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Antidepressant-Like Effects of *Anacardium occidentale* L. Leaves in the Mouse Forced Swim and Tail Suspension Tests

[1]Dharamveer Panjwani, [2]Vimla Purohit and [3]H.H. Siddiqui

[1]Faculty of Pharmaceutical Sciences, Jodhpur National University, Jodhpur, Rajasthan, India

[2]Faculty of Dental Sciences, Jodhpur National University, Jodhpur, Rajasthan, India

[3]Faculty of Pharmaceutical Sciences, Integral University, Lucknow, Uttar Pradesh, India

ABSTRACT

Background and Objectives: *Anacardium occidentale* L. (Anacardiaceae) has several therapeutic application in folk medicine in curing or managing a wide range of diseases. The present study was undertaken to investigate the antidepressant-like effects of *Anacardium occidentale* L. leaves in the mouse Forced Swim Test (FST) and Tail Suspension Tests (TST). **Methodology:** Acute toxicity and neurotoxicity studies were performed. In acute study, ethanolic extract at a dose of 200 and 400 mg kg^{-1} were administered in single dose one hour prior to the test. In the chronic study, extracts were administered once daily for a period of 14 days. Imipramine (10 mg kg^{1}) and fluoxetine (10 mg kg^{1}) were used as standard antidepressant agents throughout the study. The extract did not show any type of toxicity. **Results:** The results showed that the ethanolic extract decreased immobility periods significantly in a dose dependent manner in both Tail Suspension Test (TST) and Forced Swim Test (FST). The observed results were also comparable with known standard drugs. The flavonoids apigenin and quercetin act as reversible monoamine oxidase inhibitors and selectively binds with high affinity to the central benzodiazepine receptors possesses important antidepressant activity. **Conclusion:** The literature reveals that the main constituents of *A. occidentale* are flavonoids, quercetin, apigenin, tannins and alkaloids. It was possible that the extract exerted the antidepressant activity through interaction with adrenergic, dopaminergic, serotonergic and GABAnergic system.

Key words: *Anacardium occidentale,* antidepressants, serotonin, flavonoids

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INTRODUCTION

Depression is a disorder that is recognized to be heterogeneous, symptomatically, psychologically and biologically and affects a person's mood, physical health and also behavior. According to WHO, approximately 450 million people suffer from mental and behavioural disorder[1] which accounts to 12.3% of global burden of disease and this will rise to 15% by 2020[2]. An estimated 5.8% men and 9.5% women experience the depressive episodes in their lifetime[3]. Approximately, two third of depressed patients experience suicidal thoughts and 10-15% of them attempt suicide[4].

Although, several classes of antidepressants like Tricyclic Antidepressants (TCAs), selective reversible inhibitors of monoamine oxidase A (SRIMAO-A), Selective Serotonin Reuptake Inhibitors (SSRIs) and specific Serotonin Nor-adrenaline Reuptake Inhibitors (SNRIs) are currently being used but these are mainly associated with adverse effects such as problematic interaction and relatively low response[5]. Due to clinical limitations and adverse effects, there is a critical interest in the development of efficient and safe drugs for the treatment of depression[6]. This consideration implicates search for the new antidepressant agents having lesser side effects and quick on set of action. Recently, researchers have focused significantly on novel pharmacotherapy from medicinal plants for their psychopharmacological disorders.

Herbal medicines have been the basis of health-care worldwide since the earliest days of man kind. Even in developed countries, patients rely on medicinal plants and herbal medicines for their primary care. In Germany, herbal medicines are fully recognized as medicines, whereas most herbal products in the USA are regulated as food or dietary supplements, even though many are used by consumers as folk remedies[7]. In China

Corresponding Author: Dharamveer Panjwani, Faculty of Pharmaceutical Sciences, Jodhpur National University, Jodhpur, Rajasthan, India Tel: +91-9336010647

and other oriental countries, there are lots of natural products such as peoniflorin, berberine, glycyrrhizin, rosmarinic acid and hypericin that were involved with the treatment of depression disorders.

Anacardium Occidentale (AO) is a tree native to tropical America (Mexico, Peru, Brazil, etc.) and belongs to the Family Anacardiaceae. Despite that, it is widely cultivated in India and East Africa. India being its largest producer[8] in Folk medicine, in West Africa, as well as in South America, decoction or the leaves has been used to treat gastrointestinal disorders. The cardiovascular effects of the aqueous extract of the cashew tree leaves have been studied on the arterial blood pressure of rabbit. The anti-microbial effect of an 80% ethanol extract on cashew tree leaves, has been described by Kudi *et al.*[9] Furthermore, cashew nut occupies a central position in the diets of the human population throughout the world and it has been proved that its consumption has a cardioprotective, anti-obesity, anti-cancer and antioxidant effects[10]. In fact, generally nuts including cashew nuts have been suggested as a natural source of antioxidants such as phenolics, flavonoids, tocopherols and alkyl-phenols[11]. The leaves possess antidiabetic[12], antiulcer[13] and anti-inflammatory[14] activities. The present study was undertaken to investigate the antidepressant-like effects of *Anacardium occidentale* leaves in the mouse forced-swim and tail-suspension tests.

MATERIAL AND METHODS

Plant material: The leaves of cashew tree (AO) were collected from Goa during the 2011 season and authenticated by Prof. Singh and Goel[15], Head of Department of Botany, Lucknow University, Lucknow, Uttar Pradesh, India and the voucher herbarium specimen was deposited in the Department of Botany, Lucknow University, Lucknow, for future reference. The samples were washed and air dried and this was followed by complete drying in an oven at 400°C. The dried sample was crushed mechanically to powder, sieved and stored in an air-tight container for further analysis.

Preparation of the extract: The powdered was extracted with different solvents of varying polarity by soxhlet apparatus at room temperature. The extracts were evaporated to dryness on the rotary evaporator at 37°C and the residues were kept for further analysis.

Animals: Swiss albino male mice (weighing between 18-25 g) obtained from the animal house of Babu Banarasi Das National Institute of Technology and Management (BBDNITM), Lucknow were used in the study. They were maintained at a temperature of 22±5°C, relative humidity 55±5°C with free access to food and water *ad libitum*, under a 12:12 light/dark cycle (light on at 8:00 h). All manipulations were carried out only once between 9:00 and 15:00 h. with each animal used.

The experimental protocol was approved by the Institutional Animal Ethics Committee as per the direction of the Committee for the Purpose of Control and Supervision of Experimental on Animals (approval no BBDGEI/IAEC/29/2011). The animals were acclimatized for a period of 7 days before the study. All efforts were made to reduce the number of animals used and treated humanely to minimize their pain and discomfort.

Drugs and chemicals: Fluoxetine hydrochloride (FLUDAC®, Cadila Pharmaceuticals, Ahmedabad, India) and imipramine hydrochloride (Zenith healthcare, Mehsana, Gujrat) were used as standard drugs. All other chemicals and reagents used for the study were of analytical grade.

Acute toxicity study: Mice were kept on overnight fasting and water was withdrawn 3-4 h before administration of test compound. Ethanolic extract of *A. occidentale* (EEAO) was administered orally in increasing doses of 100, 500, 1000, 2000 and 4000 mg kg^{-1} of body weight. Immediately after dosing, the mice were observed continuously for 4 h for symptoms of toxicity like motor activity, tremors, convulsions, tonic extension, muscle spasm, loss of frightening reflex, ataxia, sedation, hypnosis, lacrimation, diarrhea, salivation and writhing. Mice were then kept under observation upto 72 h for any mortality[16]. Locomotor activity was monitored by using actophotometer (IMCORP, India). Animals were individually placed in activity meter after 60 min of treatment and total activity count was registered for 5 min. The locomotor activity was expressed in terms of total photobeam interruption counts/5min[17].

Neurotoxicological studies: Neurotoxicity was determined using rotarod test. Mice which were able to remain on the rotating rod, with a speed of 10 rpm for 5 min or more were selected and divided into three groups (n = 6). The experimental groups received varying doses of extract 200, 400 mg kg^{-1} (p.o.). One group received only vehicle and served as control. All animals were placed on the rotarod after 30 min of treatment and average retention time on the rod was calculated. Neurotoxicity was assessed as inability of the animal to maintain equilibrium on the rotating rod for at least 3 min[15].

Experimental protocol: In acute study, animals were randomly divided into five groups with six animals each. Group I received only vehicle and served as control; group II and III received standard antidepressant drug imipramine (10 mg kg^{-1}) and fluoxetine (10 mg kg^{-1}), group IV and V received 200 and 400 mg kg^{-1} b.wt. of ethanolic extract of *A. occidentale* (EEAO) orally. Drugs and extract were administered 60 min before the test. For chronic, study the protocol of drug administration was the same as of acute study except that in chronic study treatments were administered for 14 days.

Forced-swimming Test (FST): The forced-swimming test was conducted as previously described by Porsolt *et al.*[18]. Mice were individually forced to swim in an open cylindrical container (diameter 10 cm, height 25 cm), containing 15 cm of water (depth) at 25±1°C. The total duration of immobility was recorded during a 6 min period. Each mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water. A decrease in the duration of immobility is indicative of an antidepressant-like effect.

Tail-suspension Test (TST): The test was carried out according to the method described by Steru *et al*[19]. Mice were suspended on the edge of a table 50 cm above the floor by the adhesive tape, placed approximately 1 cm from tip of the tail. Immobility time was recorded during a 6 min period. Animal was considered to be immobile when it did not show any movement of the body, hanged passively and completely motionless .

Statistical analysis: All the data represent Mean±SEM. The data were analyzed by means of Analysis of Variance (ANOVA). Whenever ANOVA was significant, further multiple comparisons were made using Tukey's test as the post hoc test. All analyses were performed using the Prism statistical software. The levels of statistical significance ranged from $p<0.05$ to $p<0.001$.

RESULTS

Acute toxicity: In acute toxicity study, EEAO did not show any mortality in mice. Even at this higher dose ie 4000 mg kg^{-1}, there were no gross behavioral changes were observed and 200 and 400 mg kg^{-1} dose were used for evaluation of antidepressant activity (Table 1).

Table 1: Acute toxicity and neurotoxicity screening of EEAO

Treatments	Acute toxicity test: Locomotor activity Count 5 min^{-1}	Acute toxicity test: Mortality (%)	Neurotoxicity screening: Retention time (sec)
Vehicle control	358.00±4.89	0	312.17±1.68
Extract 200 mg kg^{-1}	346.50±6.27	0	309.17±.720
Extract 200 mg kg^{-1}	371.00±3.48	0	305.33±1.65

Table 2: Effect of ethanolic extract of leaves of *Anacardium occidentale* on TST in mice

Group	Treatments	Period of immobility: Acute study	Period of immobility: Chronic study (14 days)
I	Control	194.00±8.49	210.17±8.42
II	Imipramine	79.500±2.23***	58.17±2.94***
III	Fluoxetine	78.500±2.41***	64.33±2.23***
IV	EEAO 200 mg kg^{-1}	157.17±7.39	102.67±0.69***
V	EEAO 400 mg kg^{-1}	125.67±5.81*	82.33±3.33***

Values are expressed as Mean±SEM, *p<0.05, **p<0.01, ***p<0.001 compared with control (group I)

Neurotoxicological studies: In the rotarod test, the vehicle-treated mice did not demonstrate any signs of impaired motor co-ordination. Each control mouse was capable of performing the test, i.e., the mean time spent on the rotarod apparatus was 180 sec. Similarly EEAO did not effect the motor coordination of mice in the rotarod test at any dose. Thus, the extract was found to have no neurotoxic effects up to 400 mg kg^{-1} dose (Table 1).

Effect of EEAO on immobility period in TST: In acute study EEAO at a dose of 400 mg kg^{-1} showed a significant ($p<0.05$) decrease in the period of immobility when compared with the control group where as EEAO at 200 mg kg^{-1} showed no significant decrease in immobility period as compared to the control group. Fluoxetine and imipramine showed a significant ($p<0.001$) decrease in immobility period when compared with control (Table 2).

In chronic study, EEAO at a dose of 200 and 400 mg kg^{-1}, imipramine and fluoxetine was administered for 14 successive days to mice and it was found that all the treatments showed significant ($p<0.001$) decrease in the period of immobility as compared to the control. ANOVA showed no significant differences in the TST results between the two groups of EEAO nor any significant differences between the EEAO-treated and imipramine and fluoxetine treated ones. The efficacy of EEAO was found to be comparable to imipramine and fluoxetine in the FST (Table 2).

Table 3: Effect of ethanolic extract of leaves of *Anacardium occidentale* on FST in mice

Group	Treatment	Period of immobility	
		Acute study	Chronic study(14 days)
I	Control	192.83±4.07	210.33±3.89
II	Imipramine	56.17±1.78***	34.17±3.31***
III	Fluoxetine	56.67±1.78***	30.33±2.65***
IV	EEAO 200 mg kg^{-1}	145.33±4.08**	88.67±2.83***
V	EEAO 400 mg kg^{-1}	126.67±2.38***	42.17±3.08***

Values are expressed as Mean±SEM; *p<0.05, **p<0.01, ***p<0.001 compared with control (group I)

Effect of EEAO on immobility period in FST: In acute study, EEAO at a dose of 200 and 400 mg kg^{-1} showed significant ($p<0.01$, $p<0.001$) decrease in the period of immobility when compared with the control. Fluoxetine and imipramine showed a significant ($p<0.001$) decrease in immobility period as compared to control group (Table 3).

In the chronic study, EEAO, imipramine and fluoxetine showed significant ($p<0.001$) decrease in period of immobility when compared with the control group. ANOVA showed no significant differences in the FST results between the two EEAO groups, nor any significant differences between the EEAO treated and imipramine and fluoxetine treated mice. The efficacy of EEAO was found to be comparable to imipramine and fluoxetine in the FST (Table 3).

DISCUSSION

The incidence of depression in the community is very high and is associated with a lot of morbidity. Hence, it is very important to address these problems and find effective remedies. Though several drugs are available, all are associated with some limitations and there is an urgent need for alternative medications for these disorders. Despite the widely popular use of *A. occidentale* for treating various disorders, there is an absence of scientific reports about the evaluation of its antidepressant effects. In this work, it was demonstrated that the administration of different doses of the ethanolic extract of *A. occidentale* in mice induced antidepressant effects. On the basis of the clinical association of depressive episodes and stressful life events, many of the animal models for the evaluation of antidepressant drug activity assess stress precipitated behaviors. The two most widely used animal models for antidepressant screening are the forced-swimming and tail-suspension tests. These tests are quite sensitive and relatively specific to all major classes of antidepressants[18]. In TST, immobility reflects a state of despair which can be reduced by several agents which are therapeutically effective in human depression. Similarly in the FST, mice are forced to swim in restricted space from which they cannot escape. This induces a state of behavioral despair in animals which is claimed to reproduce a condition similar to human depression[20]. It has been seen that the TST is less stressful and has higher pharmacological sensitivity than FST[21]. Results showed that the administration of the EEAO produced a diminution of period of immobility (a posture thought to reflect a state of "behavior despair" in which animals have given up the hope to escape) of mice exposed to both, the forced-swimming and the tail-suspension tests. In the present study, ethanolic extract (200 and 400 mg kg^{-1}) produced significant antidepressant like effect in both the TST and the FST and their efficacies were found to be comparable to imipramine (15 mg kg^{-1}) and fluoxetine (20 mg kg^{-1}). It has been established that the shortening of immobility time in the forced-swimming and the tail-suspension tests depends mainly on the enhancement of central 5 HT and catecholamine neurotransmission[22]. Early evidence of a role for noradrenaline in depression came from the discovery that drugs, either causing or alleviating depression, acted to alter the noradrenaline metabolism. Furthermore, depletion studies carried out in treated and untreated patients indicated a role for serotonin and noradrenaline in depression[23].

Monoamine Oxidase (MAO) is a flavoenzyme found in the outer membrane of mitochondria. MAO catalyzes the oxidative deamination of primary, secondary and some tertiary amines[24]. Two isoforms of MAO exists: MAO-A and MAO-B, where MAO-A preferentially oxidizes serotonin (5-hydroxytryptamine) and noradrenaline, whereas MAO-B preferentially oxidizes phenylethylamine[25]. Dopamine and tyramine appears to be substrates for both isoenzymes. In the CNS, MAO-A is present in the extraneuronal compartment and within the dopaminergic, serotonergic and noradrenergic nerve terminals while MAO-B is mainly localized in the glial cells[26]. The primary roles of MAO-A and MAO-B lie in the metabolism of exogenous amines and in the regulation of intracellular amine stores[25]. It is believed that the pathology of depression involves a deficiency of 5-hydroxytryptamine and noradrenaline and inhibition of this enzyme causes a reduction in metabolism and subsequent increase in the concentration of biogenic amines. Selective inhibitors of MAO-A are therefore, used in the treatment of depression.

The flavonoids apigenin[27] and quercetin act as reversible monoamine oxidase inhibitors and selectively

binds with high affinity to the central benzodiazepine receptors possesses important antidepressant activity. The literature reveals that the main constituents of *A. occidentale* are flavonoids, quercetin, apigenin, tannins and alkaloids[28]. It was possible that EEAO exerted the antidepressant activity through interaction with adrenergic, dopaminergic, serotonergic and GABAnergic system. However, the precise mechanisms by which the extract produced antidepressant like effect were not completely understood. Further studies would be necessary to evaluate the contribution of active chemical constituents for the observed antidepressant activity.

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