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Methanol extract of *Caralluma dalzielli* N.E. Br (Asclepiadaceae) possesses antidepressant activity in mice

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Abstract

Caralluma dalzielli N.E. Br (Asclepidiaceae) is reportedly used in the management of depression by traditional practitioners in Northwestern Nigeria. However, there is paucity of data in the literature on the antidepressant potential of the plant scientifically. The aim of the work was to provide scientific rationale for the use of the plant *Caralluma dalzielli* in the management of depressive illnesses locally. Preliminary phytochemical screening was conducted using thin layer chromatography (TLC), acute toxicity study (LD_{50}) using OECD guideline 425 and beam walking assay (BWA) was used to assess effect of the extract on motor coordination deficit. Antidepressant activity of methanol extract of *Caralluma dalzielli* (125-500 mg/kg) was evaluated using tail suspension test (TST) and forced swim test (FST). The effect of extract on locomotor activity and cognitive behaviour was assessed using open field test (OFT) and novel object recognition test (NORT) respectively. Preliminary phytochemical tests revealed the presence of carbohydrates, saponins, tannins and flavonoids. The LD_{50} was found to be ≤2000 mg/kg. The extract at all doses tested significantly (p<0.05) and dose dependently decreased the duration of immobility in the TST and FST. Additionally, the extract significantly (p<0.001) increased climbing activity of mice in the FST. There was significant decrease in number of lines crossed from the OFT at the dose of 125 mg/kg. However, the extract neither increase nor decrease the discrimination index of mice in the NORT. The methanol extract of *Caralluma dalzielli* possesses significant antidepressant activity with no motor coordination deficit.

Keywords: *Caralluma dalzielli*; Tail suspension test; Forced swim test; Open field test

INTRODUCTION

Depression is a serious mood disorder, which affects millions of the world population. It is the fourth leading cause of disability worldwide [1] with approximately two third of depressed patients experiencing suicidal thoughts and suicidal attempts [2]. Antidepressant drugs used in the treatment of depression are proven effective but they are burdened with adverse effects, problematic interactions and relatively low response [3]. In addition, reports showed that some patients do not respond to any given treatment [4]. However, drugs obtained from natural sources were found to be efficacious and minimal side effects profile [5]. This has made search for novel pharmacotherapy from medicinal plants for management of psychiatric illnesses to progress significantly. Herbal therapies with folkloric claim were reported to be used in the management of depression. Medicinal plants like *Caralluma dalzielli* were reported useful in management of depression locally [6]. The plant *Caralluma dalzielli* (CD) N.E. Br. belongs to the family Asclepiadaceae. It is called "Karan masallaci" in Hausa, ''Mosque

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reed'' in English and ''Gubehi'' in Fulfulde [7]. It is succulent perennial, branched and erect up to 40 cm high, with branches, found from dry Sahel regions in Senegal to North Western Nigeria, and to the Sahara region as well. The plant is easily grown from cuttings in Northern Nigerian towns and sometimes close by houses as a charm against evil. The stems of the plant are the parts reported to be mostly used for medical purposes, although the plant can be used whole. In view of the reported use locally in the management of depression, the antidepressant property of the methanol stem bark extract of the plant was evaluated.

EXPERIMENTAL

Plant collection and extraction. The whole plant (CD) 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 previously deposited in the Department (voucher number 217). The methanol extract of *Caralluma dalzielli* (MCD) were transported to the Department of Pharmacognosy and Drug Development where it was dried and size-reduced using mortar and pestle. The powdered MCD was extracted with methanol via Soxhlet extraction. The solution was concentrated at a temperature of 45ºC. The extract was stored in desiccator until needed for the work. Aqueous solution was freshly prepared for each study using distilled water.

Animals. Swiss albino mice of either sexs were obtained from the Animal House Facility of the Department of the Pharmacology and Therapeutics, Ahmadu Bello University Zaria. They were housed in propylene cages and kept under natural day and light cycle. The animals were fed on standard laboratory animal diet and water *ad libitum*. All experimental protocols were as approved by the University Animal ethics committee with Approval number ABUCAUC/2017/022.

Drugs and chemicals. The followings are some of the chemicals used for the experiment. Imipramine (Tofranil GSK brand), Diazepam (Roche, France), Methanol (Fluka-Aldrich)

Phytochemical screening. Phytochemical screening was carried out on the methanol extract of the plant *Caralluma dalzielli* (MCD) using standard protocols [8].

Acute toxicity studies. LD_{50} determination was conducted using Organization for Economic Co-operation and Development (OECD 425) guidelines in rats and mice [9]. 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. The extract MCD was administered in a single oral dose using a cannula. A start dose of 5000 mg/kg was used for one mouse and observed for 48 hours. The mouse died and the main test was conducted where another mouse was dosed at 2000 mg/kg then observed as above. The mouse survived, and an additional four mice were dosed and all five 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.

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 divided into five groups each containing eight mice. The first, second and

third groups received 125, 250 and 500 mg/kg of MCD 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 [10].

Tail suspension test in mice. Mice were taken to neurobehavioral laboratory and adapted for 1 hour. Forty mice were divided into five groups of eight animals each. Groups 1, 2 and 3 mice were treated with 125, 250 and 500 mg/kg of methanol extract *Caralluma dalzielli* orally 1 hour prior to test. Group 4 and 5 mice were treated with distilled water (10 ml/kg) and imipramine (15 mg/kg) respectively. For the test, mice were suspended on the edge of the shelf 58 cm above a tabletop 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 [11].

Forced swim test. Forty mice were divided into five groups of eight animals each. Groups 1, 2 and 3 were treated with 125, 250 and 500 mg/kg of methanol extract of *Caralluma dalzielli* orally. Group 4 and 5 were treated with distilled water (10 ml/kg) and Imipramine (10 mg/kg) respectively. One hour after treatment, mice were individually forced to swim inside a vertical plexiglass cylinder (height: 24cm, diameter: 12cm, containing 12cm of water maintained at 25°C). The total duration immobility is measured for 5 minutes. An animal is judged immobile whenever it remains floating passively in the water in a slightly hunched but upright position, its nose just above the surface [12].

Open field test in mice. Forty mice were divided into five groups of eight animals each. Group 1, 2 and 3 mice were treated with 125, 250 and 500 mg/kg of MCD orally 1 hour prior to test. Group 4 and 5 mice were treated with distilled water (10 ml/kg) and imipramine (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 plexiglass. The plexiglass floor was 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 [13].

Novel Object Recognition Test (NORT). It involved the use of open field arenas (44×44×17cm) kept in a room with low level of light and sound. The NORT was taken in three different sessions (habituation, training, and test), one session per day. On day 1, mice were habituated to open field arena for 3 minutes. On day two, mice were exposed to a 10 minutes' familiarization trial in the presence of two identical objects made up of cylindrical glassware filled with white cotton wool, placed in the opposite corner of the open field arena, 12cm from the walls. The time spent exploring the object was hand-scored using stopwatches. On day 3, mice were subjected to a 10 minutes' choice trial in the presence of the familiar object F and a novel object N placed in the opposite corner. The time spent exploring the two object A and B were manually scored for 5 minutes. Arenas and objects were cleaned with 70% ethanol solution between each test. Each mouse was placed in the middle of the box facing the wall and was allowed to freely explore the apparatus and the objects [14]. Results of this test was expressed a discrimination index (DI) between objects during the test session, calculated as the

difference between the time spent exploring the novel object (N) and the familiar object (F) divided by the total time exploring both objects (DI=(N-F)/(N+F). A discrimination index of 50% corresponds to chance level and a significantly higher discrimination index reflect good recognition memory.

Statistical analysis. All values were expressed as mean ± SEM. The data for TST, FST, BWA, OFT and NORT among different treated groups were analyzed using one-way ANOVA followed by Bonferroni post hoc test using SPSS version 23.0. A significant level of $p < 0.05$ was considered significant.

RESULTS

Phytochemical constituents present in methanol extract of *Caralluma dalzielli.* The phytochemical constituents of the MCD include glycosides, carbohydrates, saponins, flavonoids, tannins and alkaloids (Table 1).

Median lethal dose (LD_{50}) for methanol extract of *Caralluma dalzielli.* The LD_{50} was estimated to be ≥2000 mg/kg orally in mice. There was no significant increase in the body weights of mice treated with the extract. Symptoms of coma and death were reported when the extract was given at 5000 mg/kg (Table 1).

Effect of methanol extract of *Caralluma dalzielli* on motor coordination deficit. The methanol extract of *Caralluma dalzielli* does not impair motor coordination deficit at all tested oral doses. The standard drug, diazepam, significantly impaired motor coordination ($p < 0.001$) at the dose of 10 mg/kg orally (Figure 1).

Effect of methanol extract of the plant *Caralluma dalzielli* on the TST. The methanol extract of the plant *Caralluma dalzielli* 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, imipramine (15 mg/kg), also significantly ($p < 0.001$) decreased the duration immobility time (Figure 2).

Effect of methanol extract of *Caralluma dalzielli* on the FST. The methanol extract of *Caralluma dalzielli* significantly ($p < 0.001$) decreased the duration immobility in the treated mice when compared to the distilled water (10 ml/kg) group. Similarly, the standard drug, imipramine (15 mg/kg), also significantly ($p < 0.001$) decreased the duration immobility time (Figure 3). So also, the methanol extract of *Caralluma dalzielli* significantly ($p \leq 0.001$) decreased swimming time as well as increased the climbing time of mice when compared to the distilled water (10 ml/kg) group. On the other hand, the standard drug imipramine (15 mg/kg) significantly ($p \leq 0.001$) increased climbing time without modification of swimming time (Figure 4).

Effect of methanol extract of *Caralluma dalzielli* on the Open Field Test. In the open field test, the distilled water treated animals exhibited ambulatory activities marked by the number of line crossing (108.75 ± 8.39). The methanol extract of *Caralluma dalzielli* significantly ($p < 0.05$) decreased the number of line crossing activity at dose of 125 mg/kg. So also, the inhibitory effect of diazepam on the number of line crossing activity was significant ($p < 0.01$) (Figure 5).

Effect of methanol extract of *Caralluma dalzielli* on cognitive behaviour of mice in the NORT. The methanol extract of *Caralluma dalzielli* (125, 250 and 500 mg/kg) did not significantly alter exploration time of mice in the novel object recognition task. Conversely, imipramine (15 mg/kg) insignificantly decreased the exploration time in the NORT (Figure 6).

DISCUSSION

Caralluma dalzielli has been used in the management of depression in traditional medicine; this study therefore provides pharmacological rationale for its use locally. The methanol extract of *Caralluma dalzielli* was studied and shown to possess antidepressant activity. Extract was found to be relatively safe at dose of 2000 mg/kg.

Tail suspension test (TST) is a model used over the years to screen for antidepressant properties, where immobility is assessed from suspending rodents by their tail for a period of time [15]. Acute administration of most antidepressants were found to decrease duration of immobility in TST [16] which is a reflection of a behavioural despair resembling a depressive state in humans [17]. Thus, the ability of MCD to reduce the duration immobility is an indication of its antidepressant properties.

Table 1: Phytochemical Constituents Present in the Methanol Extract of the Plant *Caralluma dalzielli*

S/No	Phytoconstituents	Inference
1	Alkaloids	+
2	Flavonoids	+
3	Tannins	+
4	Saponin glycoside	+
5	Cardiac glycoside	+
7	Anthraquinones	-

+= present, -=absent

Table 2: LD_{50} Values for Methanol Extract of the Plant *Caralluma dalzielli*

S/n	LD_{50} values	Onset of toxicity	Duration of toxicity	Mean body Weight (g)		Signs and symptoms of toxicity
				Day 0	Day 14	
1	≥5000	Immediately	≤24 hours	18.6±2.1	0	Coma, Death
2	≥2000	Nil	Nil	19.33±2.9	17.67±2.96	Nil

n=5.

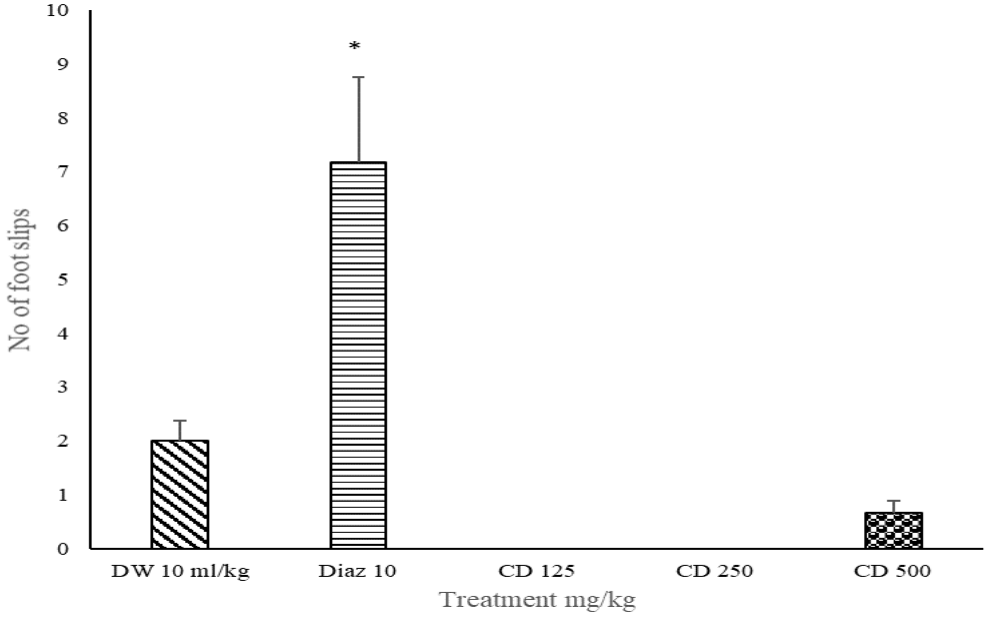

Figure 1: Effect of Methanol Extract of *Caralluma dalzielli* on Motor Coordination in Mice Beam Walking Assay Each column represents the mean ± S.E.M. of 6 animals. Data analysis was performed using One-way ANOVA followed by Bonferroni post hoc test, **P*≤ 0.001, significantly different from distilled water treated animals. CD= *Caralluma dalzielli*, DW= Distilled water, Diaz= Diazepam

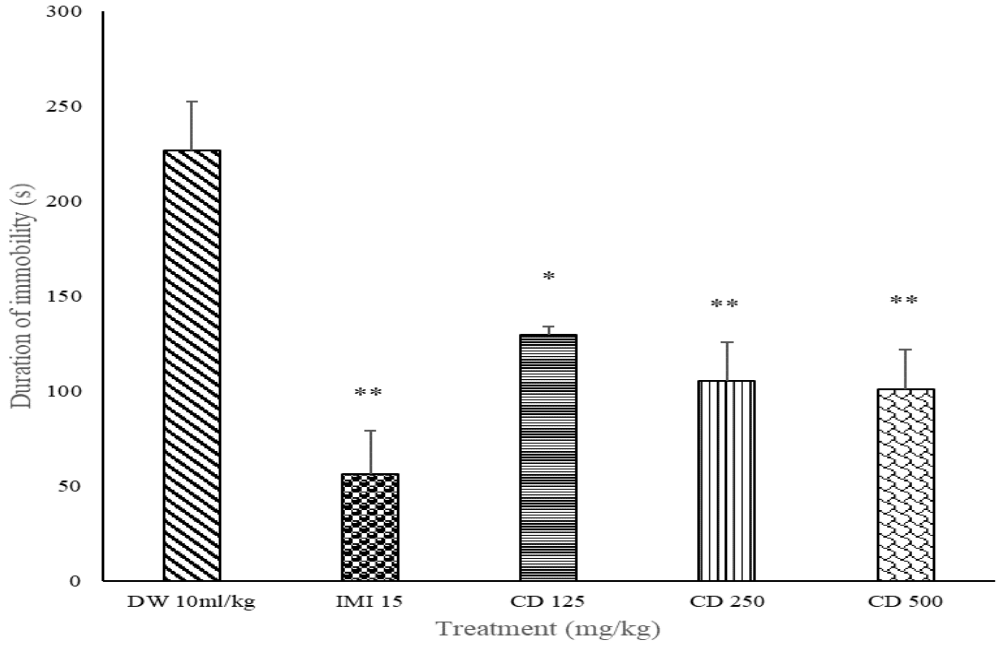

Figure 2: Effect of Methanol Extract of *Caralluma dalzielli* on Duration Immobility of Mice in Tail Suspension Test

Animals were acutely treated with CD (125, 250 or 500 mg/kg, *po*), distilled water (10 ml/kg, *po*), or imipramine (15 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, *$p \leq 0.05$, **$p \leq 0.001$ significantly different from distilled water treated group. CD= *Caralluma dalzielli*, DW= Distilled water, IMI= Imipramine

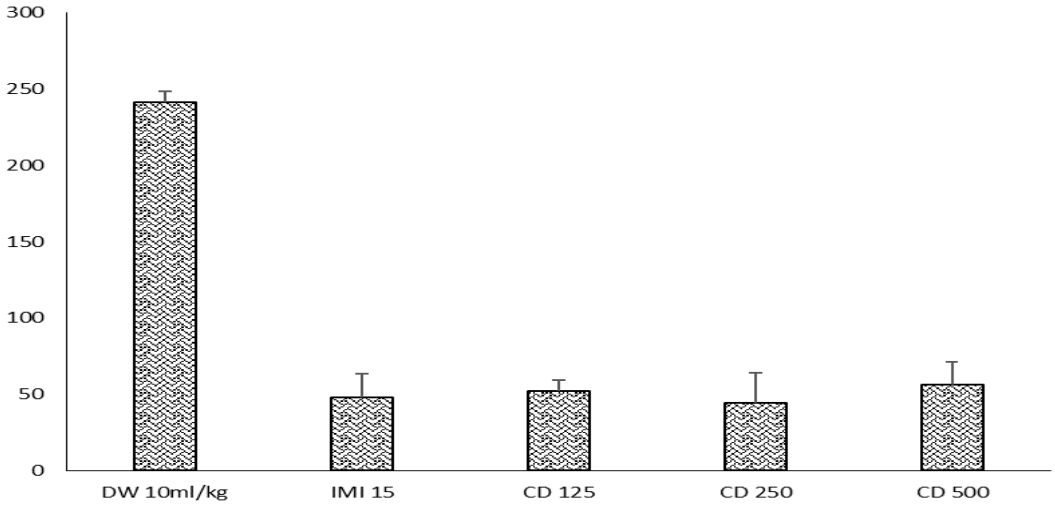

Figure 3: Effect of Methanol Extract of *Caralluma dalzielli* on Duration Immobility of Mice in Forced Swim Test

Animals were acutely treated with CD (125, 250 or 500 mg/kg, *po*), distilled water (10 ml/kg, *po*), or imipramine (15 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, *$p \leq 0.001$ significantly different from distilled water treated group. CD= *Caralluma dalzielli*, DW= Distilled water, IMI= Imipramine

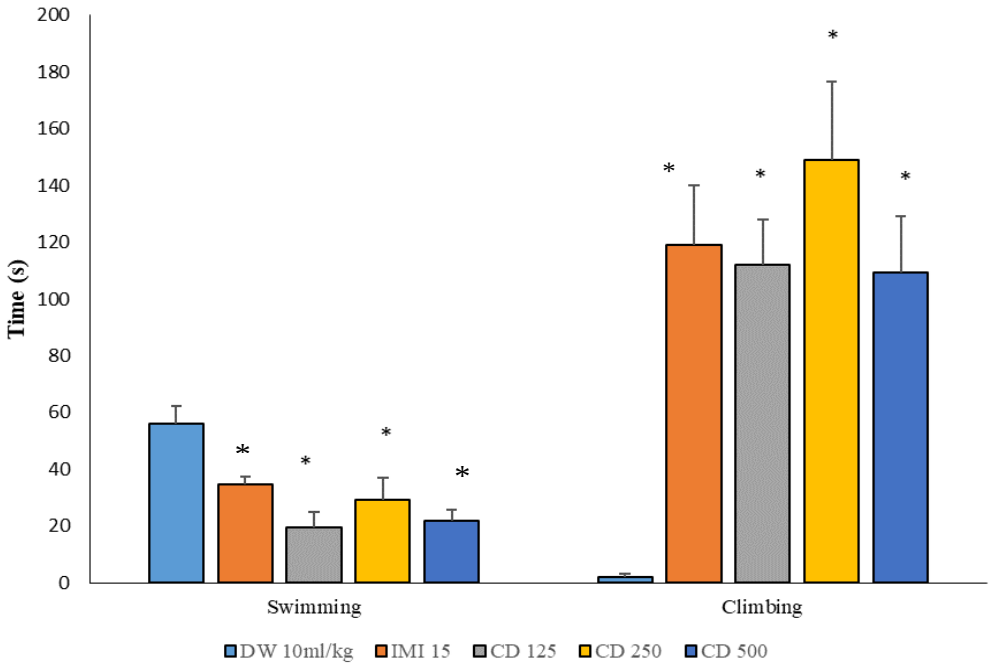

Figure 4: Effect of Methanol Extract of *Caralluma dalzielli* on Swimming and Climbing Time on FST in Mice Animals were acutely treated with *Caralluma dalzielli* (CD 125, 250 or 500 mg/kg, *po*), distilled water (10 ml/kg), or imipramine (15 mg/kg, *po*). Each column represents the mean ± S.E.M. of 8 animals. Data was analysed using one-way ANOVA followed by Bonferroni post hoc test, **P* <0.001 significantly different from distilled water treated group.

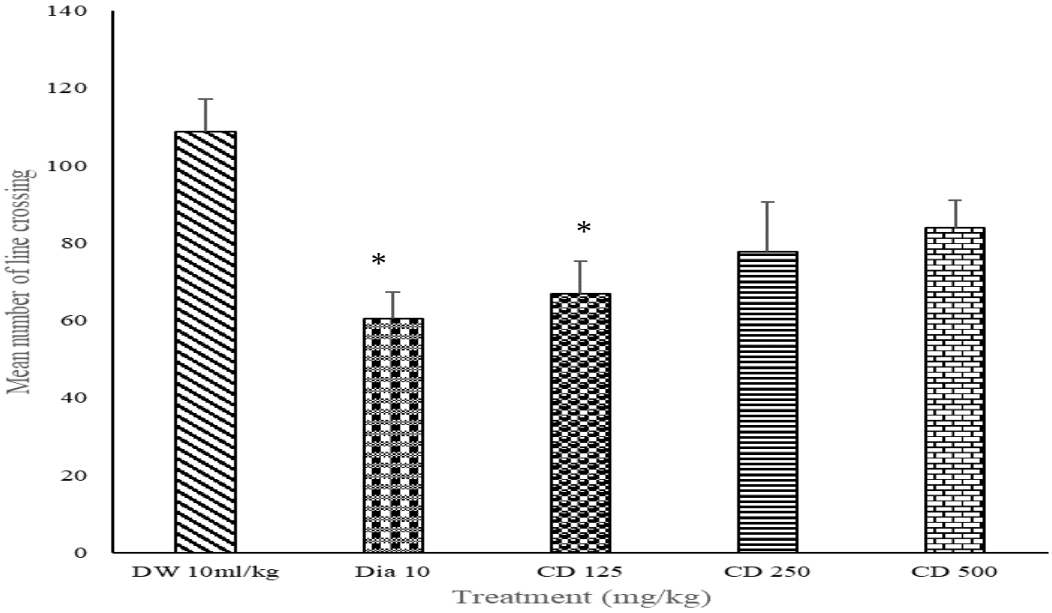

Figure 5: Effect of Methanol Extract of *Caralluma dalzielli* on Line Crosses Activity of Mice in the Open Field Test Animals were acutely treated with CD (125, 250, or 500 mg/kg, *po*), distilled water (10 ml/kg, *po*), or diazepam (10 mg/kg, *po*). Each column represents the mean ± SEM of 8 animals. Data analysis was performed using One-way ANOVA followed by Bonferroni post hoc test, **p*≤ 0.01, significantly different from distilled water treated animals. CD= *Caralluma dalzielli*, DW= Distilled water, Dia= Diazepam

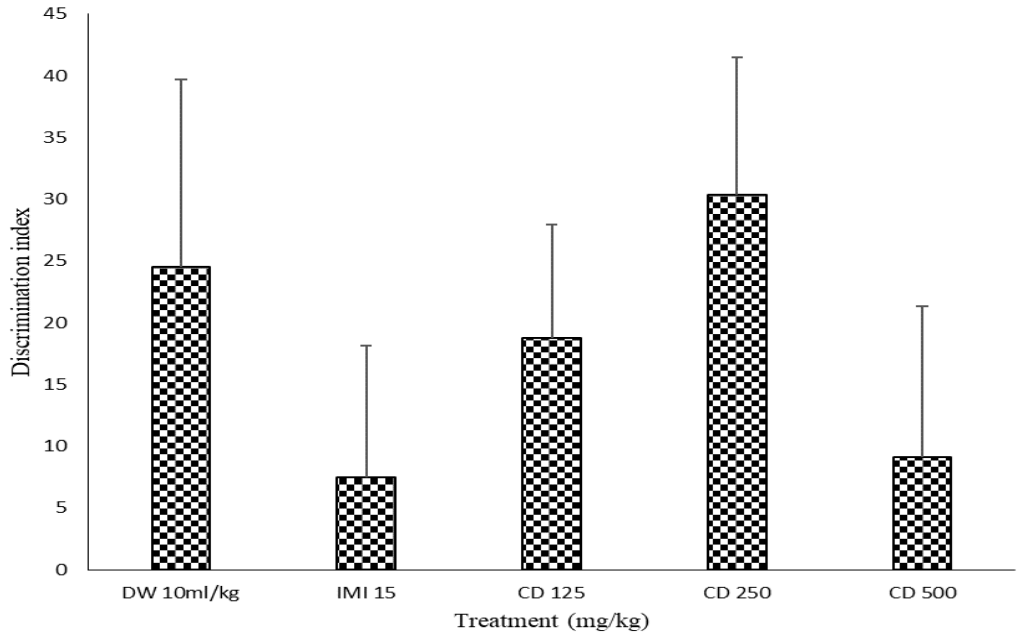

Figure 6: Effect of Methanol Extract of *Caralluma dalzielli* on Discrimination Index in the Novel Object Recognition Task in Mice
Data expressed as mean±SEM (*n*=8) and were analysed by one-way ANOVA followed by Bonferroni post hoc test. DW= Distilled water (10 ml/kg, *po*); IMI= Imipramine (15 mg/kg, *po*); CD= methanol extract of *Caralluma dalzielli* (125, 250 and 500 mg/kg, *po*)

Forced swim test is also a model used to screen for antidepressant activity of compounds [18]. Behaviour of mice in the FST is divided in to swimming and climbing, each of which correlates with a particular neuromodulator activity. One or both behaviours could be enhanced by different classes of antidepressant agents. Tricyclic agents like imipramine selectively inhibits noradrenaline uptake to enhance climbing behaviour whereas the selective serotonin reuptake inhibitors like fluoxetine enhances swimming but not climbing behaviour. In addition, dopaminergic pathways were also recently shown to be activated in climbing behaviour. The extract markedly modified climbing but not swimming behaviour, just like imipramine, which also enhanced climbing time. Thus, the antidepressant activity of MECD may be as a result of activation of dopaminergic pathways.

Psychostimulants such as amphetamine and methylphenidate known to stimulate the CNS and increase locomotion also decreases immobility in the TST [19] thereby producing false positive result. It is in view of this that the extract MCD was screened for its effect on locomotion. The extract MCD showed effect similar to imipramine in TST and had inhibitory effect on locomotion at the highest dose of 125 mg/kg like diazepam. There was no significant effect on motor coordination from the beam walking assay test, indicating that the extract of MCD is devoid of impairment of motor coordination.

There exists a connection between behaviour and novelty, which makes perception of rodents affected by a novel stimulus [20]. This assumption made novel tests useful in determining effects of various pharmacological therapies as well as brain alterations [21]. The three tested doses of MCD did not show any effect on exploratory behaviour as well as on the discrimination index of mice in this study. This is an

indication that the extract neither increase nor decrease the cognitive behaviour of mice at the tested doses.

Phytoconstituents like alkaloids, flavonoids, steroids, phenolics, terpenoids, saponins were reported to have neuroprotective potentials. They are able to protect the central nervous system against neuronal injury due to neuropsychiatric and neurodegenerative disorders such as Parkinson's disease, Alzheimer's disease, depression, psychosis, anxiety, epilepsy and cerebrovascular impairment [22]. It is plausible to suggest that the antidepressant activity of MCD is due to the presence of some secondary metabolites like saponins and terpenoids which were previously reported to possess antidepressant activity [23].

Conclusion. The methanol extract of the plant *Caralluma dalzielli* possesses antidepressant activity.

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