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Acute Toxicity Studies and Evaluation of Analgesic Property of the Methanol Stem Bark Extract of *Neocarya macrophylla*

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ABSTRACT

The methanol stem bark extract of *Neocarya macrophylla* was screened for its analgesic activity using acetic acid-induced writhing in mice and formalin-induced pain in rats. The results of the study showed that the extract (60mg/kg, *i.p.*) decreased writhing response with 63.9% inhibition. The methanol extract also exhibited significant analgesic effect ($P<0.05$) in the formalin test which is in the same order of magnitude as that observed after administration of pentazocine (10mg/kg, *i.p.*) the standard drug. The intraperitoneal median lethal dose (LD_{50}) of the methanol extract of *N. macrophylla* was found to be 283mg/kg in mice suggesting the plant is fairly toxic. The results of the study have shown that the methanol extract of *N. macrophylla* possesses analgesic activity which rationalizes the traditional use of the plant in the management of pain.

INTRODUCTION

Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage (Bauman, 2002). Analgesics are drugs used to relieve pain without loss of consciousness (Romulo *et al*., 2013). Narcotic analgesics otherwise called opioid analgesics are natural or synthetic compounds that produce morphine-like effects. Their major drawbacks are tolerance, dependence and respiratory depression (Howland and Mycek, 2006). Non-steroidal anti-inflammatory drugs (NSAIDs) are analgesics that work to decrease blood levels of prostaglandins, chemicals that promote inflammation and pain (Insel, 1996). They are effective for treatment of inflammation, mild to moderate pain and fever. The use of these drugs is limited due to their side effects such as gastric irritation leading to hyperacidity and aggravation of ulcer (Insel, 1996). All of the above hazards and drawbacks necessitate the search for readily available effective alternatives with safer therapeutic profile. For thousands of years natural products from medicinal plants and other sources are known to be used extensively as remedies for human health (Thitiya, 2000). This is due to the presence of active chemical compounds/constituents which produce definite physiological action on human body (Borris, 1996; Thitiya, 2000; Sasidharan *et al*., 2011).

WHO encourages the use traditional medicines in health care programs in developing countries because of the great potential they hold in prevention of various diseases (Amos *et al*., 2001). Many medicinal plants have been screened for their use in the management of pain (Agunu *et al*., 2009; Wambagu *et al*., 2011; Sreekeesoon *et al*., 2014). *Neocarya macropylla* is a plant employed in traditional medicine to manage pain conditions in Northern Nigeria. It is commonly known as Gingerbread plum or Neou oil tree and Gawasa or Farar rura in Hausa language. It is a shrub or small tree (6-10m) high with densely pubescent and russet brown stems and alternate or ovate leaves (10-25cm) long (Burkil, 1985; Arbonnier, 2004). In ethnomedicine, the decoction of the bark is used to treat diarrheoa, dysentery, cancer, snakebite, pain and inflammation (Personal Communication). The leaves may also be chewed or applied topically for the relief of pain (Tidjani *et al*., 2010). Literature search revealed that no work has been reported on the analgesic activity of the plant. The aim of this study was therefore to evaluate the effect of the methanol stem bark extract of *N. macropylla* on pain using animal models.

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MATERIALS AND METHODS

Collection and Identification of Plant material

The plant sample of *Neocarya macrophylla* was collected in November 2012 at Jega, Jega Local Government Area of Kebbi State. It was identified by U.S Gallah of the Herbarium unit, Department of Biological Sciences, Ahmadu Bello University by comparing with herbarium reference voucher specimen (No. 3197).

Preparation of the extract

The stem bark was shade dried, pulverized, labelled and stored at room temperature for use. The powdered stem bark (3000g) was extracted with methanol using maceration method for 3 days (3 times) with occasional shaking. The extract was evaporated *in-vacuo* using rotary evaporator to afford a reddish-brown residue (396g) subsequently referred to as the crude methanol extract (ME).

Experimental Animals

Swiss albino mice and adult Wister rats of either sex (15-38g) and (121-240g) respectively obtained from the Animal House Facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria, were used for the study. They were fed with laboratory diet and water *ad libutum* and maintained under standard conditions (12hr light and 12hr dark cycle) in propylene cages at room temperature. All experimental procedures were approved by the Animal right Ethic Community of the university.

Drugs and Chemicals

Pentazocine injection Bp manufactured by Alpha Laboratories LTD, Indore M.P, India, Piroxicam capsules U.S.P manufactured by Yangzhou Pharmaceutical Co. LTD, Yiling Town, Jiangdu City, Jiangsu, China. All other chemicals were of analytical grade.

Median lethal dose (LD_{50}) Determination

The method described by Lorke (1983) was employed. In the first phase, nine mice of either sex were divided into three groups containing three mice each. The first, second and third groups received 10mg/kg, 100mg/kg and 1000mg/kg (*i.p.*) respectively and observed for 24hrs.

In the second phase, four groups of one mouse each were injected *i.p.* with the methanol extract at doses 50 mg/kg, 100mg/kg, 200mg/kg and 400mg/kg and observed for signs and symptoms of toxicity for 24hrs. The median lethal dose was calculated using the following formula;

$$LD_{50} = \sqrt{Minimal\ lethal\ dose \times Maximal\ survival\ dose}$$

Analgesic Studies

Acetic acid-induced abdominal constrictions in mice

The method described by Koster *et al* (1959) was adopted; 30 albino mice were divided into 5 groups of 6 mice each. Groups 1, 2 and 3 were injected *i.p.* with 30mg/kg, 60mg/kg and 90mg/kg of the methanol extract (ME) respectively. While group 4 was injected *i.p.* with piroxicam 10mg/kg (positive control) and group 5 was injected with 10ml/kg *i.p.* of normal saline (negative control).

Thirty minutes later, each mouse was injected with 10ml/kg of aqueous solution of acetic acid (0.6%). The number of abdominal constrictions for each mouse was counted 5 min after injection of acetic acid for a period of 10 min. The percentage inhibition of abdominal constrictions was calculated using the following formula:

$$\text{Inhibition (\%)} = \frac{\text{Mean no. of writhes (negative control)} - \text{Mean no. of writhes (Test)}}{\text{Mean no. of writhes (negative control)}} \times 100$$

Formalin test in rats

The test was conducted in accordance with the method described by Dubuisson and Dennis (1977). Twenty five rats were used. The rats were divided into 5 groups each containing 5 rats. Groups 1, 2 and 3 were injected with 30mg/kg, 60mg/kg and 90mg/kg *i.p.* of the methanol extract (ME) respectively. While group 4 was injected with pentazocine 10mg/kg *i.p.* (positive control) and group 5 was injected with 1ml/kg of normal saline *i.p.* (negative control).

Thirty minutes after this treatment; 50µl of freshly prepared 2.5% solution of formalin was injected subcutaneously under the planter surface of the left hind paw of each rat. The rats were placed individually in an observation chamber and monitored for 1hr.

The severity of pain response was recorded for each rat based on the following scale:

(0) rat walked or stood firmly on the injected paw.
(1) the injected paw was partially elevated.
(2) the injected paw was clearly lifted off the floor.
(3) the rat licked, chewed or shook the injected paw.

Anti-nociceptive effect was determined in two phases. The early phase (phase 1) was recorded during the first 5 minutes, while the late phase (phase 2) was recorded during the last 45 minutes (Dubuisson and Dennis, 1977).

Statistical analysis

All data were expressed as mean ± SEM. The mean values of control groups were compared with the mean values of treated groups using student's *t-test.* Results were considered significant at $P<0.05$.

RESULTS

Acute Toxicity Studies

The intraperitoneal LD_{50} of the methanol stem bark extract in mice was found to be 283mg/kg suggesting the extract is fairly toxic (Table 1).

Table 1: Determination of median lethal dose (LD_{50}) of methanol extract of *N. macrophylla*.

First Phase		
Dose (mg/kg)	Number of mice used	Mortality
10	**3**	**0/3**
100	**3**	**0/3**
1000	**3**	**3/3**
Second Phase		
Dose (mg/kg)	Number of mice used	Mortality
50	**1**	**0/1**
100	**1**	**0/1**
200	**1**	**0/1**
400	**1**	**1/1**

Table 2: Effect of methanol extract of *N. macrophylla* on acetic acid-induced writhing in mice.

Treatment (mg/kg)	**Mean no. of writhes**	**% inhibition**
Normal saline	13.8 ±1.4	
ME 30mg/kg	7.50± 2.1*	45.8
ME 60mg/kg	5.00±1.4*	63.9
ME 90mg/kg	5.83±2.5*	57.9
Piroxicam10mg/kg)	5.00±1.8	63.9

Each value represents mean ± SEM. *P< 0.05 compared with control (student's t-test); n=6

Analgesic studies

Acetic acid-induced writhing test

The extract significantly (P<0.05) inhibited abdominal constrictions induced by acetic acid. 63.9% inhibition was observed at 60mg/kg of the extract which was in the same order of magnitude to that of piroxicam, the standard drug (63.9%). The methanol extract at doses of 90 and 30mg/kg produced 57.9% and 45.8% reduction in abdominal constriction respectively (Table 2).

Formalin test

The formalin test on rats has shown significant reduction in both phases. The extract at dose of 60mg/kg significantly inhibited the first phase of formalin-induced pain which was similar to the standard drug, pentazocine (Table 3).

Table. 3: Effect of methanol extract of *N. macrophylla* on Formalin induced pain in rats.

	Mean Pain Scores	
Treatment (mg/kg)	**First Phase**	**Second Phase**
Normal Saline	2.20±0.2	2.20±0.2
ME 30mg/kg	1.20±0.6	1.00±0.5
ME 60mg/kg	0.00±0.0*	0.80±0.5*
ME 90mg/kg	0.60±0.4*	0.20±0.2*
Pentazocine (10mg/kg)	0.00±0.0*	0.80±0.5*

Each value represents mean ± SEM. *P< 0.05 compared with control (student's t-test); n=5

DISCUSSION

Acetic acid-induced writhing test is very sensitive and used to evaluate peripheral analgesic activity (Koster *et al*., 1959; Gene *et al*.., 1998). Local peripheral receptors are postulated to be partly involved in the abdominal constriction response (Bentley *et al*., 1983). The methanol extract of *N. macrophylla* showed analgesic activity in acetic-induced writhing test in mice suggesting that it possess peripherally-mediated analgesic activity. The mechanism may be mediated via inhibition of cyclooxygenases and/or lipooxygenases (Deradt *et al*., 2000).

Formalin test involves biphasic responses. The first phase is the direct effect of formalin which involves neurogenic pain initiated when harmful mechanical, thermal or chemical stimuli agitate the peripheral terminals of neuron named nociceptors (Reeve and Dickenson, 1995; Tominaga *et al*., 2004). The second phase is involved in the inflammatory reaction. Central acting drugs can inhibit both phases while peripheral acting drugs such as NSAIDs only inhibit the second phase. The methanol extract of *N. macrophylla* (60 and 90mg/kg) and the standard drug, pentazocine (10mg/kg) were able to diminish the nociceptive response induced by formalin injection in both phases. However, the ability of the extract to inhibit both phases suggests that it may possess both central and peripheral analgesic activities. The analgesic activity of the extract could be attributed to the presence of bioactive principles including steroids, flavonoids, saponins and tannins (Das *et al*., 1989).

Similarly, analgesic effects of terpenoids, Saponins and tannins have been reported (Das *et al*., 1989; Pateh *et al*., 2009) most of which were detected in the methanol extract (Yusuf, 2014).

CONCLUSION

The results of the investigation showed that the methanol stem bark extract of *N. macropylla* possesses analgesic activity in experimental animals validating its ethno medicinal use in the management of pain.

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