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Anti-inflammatory and Analgesic Effects of the Methanol Stem Bark Extract of *Brachystegia eurycoma* Harms (Fabaceae)

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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 BAA and AI managed the analyses of the study. Author II performed the statistical analysis. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: The aim of the study was to investigate the analgesic and anti-inflammatory activities of the methanol stem bark extract of *Brachystegia eurycoma* Harms.
Place and Duration of Study: Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin.
Methodology: Analgesic activity was examined using the hot plate and acetic acid-induced writhing test while acute anti-inflammatory effect was studied using carrageenan and dextran-induced paw edema.
Results: Oral administration of the extract produced significant ($P<0.01$) antiedematogenic effect with a dose of 100 mg/kg within the 2nd and 4th h of the experiment in the dextran-induced paw edema and within the 1st and 3rd h in the carrageenan model. The extract exhibited a significant ($P<0.001$) inhibition of acetic acid-induced writhing. In the hot plate test, the extract (100 mg/kg) significantly ($P<0.05$) prolonged the reaction latency to pain at the 60 min. Oral acute toxicity studies did not show any mortality at 5 g/kg of the plant extract.
Conclusion: This study showed that *Brachystegia eurycoma* possesses significant anti-inflammatory and analgesic properties.

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Keywords: Brachystegia eurycoma; analgesic; anti-inflammatory; prostaglandins.

1. INTRODUCTION

Brachystegia eurycoma Harms is a woody plant mostly found in the forest zone. The tree is about 35m tall, with bole of 2m diameter. It is vaguely buttressed, has low branching, large, flat crown common on river banks of the forest zone in southern Nigeria and Cameroun (Dalziel, 1937; Keay et al., 1964). It is also a very popular plant in the Eastern part of Nigeria. It flowers between April and May and bears fruits between September and January. The fruits are very conspicuous and persistently woody. The sap wood is white, not durable and quickly rotting on exposure. It may be readily recognized by its large size, irregular bole and huge twisted spreading branches and by the rough fibrous bark which peels off in untidy patches and often exudes a brownish buttery gum. In Nigeria, it is commonly called Achi (Igbo), Akalado or Eku (Yoruba), Akpakpa or Apaupan (Ijaw), Dewen (Bini), Okwen (Edo), Okung (Efik) (Ikegwu et al., 2010).

Various part of *Brachystegia eurycoma* has been widely used in Eastern part of Nigeria and some African countries for example, products from the tree bark have found application as fibres, food wrappers and have been used to make containers. The timber products are used as building materials in carpentry and related applications. The seed is used in food majorly in soup making as a soup condiment, flavouring agent and for soup thickening as emulsifiers and thickeners in traditional soups. The seeds have been shown to have alkaloids, flavonoids, saponins and tannins (Okwu and Okoro, 2005) while the stem bark extract contains tannins and flavanoids (Adekunle, 2000). The leaves have been found to contain volatile oil such as oxygenated monoterpenoids and sesquiterpenoid hydrocarbons. Other compounds identified in the leaves and stem bark are 1, 8 – cineole, acorenone, caryophyllene and geranyl acetone (Ogunbinu and Ogunwande, 2006). The extracts of *B euroycoma* is used by some Nigerian natives to cure infections like toothache, scabies, asthma, tuberculosis, bronchitis, catarrh, sore throat, phlegm, guinea worm infections and other inflammatory conditions (Burkhil, 1997). The growth and cytotoxic effect of the methanolic leave extract has been reported (Ayinde, 2010). However, the use of this legume has been limited to local traditional culinary practices which makes them under exploited and has also received little attention and recognition from researchers (Ikegwu et al., 2009). Based on its folkloric use in the treatment of pain and inflammation, the present study was designed to evaluate the analgesic and anti-inflammatory effect of the stem bark of *B euroycoma* in some experimental models.

2. MATERIALS AND METHODS

2.1 Plant Collection and Extraction

The stem bark of *B. euroycoma* were collected in September, 2011 beside a river in Iwo, Osun State. The plant was identified by Prof. Idu, Department of Plant Biology and Biotechnology, University of Benin, Benin City. The pieces of fresh stem barks were air-dried for 5 days and later oven-dried at 50ºC for 6 h to dryness after which they were reduced to powder using an electric mill. The powdered plant material (500 g) was extracted with 3 L of methanol using Soxhlet apparatus. The methanol extract obtained was concentrated to dryness using a rotary evaporator (yield=16.8%). The dried extract was stored in clean glass containers in the refrigerator until use.

2.2 Animal

All experiments were performed using male Swiss albino mice (22–30 g) and Wistar rats (190–280 g) of either sex. All the animals were obtained from the Laboratory Animal Centre of the College of Medicine, University of Ibadan. The animals were fed standard rodent cubes (Ladokun Feeds, Ibadan) and water *ad libitum*. Animals were exposed to natural lighting conditions and 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 were used for this study. The animals were divided into four groups of five mice each. Three groups were administered, via an orogastric syringe, the methanol extract of *Brachystegia eurycoma* (BE) at doses of 1, 2.5 and 5 g/kg respectively. The control group received 10 mL/kg of distilled water. General symptoms of toxicity and mortality in each group were observed within 24 hours. Animals that survived after 24 hours were observed for any signs of delayed toxicity or mortality for 14 days.

2.4 Anti-Inflammatory Activity

2.4.1 Carageenan-paw induced edema

In determining carrageenan-induced paw oedema, Wistar rats were randomly divided into five groups of five animals each. The test groups were treated orally with 100, 200 and 400 mg/kg of the extract while the reference group received indomethacin (10 mg/kg) orally. The control group received 10 mL/kg of distilled water *p.o*. The animals were treated 1 h before injection of 0.1 mL of 1% carrageenan into the subplantar tissue of the right hind paw (Winter et al., 1962). Paw thickness was measured using a vernier caliper (Thambi et al., 2006) at 0, 1, 2, 3, 4 and 5 h.

2.4.2 Dextran-paw induced edema

Wistar rats were randomly divided into five groups of five animals each. Animals were treated orally with the extract (100, 200 and 400 mg/kg), diphenhydramine (60 mg/kg) and distilled water (10 mL/kg). The animals were treated 1 h before injection of 0.1ml of 1.5% dextran into the subplantar tissue of the right hind paw (Glauce et al., 1998). Paw thickness was measured using venier calipers at 0, 1, 2, 3, 4 and 5 h.

2.5 Test for Analgesic Activity

2.5.1 Acetic acid-induced writhing in mice

This was based on the modification of the method described by Koster et al (1959). Swiss albino mice were randomly divided into five groups of five animals each. The different groups of animals received extract (100, 200 and 400 mg/kg) or acetyl salicylic acid (ASA) (100 mg/kg) or distilled water (10 mL/kg) orally. The animals were treated 1 h prior to injection of 0.6% acetic acid (10 mL/kg) intraperitoneally. The number of writhes by each mouse was counted immediately after acetic acid administration at intervals of 5 min for a period of 30 min. (Igbe et al, 2009a)

2.5.2 Hot plate test

The hot plate test was used to measure the latencies of pain response according to method described by Eddy and Leimback (1953). Swiss mice were divided into five groups of five mice each. The animals were individually placed on a hot plate maintained at a constant temperature of 55 ± 1ºC, the time interval from placement and shaking/licking of the paw or jumping was recorded as an index of response latency. The initial reaction time of each animal was determined and the cut off time was set at 30 secs. Group I was treated orally with distilled water, 10 mL/kg (negative control). The different groups of animals received extract (100, 200 and 400 mg/kg) or distilled water (10 mL/kg) orally. Morphine (4 mg/kg) given intraperitoneally, was used as a standard. The animals were placed on the hot plate at 30, 60, 90 and 120 min after treatment and the time taken for either paw licking or jumping was recorded.

2.6 Statistical Analysis

Data were expressed as the mean ± standard error of the mean (S.E.M). Comparison between the treatment groups was carried out using one way ANOVA followed by Tukey's *post hoc* test. Analysis was done using Graph pad prism version 5.0. Results were considered significant when $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Acute Toxicity Studies

At 5 g/kg of the extract, there was no mortality nor were toxic symptoms in any of the groups.

3.2 Anti-Inflammatory Activity of *B. eurycoma*

The result presented in Fig. 1 show that the extract at 100 mg/kg significantly ($P<0.05$) inhibited paw edema within three hours (1 – 3 h) of administration compared to the negative control. The extract at 200 and 400 mg/kg only produced inhibitory effects ($P<0.01$ and $P<0.05$ respectively) at the 1st h compared to the negative control. The inhibitory effects were comparatively less than that of indomethacin. This study showed that the methanol bark extract of *Brachystegia eurycoma* possesses analgesic and anti-inflammatory activities. Carrageenan-induced hind paw edema is an established experimental model for acute inflammation. Carrageenan is the phlogistic agent of choice for testing anti-inflammatory drug as it is none antigenic and is devoid of apparent systemic effect (Chakraborty et al., 2006). The development of carrageenan-induced edema is biphasic (Vinegar et al., 1987); the first phase occurs within one hour of carrageenan inflammation is attributed to the release of cytoplasmic enzymes, histamine and serotonin, from mast cells. However, platelet activating factor and arachidonic acid metabolites also play a role (Boughton-Smith et al., 1993). The second phase (>1 h) is mediated by release of prostaglandins, arachidonate metabolites, neutrophil migration, release of oxygen free radicals, proteolytic enzymes, as well as other neutrophil-derived mediators (Boughton-Smith et al., 1993; Bouriche et al., 2003) and the continuity between the two phases is maintained by kinins (Vinegar et al., 1987).

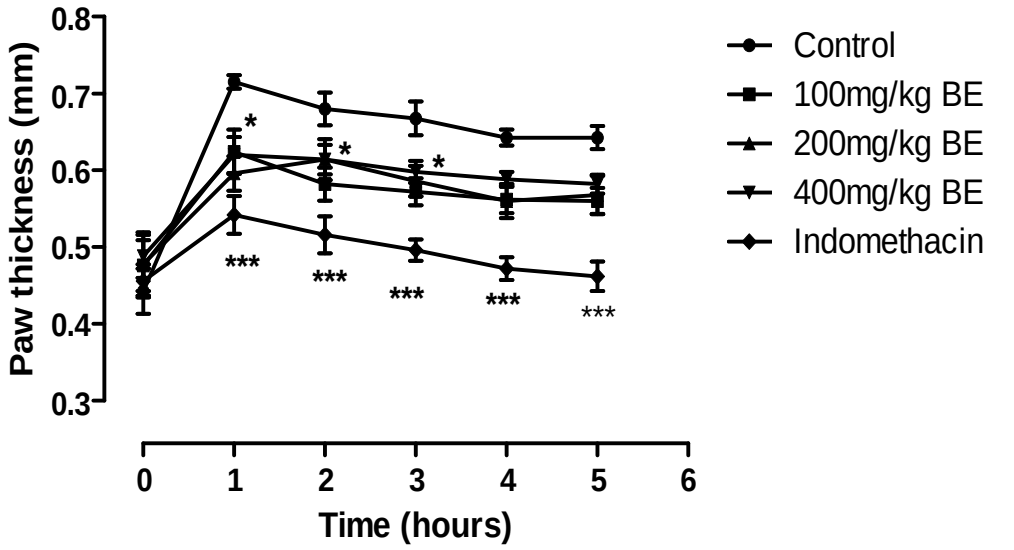

Fig. 1. Effect of methanol extract of *Brachystegia eurycoma* (BE) on carrageenan Induced paw edema in rats. *P<0.05, **P<0.01, ***P<0.001 as compared to the control (n =5 for each group)

The methanol extract of *Brachystegia eurycoma* significantly inhibited paw edema 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 (Kuriyama et al., 2000). Histamine stimulates vessel endothelial cells to increase vascular permeability (Kuriyama et al., 2000) resulting in the outpouring of cells and fluid. The effect of the extract in the second phase suggests a possible inhibition of cyclooxygenase synthesis because the carrageenan inflammatory model basically reflects the actions of prostaglandins (Di Rosa et al., 1971; Ferreira et al., 1974).

In Fig. 2, the extract (100 mg/kg) produced a significant (P<0.01) inhibition of paw edema induced by dextran between the 2^{nd} h and the 4^{th} h. The extract's inhibitory effect was comparable to the positive control, diphenhydramine at the 4^{th} h (P <0.001).

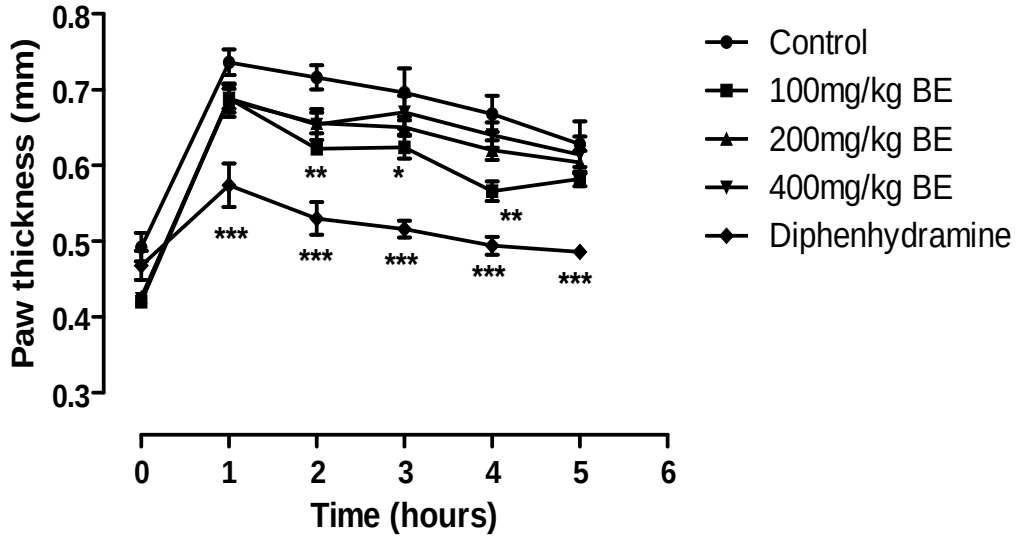

Fig. 2. Effect of methanol extract of *Brachystegia eurycoma* (BE) on dextra-induced paw edema in rats. *P<0.05, **P<0.01, ***P<0.001 as compared to the control (n =5 for each group)

Dextran-induced paw edema has been reported to be mediated mainly by histamine and serotonin released by the mast cells (Lo et al., 1982). The release of these inflammatory mediators results in marked vascular changes: including vasodilatation, increased permeability and the slowing of blood flow, eventually leading to an increase in paw size. The methanol extract of *B. eurycoma* showed significant inhibition of dextran-induced paw edema at the lowest dose (100 mg/kg) used for the experiment.

3.3 Analgesic Activity of *B. eurycoma*

Fig. 3 shows the effects of the methanol extract on acetic acid-induced mouse writhing. The extract (100, 200 and 400 mg/kg) produced a significant (P<0.001) decrease in the number of writhes compared to the negative control. The inhibitory effect at the 30^{th} min was comparable to acetyl salicylic acid (ASA). In the hot plate test (Table 1), the methanol extract of *B. eurycoma* increased the reaction time of the mice at 100 mg/kg (P<0.05) at the 60^{th} min compared to the negative control. The effect was not comparable to that of positive control, morphine.

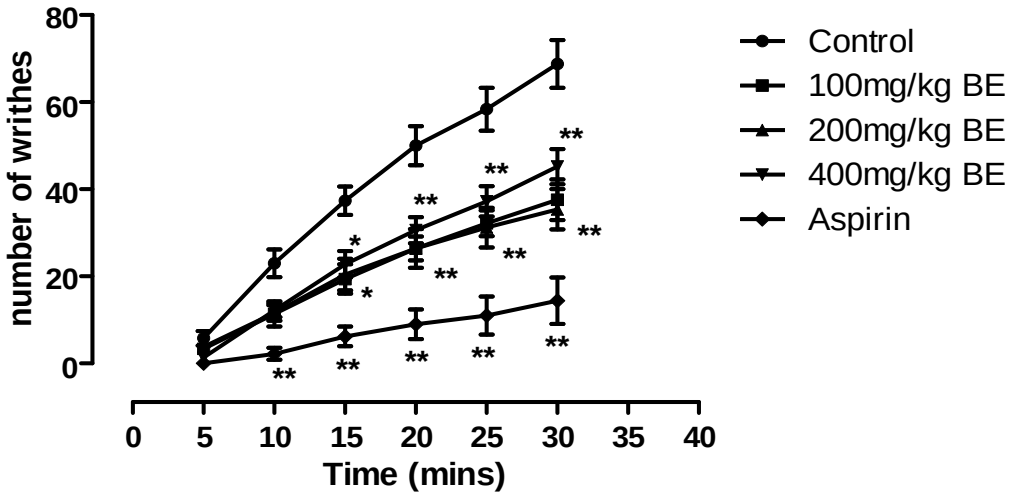

Fig. 3. Effect of methanol extract of *Brachystegia eurycoma* (BE) on acetic acid-induced writhing in mice. *P<0.05, **P<0.01 as compared to the control (n =5 for each group)

The acetic acid-induced writhing in mice is widely used for the evaluation of peripheral anti-nociceptive activity (Gene' et al., 1998). It is very sensitive and able to detect anti-nociceptive effects of compounds at dose levels that may appear inactive in other methods like the tail-flick test (Collier et al., 1968; Bentley et al., 1981). Local peritoneal receptors are postulated to be partly involved in the abdominal constriction response (Bentley et al., 1981). Oral administration of the methanol extract of *B. eurycoma* was shown to significantly reduce writhes induced by acetic acid at all doses and it was sustained throughout the 30 min period suggesting that analgesic effect of the extract maybe peripherally mediated. The hot plate method is one of the most common tests of nociception based on a phasic stimulus of high intensity (Mandegary et al., 2004). Pain induced by thermal stimulus of the hot plate is specific for centrally mediated nociception (Heidari et al., 2009). The ability of the extract to prolong the reaction latency to pain thermally-induced in mice by the hot plate further suggests central analgesic activity. The extract at 100 mg/kg, significantly reduced reaction time in hot plate induced pain. This effect was however short-lived and much less than that of morphine (4 mg/kg), a known centrally active analgesic drug. The possible explanation why the lowest dose of the extract, 100 mg/kg showed a significant analgesic and anti-inflammatory effect compared to the higher doses (200 and 400 mg/kg) maybe that at higher doses, constituents of the crude extract opposed to the desired effect (analgesic and anti-inflammatory) increases and this result in the blunting of the desired effects.

Table 1. Anti-nociceptive effect of methanol extract of *Brachystegia eurycoma* (BE) in the hot plate test

Treatment	Reaction Time (sec) / Dose	0 min	30 min	60 min	90 min	120 min
Control	10 ml/kg	4.22 ± 0.43	4.22 ± 0.94	4.58 ± 0.59	3.73 ± 0.37	4.05 ± 0.53
BE	100	4.34 ± 0.44	6.10 ± 0.50	6.84 ± 0.26*	5.66 ± 0.76	5.04 ± 0.39
(mg/kg)	200	3.92 ± 0.48	5.36 ± 0.28	4.96 ± 0.55	4.42 ± 0.47	4.22 ± 0.38
	400	5.08 ± 0.16	5.68 ± 0.94	6.50 ± 0.75	4.10 ± 0.31	4.64 ± 0.23
Morphine (mg/kg)	4	4.34 ± 0.37	10.22 ±0.97***	9.26 ± 0.59***	9.72 ± 0.66***	7.24 ± 0.10***

*Values are mean ± S.E.M. *P<0.005, ***P<0.001, as compared with the control (n=5 for each group).*

4. CONCLUSION

The present results demonstrate that single oral doses of the methanol stem bark extract of *B. eurycoma* produce effective analgesic and anti-inflammatory activity, therefore supporting the claim in the ethnomedical use of the stem bark extract of *B. eurycoma* for the treatment of conditions which may be interpreted as involving inflammation and pain.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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