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Anti-inflammatory and anti-oxidant activities of the methanolic extracts of the stalk of *Parkia biglobosa* (jacq.) Benth.

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Abstract

Oral administration of various doses (50, 100 and 250 mg/kg) of the methanol extracts of the stalk of *Parkia biglobosa* produced anti-inflammatory activities by reducing the croton oil ear inflammation, though not statistically relevant at $p<0.01$ level of significance. It also antagonised the oedema produced by carrageenin and arachidonic acid as well as the granuloma by cotton pellet in rats. The stalk also showed *in vitro* anti-oxidant activities using the DPPH. The extract is suspected to produce its anti-inflammatory activity by inhibiting both the lipo-oxygenase and cyclo-oxygenase pathways of the arachidonic acid metabolism.

Key words: Anti-inflammatory, Anti-oxidant, *Parkia biglobosa*

1. Introduction

Parkia biglobosa, a Fabaceae, commonly known as 'African locust bean', is a plant used extensively in West Africa for timber, food and medicine [1]. A decoction of the bark, root and leaves is used in treating toothaches, leprosy, eye sores, hypertension and fevers [2]. *P. biglobosa* pulped bark is used along with lemon for wound and ulcers [3]. It is also used against bronchitis, pneumonia, ulcers, bilharzias, malaria, diarrhoea, violent colic, venereal diseases, sterility, rickets, oedema, haemorrhoids and toothaches [4]. The pulp is used as a diuretic and mild purgative [5]. The fermented seeds of *P. biglobosa* are used in all parts of Nigeria and indeed the West Coast of Africa for seasoning traditional soups [2]. Previous pharmacological studies [6] showed the stem bark as potent anti-snake venom.

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P. biglobosa have been shown to contain glycosides, tannins, slight presence of alkaloids, steroids were negligible. However, there was complete absence of saponins and anthraquinones [7].

A decoction of the stalk is traditionally used in the treatment of arthritic pains. This present work therefore, was undertaken to investigate anti-inflammatory and anti-oxidant effects of the methanol stalk extracts of *P. biglobosa.*

2. **Materials and Methods**

2.1. **Plant Material**

The stalks of *P. biglobosa* were collected from Nsukka Urban Area, Enugu State, Nigeria in November, 2009 and plant parts were identified and authenticated by Mr. A.O. Ozioko, a taxonomist with the International Centre for Ethnomedicine and Drug Development, Nsukka. A voucher specimen with number MOUAU/CVM/VPP/HB503 was deposited in the herbarium of the Department of Veterinary Physiology and Pharmacology, Michael Okpara University of Agriculture, Umudike for reference.

2.2. **Preparation of the extract**

The dried plants were pulverized into coarse powder and were extracted in 80% methanol for 48 hours with intermittent shaking. Thereafter, filtration was done using filter papers and funnel into an already weighed beaker. The solvent was allowed to evaporate in a rotary evaporator at 40^{o}C and water using a lyophilizer.

2.3. **Animals**

Mature out-bred Albino rats of both sexes weighing 150–175g were purchased from the Laboratory Animal Facility of the Department of Veterinary Physiology and Pharmacology, University of Nigeria, Nsukka and used. They were kept in clean stainless steel wire mesh cages, maintained at normal temperature and natural daylight/night conditions. They were allowed free access to standard commercial pelleted feed and clean drinking water. Ethical conditions as stipulated by Ward and Elsea [8] in the conducts of experiments with life animals were adhered to strictly. The study protocol was approved by the University's ethical committee.

3. **Anti-inflammatory activity**

3.1**.** *Cotton pellet-induced granuloma*

Five groups of six rats each were used. Two cotton pellets weighing 10 mg were autoclaved and implanted subcutaneously into both sides of the interscapular region of each anaesthetized rat with the incision closed by interrupted sutures after expelling air from the tunnels [9].

P.biglobosa at doses of 50, 100 and 250 mg/kg, ibuprofen 100 mg/kg and distilled water were administered through the intraperitoneal route starting from the day of pellet implantation. They were treated daily for 7 days. On the 8th day, animals were sacrificed with ether; the pellets together with the granuloma tissues were carefully removed, dried in the oven at 60°C, weighed and compared with the control.

3.2. *Carrageenin induced rat paw oedema*

Oedema was induced by the methods of Winter *et al* [10]. Six rats of either sex were divided into five groups and treated with 50, 100 and 250 mg/kg of the extracts, ibuprofen 100 mg/kg and the vehicle, distilled water (1 ml/kg) respectively, 30 minutes prior to an injection of Carrageenin (0.1ml/100g from a 10mg/ml solution) into the planter aponeurosis of the right hind paw of the rats. The left hind paw served as control receiving 0.1 ml of saline. The paw volume was measured plethysmographically 4 h after carrageenin injection.

3.3. *Croton oil-induced ear inflammation*

Croton oil irritant solution (0.1ml) was applied externally to the outer surface of the right ear of test rats according to the methods of Brooks *et al* [11]. Thirty rats were employed, divided into 5 groups containing 6 animals each. Group 1 (negative control) received 0.1mg/10g of normal saline intraperitoneally; group 2 received 100mg/kg of Ibuprofen, i.p.; groups 3, 4 and 5 received 50, 100 and 250 mg/kg, i.p. respectively, of the extracts (PB), 30 minutes prior to croton oil application. The rats were sacrificed with ether after 4 h and 7mm punches were made in the ear with a cork borer. Each ear disc was weighed and compared with the control.

3.4. *Arachidonic acid-induced paw oedema*

The methods of DiMartino *et al* [12] were employed in the experiment. Thirty six male albino rats grouped into 6 of 6 animals each and treated intraperitoneally with the vehicle, normal saline (0.1 ml/10g), a double blocker, phenidone (100 mg/kg), indomethacin (10 mg/kg) and the extracts (50, 100 and 250 mg/kg). Paw oedema was induced in the right plantar surface of the hind paw, 30 minutes post-treatment by a single injection of 0.1 ml of 0.5% arachidonic acid in 0.2M carbonate buffer (pH 8.4). The hind paw volume was measured 1 h after arachidonic acid injection.

4. **Anti-oxidant activity.**

DPPH photometric assay

The free radical scavenging activity of the extract was analyzed by the 1, 1–diphenyl-2-picryl-hydrazyl (DPPH) assay described by Iwalewa *et al* [13].

Two (2) ml of the test extract, at concentrations of 10 µg/ml, 50 µg/ml, 100 µg/ml, 200 µg/ml and 400 µg/ml each was mixed with 1 ml of 0.5 mM DPPH (in methanol). The absorbance of the resulting solution was read at 517 nm after 30 minutes of incubation in the dark at room temperature using a spectrophotometer.

One (1) ml of 1000 µM ascorbic acid in 1 ml of DPPH was used as reference standard antioxidant while a blank of 1ml methanol plus 2 ml of extract was ran with each assay. All determinations were carried out in triplicate. The same procedure was repeated using control sample (DPPH without extracts). Mean values were obtained and used for the following calculation:

% Antioxidant Activity [AA] = [{(Abs $_{sample}$ – Abs $_{blank}$) **X** 100}/ Abs $_{control}$].

5. **Data analysis**

Data obtained were presented as mean ± SEM and analysed using one-way analysis of variance (ANOVA) and post-hoc comparisons were carried out using Dunnett's *t*-test. Values of $p<0.05$ were considered significant in the study.

6. **Results and Discussion**

The yield of the extract obtained was 14.5%. Intraperitoneal administration of the methanolic extracts of *Parkia biglobosa* stalk significantly antagonized the formation of croton pellet granuloma (Table 1) in a dose-dependent manner.

The extract also showed a dose-dependent inhibition of the croton oil ear inflammation in test animals (Table 3). There was also appreciable inhibition of carrageenin-induced rat paw oedema compared with controls. The difference observed between the 100 and 250 mg/kg was not statistically relevant at $p<0.05$ level of significance (Table 2).

The extracts of PB further inhibited the arachidonic acid induced paw oedema in a dose-dependent manner comparable to the dual-blocker, phenidone (Table 4). Thus, suggesting that the observed anti-inflammatory activities may be produced by the inhibition of the lipo-oxygenase pathways, the cyclo-oxygenase pathways or both which are involved in metabolism of arachidonic acid.

Findings from this study unearths the potential of the stalk of *P. biglobosa* as anti-oxidant (Figure 1) which could be exploited in drug development urgently needed to challenge free radicals in biological systems and consequently prevent the body from reactive oxygen species (ROS) originated ailments, which may include among others cancer, arthritis, diabetes.

7. **Conclusion**

Based on these results, we concluded that administration of the stalk of *P. biglobosa* either orally or parenterally results in anti-inflammatory activity. The *in vitro* anti-oxidant activity of the extract gives credence to the folkloric use of the plant as an agent against inflammatory conditions such as arthritis.

Table 1. Effect of *P. biglobosa* stalk extracts on Cotton pellet-induced granuloma

Drug	**No. of animals**	**Dose (p. o.)**	**Weight of granuloma (mg)**
Normal saline	6	0.1ml/10g	70.0 ± 4.0
Ibuprofen	6	100mg/kg	48.0 ± 2.0*
PB	6	50mg/kg	48.0 ± 2.0*
PB	6	100mg/kg	43.0 ± 1.0*
PB	6	250mg/kg	35.0 ± 1.0*

Values are means ± SEM. *p<0.05

Table 2. Effect of *P. biglobosa* stalk extracts on Carrageenin-induced rat paw oedema

Drug	**No. of animals**	**Dose (p. o.)**	**Paw volume (ml)**
Normal saline	6	0.1ml/10g	0.61 ± 0.006
Ibuprofen	6	100mg/kg	0.21 ± 0.02*
PB	6	50mg/kg	0.29 ± 0.005*
PB	6	100mg/kg	0.32 ± 0.005
PB	6	250mg/kg	0.33 ± 0.005

Values are means ± SEM. *p<0.05

Table 3. Effect of *P. biglobosa* stalk extracts on Croton oil-induced ear inflammation

Drug	No. of animals	Dose (p. o.)	Weight of ear disc (mg)
Normal saline	6	0.1ml/10g	13.0 ± 5.0
Ibuprofen	6	100mg/kg	11.0 ± 2.0*
PB	6	50mg/kg	12.0 ± 5.0*
PB	6	100mg/kg	11.0 ± 4.0*
PB	6	250mg/kg	10.0 ± 4.0*

Values are means ± SEM. *p<0.05

Table 4. Effect of *P. biglobosa* stalk extracts on Arachidonic acid induced paw oedema

Drug	No.	Dose (i.p.)	Paw volume difference (Mean ± SEM) ml	% inhibition as compared to control
Normal Saline	6	0.1ml/10g	4.22 ± 0.25	-
Indomethacin	6	10 mg/kg	2.51 ± 0.39*	40.52%
Phenidone	6	100 mg/kg	0.94 ± 0.15*	77.73%
PB	6	50 mg/kg	1.85 ± 0.34*	56.16%
"	6	100 mg/kg	1.01 ± 0.25*	76.07%
"	6	200 mg/kg	0.64 ± 0.12*	84.83%

Values are means ± SEM. *p<0.01 vs. group 2.

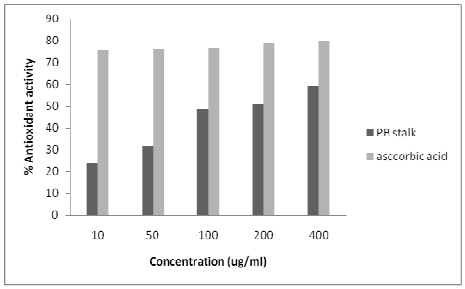

Figure 1. The antioxidant activities of methanol stalk extracts of *P. biglobosa* using the DPPH assay.

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