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Antipsychotic and sedative effects of residual aqueous fraction of ethanol root bark extract of *Carissa edulis* (Vahl) in mice

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Abstract
Carissa edulis (Vahl) (Family Apocynaceae) is used traditionally for the treatment of headache, gonorrhea, syphilis, rheumatism, epilepsy and mental disorders. This study investigated the antipsychotic and sedative effects of the residual aqueous fraction of ethanolic root bark extract of *Carissa edulis* using mice models: apomorphine-induced stereotypic climbing behaviour, amphetamine-induced hyperlocomotion in open field, walking beam assay for motor coordination deficit, hole board test for exploratory behavior and diazepam-induced sleep. The residual aqueous fraction (RAF) at 150, 300 and 600 mg/kg, produced significant dose dependent decrease ($p<0.05$) in stereotypic climbing behaviour induced by apomorphine; while at doses of 300 and 600 mg/kg, it produced a significant ($p<0.05$) reduction in locomotor activity induced by amphetamine. In addition, it exhibited (150, 300 and 600 mg/kg) significant dose dependent increase ($p<0.05$) in the time taken to cross the beam. Similarly, at doses of 300 and 600 mg/kg significantly ($p<0.05$) potentiated the duration of sleep, but there was no significant difference in the number of head dips in the hole board experiment. Therefore, the results of this study suggest that the residual aqueous fraction of *Carissa edulis* contains bioactive principles with antipsychotic and sedative property. Thus, justify the traditional use of the plant in mental illness.

Keywords: *Carissa edulis*; Antipsychotic; Sedative; Apomorphine; Amphetamine

INTRODUCTION

Schizophrenia is a chronic disabling psychiatric disorder affecting 1% of the population worldwide [1]. It is characterized by hallucinations, delusions, affective flattening, alogia, avolition, anhedonia and cognitive deficit [2]. Majority of people in the developing countries rely on traditional healing practices and medicinal plants for the treatment of their ailments [3]. Recently, interests have geared toward developing therapeutic agents from plant sources for their safety, cost and therapeutic effectiveness [4]. Hence, there is need to source for other alternatives to control the severity and progression of psychiatric symptoms and some adverse effects of the conventional antipsychotic drugs [1].

Carissa edulis Vahl belongs to the family Apocynaceae; a native of South Africa; commonly called *Cizaki* in Hausa; *Kanboro* in Fulfulde; *Emir* in Arabic; *Muyunzo* in

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Luganda, *Endelkoring-neominoem* in Africana; *Agam* in Amharic and *Mlanoa-mboo* in Swahili [5]. The plant has various ethnomedical uses such as treatment of fever, sickle cell anemia and hernia, treatment of edema, toothache, cough, ulcer, worm infestation; management of epilepsy, mental illness and cancer. The plant extracts have been previously shown to possess antidiuretic effect [6]; analgesic and antimicrobial activity [7,8]; hypoglycemic [9], antiviral [10], anticancer [11] effects; and anticonvulsant, anxiolytic, and sedative activities [12-15]. The present research studied the antipsychotic and sedative properties of the residual aqueous fraction obtained from the ethanol root bark extract.

EXPERIMENTAL

Plant material. The root bark of *C. edulis* was collected by a herbalist in July 2010, at Basawa Village, Zaria, Nigeria. The plant sample was identified and authenticated by Malam Umar Gallah, a botanist in the Herbarium section of the Department of Biological Sciences, Ahmadu Bello University, Zaria. This was made by comparing the sample with the already deposited specimen (voucher number 601). Large quantity of the root bark part was air-dried at room temperature under shade and closely monitored until a constant weight was obtained. The dried samples were size reduced with wooden mortar and pestle.

Extraction and fractionation of the crude extract. The powdered sample (500 g) was cold-macerated with 2 L of 70%$^{v}/_{v}$ ethanol in water and the filtrate was concentrated to dryness using water bath regulated at low temperature (40^{o}C). The dried extract was brownish in appearance with a pleasant smell. The crude extract was dissolved in distilled water and defatted with petroleum ether to obtain petroleum ether fraction and aqueous portion. The aqueous portion was partitioned with ethyl acetate and thus obtained its fraction and aqueous portion. Similarly, the aqueous portion was subsequently partitioned with N-butanol to obtain N-butanol fraction and residual aqueous fraction (RAF).

Animals. One hundred and forty four Swiss Albino mice (18–24 g) were obtained from Animal House Facility, Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria. They were maintained under good laboratory care and fed with standard animal feeds (Feeds Masters, Ilorin, Nigeria), and were allowed access to uninterrupted source of water. The animals were used in compliance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (Publication nos. 85-23, revised 1985). The institutional approval number for the protocol was given as DAC/IW-OT/003-10.

Apomorphine-induced stereotypic behaviour in mice. The effect of the residual aqueous fraction of the ethanol root bark extract of *C. edulis* was investigated as described by [16]. Thirty Swiss albino mice of either sex were randomly divided into five groups each containing six mice. The first group received distilled water (10 ml/kg). The second, third and fourth groups received the RAF at doses of 600, 300 and 150 mg/kg, respectively, while the fifth group received 1 mg/kg of haloperidol. All the administrations were via intraperitoneal route (*i.p.*). Thirty minutes post administration, mice in all the groups were administered apomorphine (1 mg/kg, s.c.) and each mouse was placed singly in a wire mesh cage (about 18 cm in diameter with wall of vertical bars, 2 mm in diameter, 1 cm apart surmounted by a smooth surface). The climbing behavior of each mouse was observed at ten-minute intervals after apomorphine administration and scored as follows:

0 = four paws on the floor
1 = fore feet holding the vertical bars
2 = four feet holding the vertical bars

Amphetamine-induced hyperlocomotion in open field. The experiment was performed by employing the procedure described by [17]. The open field board was a wooden board (50 × 50 cm) with 25 squares (50 × 5 cm). Swiss albino mice of both sex were randomly divided into five groups each containing six mice. The first and fifth groups received distilled water (10 ml/kg, i.p.) and haloperidol (1 mg/kg, i.p.), each respectively. The second, third and fourth groups received graded doses of RAF at 600, 300 and 150 mg/kg, via *i.p.* route, respectively. Amphetamine (1.5 mg/kg, *i.p.*) was administered 30 minutes post-treatment. Fifteen minutes later, each mouse was placed singly at a corner of the open field board. The number of lines crossed was counted with the aid of a tally counter during a 5 minutes period.

Beam walking test for motor coordination deficits in mice. The study was conducted according to the method described by [18]. Mice were trained to travel from a start platform along a ruler (80 cm long, 3 cm wide) elevated 30 cm above the bench by metal supports, to a goal box. Trials were performed for each mouse, and were designed such that the mice tested would be aware that there was a goal box that could be reached. Mice of either sex were divided into five groups of six mice each. Group 1 received distilled water (10 ml/kg, *i.p.*) and served as control, while Groups 2, 3 and 4 received 600, 300 and 150 mg/kg *i.p*. doses of RAF, respectively. Group 5 received diazepam at a dose of 0.5 mg/kg. *i.p.* Thirty minutes later, each mouse was placed at one end of wooden beam (8 mm in diameter, 60 cm long and elevated 30 cm above the bench by metal supports), and allowed to walk to the box within a maximum of 60 s. The time taken on the beam, number of falls and the number of foot slips were counted and recorded.

Exploratory behavior in mice. This study was done using the head-dip test on the hole-board [19]. It was carried out using wooden board (40 × 40 cm) with four equidistant holes (1 cm diameter, 2 cm depth). Mice of either sex were divided into five groups of six mice each. Animals in Group 1 received normal saline (10 ml/kg, *i.p.*) and served as control, while those in Groups 2, 3 and 4 received RAF at doses of 600, 300 and 150 mg/kg *i.p.* respectively. Mice in Group 5 received diazepam (0.5 mg/kg, *i.p.*). Thirty minutes after treatment, each mouse was placed at one corner of the board and allowed to move about and dipped its head into the holes indicating exploratory behaviour. The number of times the mice dipped their heads into the holes during the 5-minutes period was counted and recorded.

Diazepam-induced sleeping time in mice. Mice of either sex were divided into four groups of six each. Group 1 received normal saline (10 ml/kg) and served as control, while those in Groups 2, 3 and 4 received the RAF at doses of 600, 300 and 150 mg/kg (*i.p.*) respectively. Thirty minutes post-treatment, all the animals were administered diazepam (30 mg/kg, *i.p.*). Each mouse was observed for the onset and duration of sleep, with the criterion of sleep being loss of righting reflex [20,21]. The time from the loss of righting reflex to recovery was recorded as sleeping time [22].

Statistical analysis. Results were presented in tables and charts; and expressed as Mean ± SEM. The level of significance between means was tested by one-way ANOVA followed by post hoc test and results were regarded as statistically significant from $p \leq 0.05$.

RESULTS

The residual aqueous fraction (RAF) of ethanol root bark extract of *C. edulis* (150, 300 and 600 mg/kg) and haloperidol (1 mg/kg) showed dose dependent decrease in stereotypic climbing behaviour induced by apomorphine; the decrease was only significant ($p<0.05$) with haloperidol and the

highest dose of RAF. In addition, RAF at doses of 300 and 600 mg/kg, and haloperidol at a dose of 1 mg/kg, produced a significant ($p<0.05$) reduction in locomotor activity induced by amphetamine when compared to the control group. The reduction produced by RAF was in dose dependent manner.

In beam walking assay for motor coordination deficit, diazepam (0.5 mg/kg) and RAF (150, 300 and 600 mg/kg), exhibited significant dose dependent increase ($p<0.05$) in the time taken to cross the beam when compared to the control group. However, there was no significant difference in the number of hind limb slips as compared with the control group.

For the hole-board test for exploratory behaviour, RAF did not show significant difference in mean number of head dips as compared to the control group. However, diazepam used as standard control produced significant ($p<0.05$) reduction in the number of head dips when similarly compared to the control group. There was no significant decrease in the onset of sleep at all the RAF-treated groups (150, 300 and 600 mg/kg) when compared to the control group. However, there was significant ($p<0.05$) and dose dependent increase in the duration of sleep (300 and 600 mg/kg) as compared to the control group.

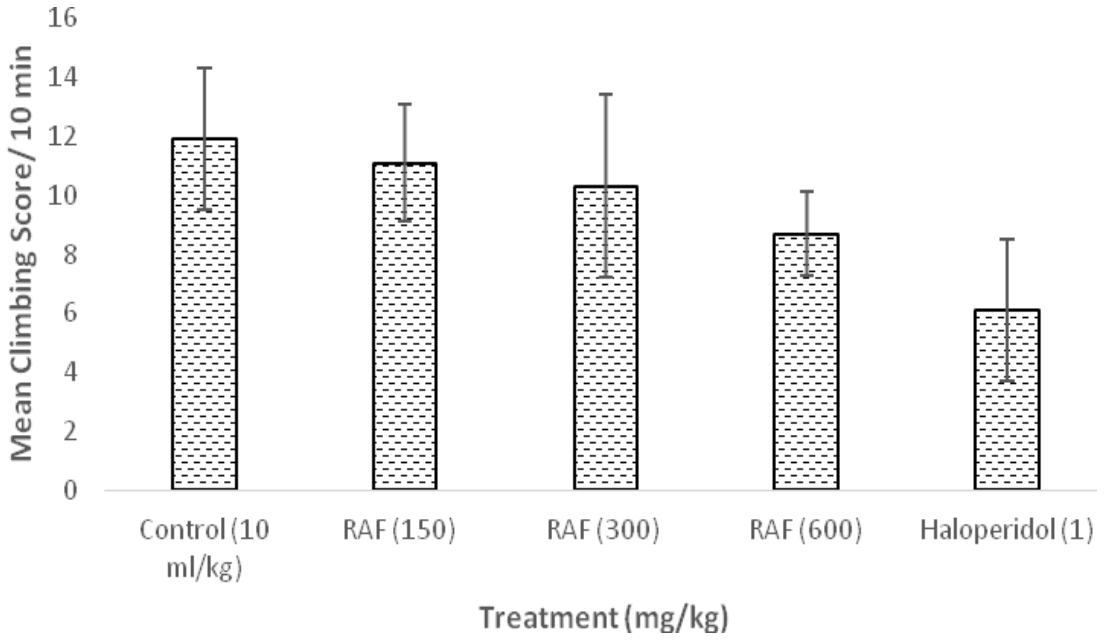

Figure 1: Effect of Residual Aqueous Fraction (RAF) of *C. Edulis* on apomorphine-induced stereotypic climbing behaviour in mice

Values are presented as Mean ± SEM, n = 6 mice per group, RAF = Residual aqueous fraction of ethanol root bark extract of *C. edulis*, Control = Distilled water, Significant difference at $^{*}p<0.05$ (ANOVA) followed by post hoc test.

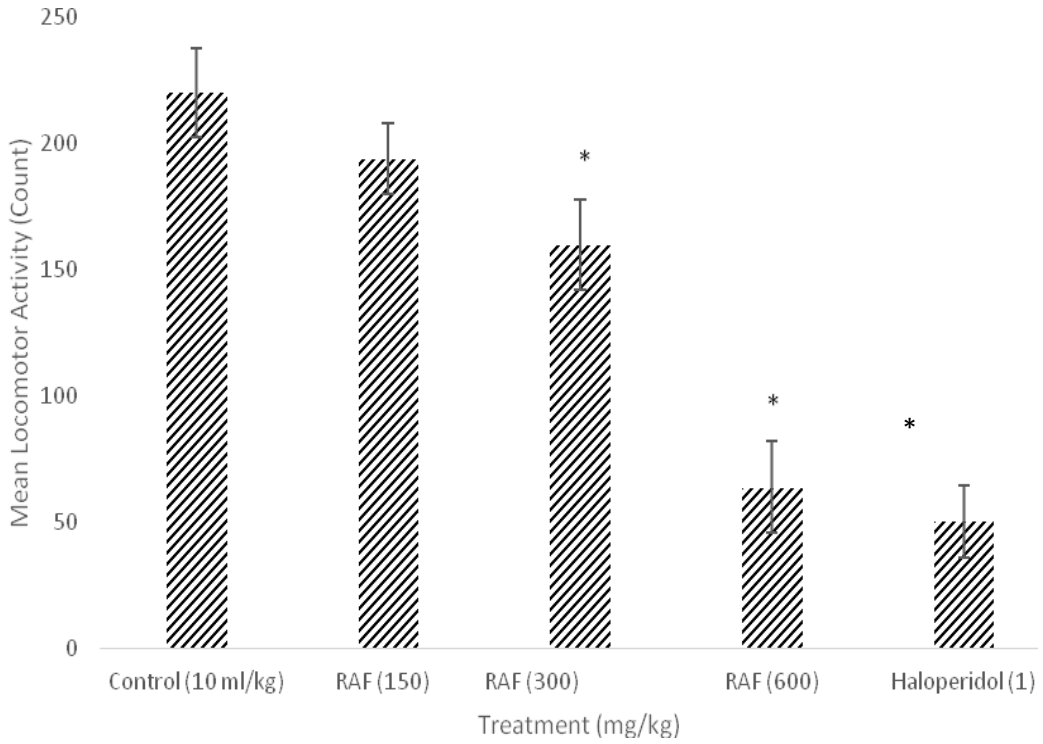

Figure 2: Effect of Residual Aqueous Fraction (RAF) of *C. edulis* on amphetamine-induced hyperlocomotion in open field in mice

Values are presented as Mean ± SEM, n = 6 mice per group, RAF = Residual aqueous fraction of ethanol root bark extract of *C. edulis*, Control = Distilled water, Significant difference at $^{*}p<0.05$ (ANOVA) followed by post hoc test.

Table 1: Effect of Residual Aqueous Fraction (RAF) of *C. edulis* and diazepam (DZ) on motor coordination deficit in mice

Treatment (mg/kg)	Number of Hind Limb Slips	Time Taken for the Task (s)	Number of Falls
Control	1.4 ± 0.24	8.0± 0.63	0.0
RAF (150)	2.0± 0.37	17.1± 1.87*	0.0
RAF (300)	2.6 ± 0.24	19.3 ± 4.00*	0.0
RAF (600)	2.0 ± 0.54	23.7 ± 4.49*	0.0
DZ (1)	4.6 ± 0.40	20.6 ± 2.18*	0.0

Values are presented as Mean ± SEM, n = 6 mice per group, RAF = Residual aqueous fraction of ethanol root bark extract of *C. edulis*, DZ = Diazepam, Control = Distilled water (10 ml/kg), Significant difference at $^{*}p<0.05$ (ANOVA) followed by post hoc test.

Table 2: Effect of Residual Aqueous Fraction (RAF) of *C. edulis* and diazepam (DZ) on exploratory behaviour in mice

Treatment (mg/kg)	Mean Number of Head Dips/5 minutes
Control (10 ml/kg)	13.0 ± 0.97
RAF(150)	15.0 ± 1.77
RAF (300)	11.2 ± 1.70
RAF(600)	9.67 ± 2.86
DZ 0.5	8.67 ± 0.88*

Values are presented as Mean ± SEM, n = 6 per group, RAF = Residual aqueous fraction of ethanol root bark extract of *C. edulis*, DZ = Diazepam, Control = Distilled water (10 ml/kg), Significant difference at $^{*}p<0.05$ (ANOVA) followed by post hoc test.

Table 3: Effect of Residual Aqueous Fraction (RAF) of *C. edulis* on diazepam-induced sleep in mice

Treatment(mg/kg)	Mean onset of sleep (min)	Mean duration of sleep (min)
N/saline (10 ml/kg)	3.4 ± 0.87	108.0 ± 4.30
RAF(150)	3.0 ± 0.55	128.2 ± 11.04
RAF (300)	2.55 ± 0.50	148.8 ± 22.83*
RAF (600)	2.8 ± 0.58	230.8 ± 3.78*

Values are presented as Mean ± SEM, n = 6 per group, RAF = Residual aqueous fraction of ethanol root bark extract of *C. edulis*, DZ = Diazepam, Control = Distilled water (10 ml/kg), Significant difference at $^{*}p<0.05$ (ANOVA) followed by post hoc test).

DISCUSSION

Dopamine and dopaminergic mechanisms are central to psychosis and have been one of the most enduring ideas about the illness [23]. Dopamine (D_2) receptor blockade in the brain is a general pharmacodynamic property of all typical antipsychotics [24]. The model of apomorphine-induced climbing behaviour detects motor deficits associated with Parkinson-like side effect of these drugs, thus, the deficits represent toxicities of antipsychotic drugs and an indicator of D_2 receptor blockade [25]. The ability of a drug to antagonize apomorphine-induced climbing behaviour in the mouse has been correlated to anti-dopaminergic actions on the limbic system [26]. The observed reduction in the climbing episodes as demonstrated by the RAF suggests its antidopaminergic action and possesses antipsychotic property related to haloperidol.

RAF significantly decreased the hyperlocomotion induced by amphetamine. Amphetamine inhibits intracellular monoamine oxidase responsible for metabolism of dopamine [27]. Administration of amphetamine and methamphetamine produces hyperlocomotion and increased grooming in experimental animals; these psychiatric-like behaviours depend on dopaminergic system [28]. Changes such as hyperactivity, and increased locomotor, are stereotypic to clinical image of psychosis [29]. Similarly, the hyperlocomotion as a result of hyperdopaminergic condition in the mesolimbic system may be correlated with positive symptoms in schizophrenia [30], and the inhibition is related to the D_2 receptor blocking action of antipsychotics [31]. Therefore, the ability of RAF to decrease the observed hyperactivity suggests its antidopaminergic activity and thus, indicates its antipsychotic potential.

Benzodiazepine-induced ataxia serves as an indicator of motor coordination deficit caused by functional depression to motor cortex [18]. RAF produced a significant delay in the time taken by the mice to cross the beam, but it did not cause significant increase in the number of foot slips. This suggested mild damage to the motor cortex by RAF, and also indicated centrally mediated neuromuscular blockade [32]. Therefore, RAF could be said to possess benzodiazepine-like property.

The doses of RAF used to test for exploratory behaviour did not produce significant change in the number of head dips. This test was to evaluate sedative and anxiety condition in animals [33]. Increase and decrease in the number of head dips indicate sedative and anxiolytic behaviours, respectively [34,35].Thus, RAF might not possess either of the two indices at the tested doses.

Hypnotic effect is an important index for depression of central nervous system [36]; and $GABA_A$ receptor in the CNS is known to favour sleep [37]. Benzodiazepines and barbiturates are examples of widely used therapeutic agents that act as positive allosteric modulators at $GABA_A$ receptors [38]. It could therefore be said that RAF potentiated sleep induced by benzodiazepine and thus, may partly possess GABA-mediated property.

Conclusion. This study, therefore, suggests that the residual aqueous fraction of *Carissa edulis* possesses antipsychotic and sedative properties and thus, provides scientific rationale for the traditional use of the plant in mental and neurological illnesses.

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