 16 visible squares (15 × 15 cm) with a central square. Peripheral and central square crossing behaviour of each mouse was recorded for 6 min with the aid of a camera suspended at the top of the apparatus [15]. This test was done at 4, 24 and 48 h after BCG administration.

Statistical analysis

All values are expressed as mean ± SEM. Values for rectal temperature were analysed using repeated measures ANOVA followed with Bonferroni post hoc test. The differences in the mean of immobility from tail suspension test, and mean number of line crossing activities from open field studies among different treated groups were analysed using one-way ANOVA followed by Bonferroni post hoc test using SPSS version 20.0. A value of $p < 0.05$ was considered significant.

RESULTS

Median lethal dose (LD₅₀) for methanol root bark extract of *Acacia seyal*

The LD₅₀ was ≥ 5000 mg/kg orally in mice.

Effect of BCG on body temperature at 48 h post-administration

The changes in core body temperature was measured at 4, 24 and 48 h post-BCG injection. There was a significant ($p < 0.001$) decrease in rectal temperature of mice at all tested doses 4 hours post BCG injection when compared with the rectal temperature at 0 h. There was also a significant ($p < 0.05$) change in rectal temperature 24 h post-BCG injection at 1000 mg/kg dose of extract. There was also a decrease in rectal temperature at all tested doses 48 h post BCG injection, when compared to the distilled water group (Figure 1).

Effect of methanol root extract of *acacia seyal* on duration of immobility in BCG-induced depression in mice

There were a significant ($p < 0.001$) decrease in the duration of immobility in mice treated with

fluoxetine (20 mg/kg) and *Acacia seyal* (AS 500 and 1000 mg/kg) 4 h post-BCG administration. There were also significant ($p \leq 0.01$) and dose-dependent decrease in the duration of immobility at all the tested doses when compared to the distilled water group 24 hours post-BCG injection. However, only the highest dose of extract and fluoxetine were able to significantly ($p \leq 0.05$) decrease the duration of immobility 48 h post-BCG injection when compared to the distilled water group (Figure 2).

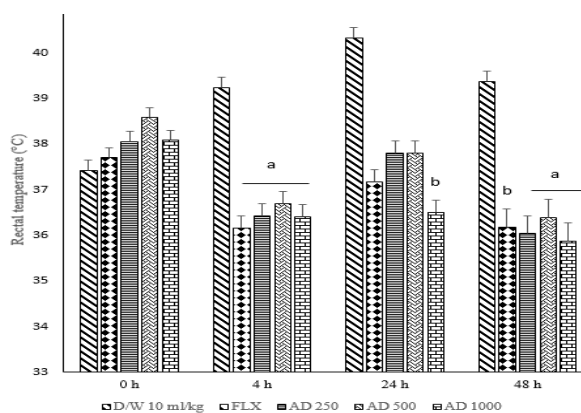


Figure 1: Effect of methanol root bark extract of *Acacia seyal* on BCG-induced changes in rectal temperature of mice. **Note:** Each column represents mean ± S.E.M. of 8 mice. Data analysis was performed using repeated measures ANOVA followed by Bonferroni post hoc test, ^a $p < 0.001$ and ^b $p < 0.05$, significantly different from distilled water group at various times. AS = *Acacia seyal*, DW = Distilled water, FLX = Fluoxetine, h = hour

Effect of methanol root extract of *acacia seyal* on locomotory behaviour in mice on BCG-induced depression

Following BCG administration, there was a decrease in locomotor activity of mice. Treatment with methanol root extract of *Acacia seyal* (1000 mg/kg) significantly ($p \leq 0.001$) increased the number of line crossing in mice at 4 h post-BCG injection. However, significant ($p \leq 0.001$) increased number of line crossing at 24 h post-BCG was exhibited by the mice treated with methanol root extract of *Acacia seyal* (250, 500 and 1,000 mg/kg) as compared to the distilled water group. At the end 48 h post-BCG, only the standard drug fluoxetine (20 mg/kg) significantly increased locomotor activity of mice when compared with mice treated with distilled water (Figure 3).

DISCUSSION

This study established the involvement of anti-inflammatory mechanism in the antidepressant activity of *Acacia seyal* using BCG-induced

depression model in mice. Bacillus Calmette-Guerin (BCG) is a vaccine primarily used against tuberculosis in healthy babies in countries where tuberculosis or leprosy is common [16]. The BCG acts by activating indoleamine 2, 3-dioxygenase (IDO) in the periphery and brain which induces pro-inflammatory cytokines well known to cause clinical signs of depressive-like behaviour in both humans and animal models [17,18].

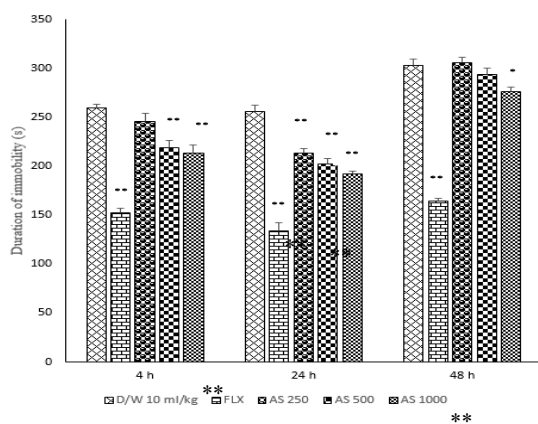


Figure 2: Effect of methanol root extract of *Acacia seyal* on duration of immobility in the BCG-induced depression in mice. **Note:** All results are expressed as mean \pm SEM ($n = 8$). Data were analysed using one-way ANOVA followed by Bonferoni post hoc test, $*p < 0.05$ and $**p \leq 0.001$ significant difference when compared to distilled water group. DW = distilled water (10 ml/kg, po), AS = *Acacia seyal* (250, 500 & 1,000 mg/kg, po), FLX = Fluoxetine (20 mg/kg, po)

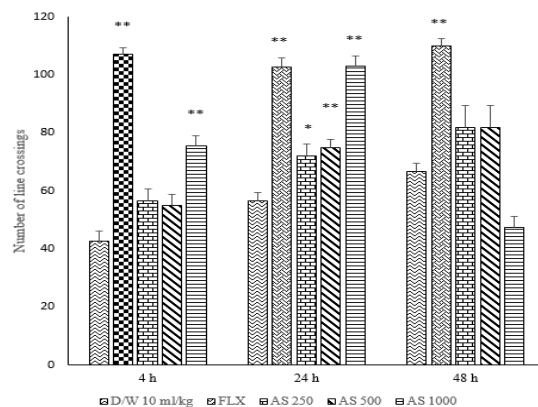


Figure 3: Effect of methanol root extract of *Acacia seyal* on line crossing behaviour of mice following BCG-induced depression. **Note:** Data were analysed using one-way ANOVA followed by Bonferoni post hoc test, expressed as mean \pm SEM ($n = 8$). $*p \leq 0.05$ and $**p \leq 0.001$ significant difference when compared with distilled water group. DW = distilled water (10 ml/kg, po), AS = *Acacia seyal* (250, 500 & 1,000 mg/kg, po), FLX = Fluoxetine (20 mg/kg, po), h = hour

Mice injected with BCG shows high levels of circulatory pro-inflammatory cytokines which was

observable as sickness behaviour and increased rectal temperature usually within 4 h post injection. Several researches showed a close linkage between inflammation and depression [19,20] and drugs with antidepressant effect decrease rectal temperature. Administration of *Acacia seyal* extract at 3 h post-BCG produced a decrease in rectal temperature indicating the effect of *Acacia seyal* on BCG-induced depression.

There was a decrease in duration of immobility in mice treated with methanol root bark extract of *Acacia seyal* extract in the tail suspension test. The tail suspension test (TST) is utilized to evaluate antidepressant drugs [21] and is able to detect most classes of antidepressant drugs like tricyclics, selective serotonin reuptake inhibitors (SSRIs) and monoamine oxidase inhibitors. In TST, duration immobility is the indicator behaviour which reflects behavioural despair similar to the case in human depression [22]. Thus, reduction of immobility by AS is an indication of its antidepressant activity. The concept of cytokine-induced depression-like behaviour in rodents using BCG vaccine is well established and it explains resistance to antidepressants treatment in some patients [5]. This resistance warrants the screening of alternative drugs for wider efficacy [23].

Acacia seyal extract was able to ameliorate BCG-induced depression by blocking the development of both sickness and depressive-like behaviour in treated mice. Since accumulating evidence suggests that depression is associated with an up-regulation of pro-inflammatory cytokines induced by BCG administration, thus AS to down regulated these inflammatory cytokines and ameliorated BCG-induced depression [24]. So also, AS was reported to possess anti-inflammatory activity in models of pain further supporting the finding in this study the additional benefits in inflammation associated depression [12].

The Open field study showed that rodent develop fear for open areas against their desire to explore new environments [25]. The open field test was carried out to determine the effect of the extract on spontaneous locomotor activity since the BCG has the ability to impair locomotor behaviour in addition to the depressive episodes [26]. The number of squares crossed are usually used as measures of locomotor activity, exploration and anxiety. In this study, treatment with AS increased line crossing activity indicating its ability to reverse the altered locomotion by BCG. The oral median lethal dose of the extract was

found to be 5000 mg/kg suggesting its relative safety as described by the OECD guideline [13].

CONCLUSION

The methanol root bark extract of *Acacia seyal* possesses significant antidepressant activity possibly mediated via anti-inflammatory mechanisms. The root bark of the plant *Acacia seyal* can be used in the management of depression resistant to other conventional antidepressant agents.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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