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Evaluation of the Stem Bark Extract *Picralima nitida* for Antinociceptive Property

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Authors' contributions

This work was carried out in collaboration among all authors. Author KKI did literature search, managed the animals and helped in data collection. Author MIE wrote part of the protocol, helped in data collection and also wrote first draft of the manuscript and did the statistical analysis. Author OVI designed the work and wrote part of the protocol. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To investigate the antinociceptive property of *Picralima nitida* stem bark methanol extract. Place and duration of study: Department of Veterinary Physiology and Pharmacology, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria from February to July, 2020.
Methodology: The extraction was done by cold maceration of the pulverised *P. nitida* stem bark in 80% methanol for 48 hours. Acute toxicity study was done using up and down method. The antinociceptive study was carried out using tail flick and hot plate antinociceptive models. The extract was used at the doses of 100, 200 and 400 mg/kg while pentazocine 3 mg/kg was used as the standard reference drug (positive group) and 5 ml/kg distilled water was used for the negative group.
Results: In the tail flick test, *P. nitida* at the doses of 100 and 400 mg/kg with the standard drug pentazocine significantly (P = 0.05) increased the pain reaction time (PRT), increasing the PRT from 1.80 ± 0.08 sec in the negative group (distilled water 5 ml/kg) to 2.90 ± 0.18 sec at the dose of 200 mg/kg group of mice representing 62.06% increase. Also, in the hot plate model, the PRT was

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increased from 2.03 ± 0.02 sec in distilled water treated group of mice to 9.58 ± 0.99 sec in the 400 mg/kg dose of the extract.
Conclusion: The stem bark methanol extract of *P. nitida* demonstrated a good level of antinociceptive activity in the models used in this study.

Keywords: P. nitida; nociception; tail flick; hot plate; pentazocine.

1. INTRODUCTION

Traditional medicine has been used by the majority of the world population for thousands of years and the use of natural products with therapeutic properties is as ancient as human civilization [1]. World Health Organisation (WHO) Expert Group defined Traditional Medicine as the sum total of all knowledge and practices, whether explicable or not, used in diagnosis, prevention and elimination of physical, mental, or social imbalance and relying exclusively on practical experience and observation handed down from generation to generation, whether verbally or in writing [2]. Over 90% of traditional medicine recipes/remedies contain medicinal plants and according to WHO, an estimated 80 % of the population in developing countries depend on traditionally used medicinal plants for their primary health care [3].

Ethnobotanic medicine may be defined as the use of plants by humans as medicines which fossil records date back at least to the middle paleolithic age some 60,000 years ago [4].

Natural products especially of plant origin have been used in the treatment of diseases for a very long time and ethno-medical studies are today recognized as the most viable methods of identifying new medicinal plants or refocusing on those earlier reported for bioactive constituents [5].

Over time, the development of traditional medical systems incorporating plants as a means of therapy and a source of medicine have increased throughout history and continues to serve as the basis for many pharmaceuticals used today [6].

Medicinal plants may not have standardized dosage regimen but they contain natural ingredients and have a variety of other advantages including; lower cost when compared to prescription medication, multiple effects, low side effects, widespread availability and acceptability within the cultures among others [7].

Picralima nitida is one of the plants used in African traditional medicine. *Picralima nitida* (Stapf) belongs to the family Apocynaceae. It is found mostly in forest areas of Africa such as Ivory coast, Uganda, Cameroun, Democratic Republic of Congo, Nigeria etc [8]. The local names in Nigeria include: osi or osu-iwe in Igbo; otosi in Idoma and erin in Yoruba. It is a shrub or a deciduous tree that can grow up to 35 meters in height. It has a cylindrical trunk measuring about 60 cm in diameter with white latex in its parts. The bark of the trunk is fragile and is greyish brown or black in colour. The fruits are ovoid and yellowish when mature with each fruit containing three seeds which are covered in a pulp. The leaves are opposite, simple, entire and pinnately veined while the flowers are bisexual and regular [9].

Various parts (leaves, fruits and stem bark) have been used in African ethnomedicine for the treatment of some ailments such as fever, hypertension, jaundice, dysmenorrhea, malaria, pains and gastrointestinal disorders among others [10]. There is no scientific verification of the use of the stem bark for management of pain despite its use in Nigerian traditional medicine for that purpose. This study was therefore designed to investigate the antinociceptive activity of the stem back extract of *P. nitida*.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Materials

Fresh stem bark of *P. nitida* were collected from Owerri in Imo State, Nigeria and identified by Prof. M.C. Dike, a taxonomist of the college of Natural and Environmental Sciences, Michael Okpara University of Agriculture, Umudike (MOUAU). A voucher specimen number MOUAU/VPP/2020/23 was deposited in the Department of Veterinary Physiology and Pharmacology, College of Veterinary Medicine, MOUAU herbarium.

2.2 Extraction of Plant Materials

Extraction was done by cold maceration method. The stem bark of *P. nitida* was chopped into small pieces, dried under room temperature, pulverised into a coarse powder and macerated with 80% methanol for 48 hrs with intermittent shaking at 2 hours internal. The extract was filtered with Watman No 1 filter papers and was concentrated in a hot air oven at 40°C. The extract was stored in a refrigerator at 4°C as *P. nitida* stem bark extract (PNSBE) until time of use. The percentage yield of the extract was calculated using the formula below:

$$\frac{\text{Weight of extracted material}}{\text{Weight of starting material}} \times \frac{100}{1}$$

2.3 Experimental Animals

A total of 50 mature mice of mixed sexes weighing between 30 – 37 g obtained from the laboratory animal unit of the Department of Veterinary Physiology and Pharmacology, MOUAU were used for the experiment. They were housed in stainless steel cages and fed with standard commercial pelleted feed (Vital feed®, Nigeria). Clean drinking water was provided *ad libitum*. Ethical conditions governing the conducts of experiments with life animals were strictly observed as stipulated by Ward and Esea [11].

2.4 Acute Toxicity Test

The acute toxicity study was done using the up and down method as described by QECD [12].

2.5 Antinociceptive Study

2.5.1 Tail flick test

Twenty-five (25) albino mice were randomly divided into five groups (A-E) of five mice each. The rats were fasted for 24 hours with clean drinking water provided *ad libitum.* The animals were pre-treated 1 hour before tail immersion as follows: 5 mg/kg distilled water group A (negative control), Pentazocine, 3 mg/kg for group B (positive control) and 100, 200, 400 mg/kg of *P. nitida* extract for groups C, D and E respectively. Then about 2-3 cm of the tail of each of the mice was dipped into a water bath containing warm water maintained at a temperature of 50 ± 1° C and the time taken for the mice to flick its tail or withdraw it from the warm water known as the pain reaction time (PRT) was recorded for all the mice [13].

2.5.2 Hot plate method

Twenty (25) mature albino mice of both sexes were randomly divided into 5 groups (A-E) of 5 mice per group. The mice were fasted for 12 hours with provision of clean water ad libitum. The mice were treated as follows: Group A mice received 5 ml/kg of distilled water (negative control). Group B mice were given Pentazocine, 3 mg/kg (positive control) while groups C, D and E received 100, 200 and 400 mg/kg of *P. nitida* stem bark extract respectively per os all by gastric gavage. Thirty *(*30) minutes after drug administration each mouse placed upon the heated metal plate (Hot plate) maintained at the temperature of about 50-55°C within the restraining plastic cylinder. The pain reaction time (PRT) period, which is the time taken for the mice to react to the pain stimulus was determined for each mouse in all the groups with a stop watch. The response to the heat stimulus varied with the animals and consisted of the following: kicking its hind foot and jumping about, shaking a foot and licking it or raising one or the other of the hind foot and holding it tightly against the body. Responses involving the fore foot are not considered since they are difficult to distinguish from the normal grooming behaviour of the mice [14].

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Tail flick test

The result of the tail immersion test in mice is presented in Table 1. *P. nitida* at the doses of 100 and 200 mg/kg with the standard drug pentazocine significantly (P = 0.05) increased the pain reaction time (PRT) from 1.80 ± 0.08 sec in the negative group (distilled water 5 ml/kg) to 2.90 ± 0.18 sec at the dose of 200 mg/kg group of mice representing 62.06% increase. There was no significant difference between the PRT of mice treated with the dose of 400 mg/kg *P. nitida* and the distilled water treated group.

3.1.2 Hot plate method

P. nitida at all the doses used and the standard drug pentazocine significantly (P = 0.05) increased the mean pain reaction time (PRT). The PRT was increased from 2.03 ± 0.02 sec in distilled water treated group of mice to 9.58 ± 0.99 sec in the 400 mg/kg dose of the extract. The extract at the dose of 100 and 400 mg/kg

had a better antinociceptive action than the standard drug (pentazocine 3 mg/kg) (Table 2).

Table 1. Effects of *P. nitida* stem bark extract on tail flick test

Treatment	Pain reaction time (sec)
Distilled water, 5 ml/kg	1.80 ± 0.08
Pentazocine, 3 mg/kg	2.88 ± 0.17*
Extract, 100 mg/kg	2.68 ± 0.09*
Extract, 200 mg/kg	2.90 ± 0.18*
Extract, 400 mg/kg	1.83 ± 0.17

**P = 0.05 when compared to the distilled water treated group*

3.2 Discussion

Nociception (from Latin word 'nocere' meaning to harm or hurt) is the sensory nervous system's process of encoding noxious stimuli. It is a mechanism where noxious peripheral stimuli are transmitted to the central nervous system and is a component of pain. Nociception triggers a variety of physiological and behavioural responses and usually results in a subjective experience, or perception, of pain. The peripheral nociceptive afferent neuron which is activated by noxious stimuli and the central mechanism by which the afferent input generates pain are the two components of pain either or both of which may be involved in pathological pain state [15].

Certain noxious stimuli are painful and reflex movements or behaviours resulting from such stimuli are indicative of pain threshold [16]. The stimulus may be either thermal, electrical or chemical or a combination [17]. This informed the choice of the antinociceptive models (tail flick and hot plate methods) employed in this study and also because of their sensitivity in the measurement of pathological pain.

Table 2. Effects of *P. nitida* stem bark extract on hot plate test

Treatment	Pain reaction time (sec)
Distilled water, 5 ml/kg	2.03 ± 0.02
Pentazocine, 3 mg/kg	3.98 ± 0.22*
Extract, 100 mg/kg	5.13 ± 0.35*
Extract, 200 mg/kg	3.63 ± 0.33*
Extract, 400 mg/kg	9.58 ± 0.99*

**P =0.05 when compared to the distilled water treated group*

The procedure in tail flick method is based on the observation that morphine-like drugs selectively prolongs the reaction time of the typical tail withdrawal reflex of mice [18] while in hot plate method, the paws of mice are very sensitive to temperatures between 55-60°C [19]. From the results of the this study the *P. nitida* extract significantly increased the pain reaction time (PRT) in both the tail flick and hot plate methods when compared to the distilled water treated group. The extract at the dose of 200 mg/kg and 400 mg/kg had a better antinociceptive effect on the mice than the standard drug (pentazocine 3 mg/kg) (Tables 1 and 2). In the tail flick and hot plate models increase in PRT indicates the level of antinociception (analgesia) of the drug or extract [18]. Therefore, the increase in PRT by *P. nitida* extract in both models shows that the extract possesses antinociceptive activity. The less activity seen at the dose of 400 mg/kg in hot plate method may be as a result of receptor site saturation by the extract with increase in dose.

The hot plat and tail flick models are used to test pains mediated by central activity [19] and increase in stress tolerance of animals in these models indicates the possible involvement of higher centre [20]. The above assertions suggest that the increase in pain reaction time of *P. nitida* in these models may have been mediated through the central nervous system.

Pentazocine is a synthetic mixed agonist-antagonist which has its analgesic activity similar to that of morphine and acts by changing the way the brain and nervous system respond to pain [15]. *P. nitida* may have acted through the same mechanism as pentazocine. Another possible mechanism of the antinociceptive effects of the plant may be by the inhibition of cyclooxygenase activities which catalyzes the biosynthesis of prostaglandins from arachidonic acid, just as the aspirin,[21] or may be through the desensitization of the nociceptor and/or increase in the pain threshold in the hypothalamus [15]. Phytochemical analysis of *P. nitida* showed it contains alkaloid, flavonoids, tannins saponins and sterols [22]. The antinociceptive activity of the plant extract may be as a result of the phytoconstituents.

4. CONCLUSION

The stem bark methanol extract of *P. nitida* demonstrated a good level of antinociceptive activity in the models used and the action may involve central mechanism among others. However, more work is required to isolate the active principle responsible for the

antinociceptive activity and to determine the exact mechanism.

ETHICAL APPROVAL

The study protocol was approved by the ethics committee of college of Veterinary medicine of Michael Okpara University of Agriculture, Umudike and the experiment was performed in accordance with the ethical standard laid down in the 1964 declaration of Helsinki and followed by Michael Okpara University of Agriculture, Umudike.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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