British Journal of Pharmaceutical Research
3(3): 391-400, 2013

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The Effect of Leaf Aqueous Extract of *Brachystegia eurycoma* Harms (Fabaceae) in Acute and Chronic Inflammatory Animal Models

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Authors' contributions

This work was carried out in collaboration between all authors. Author II designed the study, managed the literature searches, wrote the protocol, and the first draft of the manuscript. Author GOI performed the extraction, Authors II and GOI carried out the anti-inflammatory evaluation. Author GOI performed the statistical analysis. All authors read and approved the final manuscript

Research Article

Received 26th December 2012
Accepted 5th March 2013
Published 12th April 2013

ABSTRACT

Aims: To investigate the effect of the leaf extract of *Brachystegia eurycoma* Harms (Leguminosae) in acute and chronic Inflammatory animal models.
Study Design: Extraction and anti-inflammatory evaluation.
Place and Duration of Study: Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin.
Methodology: The anti-inflammatory effect of the aqueous leaf extract of *B. eurycoma* Harm (Leguminosae) was evaluated using the carrageenan- and dextran-induced rat paw edema, xylene-induced ear edema and formalin-induced arthritis inflammation test.
Results: In the carrageenan-induced paw edema, *B. eurycoma* at 100 mg/kg significantly ($P<0.001$) inhibited paw edema within four hours (1–4 h) The extract also produced significant inhibitory effects ($P<0.05$) at 200 mg/kg (1h and 4 h) and 400 mg/kg (2 h). *B. eurycoma* (100 mg/kg) produced a significant ($P<0.01$) inhibition of paw edema induced by dextran from 0 h and 4th h and at the 5th h ($P<0.05$) The extract (100 mg/kg) produced a significant inhibition (41.84 %, $p<0.05$) in the xylene induced ear edema model but in chronic inflammation (formalin induced arthritis) did not show any significant anti-inflammatory activity after seven (7) days. Oral acute toxicity assays did not show any

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mortality at 8 g/kg of the plant extract.
Conclusion: These results suggest that the aqueous leaf extract of *B. eurycoma* possesses significant anti-inflammatory activity on acute inflammation but no effect on chronic inflammation, thus supporting the usage of the plant in traditional medicine treatment of inflammation.

Keywords: Anti-inflammatory; arthritis; edema; Brachystegia eurycoma.

1. INTRODUCTION

The use of plants as herbal drugs has provided us with some of the very important life-saving drugs used in the armamentarium of modern medicine [1]. However, among the estimated 250,000-400,000 plant species, only 6% have been studied for biological activity, and about 15% have been investigated phytochemically [2,3]. This shows a need for planned activity guided phyto-pharmacological evaluation of herbal drugs. Several modern drugs are used to treat inflammatory disorders but, their prolonged use may cause severe adverse side effects, the most common being gastrointestinal bleeding and peptic ulcers [4]. Consequently, there is a need to develop new anti-inflammatory agents with minimum side effects. Many herbal preparations are being prescribed widely for the treatment of inflammatory conditions [5].

B. eurycoma Harms (Leguminosae) is a tree common along river banks of the forest zone in southern Nigeria and Cameroun [6,7]. It is also a very popular plant in the Eastern part of Nigeria. Its local names are; Achi (Igbo), Akalado or Eku (Yoruba), Akpakpa (Ijaw), Dewen (Bini), Okwen (Edo), Okung (Efik).The leaves are stipulate, nearly always alternate, and range from bipinnately to palmately compound to simple. The fruits are legumes, very conspicuous and persistently woody. The seeds often have a hard coat with hourglass-shaped cells, and sometimes bear a U-shaped line called a pluerogram (Carr) [7,8]. The seeds have been shown to contain alkaloids, flavonoids, saponins and tannins [9]. The leaves extract of *B. eurycoma* have been found to contain volatile oils such as oxygenated monoterpenoids and sesquiterpenoid hydrocarbon. Other compounds identified in the leaves and stem bark are 1,8 – cineole, acorenone, β- caryophyllene and geranyl acetone [10]. The stem bark extract also contains tannins and flavonoids [11] and has been shown to posses both analgesic and anti-inflammatory activity [12]. *B. eurycoma* has been widely used in the Eastern part of Nigeria and some African countries as emulsifiers and thickners in traditional soups [8]. Products from the tree bark have found application as fibres, food wrappers and in making containers. The timber products are used as building materials in carpentry. The seeds and leaves are used traditionally in the treatment of various inflammation-related disorders like scabies, asthma, tuberculosis, bronchitis, and guinea worm infections [13]. This has therefore created the need for the present study which is to investigate the anti-inflammatory activities of the aqueous extract of the leaves of *B. eurycoma* using various experimental animal models of inflammation.

2. MATERIALS AND METHODS

2.1 Sample Collection and Extraction

The leaves of *B. eurycoma* were collected in the month of June 2012 on the bank of a river in Iwo, Osun State. The plant was identified by Prof. Idu of the Department Plant Biology and

Biotechnology, University of Benin, Benin City. The fresh leaves were air- dried for 2-3 days in the laboratory and later oven-dried at 50^{o}C for 6 h after which they were reduced to powder form using an electric mill. The powdered plant material (250 g) was extracted with 1500 ml of water using soxhlet apparatus. The extract obtained was concentrated to dryness using a rotary evaporator. The weight of the dried extract obtained was 52.23 g giving a yield of 20.9%. The dried extract was stored in a clean glass container in the refrigerator until use.

2.1.1 Chemicals

λ-Carrageenan, dextran- A, indomethacin and diphenhydramine were obtained from Sigma Chemical Co. (St. Lious, MO, U.S.A), xylene and formaldehyde were obtained from BDH Chemicals, UK, while dexamethasone was obtained from Vardhman Exports, India.

2.2 Experimental Animals

All experiments were performed using Wistar rats (160- 220 g) and Swiss albino mice (24-35 g). All the animals were obtained from the Animal house of the Department of Pharmacology and Toxicology, University of Benin. All animals were maintained under standard environmental conditions and had free access to standard rodent cubes and water *ad libitum*. Experimental protocol was approved by Institutional Ethics Committee of the Faculty of Pharmacy, University of Benin (approval no. EC/FP/12/14). Animals were handled in accordance with international principles guiding the use and handling of experimental animals (United States National Institutes for Health Publication, 1985).

2.3 Acute Toxicity Study

Overnight-fasted Swiss albino mice (24-35 g) of either sex were used for the study. The animals were divided into five groups of five animals each. Groups A to D received orally 1, 2, 4 and 8 g/kg of the extract, respectively, while the control (group E), received distilled water (3 mL/kg) by the same route. General symptoms of toxicity and mortality in each group were observed within 24 h. Animals that survived after 24 h were observed for any signs of delayed toxicity for two weeks.

2.4 Tests for Anti-inflammatory Activity

2.4.1 Carrageenan-induced paw edema

In determining carrageenan-induced paw edema, Wistar rats (160-220g) were divided into five groups of five rats each. The test groups received 100, 200 and 400 mg/kg (*p.o*) of the extract. The reference group received indomethacin (10 mg/kg, *p.o.*) while the control group received 3 ml/kg of distilled water. After 1 h, 0.1ml of 1% w/v carrageenan suspension in normal saline was injected into the subplantar tissue of the right hind paw. Paw thickness was measured using a vernier caliper at hourly intervals for 5 h [14,15].

2.4.2 Dextran-induced paw edema

In determining dextran-induced paw edema, Wistar rats (160-220g) were divided into five groups of five rats each. The test groups received 100, 200 and 400 mg/kg (*p.o*) of the extract. The reference group received diphenhydramine (60 mg/kg, *p.o.*) while the control group received 3 ml/kg of distilled water. After 1 h, 0.1ml of 1.5% w/v dextran in normal

saline was injected into the subplantar tissue of the right hind paw [16] Paw thickness was measured using a vernier caliper at hourly intervals for 5 h.

2.4.3 Xylene-induced ear edema in mice

Swiss albino mice (25-35 g) were allotted to five groups of five mice each. The test groups received 100, 200 and 400 mg/kg, *p.o.* of the extract. The reference group received dexamethasone (1 mg/kg, *p.o.*) while the control group received 3 ml/kg of distilled water. Thirty minutes after, edema was induced in each mouse by applying a drop of xylene to the inner surface of the right ear. Fifteen minutes later, the animals were sacrificed under chloroform anaesthesia and both ears cut off, sized and weighed. The mean difference between the ear weights was taken as the edema induced by xylene [17].

2.4.4 Formalin-induced arthritis

In determining formalin-induced arthritis, Wistar rats (160-220g) were divided into five groups of seven rats each. Inflammation was produced by subaponeurotic injection of 0.1 ml of 2% w/v formalin in normal saline in the right hind paw of rats on the first and third day. The extract (100, 200 and 400 mg/kg) and distilled water (3 ml/kg) were administered orally once a day for 7 days. Indomethacin (5 mg/kg) given orally, was used as standard. The rat paw thickness was measured daily for 7 days using vernier caliper [15,18]. The percentage reduction of paw thickness of each group was calculated on the seventh day and compared with the control.

2.5 Statistical Analysis

Experimental data were expressed as the mean ± standard deviation error of the mean (S.E.M). Differences between the treatment groups were ascertained using one way ANOVA followed by the Bonferroni test performed using GraphPad Prism 5.00.288, GraphPad Software Inc., San Diego, California, USA. ***P*** values less than 0.5 were considered as indicative of significance.

3. RESULTS AND DISCUSSION

Acute toxicity studies showed that all the doses (1, 2, 4, and 8 g/kg) of the *B. eurycoma* extract used for the study were non-toxic. In the carrageenan-induced paw edema (Fig. 1) the aqueous leaf extract of *B. eurycoma* at 100 mg/kg significantly ($P< 0.001$) inhibited paw edema within four hours (1 – 4 h) of the inflammatory response compared to the negative control. The extract also produced significant inhibitory effects ($P<0.05$) at 200 mg/kg (1h and 4 h) and 400 mg/kg (2 h) when compared to the negative control. The inhibitory effect was comparatively less than for indomethacin.

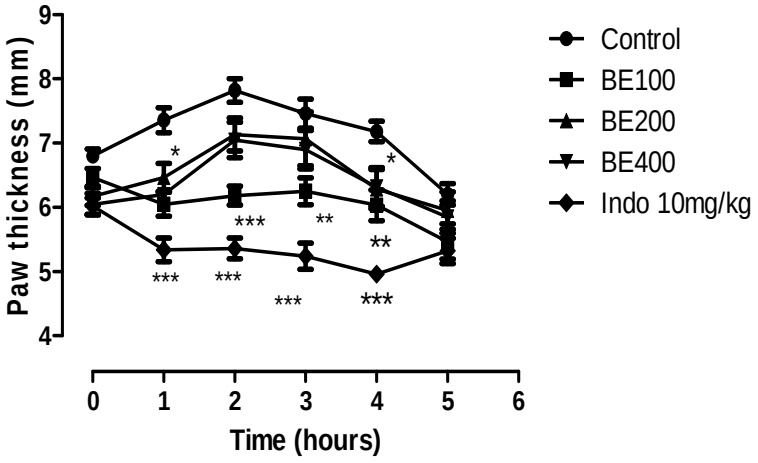

Fig. 1. Effect of Aqueous leaf extract of *B. eurycoma* on carrageenan-induced paw edema in rats

**P<0.05, **P<0.01, ***P<0.001 as compared to the control (n =5 for each group). BE = Brachystegia eurycoma, Indo = Indomethacin.*

In the dextran-induced paw edema (Fig. 2) the extract at 100 mg/kg produced a significant (P<0.01) inhibition of paw edema from 0 h and 4th h and at the 5th h (P<0.05) when compared to the negative control. The extract's inhibitory effect was comparable to the positive control; diphenhydramine.

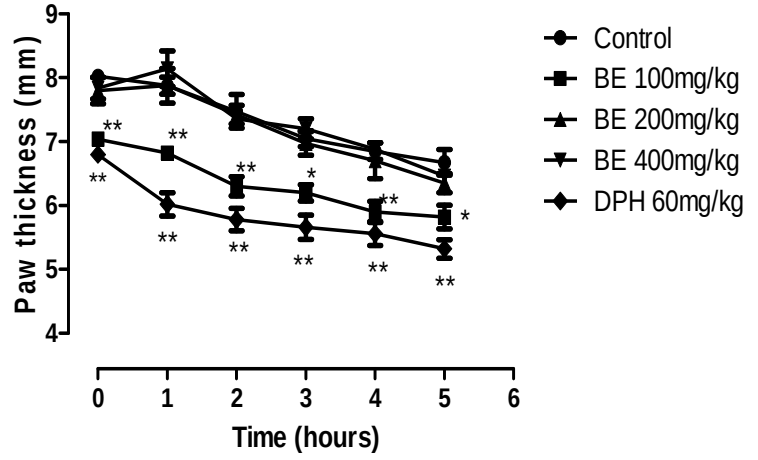

Fig. 2. Effect of Aqueous leaf extract of *B. eurycoma* on dextran-induced paw edema in rats

**P<0.05, **P<0.01, ***P<0.001 as compared to the control (n=5 for each group). BE = Brachystegia eurycoma, DPH = Diphenhydramine.*

On xylene-induced ear edema (Table 1), the extract (100 mg/kg) significantly (p<0.05) inhibited edema. Table 2 shows the effect of the extract on formalin-induced arthritis. The extract (100, 200 and 400 mg/kg) did not produce any significant inhibition (P>0.05).

Table 1. Effect of aqueous extract of *B. eurycoma* on xylene-induced ear edema in mice

Treatment	Dose (mg/kg)	Weight of left ear (mg)	Weight of right ear (mg)	Difference (mg)	% Inhibition
Control	3 ml/kg	51.47±3.51	41.67±4.24	9.8±1.55	-
B. eurycoma	100	56.10±7.78	52.23±7.45	5.70±0.51*	41.84
	200	67.13±4.89	60.55±6.60	8.58±3.62	12.44
	400	69.75±2.62	60.15±1.37	9.60±2.42	2.04
Dexamethasone	1	44.28±5.30	39.06±4.64	4.32±1.94*	55.92

**P<0.05 as compared to the control (n =5 for each group). Values are mean ± S.E.M. as compared to the control (n=5).*

Table 2. Effect of aqueous leaf extract of *B. eurycoma* on formalin-induced arthritis in rats

Treatment	Dose (mg/kg)	Paw Thickness(mm) on Day 7	% Inhibition
Control	3 ml/kg	7.05±0.05	-
B. eurycoma	100	7.68±0.18	-
	200	7.25±0.21	-
	400	7.28±0.19	-
Indomethacin	5	5.73±0.30*	18.70

**P<0.05 as compared to the control. Values are mean ± S.E.M. as compared to the control (n=7).*

Although traditional medicine practitioners claim to treat infections, pain and inflammation with *B. eurycoma* [13] but no documented results have been reported on the anti-inflammatory properties of the aqueous leaf extract of this plant; hence, the need to evaluate this claim and determine the possible mechanism(s) of anti-inflammatory activities.

In this study, the anti-inflammatory activity of the aqueous leaf extract of *B. eurycoma* has been evaluated in both acute and chronic inflammatory models. The inhibition of carrageenan-induced inflammation in rats is an established model for evaluating anti-inflammatory drugs, which has been used frequently to assess anti-edematous effect of natural products [15]. Carrageenan as a phlogistic agent is non antigenic and is devoid of apparent systemic effect [19]. This model is based on the principle of release of various inflammatory mediators by carrageenan. Edema formation due to carrageenan in the rat paw is a biphasic event, with the initial phase (occurs within the first one hour) being attributed to the release of histamine and serotonin, while the second phase (> 1.0h) of edema is due to the release of prostaglandins, protease and lysosome [20,21]. Platelet activating factor and arachidonic acid metabolites also play a role in the first phase [22]. The second phase is mediated by an increased release of prostaglandins, arachidonate metabolites, neutrophil migration, release of oxygen free radicals, proteolytic enzymes, as well as other neutrophil-derived mediators [22,23]. The edema maintained between the first and second phase (1-2h) is due to kinin-like substances, especially bradykinin [21].

The aqueous leaf extract of *B. eurycoma* significantly inhibited paw edema in a dose-dependent manner with the lowest dose (100 mg/kg) producing inhibitory effect in the first, second as well as the continuity phases of the inflammation. Inhibition of the first phase suggests antihistamine activity of the extract that could impair microvascular leakage induced by carrageenan [24]. Histamine stimulates vessel endothelial cells to increase

vascular permeability [24] resulting in the outpouring of cells and fluid. Inhibition of the second phase suggests a possible inhibition of cyclooxygenase synthesis because the carrageenan inflammatory model basically reflects the actions of prostaglandins [25,26]. The effect of the extract on the maintenance phase indicates that the extract had blocked bradykinin release and/or its vascular permeability promoting action. This effect is similar to that produced by non-steroidal anti-inflammatory drugs such as indomethacin whose mechanism of action is inhibition of cyclooxygenase enzyme which catalyses the synthesis of cyclic endoperoxides important in the formation of prostaglandins [27]. Dextran-induced paw edema has been reported to be mediated mainly by histamine and serotonin release by the mast cells [28]. The release of these inflammatory mediators result in marked vascular changes including; vasodilatation, increased permeability and reduction of blood flow, eventually leading to an increase in paw size. The aqueous leaf extract of *B. eurycoma* inhibited paw edema in a dose-dependent manner with the lowest dose (100 mg/kg) having the significant effect. This finding suggests an inhibitory effect on H_1-receptors by the extract similar to known H_1-receptor antagonists such as diphenhydramine which was used as the positive control. However, the reason why the lowest dose (100 mg/kg) produced the greatest effect may be attributed to an increase in the concentrations of constituents that may interfere with its anti-inflammatory action as the dose of the crude plant extract increased.

The xylene ear edema model permits the evaluation of anti-inflammatory steroids and is less sensitive to non-steroidal anti-inflammatory agents [29]. After topical application of xylene, marked increases in ear weight were detected as a result of the acute inflammation response; these increases in ear weight have been used as valuable markers for anti-inflammatory effects [30,31]. Histopathologically, severe vasodilation, edematous changes of skin and infiltration of inflammatory cells were detected as signs of acute inflammation after topical application of xylene [32]. In the present study, the increases in ear weight was inhibited by the extract at 100 mg/kg suggesting there was inhibition of phospholipase A_2 similar to that produced by anti-steroids such as dexamethasone. These anti-steriod agents facilitate the production of lipocortin which then blocks phospholipase A_2, an enzyme responsible for the conversion of phospholipids to arachidonic acid [33]. The aqueous extract produced no significant inhibitory effect in formalin-induced arthritis at doses 100, 200 and 400 mg/kg in this study. The inhibition of edema induced by formalin in rats is one of the most suitable test procedures to screen anti-arthritis and anti-inflammatory agents, as it closely resembles human arthritis [34]. Arthritis induced by formalin is a model used for the evaluation of an agent with probable anti-proliferative activity [35]. The data ruled out any possible effect of the extract on formalin induced cell damage and accordingly, arthritic conditions.

4. CONCLUSION

In conclusion, the aqueous leaf extract of *B. eurycoma* possess dose-dependent anti-inflammatory properties to acute inflammation (carrageenan and dextran-induced paw edema) but has no significant inhibitory effect on chronic inflammation (formalin-induced arthritis). These results therefore support the ethnomedical use of the leaf extract of *B. eurycoma* for treatment of conditions involving acute inflammation.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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