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Analgesic and anti-inflammatory studies of methanol stem bark extract of *Burkea africana* Hook (Fabaceae)

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ABSTRACT

Burkea africana Hook (Fabaceae) is a plant used in traditional medicine for the treatment of pain and inflammation in Africa. The methanol stem bark extract was evaluated for analgesic property using acetic acid induced writhing and hot plate test in mice while the anti-inflammatory property was evaluated using carrageenan- induced paw oedema. Acute toxicity studies and phytochemical screening using standard protocol was also conducted. Preliminary phytochemical screening of the extract revealed the presence of flavonoids, tannins, steroids and triterpenes, cardiac glycosides and saponins. The intraperitoneal median lethal dose (LD_{50}) in mice was found to be 471.17 mg/kg body weight. The extract at all tested doses (20, 40 and 80 mg/kg) significantly ($P \leq 0.001$) decreased abdominal contractions induced by acetic acid and also significantly ($P \leq 0.01$) delayed the mean reaction time of the mice. The extract at all doses showed no significant anti-inflammatory activity. The results obtained from this study showed that the methanol stem bark extract of *Burkea africana* possesses both central and peripheral analgesic activities. This provides a rationale for its traditional use against pain.

Keywords: *Burkea africana*, analgesic, anti-inflammatory, carrageenan.

Introduction

Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage as defined by the International Association for the study of pain (IASP).[1] It is a disabling accompaniment of many medical conditions and pain control is one of the most important therapeutic priorities.[2] Inflammation is a local response of living mammalian tissues to injury.[3] It is the body defense reaction to eliminate or limit the spread of injurious agent followed by removal of the necrotic cells.[4] Common drugs for the relief of pain and inflammation such as aspirin and morphine have been widely used for decades.[5-8] In most cases, these analgesics and anti-inflammatory drugs particularly opioids and nonsteroidal anti-inflammatory drugs (NSAIDs), cause serious side effects such as, physical dependency, tolerance, gastro intestinal disorders and cardiovascular problems.[9] This necessitate the search for alternatives therapeutic agents against pain and inflammations especially from natural sources.

Overwhelming evidence has accumulated to demonstrate the potentials of medicinal plants used in various traditional, complementary and alternative systems of treatment of diseases.[10-12] There are over hundreds of plants and natural anti-inflammatory herbs all over the world, which are used in herbal medicine as treatments for pain and inflammation.[13] One of such plants is *Burkea africana* Hook (Fabaceae), commonly known in Northern Nigeria as 'Bakin makaarho' (Hausa), 'Apasa' in South west (Yoruba) and 'Ofo' in South east (Igbo).[14] The bark, root and leaves are used in traditional medicine to treat fever, scabies, stomach aches, abscesses, edema, epilepsy, bloody diarrhea, gonorrhea, syphilis, ulcers and wounds, cough, catarrh, menorrhea, pneumonia, toothache, headache and inflammation of tongue and gums, poisoning and skin diseases.[15] Previous studies have reported the anti-cancer,[16] antidiarrheal,[17] sedative and anxiolytic[18] properties of the plant. The polyphenolic rich extract of *B. africana* was found to decrease oxidative stress related parameters.[19] The phytochemistry of *B. africana* was reported to contain largely polyhydroxylated compounds such as fisetinidol- catechin 3-gallate and bis-fisetinidol catechin-3-gallate, monomeric flavan-3-ols (catechin, epicatechin and fisetinidol).[20] To establish the scientific basis of the ethno medicinal uses of *B. africana* as anti-inflammatory agent; this study reports the analgesic and anti-inflammatory properties of the plant which had not been reported hitherto.

Materials and Methods

Experimental animals

Adult Swiss albino mice (16-28 g) and Wistar rats (120 - 162 g) were obtained from the Animal House Facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. The animals were kept in cages in a well-ventilated room under standard conditions of temperature and light. Feed and water were provided to the rats and mice *ad libitum*. All experimental protocols were in accordance with Ahmadu Bello University, Zaria Research policy; and ethics and regulations governing the care and use of experimental animals as contained in "Principles of Laboratory Animal Care.[21]

Plant Material

Fresh stem bark and leaf of *Burkea africana* were obtained at Galadimawa village near Zaria in July 2016. The plant was identified and authenticated by Namadi Sunusi at the Herbarium unit of the Department of Botany, Ahmadu Bello University, Zaria, Nigeria. This

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was compared with the already deposited specimen (voucher number 900227) in the herbarium.

Preparation of extract
The powdered stem bark (200 g) of *B. africana* was extracted with 95% methanol (2 L) by simple percolation in a separating funnel, for 24 hr. The extract was drained into evaporating dishes and heated on a water bath at 40- 45°C to dryness. The dried plant extract was weighed and stored in a refrigerator until needed for use.

Phytochemical screening
The phytochemical tests were carried out to detect the presence of secondary metabolites saponin, tannins, flavonoids, steroids and triterpenes, alkaloids and cardiac glycosides, according to standard protocols.[22-25]

Acute toxicity study
Acute toxicity study was carried out according to standard method.[26] The procedure was divided into two phases. In the initial phase, nine mice were divided into three groups of three mice each and treated with the extract at doses of 10, 100 and 1000 mg/kg body intraperitoneally. In the second phase, the mice were grouped into four groups of one mouse each and treated with the extract of four different doses (140, 225, 370 and 600 mg/kg body weight) intraperitoneally and the animals were observed for 24 hr in each of the phases. The LD_{50} value was calculated as the square root of the product of the lowest dose that death occurred and the highest dose at which animal survived from the second phase.
LD_{50}= √ (Highest nonlethal dose) x (Lowest lethal dose)

Acetic acid- induced writhing in mice
The experiment was conducted according to previously described method.[27]A total of 25 mice of both sexes were randomly divided into five groups of five mice each. The first group was given normal saline 10 ml/kg body weight intraperitoneally. The second, third and fourth groups were treated with the extract at doses of 20, 40 and 80 mg/kg body weight (dissolved in distilled water) via intraperitoneal route respectively. The fifth group was treated with piroxicam 5 mg/kg (dissolved in distilled water) via the same route.
Thirty minutes post treated mice in all the groups were injected with 10 mL/kg of 0.6% v/v acetic acid intraperitoneally. Mice were placed individually in transparent plastic cages, and 5 min post acetic acid administrations, the numbers of abdominal contractions were recorded for the next 10 minutes.

Hot plate method
The test was carried out using an electric hot plate maintained at 45±1°C as previously described.[28] The reaction time of the mice to thermal stimulus taken to be the interval between the time the animal reached the hot plate to the time it kicks its hind paw or jump off the hot plate. The average basal time was noted at 0, 30, 60 and 90 minutes following intraperitoneal administration of the drug and test extract. A total of 25 mice were divided into 5 groups of 5mice each. The first group was given normal saline 10 ml/kg body weight. The second, third and fourth groups were treated with the extract at doses of 20, 40 and 80 mg/kg body weight via intraperitoneal route respectively. The fifth group was treated with morphine 4 mg/kg via the same route.[29]

Carrageenan-induced paw oedema
The test was carried out according to previously described method.[30] Wistar rats of either sex were grouped into five groups with each group containing 5 rats. The first group was given normal saline (1 ml/kg) *i.p*. Groups two, three and four were given 20, 40 and 80 mg/kg body weight *i.p.* respectively of the plant extract, whereas the fifth group was treated with Piroxicam 5 mg/kg via the same route. One hour later, 0.1 ml of freshly prepared Carrageenan suspension (1% w/v in 0.9% normal saline) was injected into the sub planter region of the left hind paw of each rat. The paw thickness was measured with the aid of a vernier caliper at 0, 1, 2, 3, 4 and 5 hr after the injection of carrageenan.

Statistical analysis
The data were presented mean±SEM and results were statistically analyzed by one way and repeated measures ANOVA followed by Dunnett's and Bonferroni post hoc tests respectively using SPSS software. A difference was considered significant at $p \leq 0.05$.

Results and Discussion

The preliminary phytochemical screening of the methanol stem bark extract of *Burkea africana* revealed the presence of flavonoids, tannins, cardiac glycoside, saponins, steroids and triterpenes with the absence of alkaloids (Table 1). These findings are in accordance with the preliminary phytochemical screening of the methanol root bark extract of *B. africana.*[18] Phytochemicals such as flavonoids, steroids, saponins and tannins isolated from medicinal plants have been reported to possess significant analgesic and anti-inflammatory activities.[31-33] Since *B. africana* was reported to contain several polyhydroxylated compounds which have demonstrated antioxidant and radical scavenging properties,[20] it is reasonable to imply that flavonoids and polyphenolics detected in this study may be involved in its analgesic activity. Therefore, the methanol stem bark extract of *B. africana* has the potentials of a viable analgesic phytomedicine.
The intraperitoneal median lethal dose (LD_{50}) of the extract in mice was calculated to be 471.17 mg/kg. This suggests that the extract is relatively toxic as mortality was recorded during the acute toxicity study, this is based on the classification of toxicity by Lorke.[26] Based on these findings; the dose selected and used in this study was lower than 30% of the LD_{50} value as this has been shown to be relatively safe for pharmacological research.[34]
The acetic acid induced writhing test has been used to evaluate compounds/ substances for peripheral analgesic activity.[35, 36] It is very sensitive and able to detect anti - nociceptive effect of local peritoneal receptors which are postulated to be partly involved in the abdominal constriction response.[37] In acetic acid induced method pain is generated through the liberation of endogenous substances including prostaglandins (PGE_2 and FGF_2), serotonin, histamine, bradykinins and substance P which stimulate the nerve endings. Stimulation of these nerve endings causes pain which manifests in the mice as abdominal writhes.[38-40] The results of the acetic acid induced method revealed that the extract treated groups have statisticaly significant effect ($p<0.001$) when compared with the normal saline group. The methanol stem bark extract of *B. africana* at 40mg/kg had the highest percentage protection of 91.67% which is similar to the standard drug (piroxicam 5mg/kg). The doses of 20 and 80 mg/kg both gave 89.17% protection (Table 2). The ability of the extract to reduce the number of abdominal writhes in mice suggests that the extract possesses peripheral analgesic activity through the inhibition of prostaglandins and other endogenous substances.[41,42]
Administration of the extract at all doses increased the mean reaction time of the mice to thermal stimulus which was significant ($p<0.01$) at the time interval of 30 minutes (Table 3). The hot plate method is one of the common tests of nociception that is based on a phasic stimulus of high intensity.[43, 44] Pain induced by thermal stimulus of the hot plate is specific for centrally mediated nociception.[43, 44] The hot plate response is due to complex supraspinally integrated behaviour.[45] The ability of the extract to prolong pain reaction time to the heat stimulant suggests that it has activity via the central mechanism similar to narcotic agents. From this study, the methanol stem bark extract of *Burkea africana* at the doses tested did not produce significant ($p<0.05$) anti oedematogenic effect on induced oedema of the paw of rat induced by carrageenan (Table 4). The most widely used primary test to screen new anti-inflammatory agent's measure the ability of a compound to reduce local edema induced in the rat paw by injection of an irritant agent.[30]

Table 1: Phytochemical screening of methanol stem bark extract of *B. africana.*

Constituents	Result
Alkaloids	-
Steroids and Triterpenes	+
Cardiac glycoside	+
Saponin	+
Tannin	+
Flavonoids	+

Key: + = present, - = absent

Table 2: Effect of methanol stem bark extract of *B. africana* on acetic acid - induced abdominal writhes in mice.

Treatment (mg/kg)	Mean number of abdominal writhes	% Inhibition
DW (10 ml/kg)	24.00 ± 4.46	00.00
BAMT 20	2.60 ± 1.40*	89.17
BAMT 40	2.00 ± 1.38*	91.67
BAMT 80	2.60 ± 1.94*	89.17
PC 5	2.00 ± 0.55*	91.67

Data are presented as Mean ± S.E.M.; analyzed by one-way ANOVA followed by Dunnett's Post-hoc test; n= 5; *= $p<0.001$ versus control; DW = Distilled Water; BAMT = *Burkea africana* methanol stem bark extract; PC = Piroxicam.

Carrageenan induced rat paw oedema has been commonly used as an experimental animal model for evaluation of acute anti-inflammatory potential of natural products.[30,31] This method produces an inflammatory response which is biphasic.[46] The first phase is due to the release of histamine, serotonin and kinin in the first hour after administration of carrageenan. The second phase is more pronounced which last beyond three hours post injection and is attributed to release of bradykinin, prostaglandins and lysosomes.[47] The analgesic effect of the plant extract may therefore be due to the presence of flavonoids, tannins or saponins found present in it.

Conclusion

The results from this study suggests that the methanol stem bark extract of *Burkea africana* possess significant analgesic activity mediated through central and peripheral mechanisms. However, the extract showed no significant anti-inflammatory activity at the doses tested.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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Table 3: Effect of methanol stem bark extract of *B. africana* on thermal-induced pain in mice.

Treatment (mg/kg)	0 minute	30 minutes	60 minutes	90 minutes
DW (10 ml/kg)	2.00 ± 0.32	2.00 ± 0.32	2.60 ± 0.24	2.60 ± 0.40
BAMT 20	1.60 ± 0.24	2.40 ± 0.24	5.00 ± 0.63*	5.20 ± 1.24*
BAMT 40	2.00 ± 0.00	2.00 ± 0.32	4.60 ± 0.98*	2.80 ± 0.49
BAMT 80	2.40 ± 0.24	2.40 ± 0.24	4.60 ± 0.98*	2.80 ± 0.49
M 4	2.00 ± 0.00	9.40 ± 0.51*	5.00 ± 0.32*	5.00 ± 0.55*

Data presented as Mean ± S.E.M.; n = 5, * represents significance differences from distilled water treated group at $p<0.01$ using repeated measures ANOVA followed by Bonferroni Post-hoc test DW = Distilled Water; BAMT = *Burkeae africana* methanol stem bark extract; M = Morphine.

Table 4: Effect of methanol stem bark extract of *B. africana* on Carrageenan-induced inflammation in rats.

Treatments (mg/kg)	0hr	1hr	2hr	3hr	4hr	5hr
DW (1 ml/kg)	1.63 ± 0.03	2.29 ± 0.17	2.84 ± 0.20	2.93 ± 0.08	2.73 ± 0.15	3.05 ± 0.21
BAMT 20	1.60 ± 0.08	2.10 ± 0.19	2.50 ± 0.19	2.27 ± 0.10	2.07 ± 0.09	2.20 ± 0.07
BAMT 40	1.63 ± 0.06	2.19 ± 0.17	2.34 ± 0.05	2.31 ± 0.04	2.17 ± 0.06	2.00 ± 0.04
BAMT 80	1.58 ± 0.06	2.17 ± 0.05	2.38 ± 0.05	2.30 ± 0.04	2.18 ± 0.04	1.95 ± 0.04
PC 5	1.59 ± 0.07	2.07 ± 0.08	2.09 ± 0.16	2.11 ± 0.07	2.01 ± 0.05	2.01 ± 0.07

Data presented as Mean ± S.E.M.; n = 5, using repeated measures ANOVA. DW = Distilled Water; BAMT = *Burkea africana* methanol stem bark extract; PC = Piroxicam.

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