December 30, 2018

PharmacologyOnLine

Archives • 2018 • vol.3 • 81-89

IN VIVO ANTI-INFLAMMATORY AND ANALGESIC POTENTIALS OF METHANOL EXTRACT OF *CEIBA PENTANDRA* STEM BACK

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Abstract

The use of medicinal plant is accepted globally as the most common form of traditional medicine. The present study determined the anti-inflammatory potentials of methanol extract of *Ceiba pentandra* stem bark using different models such as xylene-induced ear oedema, egg albumin-induced rat paw oedema and vascular permeability tests. The analgesic properties were determined using acetic acid-induced writhing and tail flick latency tests. The methanol extract of *C. pentandra* stem bark showed significant ($p < 0.05$) inhibition of xylene-induced ear oedema and egg albumin-induced paw oedema, and reduction of acetic acid-induced vascular permeability. It also significantly ($p < 0.05$) reduced the number of writhes in acetic acid-induced writhing test and protected against heat-induced pain in the tail flick latency test, all in a dose-dependent manner. The presence of flavonoids in the plant extract might be responsible for the analgesic and anti-inflammatory activities of the extract.

Keywords: *Ceiba pentandra, Anti-inflammation, Acute toxicity, Oedema, Analgesics, NSAIDs*

Introduction

Inflammation was described over two thousand years ago by Celsus using the four Latin words: Rubor, calor, tumor and dolor. It constitutes body's response to injury and is characterized by a series of events that mainly occur in three distinct phases. The first phase is caused by an increase in vascular permeability resulting in exudation of fluids from the blood into the interstitial space; the second phase involves the infiltration of leukocytes from the blood into the tissue and third phase is characterized by granuloma formation and tissue repair [1-2]. Accordingly, anti-inflammatory tests are divided into: those measuring acute inflammation, sub-acute inflammation and chronic repair processes [3]. Major research efforts to find new therapeutic agents for a variety of inflammatory diseases are motivated mainly by the medical needs for drugs with fewer side effects than the ones in current use [4]. In the treatment of inflammatory diseases, basically two types of drugs are used, steroidal anti-inflammatory drugs (SAIDs) such as cortisone, and non-steroidal anti-inflammatory drugs (NSAIDs) such as diclofenac, indomethacin and aspirin. SAIDs inhibit phospholipase A_2 enzyme which converts membrane phospholipids to free fatty acids such as arachidonic acid. NSAIDs mainly inhibit the cyclo-oxygenase pathway of the arachidonic acid cascade, preventing the formation of proinflammatory prostaglandins [5].

Xylene-induced ear oedema model is useful for the evaluation of anti-inflammatory topical steroids and non-steroidal antiphlogistic agents, especially those inhibiting phospholipase A_2 [6]. Application of xylene induces acute neurogenous edema, which is partially associated with the substance P. Substance P is widely distributed in the central and peripheral nervous system and its release from sensory neurons in the periphery causes vasodilatation and plasma extravasations leading to swelling of the ear, suggesting the role of xylene in neurogenous inflammation [7]. Moreover, the ear oedema associated with xylene involves inflammatory mediators such as histamine, kinin, and fibrinolysin [8].

In some traditional practices, extracts from one part or a combination of different parts of a single plant or of different plants have been used for therapeutic purposes [9]. Scientific reports suggested synergistic and additive effects of many phytochemicals present in these plant parts, coupled with other advantages such as low doses of the individual components in the mixture [10]. *Ceiba pentandra* (Bombacaceae) is a large tree reaching a height of 50 m and 2 m of diameter. It is widely used in traditional medicine in different parts of the world [11]. Studies have shown that parts of the plant possess antidiarrhoeal [12], antibacterial [10], anti-inflammatory [13], analgesic [14], antidiabetic [15] and antispasmodic and diuretic properties [16]. Some of our earlier studies have shown that the stem bark of *Ceiba pentandra* is rich in phytochemicals and posses antimicrobial properties [17]. Also, seeds of *C. pentandra* possess antioxidant and antimicrobial properties [18]. Other pharmacological potentials of *C. pentandra* were recently reviewed [4]. It is therefore imperative to study the effect of methanol extract of the plant stem back in the treatment of induced inflammation and pain.

Methods

Collection and authentication of plant materials

Fresh stem bark of *Cieba petandra* was collected from a farm settlement in Nsukka community and was authenticated at the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, Enugu State, Nigeria. Voucher specimen was deposited in the herbarium of the Department

Management of experimental animals

Animals used for the study were male albino mice of weight of 25-35 g and albino rats of weight 150-200 g. The animals were obtained from the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. Before the experiment, the animals were acclimatized under standard laboratory condition in the animal farm of the Department of Home Science and Nutrition, Faculty of Agriculture, University of Nigeria, Nsukka for 14 days with free access to water and fed with pelletized growers feed *ad libitum*. They received human care throughout the

experimental period in accordance with the ethical rules and recommendations of the University of Nigeria committee on the care and use of laboratory animals and the revised National Institute of Health Guide for Care and Use of Laboratory Animal (Pub No.85-23, revised 1985).

Preparation of methanol extract

Stem bark of *Cieba pentandra* (1,500 g) was washed in running water and chopped into small pieces. The pieces were air-dried and ground into powder. The ground sample was weighed and soaked with 4.5 L of methanol and allowed to stand for 48 hours. This was filtered using Muslim bag followed by Whatman No. 42 filter paper. The filtrate was evaporated under reduced pressure and dried using a rotary evaporator at 55°C. The dried extract was stored in a labeled sterile screw capped bottle at 2-8°C. The percentage yield of the extract was 5.32 %.

Acute toxicity test

This study adopted the method described by Lorke [19]. Eighteen mice, divided into 6 groups of 3 mice each, were acclimatized for 14 days and were used for this experiment. Six doses (10, 100, 1000, 1900 and 2700 and 5000 mg/kg) of the methanol extract of *Cieba pentandra* stem bark were administered orally to the animals and the general behavioral, neurological and autonomic profile was observed for 24 hours.

Acetic acid-induced writhing test

The writing test was performed using a method described [20]. Twenty albino mice were acclimatized for 14 days and divided into 5 groups of 4 animals each and administered treatments orally, as shown in Table 1. After 60 minutes of administration of treatments, 1% (v/v) acetic acid (10 ml/kg) was injected intraperitoneally to all the mice. The number of writhes formed after 30 minutes of administration of acetic acid was recorded and the percentage inhibition was calculated.

$$\% \text{ Inhibition} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

Tail flick latency test

A total of twenty mice, divided into 5 groups of 4 mice each were treated as shown in Table 1. One hour later, the tail of the mice were immersed in hot water bath at 50 ± 5°c. The time it took for the test animals to completely withdraw their tail from the hot water was recorded [21]. The percentage protection was calculated as follows:

$$\% \text{ Protection} = \frac{\text{Latency of Test} - \text{Latency of control}}{\text{Latency of Control}} \times 100$$

Xylene-induced ear oedema test

Twenty mice, divided into 5 groups of 4 mice each, were treated as shown in Table 1, 30 minutes before induction of inflammation by topical application of xylene. Two drops of xylene were applied on the inner surface of the right ear and allowed to act for 15 minutes. The rats were anaesthetized with chloroform and the left and right ears were cut off and circular sections were taken using a cork borer with diameter of 7 mm and weighed. The difference between the weights of the two ears was recorded [22]. The percentage inhibition was calculated as follows:

$$\% \text{ Inhibition} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

Egg albumin-induced inflammation test

Fresh egg albumin-induced rat hind paw oedema was used as model for acute inflammation [23]. Twenty adult albino rats divided into 5 groups of 4 rats each were used in this experiment. The animals were fasted and deprived of water for 18 hours before the experiment. Deprivation of water was to ensure uniform hydration and to minimize variability in oedematous response. The right hind paw volumes were taken at time zero (t = 0) volume ($V = V_o$) using water displacement method and the rats were treated orally as shown in table 1. One hour later, inflammation of the hind paw was induced by injecting 0.1 ml of undiluted fresh egg albumin into the sub plantar surface of the right hind paw. The paw volumes were measured and recorded after 30, 60, 90, 120, 150 and 180 minutes

($t = t_n$) as V_t. Paw Oedoma Volume = $V_t - V_o$. The percentage inhibition was calculated as follows:

$$\% \text{ Inhibition} = \frac{\text{Paw oedema of control} - \text{Paw oedema of test}}{\text{Paw oedema of control}} \times 100$$

Acetic acid-induced vascular permeability test

Twenty albino rats divided into 5 groups of 4 rats each were used in this study. The animals were fasted for 10 hours prior to the experiment and treated with graded doses of extract, normal saline and indomethacin as shown in Table 1. Thirty minutes later, each animal was given an intravenous injection of 10% (w/v) solution of Evans blue (10 ml/kg b.w). Vascular permeability was induced by intravenous injection of 6% acetic acid (10 ml/kg b.w), 30 minutes after the Evans blue administration. After 20 min, the rats were sacrificed. Normal saline (10 ml) was used to wash the intraperitonium into tubes and centrifuged at 2000 rpm for 10 minutes. The absorbance of the supernatant was read spectrophotometrically at 610 nm.

Statistical analysis

The primary data were analyzed using one way analysis of variance (ANOVA) in IBM Statistical Product and Service Solution (SPSS) software, Version 16. The results were expressed as mean ± standard deviation (SD). The Fischer LSD post hoc test was used to test the difference between means of treated and control groups. Differences between means at $p < 0.05$ were regarded as statistically significant.

Results

Acute toxicity profile of the extract

At the all the doses of methanol extract of *C. pentandra* stem bark given orally to mice for acute toxicity study, there was neither clinical signs nor mortality after 24 hours post-treatment observation. Therefore, the median lethal dose (LD_{50}) value of the extract was estimated to be above 5000 mg/kg body weight (Table 2).

Effect of the plant extract on the acetic acid-induced writhing

The methanol extract of *Ceiba pentandra* stem bark at the doses of 100, 200 and 400 mg/kg body weight and Indomethacin 10 mg/kg body weight produced significant ($p < 0.05$) reduction in the number of writhes when compared to control (untreated group). The percentage reduction in number of writhes at the doses administered were 29.0%, 43.0%, 49.0% and 58.0% respectively. Hence, the three doses tested (100, 200 and 400 mg/kg) produced significant ($p < 0.05$) analgesic activity (Table 3).

Effect of the plant extract on the tail flick latency

The methanol extract of *C. pentandra* stem bark at the doses of 100, 200 and 400 mg/kg body weight and Indomethacin 10 mg/kg body weight induced significant ($p < 0.05$) reduction in pain sensation when compared to control (untreated group) in the tail flick latency method. The percentage reduction in pain sensation after oral administration of the plant extract at the doses of 100, 200 and 400 mg/kg body weight and the standard drug, Indomethacin 10 mg/kg body weight were 27.0%, 45.0%, 58.0% and 69.0% respectively. These showed that the methanol extract of *C. pentandra* stem bark posses analgesic properties (See Table 4).

Effect of the plant extract on xylene-induced ear oedema

The anti-inflammatory activity of the plant extract was measured at doses of 100, 200 and 400 mg/kg body weight against xylene-induced acute ear oedema. A strong inhibition of the ear oedema was observed with the different doses of the extract and with Indomethacin (standard drug). The three doses tested (100, 200 and 400 mg/kg) produced significant ($p < 0.05$) anti-inflammatory activity and reduced the ear oedema size by 13.6%, 19.7% and 28.8% respectively, whereas Indomethacin caused 34.8% reduction (Table 5).

Effect of the plant extract on acetic acid-induced vascular permeability

The effect of the plant extract on acetic acid-induced vascular permeability was measured at doses of 100, 200 and 400 mg/kg body weight. The percentage reduction in vascular permeability at the doses of the extract (100, 200 and 400 mg/kg b.w) and 10 mg/kg b.w Indomethacin were 38.0%, 42.1%, 54.0% and 62.3% respectively. The present result showed that methanol extract of *Ceiba pentandra* stem bark has anti-inflammatory potential which is dose-dependent (Table 6).

Effects of the extract on egg albumin-induced inflammation

The anti-inflammatory activity of the plant extract was measured at doses of 100, 200 and 400 mg/kg body weight against egg albumin-induced acute oedema. A strong reduction in the oedema volume was observed with the different doses of the extract and with Indomethacin (standard drug) and in a time-dependent manner. The 400 mg/kg produced significant ($p < 0.05$) anti-inflammatory activity and reduced the oedema volume comparable to that by Indomethacin than the untreated group and those treated with lower doses of the extract (Table 7).

Discussion

Inflammation has different phases; the first phase is caused by an increase in vascular permeability, the second one by infiltrate of leucocytes and the third one by granuloma formation. The anti-inflammatory activity of *Ceiba pentandra* stem bark was determined using inhibition of xylene-induced and egg albumin-induced oedema, and acetic acid-induced vascular permeability which are good methods to screen anti-inflammatory agents. Similar to carrageenan-induced oedema, the development of xylene-induced oedema is bi-phasic; the first phase is attributed to the release of histamine, serotonin and kinins and the second phase is related to the release of prostaglandins and bradykinins [24-26].

The anti-inflammatory potential of methanol extract of *C. pentandra* stem bark against xylene-induced ear oedema is dose-dependent. This response tendency of the extract on xylene-induced ear oedema revealed good peripheral anti-inflammatory properties of the extract, which is comparable to that of indomethacin. Indomethacin is a NSAID commonly used as anti-inflammatory, analgesic and anti-pyretic agents. Indomethacin is a non-selective inhibitor of cyclooxygenase (COX) 1 and 2; enzymes that participate in prostaglandin synthesis from arachidonic acid. Prostaglandins (PG) are hormone-like molecules normally found in the body, where they have a wide variety of effects, some of which lead to pain, fever, and inflammation [27]. Elion-Itou *et al.* [14] also showed that aqueous extract of *Ceiba pentandra* stem bark possess anti-inflammatory and analgesic potentials, though at higher doses compared to those used in this study. Also, the analgesic potential of the extract was determined using tail flick latency and acetic acid-induced writhing tests.

The anti-inflammatory effect of *C. pentandra* extract may be due to the presence of flavonoids. It has been reported that a number of flavonoids possess anti-inflammatory [28] and analgesic [29] activities. Flavonoids are known to inhibit the enzyme, prostaglandin synthetase, more specifically the endoperoxidase and reported to produce anti-inflammatory effect. Since, prostaglandins are also involved in the pain perception; inhibition of their synthesis might be the possible mechanism for the analgesic activity of the methanol extract. The presence of flavonoids, as we reported earlier from our previous study [17] may be responsible for the analgesic and anti-inflammatory activities in methanol extract. Flavonoids are known to inhibit the enzyme prostaglandin synthetase, more specifically the endoperoxidase and have been reported to produce anti-inflammatory effect. Since, prostaglandins are also involved in pain perception; inhibition of their synthesis might be the possible reason for the analgesic activity of the extract.

The presence of flavonoid earlier identified might be responsible for the analgesic and anti-inflammatory activities in methanol extract. Most NSAIDs are toxic [30], hence, being relatively safe at

the doses used and having shown significant anti-inflammatory and analgesic activities comparable to the standard drugs used, it is thus recommended that the active compounds of the methanol extract of *C. pentandra* stem bark be isolated and studied further for the treatment of inflammatory diseases.

In conclusion, result from this study showed that the methanol extract of *C. pentandra* stem bark produced significant analgesic and anti-inflammatory activities in a dose-dependent manner.

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Table 1: Experimental design for acetic acid-induced writhing test

Study Groups	Number of Animals per group	Treatment Administered
Group 1 (Normal control)	4	5 ml/kg b.w normal saline
Group 2 (Low dose)	4	100 mg/kg b.w methanol extract of *C. pentandra* stem bark (SBME)
Group 3 (Mid-dose)	4	200 mg/kg b.w of SBME
Group 4 (High dose)	4	400 mg/kg b.w of SBME
Group 5 (Standard control)	4	10 mg/kg b.w indomethacin

Table 2: Acute toxicity profile of the plant extract

Group	Dose (mg/kg body weight (b.w.))	Number of Deaths after 24 hours
Group 1	10	0
Group 2	100	0
Group 3	1000	0
Group 4	1900	0
Group 5	2700	0
Group 6	5000	0

Table 3: Effect of SBME on acetic acid-induced writhing test in albino mice

Experimental Groups	Number of Writhes	% Inhibition
Group 1 (5 ml/kg b.w Normal saline)	41.30 ± 1.75^{a}	0.0
Group 2 (100 mg/kg b.w SBME)	29.30 ± 2.03^{b}	29.0
Group 3 (200 mg/kg b.w SBME)	23.70 ± 1.30^{c}	43.0
Group 4 (400 mg/kg b.w SBME)	20.70 ± 0.89^{d}	49.0
Group 5 (10 ml/kg b.w Indomethacin)	17.30 ± 1.26^{e}	58.0

Results are Mean ± SD (n = 4). Values with different superscripts in a column are statistically significant at $p < 0.05$.

Table 4: Effect of SBME on tail flick latency test in albino mice

Experimental Groups	Flick Latency	% Protection
Group 1 (5 ml/kg b.w Normal saline)	2.88 ± 0.29^{a}	0.0
Group 2 (100 mg/kg b.w SBME)	3.66 ± 0.07^{b}	27.0
Group 3 (200 mg/kg b.w SBME)	4.17 ± 0.75^{c}	45.0
Group 4 (400 mg/kg b.w SBME)	5.55 ± 0.20^{d}	58.0
Group 5 (10 ml/kg b.w Indomethacin)	6.86 ± 0.29^{e}	69.0

Results are Mean ± SD (n = 4). Values with different superscripts down the column are statistically significant at $p < 0.05$.

Table 5: Effect of SBME on xylene-induced ear oedema test in albino mice

Experimental Groups	Oedema Size (g)	% Inhibition
Group 1 (5 ml/kg b.w Normal saline)	0.066 ± 0.004^{a}	0.0
Group 2 (100 mg/kg b.w SBME)	0.057 ± 0.006^{b}	13.6
Group 3 (200 mg/kg b.w SBME)	0.053 ± 0.007^{c}	19.7
Group 4 (400 mg/kg b.w SBME)	0.044 ± 0.009^{d}	28.8
Group 5 (10 ml/kg b.w Indomethacin)	0.043 ± 0.011^{e}	34.8

Results are Mean ± SD (n = 4). Values with different superscripts down the column are statistically significant at $p < 0.05$.

Table 6: Effect of SBME on acetic acid-induced vascular permeability test in albino rats

Experimental Groups	Absorbance	% Inhibition
Group 1 (5 ml/kg b.w Normal saline)	0.13 ± 0.003^{a}	0.0
Group 2 (100 mg/kg b.w SBME)	0.08 ± 0.000^{b}	38.0
Group 3 (200 mg/kg b.w SBME)	0.07 ± 0.000^{c}	42.1
Group 4 (400 mg/kg b.w SBME)	0.06 ± 0.001^{d}	54.0
Group 5 (10 ml/kg b.w Indomethacin)	0.05 ± 0.001^{e}	62.3

Results are Mean ± SD (n = 4). Values with different superscripts down the column are statistically significant at $p < 0.05$.

Table 7: Effect of SBME on egg albumin-induced paw oedema in albino rats

Groups	Paw Oedema Volume based on Time Duration (% Inhibition)					
	30 min	60 min	90 min	120 min	150 min	180 min
Group 1	$1.02\pm0.10^{a, A}$	$0.99\pm0.10^{a, A}$	$0.96\pm0.14^{a, A}$	$0.94\pm0.12^{a, A}$	$0.97\pm0.18^{a, A}$	$0.97\pm0.22^{a, A}$
Group 2	$0.83\pm0.09^{a, B}$ (18.8%)	$0.80\pm0.08^{b, B}$ (21.9%)	$0.75\pm0.11^{b, B}$ (22.7%)	$0.69\pm0.07^{c, B}$ (27.1%)	$0.65\pm0.06^{d, B}$ (33.0%)	$0.61\pm0.07^{d, B}$ (36.8%)
Group 3	$0.85\pm0.24^{a, B}$ (20.7%)	$0.81\pm0.25^{a, B}$ (19.3%)	$0.78\pm0.16^{a, B}$ (21.7%)	$0.72\pm0.21^{a, B}$ (24.0%)	$0.68\pm0.21^{a, B}$ (29.7%)	$0.64\pm0.07^{a, B}$ (34.0%)
Group 4	$0.73\pm0.12^{a, C}$ (29.7%)	$0.69\pm0.12^{a, C}$ (32.1%)	$0.64\pm0.13^{b, C}$ (33.6%)	$0.62\pm0.13^{b, C}$ (35.6%)	$0.58\pm0.12^{b, C}$ (40.0%)	$0.54\pm0.12^{b, C}$ (44.3%)
Group 5	$0.91\pm0.13^{a, D}$ (12.8%)	$0.84\pm0.14^{b, B}$ (17.4%)	$0.73\pm0.06^{c, B}$ (23.6%)	$0.67\pm0.10^{d, C}$ (28.6%)	$0.61\pm0.08^{e, C}$ (36.9%)	$0.52\pm0.06^{f, C}$ (46.1%)

Results are Mean ± SD (n = 4). Values with different small case alphabets as superscripts in a column are statistically significant at $p < 0.05$; Values with different upper case alphabets as superscripts across the row are statistically significant at $p < 0.05$. Values in parenthesis "()" are percentage inhibition of inflammation calculated relative to control.