British Journal of Medicine & Medical Research
3(2): 216-229, 2013

SCIENCEDOMAIN *international*
www.sciencedomain.org

Evaluation of Anti-inflammatory and Antinociceptive Potentials of *Khaya senegalensis* A. Juss (Meliaceae) Stem Bark Aqueous Extract

O. T. Kolawole[1*], M. O. Akiibinu[2], A. A. Ayankunle[1] and E. O. Awe[1]

[1]Department of Pharmacology and Therapeutics, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.
[2]Department of Chemistry and Biochemistry, Caleb University, Lagos, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author OTK designed the study, wrote the protocol and the first draft of the manuscript. Authors MOA and AAA managed the literature search. Authors MOA and EOA managed the analyses of the study. All authors read and approved the final manuscript.

Research Article

Received 30th July 2012
Accepted 23rd November 2012
Published 19th January 2013

ABSTRACT

Aim: To investigate anti-inflammatory and antinociceptive potentials of aqueous stem bark extract of *Khaya senegalensis* A. Juss (Meliaceae) in rodents.
Methodology: Anti-inflammatory activity of aqueous stem bark extract of *K. senegalensis* (AKS) was studied in different models. Effect of the extract in acute inflammation was tested in carrageenan-induced rat paw edema and its effect in chronic inflammation was evaluated using cotton pellet-induced granuloma test. Croton oil-induced ear edema in mice was used to investigate the effect of the extract on topical inflammation. Antinociceptive property of AKS was evaluated using three models of nociception: hot-plate test, acetic acid-induced writhing in mice and formalin-induced paw licking in rats. Membrane stabilizing effect of AKS was tested in heat and hypotonicity-induced hemolysis. The mechanism of antinociceptive effect of the extract was evaluated by pre-treating rats with metoclopramide, a dopamine (D2) antagonist (1.5 mg/kg body wt.) and naloxone, an opioid receptor antagonist (5 mg/kg body wt.).

**Corresponding author: Email: tymkol@yahoo.co.uk;*

One hour after these treatments, the rats were given AKS (150 mg/kg p.o) and their reaction time in hot-plate was assessed. Free-radical scavenging activity of the extract was measured by decrease in the absorbance of 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) methanol solution.

Results: The extract produced significant (P<0.05) and dose-dependent inhibition of ear edema in mice. It also caused a significant (P<0.05) reduction in granuloma formation and paw edema in rats. At concentration range of 125 - 500 µg/ml, AKS significantly (P<0.05) protects the erythrocyte membrane against lysis induced by heat and hypotonic medium. It also significantly (P<0.05) reduced the licking/biting time of the formalin-injected rat paw in the early (19 - 51 % reduction) and late (13 - 57 % reduction) phases. The extract demonstrated significant (P<0.05) antinociceptive activity in the hot-plate and writhing tests and exhibited good scavenging effect on DPPH free radical.

Conclusion: The study demonstrated that *Khaya senegalensis* stem bark aqueous extract possesses anti-inflammatory activity and antinociceptive effect mediated via central and peripheral mechanisms. Further studies on the plant may produce lead molecules for the development of new anti-inflammatory drugs.

Keywords: Khaya senegalensis; edema; anti- inflammatory; antinociceptive; extract.

1. INTRODUCTION

Inflammation is a complex protective response to tissue injury caused by physical trauma, noxious chemicals or pathogenic microbes. Inflammatory reactions occur in two phases: acute phase and chronic phase. Acute inflammation is the initial defense of the body against danger [1]. This initial phase triggers temporary local vasodilatation, intravascular activation of platelets and increased capillary permeability which leads to accumulation of fluid at the affected area [2]. If the stimulus that provokes the inflammatory response is not eliminated on time, inflammation then progresses into the chronic phase. Chronic inflammation is characterized by infiltration of leukocytes and phagocytic cells into the site of inflammation. This eventually leads to tissue deterioration and fibrosis [3].

The four classical signs of inflammation are erythema, edema, pain and heat. These signs are always observable in inflammatory diseases such as arthritis, rheumatism, cystitis, colitis and a host of other diseases which are major causes of morbidity and mortality [4]. Steroidal and non-steroidal anti-inflammatory drugs such as aspirin and dexamethasone are usually employed in the treatment or management of inflammatory diseases and pain. However these drugs are known to possess serious adverse effects and are contra-indicated under certain conditions. Therefore there is growing need for the development of novel anti-inflammatory drugs with minimal adverse effects and greater efficacy [5].

Over the last few decades, there has been an increasing interest in the use of herbal medicines. This is because medicinal plants have proved useful in the treatment of many inflammatory diseases and are usually devoid of serious adverse effects [6]. They provide considerable economic benefit to many rural and poor people who may not be able to afford the costly synthetic drugs. Medicinal plants are also sources for the development of new anti-inflammatory drugs. About 25% of modern medicines, including anti-inflammatory drugs, are derived from plants and they have continued to serve as lead molecules for the synthesis of various drugs [7].

Khaya senegalensis A. Juss (Meliaceae) is a popular medicinal plant among the Nupes and Yorubas in Nigeria. It belongs to the family Meliaceae (mahogany). The stem bark aqueous extract is traditionally used by Yoruba and Nupe tribes to treat malaria, jaundice, edema and headache. The Hausa and Fulani tribes in northern Nigeria also use *K. senegalensis* as a remedy for several human and animal ailments. It has been reported to contain scopoletin, scoparone, tannins, saponins and sterol [8]. In this study, anti-inflammatory and antinociceptive activities of the stem bark aqueous extract of *Khaya senegalensis* was investigated to justify its traditional use and explore its potential in the development of effective and safe anti-inflammatory drugs.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Healthy male Swiss mice (20 – 25 g) and Sprague Dawley rats (180 – 200 g) used in the study were obtained from the Animal House of the College of Health Sciences, Ladoke Akintola University of Technology (LAUTECH), Ogbomoso, Nigeria. They were maintained under standard laboratory conditions of humidity (40 – 60%), temperature (21 ± 1ºC) and light (12/12h light / dark cycle). The animals were acclimatized for one week, fed on rat pellets (Livestock Feeds PLC, Ibadan, Oyo State, Nigeria) and allowed free access to drinking water *ad libitum*. They were fasted overnight before the experiment was carried out. The study was approved by the Animal Welfare and Ethics Committee of LAUTECH, Ogbomoso, Nigeria. All conditions of animal use were also as approved by United States National Institute of Health (NIH) guide for Care and Use of Laboratory Animals and in accordance with the recommendation of IASP [9].

2.2 Plant Materials

Khaya senegalensis stem bark was obtained from Igbona area, Osogbo, Nigeria in the month of November. The sample was identified and authenticated by a taxonomist in the Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria. The stem bark was dried under shade for two weeks and then chopped into small pieces and pulverized into fine powder. Three hundred gram (300 g) of the powder sample was soaked in boiling distilled water (750 mL) for 10 min, allowed to cool and then filtered using Whatman filter paper (No.1). The obtained residue was further extracted twice and then concentrated using a rotary evaporator. The concentrated filtrate was evaporated to dryness in an oven at 40ºC for 48 hr under aseptic condition to produce a dark brown residue. A percentage yield (w/w) of 5.6 % was obtained. The extract was stored in a refrigerator at 4ºC until use. The study was conducted between November, 2011 and February, 2012.

2.3 Experimental Design

2.3.1 Carrageenan-induced rat paw edema

Rats used for the study were divided into five groups (n = 6). Animals in group I were treated with distilled water (10 ml/kg p.o) and served as the negative control. Groups II, III and IV were treated orally with 50, 100 and 150 mg/kg of *Khaya senegalensis* extract (AKS) respectively. These doses were chosen based on previous study of the plant [10]. Group V received diclofenac sodium, a standard anti-inflammatory drug (20 mg/kg p.o). One hour after administration of these agents, edema was induced by injection of 0.1ml carrageenan

(1% w/v in saline) into the sub-plantar region of the right hind paw [11]. The paw circumference was measured by a linear caliper just before and 3h after the injection of carrageenan [12,13]. Percentage inhibition of edema at the 3rd hour was calculated according to Ishola and Ashorobi [14] as follows:

$$\% \text{ inhibition} = \frac{Ec - Et}{Ec} \times 100$$

Ec = Edema of the control (group I) at the 3rd hour.
Et = Edema of the treated (group II – V) at the 3rd hour.

2.3.2 Croton oil-induced ear edema in mice

Mice were divided into five groups of 6 animals each. Group I (control) received distilled water (10 ml/kg p.o). Group II, III, and IV were treated orally with 50, 100 and 150 mg/kg of the extract respectively. Group V (standard) received dexamethasone (1mg/kg p.o). Thirty minutes after the treatment, croton oil (5 µg/ml) was applied to the inner surface of the right ear of each mouse to induce edema. The left ear was left untreated. The animals were sacrificed under ether anesthesia 6 h later and both ears were cut off, sized circularly using a cork borer with diameter of 7 mm. The sections were weighed and the difference between the right and left ears were determined for each group [15,16]. Percentage inhibition of ear edema was calculated relative to the untreated ear as follows:

$$\text{Inhibition (\%)} = \frac{Wc - Wt}{Wc} \times 100$$

Wc = mean of the difference in ear weight of control mice.
Wt = mean of the difference in ear weight of treated mice.

2.3.3 Cotton pellet-induced granuloma in rats

Rats were divided into five groups (n = 6) and the fur of the axilla region was shaved. The rats were anesthetized after shaving and sterile cotton pellet (10 mg) was implanted in the shaved axilla of each rat through a single incision [17]. Thereafter the animals were treated as follows:

Group I (control) received distilled water (10 ml/kg p.o). Group II, III and IV received oral administration of the extract at doses of 50, 100 and 150 mg/kg respectively. Group V received hydrocortisone (15 mg/kg p.o). The treatment was administered once a day for seven consecutive days. The animals were sacrificed on the eighth day and cotton pellets were removed and made free of extraneous tissues. The pellets were dried at 60^0C overnight. The granuloma weight was taken as the increase in dry weight of the pellets of the control and treated groups [18]. Percentage inhibition was calculated as follows:

$$\text{Inhibition (\%)} = \frac{Gc - Gt}{Gc} \times 100$$

Gc = mean granuloma of control group.
Gt = mean granuloma of treated group.

2.3.4 Hot-plate test

Rats were divided into five groups of 6 animals each. The rats were treated as follows: group I (control) received distilled water (10 ml/kg p.o), groups II, III and IV were orally treated with

50, 100 and 150 mg/kg AKS respectively while group V was treated with diclofenac (20 mg/kg p.o). One hour before drug administration and then at hourly intervals for 4 h after treatment, the reaction times of rats were measured using hot-plate method as described by Langerman et al. [19]. In the hot-plate test, rats were placed on an enclosed hot plate (Model MK 35A, Muromachi Kikai Co. Ltd., Tokyo, Japan). The hot plate was maintained at 50ºC and the time (in seconds) taken to lick the hind paw or jump from the surface of the hot plate (the reaction time) was determined using a stop watch. Rats showing a pretreatment reaction time greater than 15 s were excluded from the experiments. A cut-off time of 25 s was set to avoid tissue damage [20].

2.3.5 Formalin test

The test was performed as described by Farsam et al. [21]. Thirty rats were assigned into five groups of 6 animals each. They were treated in the following manner: group I (control) was given distilled water (10 ml/kg p.o). Groups II, III, and IV were orally treated with 50, 100 and 150 mg/kg AKS respectively. Group V received diclofenac (20 mg/kg p.o) as the reference drug. One hour after drug administration, each rat was subcutaneously injected with 0.05 ml of 2.5 % formalin solution (BDH Chemicals, Poole, UK) into the subplantar area of the left hind paw. The time spent biting/licking the injected paw was recorded in two phases: early phase (1-5 min), and late phase (15-60 min) after formalin injection.

2.3.6 Acetic acid-induced writhing

Antinociceptive activity was assessed by the acetic acid abdominal constriction test (writhing test), a chemical visceral pain model induced by intraperitoneal (i.p.) injection of acetic acid, that consists of a constriction of the abdominal muscle together with a stretching of the hind limbs [22]. Mice were injected i.p. with 10 ml/kg of 0.8% (v/v solution) acetic acid after 30 minutes of i.p. administration of aspirin (100 mg/kg), used as positive control, or AKS at the dose levels of 50, 100, and 150 mg/kg of body weight. Animals injected i.p. with the same volume of distilled water (10 ml/kg) were used as the control group. Ten minutes after the administration of the acetic acid, mice were placed in separate boxes and the number of abdominal writhes was counted for 10 minutes. The number of abdominal writhes in treated groups was compared to that in control group.

2.3.7 Membrane stabilization activities

2.3.7.1 Preparation of erythrocyte suspension

Erythrocytes suspension was prepared by the method described by Shinde et al. [23]. Whole human blood was obtained from a healthy human volunteer and transferred to heparinized centrifuge tubes, centrifuged at 3000 rpm for 5 min and washed three times with equal volume of normal saline. The volume of the blood was measured and reconstituted as a 40 % (v/v) suspension with isotonic buffer solution (10 mM sodium phosphate buffer pH 7.4, the composition of the buffer solution (g/l) was NaH_2PO_4 (0.2), Na_2HPO_4 (1.15) and NaCl (9.0).

2.3.7.2 Hypotonicity-induced hemolysis

The method of Umapathy et al. [24] was employed. Different concentrations of AKS (125, 250 and 500 µg/ml) in hypotonic solution (distilled water) was put in centrifuge tubes (5 ml). Control tubes contained 5 ml of the vehicle (distilled water) or indomethacin 100 µg/ml. Erythrocyte suspensions (0.05 ml) was added to each tube and after gentle mixing, the

mixtures were incubated for 1 h at room temperature. After incubation, the reaction mixture was centrifuged for 3 min at 1300 g and the absorbance of the supernatant was measured spectrophotometrically at 540 nm. The inhibition (%) of hemolysis was calculated as follows:

% inhibition of hemolysis = 100 × (1-A2/A1)

A1 = Absorption of the control sample.
A2 = Absorption of test sample solution.

2.3.7.3 Heat-induced hemolysis

The test was carried out as described earlier [25]. The isotonic buffer solution (5 ml) containing 125, 250 and 500 µg/ml of AKS were put in 5 sets (per concentration) of centrifuge tubes. Control tubes contained 5 ml of the vehicle or 5 ml of 100 µg/ml of indomethacin. Erythrocyte suspension (0.05 ml) was added to each tube and gently mixed. A pair of the tubes was incubated at 54ºC for 20 min in a regulated water bath. At the end of the incubation, the reaction mixture was centrifuged at 1300 g for 3 min and the absorbance of the supernatant was measured spectrophotometrically at 540 nm using. The level of inhibition of hemolysis was calculated as follows:

% inhibition of hemolysis = 100 × (1-A2/A1)

A1 = Absorption of the control sample.
A2 = Absorption of test sample solution.

2.3.8 Free radical scavenging activity

Free-radical scavenging activity of the extract was measured by decrease in the absorbance of methanol solution of DPPH [26]. Briefly, 1.5 ml of DPPH solution (0.004 % in methanol) was incubated with 1.5 ml of extracts at various concentrations (100-1000 µg/ml). The reaction mixture was shaken well and incubated in the dark for 30 min at room temperature. The control was prepared as above without extract. The absorbance of the solution was measured at 517 nm against a blank. The radical scavenging activity was measured as a decrease in the absorbance of DPPH and was calculated using the following equation:

$$\text{Scavenging effect (\%)} = \frac{\text{1- Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Catechin was used as reference drug.

2.3.9 Evaluation of involvement of dopamine and opioid receptors

Eighteen rats were randomly assigned into three equal groups. Rats in group 1 were treated orally with 1.5 mg/kg of metoclopramide, a dopamine (D2) antagonist, in 1ml of 1% methyl cellulose. Rats in group 2 were injected with 5 mg/kg of naloxone hydrochloride in 1 ml of normal saline. Animals in group 3 were treated orally with 2 ml of normal saline. One hour after these treatments, the three groups of rats were given AKS (150 mg/kg p.o) and the hot-plate test was performed.

2.4 Statistical Analysis

Data were recorded as mean ± standard error of mean (SEM) and analyzed using one-way ANOVA followed by Tukey's multiple comparison test. Results were considered significant at $P < 0.05$.

3. RESULTS AND DISCUSSION

Effect of drugs on acute inflammatory response is usually studied by carrageenan-induced edema in rat paw. In this study, aqueous extract of *Khaya senegalensis* stem bark exhibited significant anti-inflammatory effect in rat paw edema induced by carrageenan. The inflammatory response of carrageenan model is mediated by the release of histamine, serotonin and increased synthesis of prostaglandins and bradykinin by tissue macrophages [27,28]. The increase in vascular permeability produced by these inflammatory mediators promotes accumulation of fluid in tissues which manifests as edema. The aqueous extract of *Khaya senegalensis* stem bark produced significant ($P<0.05$) inhibition of paw edema in rats. This inhibition was exhibited in a dose-dependent manner. The extract caused percentage inhibition of 37.1, 52.2 and 68.2 % with 50, 100 and 150 mg/kg respectively. This compares favorably well with the reference drug, diclofenac which inhibited edema formation by 72 % (Table 1).

Table 1. Effect of aqueous extract of *Khaya senegalensis* (AKS) on carrageenan-induced rat paw edema

Treatment	Dose (mg/kg)	Edema (cm)[a]	Inhibition (%)
Control	-	1.32 ± 0.03	-
AKS	50	0.83 ± 0.05	37.1
AKS	100	0.63 ± 0.02*	52.2
AKS	150	0.42 ± 0.02*	68.2
Diclofenac	20	0.37 ± 0.01*	72.0

[a]Values represent mean ± SEM (n=6); *$P< 0.05$ compared with control

Table 2. Effect of aqueous extract of *Khaya senegalensis* (AKS) on croton oil-induced ear edema in mice

Treatment	Dose (mg/kg)	Change in ear weight (mg)[a]	Inhibition (%)
Control	-	12.1 ± 1.1	-
AKS	50	10.5 ± 0.9	13.2
AKS	100	8.4 ± 0.6*	30.6
AKS	150	5.5 ± 1.2*	54.5
Dexamethasone	1	2.8 ± 0.2*	76.9

*[a]Values represent mean ± SEM (n = 6); *P < 0.05 compared with control*

Table 3. Effect of aqueous extract of *Khaya senegalensis* (AKS) on cotton pellet-induced granuloma in rats

Treatment	Dose (mg/kg)	Weight of granuloma(mg)[a]	Inhibition (%)
Control	-	48.3 ± 4.0	-
AKS	50	42.6 ± 3.8	11.8
AKS	100	30.0 ± 3.2*	37.9
AKS	150	22.4 ± 2.6*	53.6
Hydrocortisone	15	20.5 ± 2.8*	57.6

[a]*Values represent mean ± SEM (n = 6);* *$P < 0.05$ *compared with control*

Table 4. Effect of aqueous extract of *Khaya senegalensis* stem bark (AKS) on the reaction time of rats (formalin test)

Treatment	Early Phase (sec)	Late Phase (sec)
Control	68.1± 4.4	297.8 ± 7.5
AKS (50 mg/kg)	55.7 ± 5.3	258.8 ± 5.9
AKS (100 mg/kg)	42.6 ± 3.4*	184.5 ± 6,3*
AKS (150 mg/kg)	33.2 ± 2.2*	126.6 ± 5.2*
Diclofenac (20 mg/kg)	24.8 ± 2.6*	87.4 ± 4.7*

*Values represent mean ± SEM (n=6); *P < 0.05 compared to the control.*

Table 5. Effect of AKS on hypotonicity-induced hemolysis

Treatment	Concentration (µg/ml)	Absorbance at 540 nm	% Inhibition of hemolysis
Control	-	0.35 ± 0.03	-
AKS	125	0.28 ± 0.02	20
AKS	250	0.26 ± 0.02*	25.7
AKS	500	0.24 ± 0.04*	31.4
Indomethacin	100	0.23 ± 0.03*	34.3

*Each value represents mean ± SEM (n = 6); *P < 0.05 compared to the control*

Table 6. Effect of AKS on heat-induced hemolysis

Treatment	Concentration (µg/ml)	Absorbance at 540nm	% Inhibition of hemolysis
Control	-	0.32 ± 0.04	-
AKS	125	0.23 ± 0.02*	28.1
AKS	250	0.20 ± 0.05*	37.5
AKS	500	0.18 ± 0.03*	43.8
Indomethacin	100	0.22 ± 0.02*	31.3

*Each value represents mean ± SEM (n = 6); *P < 0.05 compared to the control*

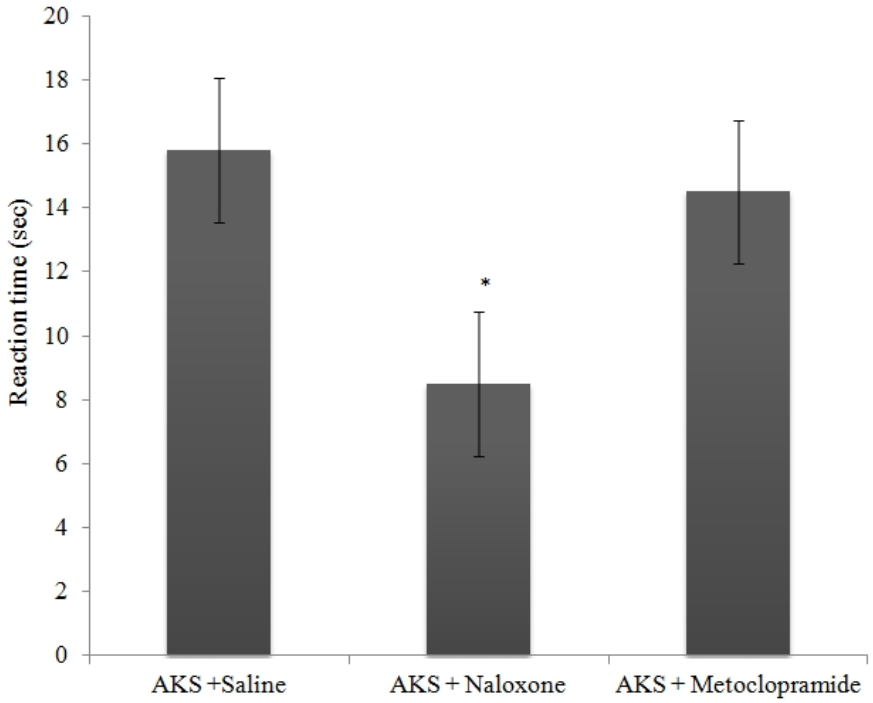

Fig. 1. Effects of naloxone and metoclopramide on reaction time of AKS-treated rats in hot-plate test

*Each value represents mean ± SEM (n = 6). *P < 0.05 compared with the control (AKS + saline)*

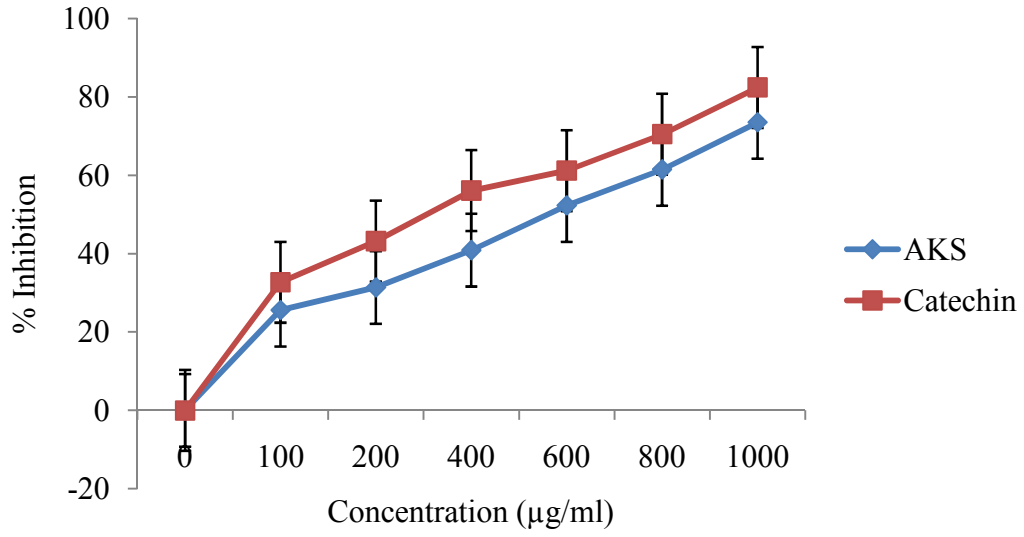

Fig. 2. DPPH free radical scavenging activity of aqueous extract of *Khaya senegalensis* (AKS)

Each value represents mean ± SEM of three replicates.

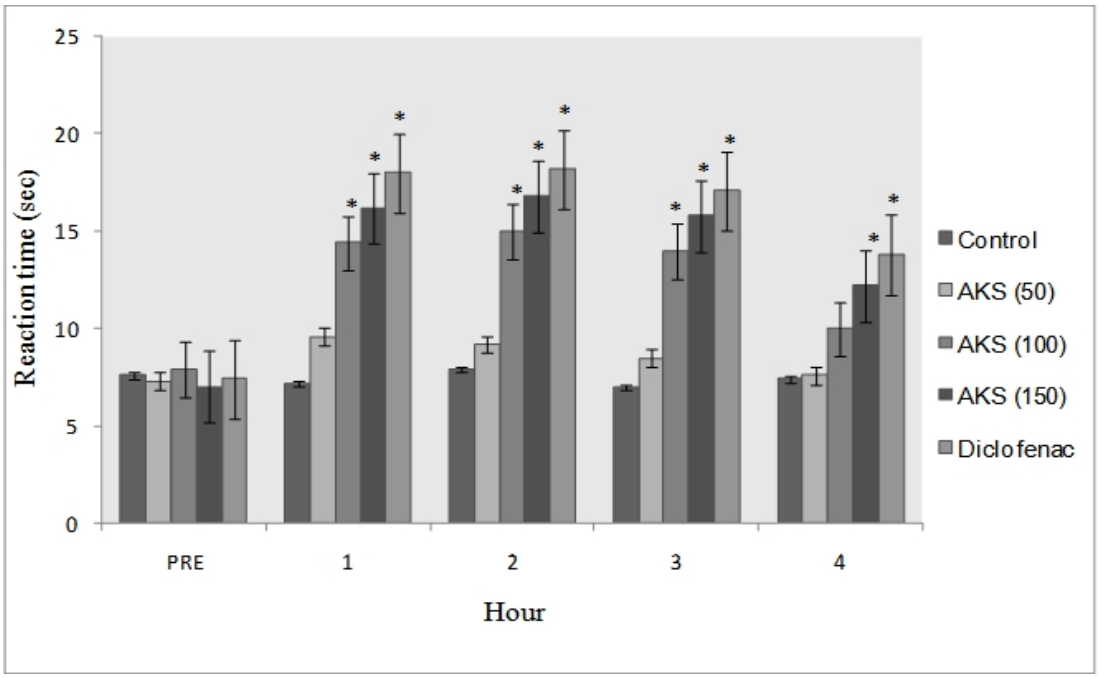

Fig. 3. Effects of aqueous extract of *Khaya senegalensis* stem bark extract on reaction time (sec) of rats (hot-plate test).

*PRE= reaction time before treatment; Values represent mean ± SEM (n = 6); *P < 0.05 compared with control.*

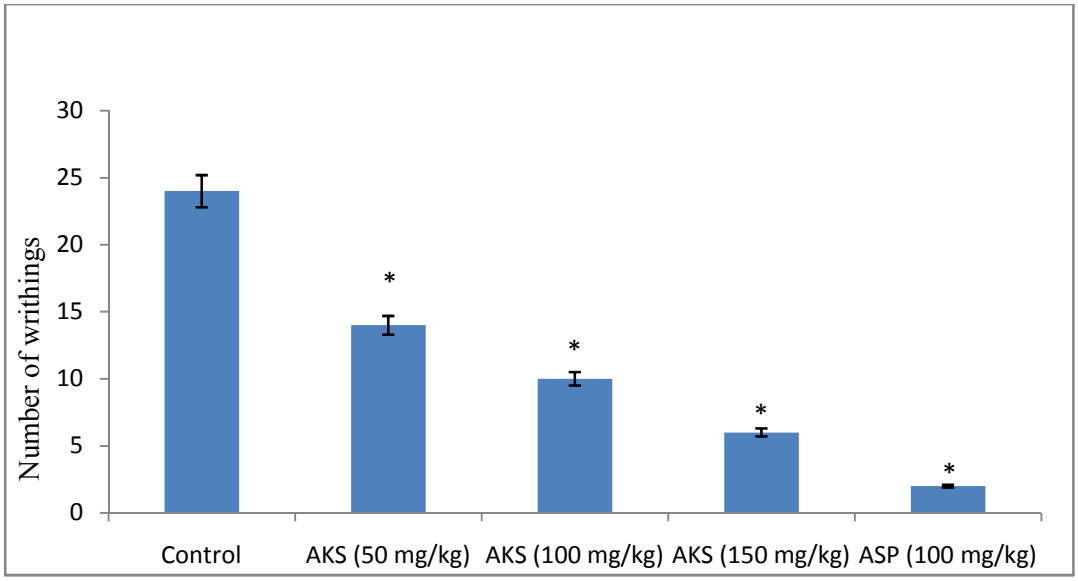

Fig. 4. Effect of aqueous extract of *K. senegalensis* (AKS) on acetic acid-induced writhing in mice

*Values are mean ± SEM (n = 6). *P < 0.05 compared with the control. ASP = aspirin*

Inhibition of rat paw edema by AKS suggests that it prevented the release or suppressed the synthesis of these mediators. Since granuloma is a typical feature of chronic inflammatory reaction, cotton pellet granuloma is commonly used as experimental model to evaluate the exudative and proliferative components of chronic inflammation [29]. Aqueous extract of *Khaya senegalensis* stem bark was able to inhibit chronic inflammation in the cotton pellet granuloma model. There was a dose-dependent inhibition of granuloma formation as shown in Table 3. Percentage inhibitions of granuloma formation were 11.8, 37.8 and 53.6% for 50, 100 and 150 mg/kg of the extract respectively. The extract probably prevented leukocyte migration which tends to prolong and maintain inflammatory reaction [30]. The extract may also have been involved in reducing the number of fibroblasts and suppression of collagen and mucopolysaccharide synthesis during granuloma tissue formation [31].

The extract caused dose-dependent significant ($P < 0.05$) inhibition of ear edema in mice. Percentage inhibitions were 13.2, 30.6 and 54.5% with 50, 100 and 150 mg/kg of the extract respectively (Table 2). Inhibition of ear edema in mice suggests that the extract has the potential to suppress topical inflammation [32].This may be due to inhibition of the release of substance P, an undecapeptide that causes vasodilatation and plasma extravasation, from the sensory neurons. Antiinflammatory activities of AKS in this study compared favorably well with the three anti-inflammatory drugs used as reference drugs. All the doses of AKS tested significantly ($P < 0.05$) reduced the licking/biting time of the formalin-injected rat paw, both in the early (19-51% reduction) and in the late (13-57 % reduction) phases (Table 4). Formalin caused pain in the early phase by directly stimulating the sensory nerve fibers while the pain of the late phase is due to the inflammatory mediators such as prostaglandin, histamine, serotonin and bradykinin [33]. Therefore the suppression of pain observed in the two phases suggests possible inhibition of sensory transmission and release of inflammatory mediators. As shown in Figs. 3 and 4, AKS also demonstrated significant ($P < 0.05$) antinociceptive activity in the hot-plate and writhing tests. This is an indication that the antinociceptive effect of the extract is peripherally and supraspinally mediated [34, 35]. Naloxone, an opioid receptor antagonist blocked the antinociceptive effect of AKS. This suggests that this effect is mediated through the stimulation of opioid receptors. Antinociception can also be mediated through dopaminergic mechanism [36]. However, this appears not to be the case in this study because metoclopramide, a dopamine receptor antagonist, failed to antagonize the antinociceptive effect of the extract (Fig. 1).

Injury to lysosomal membrane usually triggers the release of phospholipase A2 that mediates the hydrolysis of phospholipids to produce inflammatory mediators [37]. Stabilization of the membranes of these cells inhibits lysis and subsequent release of the cytoplasmic contents which in turn limits the tissue damage and exacerbation of the inflammatory response. Since the Red Blood Cell membrane is similar to that of lysosomal membrane, inhibition of RBC hemolysis will provide good insight into the inflammatory process. Therefore membrane stabilizing effect of AKS was evaluated in human erythrocytes using heat- and hypotonicity-induced hemolysis. A dose-related inhibition of hemolysis was observed in the two models as shown in Table 5 and Table 6. This might have contributed to the anti-inflammatory activity of the extract.

Free radicals have been implicated in the pathogenesis of hemodynamic disturbances and cellular damage of several inflammatory states [38]. It has also been widely reported that antioxidants play a vital role in delaying, intercepting or preventing oxidative reactions catalyzed by free radicals during inflammatory process [39]. The results of the DPPH assay show that AKS possesses free radical scavenging activity that is close to that of catechin (Fig. 2). Therefore the anti-inflammatory activity of the plant extract could be due to its

antioxidant potential and free radical scavenging activity [40]. Studies have shown that the presence of phytochemicals such as flavonoids, phenols, saponins, tannins and terpenoids in plants are responsible for their anti-inflammatory properties [41]. Interestingly, the phytoconstituents of stem bark of *Khaya senegalensis* has been reported to include saponins, tannins and phenols among others [42]. These may be responsible for its anti-inflammatory properties.

4. CONCLUSION

In all experimental models used in this study, AKS exhibited significant anti-inflammatory and antinociceptive properties which compare favorably well with the effects produced by the standard drugs used. The antinociceptive effect is mediated via central and peripheral mechanisms. These findings provide scientific basis to justify the traditional use of the plant in inflammatory diseases. The study showed that *Khaya senegalensis* is a medicinal plant with good potential for the development of new anti-inflammatory drugs.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Kumara R, Clermont G, Vodovotz Y, Chow CC. The dynamics of acute inflammation. J. Theoretical Biol. 2004;230:145-155.
2. Norman JE, Bollapragada S, Yuan M, Neison SM. Inflammatory pathways in the mechanism of parturition. BMC Pregnancy and Childbirth. 2007;7:2393-2397.
3. Cottam DR, Mattar SG, Barinas-Mictchell E, G. Eid G, Kuller I, Kelley DE, Schauer PR. The chronic inflammatory hypothesis for the mobidity associated with morbid obesity: implications and effects of weight loss. Obesity Surgery. 2004;14:589-600.
4. Sen S, Chakraborty R, De B, Ganesh T, Raghavendra HG, Debnath S. Analgesic and anti-inflammatory herbs: a potential source of modern medicine. Int J Pharm Sci Res. 2010;1(11):32-44.
5. Saha A, Ahmed M. The analgesic and anti-inflammatory activities of the extract of *Albizia lebbeck* in animal model. Pak J Pharm Sci. 2009;22:74-77.
6. Shah BS, Nayak BS, Seth AK, Jalalpure SS, Patel KN, Patel MA, Mishra AD. Search for medicinal plants as a source of anti-inflammatory and anti-arthritic agents- a review. Pharmacognosy Magazine. 2006;2:77-86
7. Verma S, Singh SP. Current and future status of herbal medicine. Veterinary World. 2006;1:347-350.
8. Gbile ZO. Ethnobotany, taxonomy and conservation of medicinal plant. In: Sofowora A.O, editor. The state of medicinal plant research in Nigeria. Ife University press; 1986.
9. Zimmermann M. Ethical guidelines for investigation of experimental pain unconscious animals. Pain. 1983;16:109-110.

10. Kolawole OT, Abiona FE, Kolawole SO, Ayankunle AA, Olaniran OI. Effect of *Momordica charantia* fruit extract in normal and alloxan-diabetic rats. Int. J Pharmacol. 2011;7(4):532-535.
11. Winter CA, Risley EA, Nuss CW. Carrageenan-induced oedema in hind paw of the rat as an assay for anti-inflammatory drugs. Proc Soc Exp Biol and Med. 1962;111:544-547.
12. Bamgbose SOA, Noamesi BK. Studies on cryptolepine II: inhibition of carrageenan-induced edema by cryptolepine. Planta Medica. 1981;42:392-396.
13. Agbaje EO, Adeneye AA, Adeleke TI. Anti-nociceptive and anti-inflammatory effects of a Nigerian polyherbal tonic (PHT) extract in rodents. Afr J Compl Alt Med. 2008;5(4):399-408.
14. Ishola IO, Ashorobi RB. Anti-stress potential of aqueous root extract of *Cnestis ferruginea*. Int J Pharmacol. 2007;3(3):295-298.
15. Akindele AJ, Adeyemi OO. Anti-inflammatory activities of the aqueous leaf extract of *Byrsocarpus coccineus*. Fitoterapia. 2007;78:25-28.
16. Agbaje EO, Fageyinbo MS. Evaluating Anti-Inflammatory activity of aqueous root extract of *Strophanthus hispidus* DC. (Apocynaceae). Int J Appl Res Nat Prod. 2012;4(4):7-14.
17. D'Arcy PF, Howard EM, Muggleton PW, Townsend SB. The anti-inflammatory action of griseofulvin in experimental animals. J Pharm Pharmacol. 1960;12:659-665.
18. Olajide OA, Kolawole OT, Fagbohun TR, Ajayi FF. Evaluation of the anti-inflammatory properties of *Chlorophora excelsa* stem bark extract. Pharmaceut Biol. 2005;43(9):746-748.
19. Langermann L, Zakouski ML, Piskoun B, Grant GJ. Hot plate versus tail flick: Evaluation of acute tolerance to continuous morphine infusion in the rat model. J Pharmacol Toxicol Methods. 1995;34:23-28.
20. Arambewela LSR, Arawwawala LDAM, Ratnasooriya WD. Antinociceptive activities of aqueous and ethanol extracts of *Piper betle* leaves in rats. Pharmaceut Biol. 2005;43(9):766-772.
21. Farsam H, Amanlou M, Dehpour AZ, Jahaniani F. Anti-inflammatory and analgesic activity of *Biebersteinia multifida* DC root extract. J Ethnopharmacol. 2000;71:443-447.
22. Tornos MP, Saenz MT, Garcia MD, Fernandez MA. Antinociceptive effects of the tubercles of *Anredera leptostachys*. Journal of Ethnopharmacology. 1999;68(1–3):229–234.
23. Shinde UA, Phadke AS, Nari AM, Mungantiwar AA, Dikshit VJ, Saraf MN. Membrane stabilization activity - a possible mechanism of action for the anti-inflammatory activity of *Cedrus deodora* wood oil. Fitoterapia. 1999;70:251-257.
24. Umapathy E, Ndebia EJ, Meeme A, Adam, Menziwa BP, Nkeh-Chungag BN, Iputo JE. An experimental evaluation of *Albuca setosa* aqueous extract on membrane stabilization, protein denaturation and white blood cell migration during acute inflammation. J Med Plants Res. 2010;4(9):789-795.
25. Okoli CO, Akah PA, Onuoha NJ, Okoye TC, Nwoye AC, Nworu CS. *Acanthus montanus*: An experimental evaluation of the antimicrobial, anti-inflammatory and immunological properties of a traditional remedy for furuncles. BMC Complementary Alternative Med. 2008;8:27.
26. Hantano T, Edamatsu R, Yoshida T, Okuda T. Phenolic constituents of licorice II. Structures of licopyranocoumarin, licoarylcoumarin and glisoflavone, and inhibitory effects of licorice phenolics on xanthine oxidase. Chem. Pharm. Bull. 1989;37:3005 – 3009.
27. Das BN, Saha A, Ahmed M. Anti-inflammatory activity of bark of *Xeromphis spinosa*. Bangladesh J. Pharmacol. 2009;4:76-78.

28. Unnisa A, Parven TD. Anti-inflammatory and acute toxicity studies of the extract from the rhizomes of *Alpinia galangal* Willd. Der Pharmacia Sinica. 2011;2(2):361-367.
29. Spector WG. The granulomatous inflammatory exudates. Int Rev Exp Path. 1969;8:1-55.
30. Wu JT, Wu LL. Acute and chronic inflammation: effect of the risk factor (s) on the progression of the early inflammatory response to oxidative and nitrosative stress. J Biomed and Lab Sci. 2007;19:71-74.
31. Garg, V.K. Paliwal, S.K. Anti-inflammatory activity of aqueous extract of *Cynodon datylon*. Int J. Pharmacol. 2011; 7: 370-375.
32. Zitterl-Eglseer K, Sosa S, Jurenitsch J, Schubert-Zsilavecz M, Della-Loggia R, Tubaro A, Bertoldi M, Franz C. Anti-edematous activities of the main triterpendiol esters of marigold (*Calendula officinalis* L.). J Ethnopharmacol. 1997;57:139-144.
33. Tjolsen A, Berge DG, Hunskaar S, Rosland JH, Hole K. The formalin test: An evolution of the method. Pain. 1992;51:5-17.
34. Lopez-Munoz FJ, Salazar LA, Castaneda-Hernandes G, Villarreal JE. A new model to assess analgesic activity: Pain induced functional impairment in the rat (PIFIR). Drug Develop Res. 1993;28:169-175.
35. Wong CH, Day P, Yamush J, Wu W, Zbuzek UK. Nifedipine-induced analgesia after epidural injections in rats. Anesth Analg. 1994;79:303-306.
36. Jensen TS, Yaksh TL. Effects of an intrathecal dopamine agonist, apomorphine, on thermal and chemical evoked noxious responses in rats. Brain Res. 1986;296:285-293.
37. Umukoro S, Ashorobi RB. Evaluation of the anti-inflammatory and membrane stabilizing effect of *Eupatorium odoratum*. Int. J. Pharmacol. 2006; 2(5): 509-512.
38. Nussler AK, Billiar TR. Inflammation, immunoregulation and inducible nitric oxide synthase. J Leukoc Biol. 1993;54:171–178.
39. Brahmachari G, Gorai D. Progress in the research on naturally occurring flavones: An overview. Curr. Org. Chem. 2006;10:873–898.
40. Gupta VK, Sharma SK. Plants as natural antioxidants. Nat. Prod. Radiance. 2006;5(4):326-334.
41. Govindappa M, Naga SS, Sadananda TS, Chandrappa CP. Antimicrobial, antioxidant and in vitro anti-inflammatory activity of ethanol extract and active phytochemical screening of *Wedelia trilobata* (L.) Hitchc. J Pharmacognosy Phytother. 2011;3(3):43-51.
42. Kubmarawa D, Khan ME, Punah AM, Hassan M. Phytochemical screening and antimicrobial efficacy of extracts from *Khaya senegalensis* against human pathogenic bacteria. Afr. J Biotechnol. 2008;7(24):4563-4566.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=177&id=12&aid=839