



## **Anti-Inflammatory Activity of the Stem Bark Methanol Extract of *Picralima nitida***

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### **Authors' contributions**

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

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### **ABSTRACT**

**Aims:** To investigate the anti-inflammatory activity of *Picralima nitida* stem bark methanol extract (PNSBE).

**Study Design:** The study was carried out using *in vivo* (carrageenan-induced paw oedema and egg albumin-induced paw oedema) and *in vitro* (Human red blood cell (HRBC) membrane stabilization assay) models in rat.

**Place and Duration of Study:** Department of Veterinary Physiology and Pharmacology, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria from March to July, 2020.

**Methodology:** The extract was used at the doses of 100, 200 and 400 mg/kg while diclofenac (20 mg/kg) was used as the standard reference drug for the *in vivo* study (carrageenan-induced paw oedema and egg albumin). For the *in vitro* study (red blood cell haemolysis), the extract was used at the concentrations of 25, 50, 100, 200 and 400 µg/ml while diclofenac 250 µg/ml was used.

**Results:** In the carrageenan-induced paw oedema model, the extract at the doses used and the

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reference drug significantly ( $p < 0.05$ ) reduced the paw edema in the rats at 1 hour in a dose dependent manner. Also, in the egg albumin model, the paw oedema of treated rats was significantly ( $P < 0.05$ ) reduced by PNSBE dose dependently reducing the mean rat paw oedema from  $0.99 \pm 0.12$  to  $0.61 \pm 0.06$  at the first hour. There was also a concentration dependent inhibition of red blood cell haemolysis by the extract in the HRBC membrane stabilization assay.

**Conclusion:** *Picralima nitida* demonstrated a significant anti-inflammatory activity in this study.

**Keywords:** *Picralima nitida*; anti-inflammatory; carrageenan; albumin; paw oedema.

## 1. INTRODUCTION

Inflammation is the response of living tissues to injury which is mostly an immunological reaction and is implicated in the pathophysiology of many disease conditions [1]. It is a complex pathophysiological process mediated by a variety of molecules produced by leukocytes, macrophages and mast cells as well as by enzyme activation and mediator release which bring about tissue breakdown and oedema formation as a result of extravasation of fluid and proteins and accumulation of leukocytes at the inflammatory site [2,3]. Inflammation is a mechanism by which the body protects itself and is triggered by damage to living tissues due to noxious stimuli, trauma, infection (bacterial, viral, fungal etc) and defective immune system [4]. The fundamental aim of inflammatory response is to localize and eliminate the harmful agents thereby protecting the body and facilitating recovery process [5]. This defence mechanism is characterised by pain, redness, heat, swelling and loss of function in affected area and in the process of inflammation both innate and acquired immune responses are involved [6,7].

Inflammation can be treated with a variety of anti-inflammatory drugs. These include a large number of steroids and non-steroidal anti-inflammatory drugs (NSAIDs) [8]. Unfortunately, despite their great number and their excellent anti-inflammatory potentials, their therapeutic efficacy seems to be hampered due to the often-associated serious adverse effects which include: gastro-intestinal irritation, ulcers, bone marrow depression, metabolic disorders, renal failure, hypertension, allergic reactions etc [9].

However, there are many reported medicinal plants with anti-inflammatory activities with low or no side effects. Some medicinal plants with anti-inflammatory activities include: *Schwenkia americana*, *Asparagus africanus*, *Dichrostachys cinerea*, *Ficus iteophylla* and *Indigofera pulchra* [10,11].

*Picralima nitida* (Stapf) commonly called Akuamma belongs to the family known as Apocynaceae. It is found mostly in forest areas of Africa such as Ivory coast, Uganda, Cameroun, Democratic Republic of Congo, Nigeria etc [12]. 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 [13].

Various parts (leaves, fruits and stem bark) have been used in African ethnomedicine for the treatment of some ailments such as fever, hypertension, arthritis, jaundice, dysmenorrhea, malaria and gastrointestinal disorders among others while the botanical uses include using the wood, called ebam in trade, for making a variety of small utensils, e.g. paddles, shuttles for weaving, dolls, combs, walking sticks, pestles and mortars, incense holders, bows and arrows, spade handles or spoons [14]. There is paucity of knowledge on the anti-inflammatory effect of the plant. Hence this study investigates the anti-inflammatory activity of *Picralima nitida* stem bark methanol extract (PNSBE).

## 2. MATERIALS AND METHODS

### 2.1 Collection and Identification of Plant Material

Fresh stem bark of *Picralima 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 (MOUUAU). A voucher specimen number MOUUAU/VPP/2020/23 was deposited in the Department of Veterinary Physiology and Pharmacology, College of Veterinary Medicine, MOUUAU herbarium.

## 2.2 Extraction of Plant Material

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 hours with intermittent shaking at 2 hours interval. 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 *Picralima nitida* stem bark extract (PNSBE) until time of use. The 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 rats of mixed sexes weighing between 130 – 155 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 Elsea [15].

## 2.4 Toxicity Study

The acute toxicity study was done using the up and down method as described by OECD [16].

## 2.5 In vivo Anti-Inflammatory Tests

### 2.5.1 Carrageenan – induced paw edema

Twenty-five albino rats were weighed and randomly divided into five groups (A-E) of 5 rats per group. Their left paw volumes were measured using the volume displacement method, as control. Paw edema was induced by injecting 0.1 ml of 0.2% solution of carrageenan into the subplantar surface of the hind right paw. After one hour of induction, group A was given 5 ml/kg of distilled water (negative control), group B was treated with 20 mg/kg of diclofenac (positive control) and groups C, D and E were treated with 100, 200 and 400 mg/kg of plant extract respectively. Thereafter, the right paw volume was determined at 1 hour, 2 hours and 3 hours post treatment using volume displacement method [17]

### 2.5.2 Egg-albumin-induced paw oedema

Twenty- five albino rats were weighed and randomly divided into five groups (A–E) of 5 rats per group. The rats were fasted overnight and were given free access to water and feed prior to the experiment. Group A was given 5 ml/kg of distilled water (negative control), group B was treated with 20 mg/kg of diclofenac (positive control) and groups C, D and E were treated with 100, 200 and 400 mg/kg of the extract respectively. One hour after treatment, paw oedema was induced by injecting 0.05 ml of fresh egg albumin into the sub-plantar surface of the hind right paw. Their left paw volumes were measured using the volume displacement method, as control. Therefore, the right paw volume was determined at 1, 2 and 3 hours post treatment. The increase in paw volume = *Right paw volume – left paw volume* [18].

## 2.6 In vitro Anti-Inflammatory Tests

### 2.6.1 Human red blood cell (HRBC) membrane stabilization assay

The effects of extract on the haemolysis of human red blood cell (HRBC) in hypotonic saline solution was evaluated as described by Anosike et al. [19]. Blood sample (5 ml) was collected from a healthy male donor (that has not received anti-inflammatory drug in the past 10 days) into EDTA sample bottle. The HRBC was repeatedly washed with normal saline by centrifugation until the supernatant was clear. Thereafter, 0.5 mL of 10 % suspension of the HRBC was added to test tubes containing different concentrations (25 – 400 µg/mL) of extract dissolved in hypotonic solution in triplicate. The mixtures were incubated for 30 min at 37 °C and later centrifuged at 3000 rpm for 5 min. The absorbance of the supernatants was recorded at 560 nm with spectrophotometer. Hypotonic solution was used as control while diclofenac (250 µg/mL) was used as reference standard.

$$\text{Inhibition (\%)} = \frac{AA - BB}{AA} \times \frac{100}{1}$$

Where: AA = absorbance of control, BB = absorbance of test substance

## 3. RESULTS

### 3.1 Carrageenan – Induced Paw Edema

The result of the carrageenan-induced paw edema is presented in Table 1. The result showed that *Picralima nitida* extract (100, 200

and 400 mg/kg) and the reference drug (diclofenac) significantly ( $p < 0.05$ ) reduced the paw edema in the rats at 1 hour in a dose dependent manner. In the 2<sup>nd</sup> hour there was various degrees of reduction in paw oedema of the rats by the extracts in the treated groups but reductions were not statistically significant when compared to the negative group. Also, at the 3<sup>rd</sup> hour the reduction in paw volume of the rats was only significantly ( $P < 0.05$ ) reduced by the standard drug (diclofenac 20 mg/kg) and the extract at the dose of 100 mg/kg.

### 3.2 Egg Albumin

The paw oedema of the treated rats was significantly ( $P < 0.05$ ) reduced by *P. nitida* extract in a dose dependent manner reducing the mean rat paw oedema from  $0.99 \pm 0.12$  to  $0.61 \pm 0.06$  at the dose of 400 mg/kg of the extract at the first hour. At the 2<sup>nd</sup> hour the reduction by the

extract was only statistically significant at the dose of 200 and 400 mg/kg while at the 3<sup>rd</sup> hour there was no significant difference between the mean paw oedema of the treated rats and the distilled water treated group of rats (Table 2).

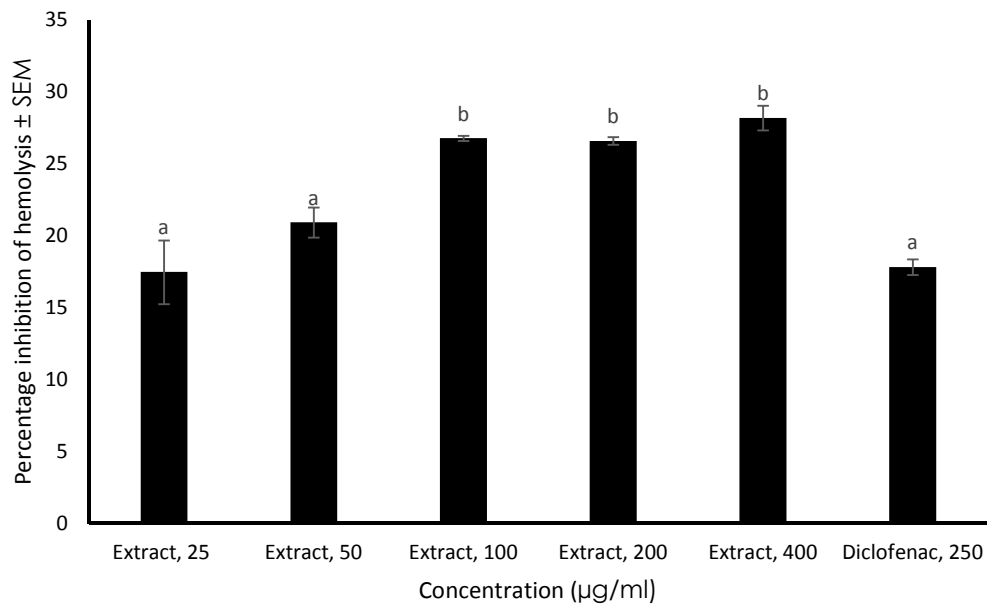
### 3.3 Human Red Blood Cell (HRBC) Membrane Stabilization Assay

The inhibition of haemolysis of the human red blood cells by *P. nitida* stem bark extract is presented in Fig. 1. The result shows a concentration dependent inhibition of red blood cell haemolysis by the extract though the concentration was statistically significant at the concentrations of 100, 200 and 400  $\mu\text{g/ml}$ , with the highest inhibition seen at the concentration of 400  $\mu\text{g/ml}$ . The extract at the concentrations of 50 – 400  $\mu\text{g/ml}$  had better inhibition activity than the standard drug diclofenac (250  $\mu\text{g/ml}$ ).

**Table 1. Effects of the extract on carrageenan induced paw-oedema**

Treatment	Increase in paw volume (ml)		
	1 h	2 h	3 h
Distilled water, 5 ml/kg	$0.32 \pm 0.06$	$0.61 \pm 0.09$	$0.73 \pm 0.10$
Diclofenac, 20 mg/kg	$0.17 \pm 0.03^*$	$0.18 \pm 0.03^*$	$0.23 \pm 0.05^*$
Extract, 100 mg/kg	$0.18 \pm 0.03^*$	$0.42 \pm 0.05$	$0.53 \pm 0.09^*$
Extract, 200 mg/kg	$0.18 \pm 0.03^*$	$0.41 \pm 0.11$	$0.66 \pm 0.09$
Extract, 400 mg/kg	$0.19 \pm 0.06^*$	$0.64 \pm 0.10$	$0.72 \pm 0.05$

\* $p < 0.05$  when compared to the distilled water treated group



**Fig. 1. Percentage inhibition of red blood cell haemolysis**

Table 2. Effects of the extract on egg albumin induced paw-edema

Treatment	Increase in paw volume (ml)		
	1 h	2 h	3 h
Distilled water, 5 ml/kg	0.99 ± 0.12	0.87 ± 0.07	0.84 ± 0.06
Diclofenac 20 mg/kg	0.70 ± 0.07*	0.73 ± 0.10	0.60 ± 0.10
Extract, 100 mg/kg	0.77 ± 0.04*	0.71 ± 0.06	0.80 ± 0.06
Extract, 200 mg/kg	0.63 ± 0.09*	0.57 ± 0.04*	0.62 ± 0.04
Extract, 400 mg/kg	0.61 ± 0.06*	0.64 ± 0.04*	0.81 ± 0.05

#### 4. DISCUSSION

This study evaluated the anti-inflammatory activity of methanol stem bark extract of *Picralima nitida*. The anti-inflammatory activity was evaluated using both *in vivo* (Carrageenan-induced paw oedema and egg albumin-induced paw oedema) and *in vitro* (Human red blood cell (HRBC) membrane stabilization assay) inflammatory models.

Acute toxicity test in rats produced no death or signs of toxicity which suggests that the extract was well tolerated and the doses used for the study were safe in the animal models used.

Carrageenan-induced paw edema model is widely used for the study of the effects of drugs and extracts on inflammation especially at the acute phase [20]. On injection of carrageenan in the sub-planter areas of rats' hind paws, development of edema results from the release of serotonin, histamine and prostaglandins [21]. The development of carrageenan-induced paw edema in rats is biphasic. The first phase occurs within one hour and is attributed to release of cytoplasmic enzymes such as histamine, serotonin and kinins from the mast cells while the second phase, which occurs after one hour is mediated by the release of prostaglandin-like substances within the inflammatory area [22].

*Picralima nitida* extract significantly ( $p < 0.05$ ) reduced the rat paw edema in dose dependent manner in the 1<sup>st</sup> hour in this study but not on the subsequent hours. This suggests that the anti-inflammatory property of *P. nitida* could be due to its effect on first phase of the inflammatory reactions. This may have been brought about by inhibition of the mediators of inflammation such as serotonin, histamine and prostaglandins by the extract through its stabilizing effects on basophils and mast cells with decrease in cellular infiltration and release of mediators of inflammation [23]. Also, the decrease in rat paw edema by PNSBE may be attributed to inhibition of cyclooxygenase and lipoxygenase enzymes since inhibitors of these enzymes play important

roles in carrageenan-induced paw edema in rats [24].

Egg albumin-induced oedema results from the release of histamine and serotonin. The extract also inhibited egg albumin-induced oedema demonstrating that it can inhibit inflammation by blocking the release of histamine and 5-HT, two mediators that are released by egg albumin [25]. Moreover, histamine induces paw oedema in rats by causing the release of prostaglandins and inflammatory mediators. *P. nitida* may also have acted on the inflammatory mediators and inhibited the release of prostaglandins and histamine mediators which causes mucus secretion and mucosal oedema [26].

*Picralima nitida* extract at concentrations of 100–400 µg/ml protected the human erythrocyte membrane against lysis. Membrane stabilization prevents leakage of serum protein and fluids into the tissues during a period of increased permeability brought about by inflammatory mediators. During inflammation, there are lyses of lysosomes which release their component enzymes that produce a variety of disorders. *Picralima* extract may have caused stabilization of the red blood cell membrane by preventing the release of lytic enzymes and active mediators of inflammation [27].

Phytochemical analysis of *P. nitida* showed that it contains alkaloid, flavonoids, tannins, terpenoids, steroids, glycosides, saponins and sterols [28]. Many reports have shown that plant flavonoids possess potent anti-inflammatory and anti-oxidant properties [29]. Their anti-inflammatory activities may probably be due to their ability to inhibit enzymes involved in the production of the chemical mediators of inflammation and metabolism of arachidonic acid [30]. The anti-inflammatory activity of PNSBE may have been due to its phytochemical constituents.

#### 5. CONCLUSION

In conclusion, *P. nitida* demonstrated reasonable anti-inflammatory activity in this study, therefore

establishing the pharmacological basis for its use in Nigeria ethnomedicine for the treatment of arthritis. However, more work is required to isolate the active principle responsible for its anti-inflammatory activity and to determine the exact mechanism of action.

## CONSENT

It is not applicable.

## 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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