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Central Nervous System Depressant Properties of *Treculia africana* Decne

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Abstract

The study was carried out to investigate the central nervous system activity of *Treculia africana*. The central nervous system depressant properties of *Treculia africana* were determined using: Novelty –Induced Rearing and Grooming, Locomotor activity, Ketamine-induced sleeping time and effect on rectal body temperature. The crude extract produced decrease in rearing, grooming and locomotor activity. It also potentiated ketamine-induced sleeping time and produced hypothermic effect in mice. The crude extract possessed sedative effect, which may be through increase in the activity of GABA in the brain.

Key words: *Treculia africana*, Ketamine-induced sleeping time, Hypothermia, Central depressant effect.

Introduction

Treculia africana Decne is native to many parts of West and Tropical Africa. It is a breadfruit species. The bread fruit is of the family Moraceae and is one of the four members of the genera *Treculia*. It grows commonly in evergreen and deciduous forests, often by streams but may sometimes be planted as in Nigeria where it is common in the Western and Eastern states (Hatchinson, 1973). It is one of the most cherished economic plants that have both food and medicinal value.

The crude extract from different parts of the plant has been used in the folk medicine in the treatment of various ailments. It is used either singly or in combination with other herbs in the traditional herbal preparation by different communities to treat various diseases. Decoction from different parts of the plant is used as an anti-inflammatory agent and in the treatment of whooping cough. The crushed leaves juice is applied on the tongue as a treatment for thrush in children; the latex is applied as an antibacterial agent in eardrops and as chewing stick. The sap of the male tree is applied locally on cotton wool to carious tooth for its removal. The root, immature leaves and bark are part of the concoction used locally for treating cough, constipation, edema and rheumatism. A decoction of the root has been used in Nigeria as a vermifuge and also in Ghana as a tonic after illness by villagers. The pulps of *Treculia africana* have been shown to be useful in the treatment of ascaris and guinea worm (Ogunleye and Parakoyi, 1992). Proximate chemical composition of the fruit and seed showed that it contains high level of carbohydrate and protein but is relatively low in fat, ash and fibre (Osabor *et al.*, 2009). *Treculia africana* leaves decoctions were reportedly used in Trinidad and Bahamas to lower blood pressure. The water soluble and ethylacetate fractions have been shown to reduce the fasting blood sugar levels (Oyelola *et al.*,

2007). Three compounds were isolated from *Treculia africana* and they are identified as Phyllocoumarin; Catechin and 6,9 – dihydroxy-megastigmane -3 –one with antibacterial and antifungal properties (Ogbonnia *et al*., 2008). The plant was claimed to be useful in the treatment of mental illness by local herbalists, this led to the present investigation.

Material and Methods

Plant Materials

Treculia africana stem barks were collected from the campus of the Obafemi Awolowo University (OAU) in March, 2009. It was scientifically identified at the Department of Pharmacognosy, Faculty of Pharmacy, and the Department of Botany, OAU, Ile- Ife, Nigeria. It was later deposited at the Herbarium, Department of Botany, OAU where it was assigned Herbarium Specimen No. UHI 4225A.

Preparation of Plant Materials

The plant was air dried for two weeks at room temperature. The dried stem bark was pulverized and 200g of the powder was extracted with 0.6 liters of 70% ethanol for 48 hr. The marc was re-extracted twice and the combined extract was concentrated *in vacuo* at a temperature of 40^{o}C to yield 15 g crude extract. The crude extract was prepared by dissolution in normal saline.

Animals

The animals used for this experiment were mice of both sexes. All the animals were bred and housed in well lit and aerated room in the Animal House, College of Medicine, Niger Delta University, Amassoma. They were maintained under natural daylight/night condition. All animals had free access to drinking water and standard commercial diet (Guinea feeds brand, Bendel Feeds Nigeria). All experiment was carried out in accordance with NIH guide for the care and use of laboratory animals.

Drugs

Ketamine (Rotexmedica, Germany), Diazepam (Roche, Basel, Switzerland).

Methodology

Toxicity Test

The method described by Lorke (1983) was used to determine the LD_{50}, which is the index of acute toxicity. Albino mice (20-25 g) of either sex were used. This method involved an initial dose finding procedure, in which the animals were divided into three groups of three animals per group. Doses of 10,100 and 1000 mg/kg were administered intraperitonealy (i.p.), one dose for each group. The treated animals were monitored for 24 h mortality and general behaviour. From the results of the above step, four different doses of (140, 225, 370 and 600 mg/kg) were chosen and administered i.p. respectively to four groups of one mouse per group. The treated animals were monitored for 24 h. The LD_{50} was then calculated as the geometric mean of the lowest dose showing death and the highest dose showing no death.

BEHAVIOURAL ASSAYS

Novelty-Induced Rearing and Grooming

The behavioural profiles of albino mice under the influence of the crude extract were assessed singly in a plexiglas cage measuring (45 cm x 25 cm x 25 cm) containing wood shavings. The animals were divided into six groups (n=5-7). Group one was given normal saline (0.2 ml/20 g, i.p.), while the remaining four groups (2-5) were given crude extract (12.5-100 mg/kg, i.p.). Thirty minutes after a single i.p. injection of extract and control, Behavioural measurements were carried by placing the animal directly from home cage into an

opaque plexiglas observation cage with only one side transparent for observation. Each animal was used only once, with the sawdust bedding changed after each assessment to remove olfactory cue from previous animal to the other. The time of the experiment was kept constant (9.00 am -1.00 pm) daily to avoid changes in biological rhythm. The behavioural component employed in this observational analysis were rearing and grooming (Ajayi and Ukponmwan, 1994: Onigbogi *et al.*, 2000). Diazepam (2 mg/kg, i.p.) group (6) served as reference drug.

The frequency of episodic rearing was quantified by using a manual counter and a stop watch. The total frequency was summed up for each animal and totaled for the 30 min of observation time. Rearing was taken as the number of times the mouse was standing on its hind limb or with its forelimbs against the wall of the observation cage or in the free air (Ajayi and Ukponmwan, 1994). Grooming was taken as the number of body cleaning with mouth and face washing with forelimbs.

Locomotor Activity

Motor activity was measured in an open field apparatus consisting a white plexiglas box (28 cm x 28 cm x 25 cm) with a painted black grid dividing the floor into 16 (7 x 7 cm) equal squares. The animals were divided into six groups (n=5-7). Group one was given normal saline (0.2 ml/20 g, i.p.), while the remaining four groups (2-5) were given crude extract (12.5-100 mg/kg, i.p.). Thirty minutes after a single i.p. injection of extract and control, the animals were placed singly in one of the corners of the box, the number of squares crossed with all four paws was counted for 5 min (Brocco *et al*., 2002). The cages were cleaned with seventy percent (70%) ethanol at intervals when the animal is removed (Haque *et al*., 2001). Diazepam (2 mg/kg, i.p.) group (6) served as reference drug.

Ketamine - Induced Sleeping Time

The effect of crude extract on ketamine-induced sleeping time was measured as described by Erden et al., 2001. The mice were divided into five groups (n=5-7). The first group served as control and was given normal saline (0.2ml/20 g, i.p.) group (1), while groups (2-4) were given different doses of the extract (50-200 mg/kg, i.p.). This was followed 30 min later by i.p. administration of Ketamine (100 mg/kg). The sleep latency and sleeping time were recorded. The sleep latency was measured as time in minute after treatment with ketamine and the loss of righting reflex. While the time in minute between losses and regaining of righting reflex was taken as sleeping time (Erden *et al.*, 2001). Diazepam (2 mg/kg, i.p.) group (5) served as reference drug.

Monitoring of Body Temperature

The recording of the body temperature was carried out using a thermoprobe. The effect of crude extract on the body temperatures was performed in five groups of male mice (n=5-7). Groups one was given normal saline (0.2 ml/20 g, i.p.), while groups (2-4) were given different doses of the extract (50-200 mg/kg, i.p.). The probe of the thermometer was inserted 1.5 cm into the rectum. The temperature of the animals was recorded immediately before the test and 30, 60, 90, 120 and 180 min after the administration of control and extract. The pre-drug recording served as the reference point for the determination of temperature changes (Parimaladevi *et al.,* 2003). Diazepam (2 mg/kg, i.p.) group (5) served as reference drug.

Statistical Analysis

Results are expressed as mean ± S.E.M. The significance of different between groups were analysed using one way analysis of variance (ANOVA), followed by post hoc analysis using the Student- Newman-keuls test.

Results and Discussion

Acute toxicity: The LD_{50} of the crude extract of *Treculia africana* was found to be 450 mg/kg i.p.

Effect of crude extract of *Treculia africana* on novelty induced rearing and grooming

The administration of crude extract of *Treculia africana* (12.5-100 mg/kg, i.p.) reduced novelty induced rearing and grooming in mice. A significant [F (5, 25) = 47.8, P < 0.001] reduction in the frequency of rearing episodes and a significant [F (5, 25) = 55.0, P < 0.001] reduction in grooming was observed dose dependently in mice when compared to normal saline. Maximal inhibition of novelty induced rearing and grooming was observed at 100 mg/kg (Table 1).

Table 1: Effect of crude extract of *Treculia africana* on novelty induced rearing, grooming and locomotor activity in mice.

Treatment	Dose (mg/kg, i.p.)	NIR/30min	NIG/30min	LA/5min
Control	0.2ml/20g	134.0±3.8	55.6±3.8	130.8±1.9
Treculia ±7.1*	12.5	137.0±2.2	61.2±1.4	97.4
Treculia	25.0	93.0±0.5*	31.0±0.7*	75.4±8.1*
Treculia	50.0	33.0±1.6*	13.8±3.2*	55.6±6.1*
Treculia	100.0	9.0±0.5*	7.1±0.4*	47.4±4.1*
Diazepam	2.0	7.0±0.7*	18.0±3.1*	48.0±3.1*

NIR: Novelty-Induced Rearing; NIG: Novelty-Induced Groming; LA: Locomotor activity. *indicate significant difference from control. P < 0.05.

The results are expressed as mean ± S.E.M, (n = 5 – 7). One way ANOVA revealed that there is significant difference between various treatment groups. Diazepam was used as standard reference drug.

Effect of crude extract of *Treculia africana* on locomotor activity

The administration of crude extract of *Treculia africana* (12.5 – 100 mg/kg, i.p.) showed a significant reduction [F (5, 25) = 24.01, P < 0.001] in the locomotor activity of mice when compared to normal saline (Table 1).

Effect of the crude extract of *Treculia africana* on ketamine – induced sleeping time and sleep latency in mice

The administration of crude extract of *Treculia africana* (50 – 200 mg/kg, i.p.) produced a significant [F (4, 20) = 140.5, P < 0.001] and moderate dose dependent prolongation of sleeping time in mice when compared to normal saline (Table 2). The crude extract also produced a significant [F (4, 20) = 52.0, P < 0.001] reduction in sleep latency when compared to normal saline (Table 2).

Table 2: Effect of the crude extract of *Treculia africana* on ketamine – induced sleeping time and sleep latency in mice.

Treatment	Dose (mg/kg, i.p.)	Sleeping time	Sleep latency

N Saline ±2.2	0.2ml/20g	22.0±0.6	2.8
Treculia ±1.3*	50.0	25.6±1.3	1.8
Treculia ±0.9*	100.0	38.0±2.2*	1..4
Treculia ±0.8*	200.0	52.2±2.6*	1.2
Diazepam	2.0	170.4±6.7*	1.0±0.7*

The results are expressed as mean ± S.E.M, (n = 5 – 7).
*indicate significant difference from control. P < 0.05 .

One way ANOVA revealed that there is significant difference between various treatment groups. Diazepam was used as standard reference drug.

Effect of the crude extract of *Treculia africana* on rectal body temperature in mice

The administration of crude extracts of *Treculia africana* (50 – 200 mg/kg, i.p.) reduced rectal body temperature dose dependently in mice. The reduction was significant [F (4, 20) = 24.44, P < 0.001] when compared to normal saline (Table 3).

Table 3: Effect of the crude extract of *Treculia africana* on rectal body temperature in mice

Treatment	Dose (mg/kg i.p)	0 min	30 min	60 min	90 min	120 min	180 min
Saline	0.2ml/20g	38.6 ±0.20	37.8±0.10	37.6±0.05	37.5±0.20	37.5±0.30	37.2± 0.10
Treculia	50	38.0±0.19	36.7±0.10*	36.5±0.10	36.8±0.12	36.8±0.13	37.5±.0.10
Treculia	100	38.1±0.15	35.4±0.09*	35.5±0.07*	36.3±.0.10	36.4±0.10*	37.2± 0.10
Treculia	200	37.9±0.16	35.2±0.06*	35.3±0.05*	36.4±0.10*	36.5±0.10*	36.8± 0.04
Diazepam	2	38.0±0.28	35.2±0.17*	35.6±0.14*	35.9±0.11*	36.4±0.12*	36.9± 0.12

The results are expressed as mean ± S.E.M, (n = 5 – 7).
*indicate significant difference from control. P < 0.05.

One way ANOVA revealed that there is significant difference between various treatment groups. Diazepam was used as standard reference drug.

The study established the acute toxicity of the crude extract of *Treculia africana* by the determination of LD_{50}. Acute toxicity test was carried out in order to determine the dose of the crude extract that will be administered to the mice. LD_{50} is the dose at which mortality occur in 50% population of the experimental animals. The higher the value of the LD_{50} for a substance, the relatively safe the substance is assumed to be. The value obtained is not toxic to the animal.

The crude extract of *Treculia africana* was examined for novelty - induced rearing (NIR) in mice. NIR is a behaviour of rodents in novel environments. The behaviour is employed by rodents as one of the survival strategies in assessing the environment for food, protection and possibly escapes (Blanchard *et al*., 2001). Measurement of the frequency of rearing in rodents and the modification can therefore be employed in assessing the crude extracts for both sedative property and central nervous system stimulation (Vogel, 2002). Rearing has been described as the vertical locomotion activity when the animal stands on its hind leg while raising up its forearm in the air or placed on the wall of the cage (Onigbogi *et al*., 2000). Drugs that stimulate the CNS increase rearing behavior, while those that depress the CNS inhibit rearing behavior. In this study, the crude extract inhibited NIR showing that it has sedative effect.

The crude extract was examined for novelty - induced grooming (NIG) in mice. Grooming is an important behavioural component in animals and is associated with de - arousal state of the central nervous system (CNS). De - arousal indicates absence of stimulation. Drugs that have depressant effect inhibit grooming behaviour. Grooming is described in animals (rat or mice) as face or head washing with forearm or body grooming with mouth (Ukponmwan *et al*., 1985). The crude extract reduced NIG this suggests that the crude extract have depressant effect on the CNS.

The novelty - induced rearing and grooming behaviour response is regulated by multiple neurotransmitter system; such transmitters include gamma-aminobutyric acid (GABA), cholinergic, adrenergic, opioid, serotonin, glutamate and dopamine receptors (Walting, 1998). The crude extract inhibit rearing and grooming behaviours suggesting that it might be acting by blockade of dopamine, potentiation of GABA, inhibition of serotonin, inhibition of cholinergic neurotransmission and inhibition of the stimulation of the excitatory neurotransmitter in the CNS. (Jones et *al.,* 1981; Strange, 1993).

The crude extract produced a reduction in locomotor activity. This reduction in locomotor activity follows the pattern obtained for NIR and NIG. The decrease of locomotor activity further confirms the depressant activity of the crude extract. Locomotion is mediated mainly through dopaminergic pathway (Rang *et al.,* 1999) but other neurochemical pathways have been reported to modulate locomotor activities in animals. Generally, CNS depressants have inhibitory effects on locomotor activities and other exploratory or inquisitiveness of animals (Haque *et al.,* 2001). A decrease in locomotor activity in rodents is suggestive of a possible CNS – depressant activity (Cooper *et al*., 1996).

Alteration of body temperature can be interpreted as an index of alteration of various central neurotransmitters acting on the receptor in the hypothalamus. Dopamine, Acetylcholine, GABA and Opioid are some of the receptors implicated in hypothalamic effect of drug in animal (Rang *et al*., 1999). Hypothermia is an effect usually observed with benzodiazepine receptor agonist that produces this effect at relatively low doses (Jackson and Nutt, 1990), hence diazepam was used as positive control. The crude extract reduced normal rectal temperature in mice when compared to control. The reduction in rectal temperature of mice was highest at 30 min after administration of crude extract and thereafter gradually returned to pretreatment temperature.

The hypothermia observed in this study suggests an implication of both central and peripheral mechanisms. The effect may be due to the decreased levels of metabolic heat production and or vasodilatation (N'gouemo *et al.,* 1996). Thus, the preoptic anterior hypothalamus is critical in the neuronal network of thermoregulation (N'gouemo *et al.,* 1996). The role of dopaminergic system in thermoregulation in mice has been highlighted and it's believed to act through D_2 receptor sites. Benzodiazepines cause hypothermia in animals even al low doses and they are thought to act through benzodiazepine receptor (Jackson and Nutt, 1990).

Ketamine – induced sleeping time is an experiment normally carried out to determine the effect of test agent on CNS depression resulting in sleep by ketamine injection. Two parameters are measured in this experiment, latency of sleep and total sleeping time. The latency of sleep is defined as the time in minute from injection time to loss of righting reflex (unconsciousness) while total sleeping time is defined as the total time in minute from loss of righting reflex (loss of consciousness) to regain of righting reflex (recovery of consciousness) (Haque *et al.,* 2001; Ayoka *et al.,* 2006). The crude extract produced a significant reduction in sleep latency and an increase in total sleeping time induced by ketamine in a dose dependent manner when compared to control. This result suggests that the crude extract possessed sedative activity.

The study concluded that the crude extract possessed central depressant properties. The sedative effect may be due to increase in the activity of GABA in the brain. It may also be due to the effect of the extract on the benzodiazepine receptors in the brain.

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