



## Studies of behavioural and analgesic properties of *Treculia africana* in mice

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### ABSTRACT

*Treculia africana* was claimed to be useful in the treatment of mental illness. The present work was carried out to evaluate the neurobiology and analgesic properties of *Treculia africana* in mice. The neurobiology and analgesic properties of *Treculia africana* was investigated by using head dip, elevated plus maze, Y-maze, tail immersion and acetic acid-induced abdominal constriction tests in mice. The results showed that *Treculia africana* reduced head dips, produced anxiogenic effect, reduced locomotor activity without effect on learning and memory and produced analgesic effect peripherally. It was concluded that *Treculia africana* possessed sedative and strong analgesic properties.

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**Keywords:** Head dip, elevated plus maze, Y-Maze, tail immersion, abdominal constriction

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### INTRODUCTION

The use of plants as medicine is very common in African society especially developing country like Nigeria. One of the plants claimed to be useful in the treatment of mental illness by local traditional herbalist is *Treculia africana*. The present work was carried out to investigate the effect of the plant on the central nervous system.

*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 plant parts are 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 (Morton, 1987). The water soluble and ethylacetate fractions have been shown to reduce the fasting blood sugar levels (Oyeleye et al., 2007). Three compounds were isolated from *Treculia africana* and they are identified as Phyllocoumarin; Catechin and 6,9-dihydroxymegastigmane-3-one with antibacterial and antifungal properties (Ogbonnia et al., 2008).

## MATERIALS AND METHODS

### Plant materials

*Treculia africana* stem barks were collected from the campus of the Obafemi Awolowo University (OAU) in March, 2009. It was identified by Mr. A. Oladele the curator of the Department of Pharmacognosy, Faculty of Pharmacy, O.A.U., Ile-Ife. It was further identified and authenticated by Dr. H. C Illoh of Department of Botany, O.A.U. Ile-Ife. A voucher specimen with no UHI 4225A was deposited at the herbarium of Department of Botany, O.A.U. Ile-Ife.

### Preparation of plant materials

The plant was air dried for two weeks at room temperature. The dried stem bark was pulverized and 200 g of the powder was

extracted with 0.6 liters of seventy percent (70%) ethanol for 48 hrs. The marc was re-extracted twice and the combined extract was concentrated *in vacuo* at a temperature of 40 °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 Swiss albino male mice. 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. Approval was given by the College of Medicine Ethics Committee on Animal Experimentation.

### Drugs

Acetylsalicylic acid (Beecham, Nig. Ltd.); Diazepam (Roche, Basel, Switzerland); Morphine, Acetic acid (BDH Chemicals Ltd., England).

### Methodology

#### Toxicity test

The method described by Lorke (1983) was used to determine the LD<sub>50</sub>, which is the index of acute toxicity. Swiss albino male mice (20-25 g) 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 body weight were administered intraperitoneally (i.p.), one dose for each group. The treated animals were monitored for 24 hrs mortality and general behaviour. From the results of the above step, four different doses of (140, 225, 370 and 600 mg/kg body weight) were chosen and

administered i.p. respectively to four groups of one mouse per group. The treated animals were monitored for 24 hrs. The LD<sub>50</sub> was then calculated as the geometric mean of the lowest dose showing death and the highest dose showing no death.

#### **Head dips test**

The effect of crude extract on the rate of head dipping was determined in the holeboard with a number of holes (usually 16) in the floor through which the animal can poke its head. The animals were divided into six groups. Group (1) was given normal saline (0.2 ml/20 g i.p.), while groups (2 - 5) was given crude extract (12.5 - 100 mg/kg i.p.). The extract was administered for 30 min into animals and placed on top of a wooden box with 16 evenly spaced holes. The number of times that each animal dipped their head into the holes in 5 min was counted (Dorr *et al.*, 1971; Lister, 1987). Seventy percent (70%) ethanol was used to clean the cages at intervals. Diazepam (2.0 mg/kg, i.p.) group (6) served as reference drug.

#### **Effect on learning and memory (Y-maze test)**

The effect of crude extract on learning and memory was determined by counting the number of times individual mouse injected with (12.5-100 mg/kg, i.p.) of crude extract groups (2-5) and normal saline (0.2 ml/20 g, i.p.) group (1) 30 min earlier entered (all four feet) an arm of a Y-maze (33 cm x 38 cm x 13 cm) during 5 min after placement at the base of the maze (Mamiya *et al.*, 2004). Diazepam (2 mg/kg, i.p.) group (6) served as reference drug. Each mouse was placed at the end of one arm (usually arm A for consistency) and allowed to move freely through the maze, and the series of arm entries was recorded for 5 min. The alternation behaviour was defined by the successive entry into the three arms an overlapping triplet sets, and such behaviour (%) was expressed as the ratio of actual alternations to possible alternation (defined as the total number of arm entries minus two) multiplied by 100. The 70% ethanol was used

to clean the Y-maze at intervals (Mandal *et al.*, 2001).

#### **Elevated plus maze**

The elevated plus maze used was a modification of the apparatus based on Montgomery's conflict test described by Lister (1987) and Millan *et al.* (2001). Mice were divided into seven groups with (n=5). Group (1) was given normal saline (0.2 ml/20 g, i.p.), groups (2-5) was given the plant extracts (12.5 - 100 mg/kg, i.p.), while group (6) was given diazepam (1 mg/kg, i.p.) which serve as reference drug. Each mouse was placed in the central square of the maze facing an open arm and its behaviours were recorded by an observer. During each 5 min test, the frequency of each of the following behaviour was scored and the duration of each behaviour was recorded.

- (i) Open arm entries; (ii) Closed arm entries;
- (iii) Time spent in the open arms; (iv) Time spent in the close arm.

The index of open arm avoidance interpreted as level of anxiety (Pellow and File, 1986) is calculated as

$$(100 - \frac{(\% \text{ time in open arm} + \% \text{ entries into open arm})}{2})$$

#### **Analgesic activity**

##### **Tail immersion test**

This test was performed as described by Sewell and Spencer (1976) and as modified by Furst *et al.*, (1993). Mice were divided into six groups (n=5-7). Group (1) received normal saline (0.2 ml/20 g, i.p.), while the crude extract (25 - 200 mg/kg, i.p.) was given to groups (2 - 5). All substances were administered 30 min before the beginning of the experiment. The analgesic activity was evaluated at 30 min interval for 2 hour (30, 60, 90 and 120 min). The tail (up to 5 cm) was dipped in a pot of water maintained at 55.0 ± 0.5 °C. The time in seconds to withdraw the tail clearly out of the water was taken as the reaction time. Morphine (5 mg/kg, i.p.) group (6) served as reference drug.

### **Acetic acid-induced writhing test**

The acetic-acid induced abdominal writhing test was performed as described by Koster et al. (1959). The mice were fasted for 12 hr and had water *ad libitum*. They were randomly divided into six groups (n = 5-7). Group (1) received normal saline (0.2 ml/20 g, i.p.), while crude extract (25-200 mg/kg, i.p.) was given to groups (2-4). Each mouse was given 1.0% aqueous solution of acetic acid and then placed in an observation box. The animals were pretreated for 30 min before acetic acid injection. Nociception was evaluated by counting the number of abdominal constrictions for 20 min after the administration of acetic acid. Antinociceptive activity was expressed as the percentage reduction or inhibition of the number of abdominal writhes. Acetylsalicylic acid (ASA, 150 mg/kg, i.p.) group (6) served as reference drug.

The percentage inhibition of writhing was calculated as follows

$$\% \text{ Inhibition} = 100 - \frac{\text{writhing in treated mice}}{\text{No of writhing in control mice}}$$

### **Statistical analysis**

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

## **RESULTS**

### **Acute toxicity**

The LD<sub>50</sub> of the crude extract was found to be 450 mg/kg i.p.

### **Effect of the crude extract of *Treculia africana* on head dips in mice**

The administration of crude extract of *Treculia africana* (12.5–100 mg/kg, i.p.) reduced head dip in mice. A significant [F (5, 24) = 33.6, P < 0.001] reduction in head dip was observed at a dose of (100 mg/kg, i.p.) when compared to normal saline (Table 1).

### **Effect of the crude extract *Treculia africana* on learning and memory exploration in mice**

The administration of crude extract of *Treculia africana* (12.5–100 mg/kg, i.p.) reduced significantly [F (5, 24) = 60.6, P < 0.001] entrance of mice in Y- maze which is an indication of reduction in locomotor activity when compared to control. The extract has no effect on learning and memory (Table 2).

### **Effect of the crude extract of *Treculia africana* on elevated plus maze**

The crude extract did not alter the frequency of open arm entries when compared to the control. The extract induced increase in time spent in the close arm with reduced locomotion activity thus indicating sedation (Table 3).

### **Effect of the crude extract of *Treculia africana* on tail immersion test in mice**

The administration of crude extract of *Treculia africana* (25–200 mg/kg, i.p.) did not increase reaction time in tail immersion test when compared to control (Data not shown).

### **Effect of the crude extract *Treculia africana* on acetic acid – induced writhes test in mice**

The administration of crude extract of *Treculia africana* (25–200 mg/kg, i.p.) produced a significant [F (5, 24) = 92.8, P < 0.001] reduction in the number of acetic acid-induced writhes in mice when compared to control (Table 4).

## **DISCUSSION**

The study established the acute toxicity of the crude extract by the determination of LD<sub>50</sub>. LD<sub>50</sub> is the dose at which mortality occur in 50% population of the experimental animals. The higher the value of the LD<sub>50</sub> for a substance, the relatively safe the substance is assumed to be. The LD<sub>50</sub> determination for the *Treculia africana* in mice via the intraperitoneal route was 450.0 mg/kg body weight. The value was not toxic to the animal.

**Table 1:** Effect of *Treculia africana* on head dips in mice.

Treatment	Dose (mg/kg, i.p.)	HD/5min
Control	0.2 ml/20g	39.8 ± 1.7
Treculia	12.5	34.0 ± 0.5
Treculia	25.0	37.0 ± 2.1
Treculia	50.0	37.8 ± 2.2
Treculia	100.0	15.8 ± 3.4*
Diazepam	2.0	13.0 ± 1.2 *

Results are expressed as Mean ± S.E.M, (n = 5-7). One way ANOVA revealed that there is significant difference between various treatment groups. HD: Head Dip.

\* indicate significant difference from control. P<0.05.

**Table 2:** Effect of the crude extract *Treculia africana* on learning and memory.

Treatment	Dose (mg/kg i.p.)	No of entrance/5min	% Alternation
Control	0.2 ml/20g	23.0 ± 0.7	60.0 ± 2.6
Treculia	12.5	21.6 ± 0.7	60.9 ± 2.7
Treculia	25.0	15.4 ± 0.5*	63.1 ± 2.8
Treculia	50.0	13.6 ± 0.5*	62.4 ± 2.7
Treculia	100.0	11.0 ± 0.7*	65.0 ± 2.9
Diazepam	2.0	12.4 ± 0.7*	41.0 ± 1.5 *

Results are expressed as Mean ± S.E.M, (n = 5-7). One way ANOVA revealed that there is significant difference between various treatment groups.

\* indicate significant difference from control. P<0.05.

**Table 3:** Effect of the crude extract of *Treculia africana* on the frequency of arm entries and time spent in the arms of an elevated plus maze.

Treatment	Dose (mg/kg i.p)	No of entries into arms		Time spent in each arm (sec)		% Time spent in open arms	Index of open Arm Avoidance
		Open	Close	Open	Close		
Control	0.2 ml/20g	4.2 ± 0.9	12.4 ± 2.1	17.2 ± 4.2	121.1 ± 14.2	17.0	81.5
Treculia	12.5	1.5 ± 0.6	8.4 ± 1.7	13.4 ± 3.1	114 ± 11.1	10.0	77.1
Treculia	25	2.1 ± 1.1	9.4 ± 1.5	15.2 ± 3.4	121.1 ± 13.4	12.1	76.1
Treculia	50	2.3 ± 2.1	8.9 ± 1.2	17.1 ± 3.2	117.1 ± 12.2	13.4	74.4
Treculia	100	2.4 ± 2.2	9.3 ± 1.4	16.1 ± 3.4	124 ± 14.4	16.0	73.7
Diazepam	1	9.6 ± 1.2*	3.1 ± 0.3	170 ± 6.7*	58.0 ± 3.5	58.0*	35.1*

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 a standard anxiolytic agent increases both open arm entries and time spent in each arm.

\* indicate significant difference from control. P<0.05.

**Table 4:** Effect of *Treculia africana* on acetic acid – induced writhing in mice.

Treatment	Dose (mg/kg i.p.)	No of Writhes/20min	% Inhibition
Control	0.2ml/20g	40.1 ±2.7	0.00
Treculia	25.0	14.4 ± 1.5*	64.1
Treculia	50.0	11.4 ± 1.2*	71.5
Treculia	100.0	6.6 ± 0.8*	81.7
Treculia	200.0	3.0 ± 0.0*	92.5
Aspirin	150.0	5.4 ± 0.7*	87.3

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. Aspirin was used as standard reference drug.

\* indicate significant difference from normal saline. P < 0.05.

The hole board is used as a model of anxiety offering a simple method for measuring the response of an animal to an unfamiliar environment. The crude extract at the doses of (12.5–50.0 mg/kg, i.p.) did not modify the number of head dips when compared to control. The extract at a dose of 100 mg/kg, i.p. produced a reduction in head dips in the hole board test. An increase or decrease in exploration, may reflect a general stimulant (or depressant) action. Since, the crude extract produced a decrease in head dipping, this suggest that the extract possesses a central depressant properties. The decrease in head dip produced by the plant resembles the effect obtained with plants such as *Cissus cornifolia*, *Ziziphus spina-christi*, *Cryptolepis sanguinolenta*, and *Cissus quadrangularis* (Musa et al., 2008; Adzu et al., 2002; Ansah et al., 2008; Viswanatha Swamy et al., 2006;)

The extract was tested in the Y- maze. The Y- maze is considered to reflect short term memory and working memory. Memory is a highly complex process that involves several brain structures as well as the role of several neurotransmitters and neuropeptides (Steckler et al., 1998). The extract showed spontaneous alternation behaviour and a decrease in total arm entries (locomotor activity). The decrease in total arm entries supports the sedative effect of the extract.

The extract did not affect working memory and learning. The decrease in locomotor activity resembles the effect of some plants such as *Cissus quadrangularis*, *Spondia mombin*, (Viswanatha Swamy et al., 2006; Ayoka et al., 2006)

The elevated plus maze represent one of the most widely used animal models for screening anxiolytic and anxiogenic drugs (Lister, 1987; Corbett et al., 1991). Administration of the crude extract produced a reduction in number of entries in open arm entry and a reduction in time spent in the open arm showing that the crude extract is anxiogenic. The animal avoids the exposed open areas of the maze and preferred the enclosed wall. The anxiogenic properties validate the CNS depressant properties of the plant. The index of open arm avoidance is very high when compared to diazepam with a low index of open arm avoidance. Benzodiazepine like diazepam at low doses has anxiolytic action and at high doses has anxiogenic effect The anxiogenic effect of the plant resembles the effect of some plants such as *Cryptolepis sanguinolenta*, *Cissus cornifolia*, *Careya anboree*, (Ansah et al., 2008; Kumar et al., 2008; Musa et al., 2008)

The tail immersion method is very effective for evaluating drugs possessing analgesic property which act centrally (Sivam

and Ho, 1983; Parimaladevi et al. 2003). The crude extract did not show an increase in reaction time when compared to morphine. The effect is at variance with some plants such as *Cleome viscosa*, *Careya arborea*, which produce analgesic properties using tail immersion (Parimaladevi et al., 2003; Kumar et al., 2008)

Acetic acid-induced writhing is another method used to evaluate drugs possessing analgesic effects or activity peripherally (N'gouemo et al., 1996). Acetic acid-induced writhing is a highly sensitive and useful test for analgesic drug development but not a selective pain test. The crude extract exhibited analgesic effects in mice by inhibiting the acetic acid-induced writhes, which is a model of visceral pain. The crude extract possessed strong peripheral analgesic properties compared to aspirin. Acetic acid causes hyperalgesia by liberating endogenous substances such as prostaglandins, leukotrienes, 5-HT, histamine, Kinnins, H<sup>+</sup> and K<sup>+</sup> which have been implicated in the mediation of pain perception (Forth et al., 1986; Rang et al., 1999). Acetylsalicylic acid induced analgesia mainly by blocking the synthesis of prostaglandins E and F. Specifically, acetic acid has been reported to cause an increase in peritoneal fluids of PGE<sub>2</sub> and PGF<sub>2</sub> (Amos et al., 2000; Nwafor and Okwuasaba, 2003). It is possible that the crude extract may be acting like acetylsalicylic acid peripherally by reducing the synthesis of prostaglandins. However, it is interesting to know that the antiwrithing effect was observed at the doses that reduced locomotor activity and exploratory behaviour activities, suggesting that analgesia may be due to the depressant action of the plant extract. The peripheral analgesic effect of the plant resemble the effect of some plants such as *Cleome viscosa*, *Melastoma malabathricum*, *Morinda citrifolia*, *Xylocarpus granatum*, *Ceslea coromandeliane*, which reduced the abdominal constriction effect of acetic acid in

mice (Younos et al., 1990; Parimaladevi et al., 2003; Sulaiman et al. 2004; Pal and Nandi, 2005; Alam et al., 2007).

### Conclusion

The present work shows that *Treulia africana* significantly reduces head dip, locomotor activity and produces anxiogenic effect thus showing the central depressant properties of the plant. The plant also possesses strong analgesic effect peripherally.

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