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Full Length Research Paper

Analgesic and anti- inflammatory activities of the ethanolic stem bark extract of *Kigelia africana* (Bignoniaceae*)*

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The ethanolic extract of *Kigelia Africana,* Family: (Bignoniaceae) was evaluated for analgesic property using acetic acid induced mouse writhing and hot plate reaction time and anti-inflammatory property using the carrageenan induced paw edema and its probable mechanism evaluated in mice and guinea pigs. *K. africana* extract showed a dose dependent significant reduction of the number of writhes (P<0.001) with 500 mg/kg body weight dose giving the highest reduction. The extract showed an insignificant elongation of the hot plate reaction time (P>0.05). In the carragenan induced paw edema, a dose dependent significant inhibition was observed (P<0.001) between the 2nd and 5th h. It is clear that the ethanolic stem bark extract of *K. africana* has significant analgesic and anti inflammatory activity. Inhibition of the synthesis of prostaglandins and other inflammatory mediators probably accounts for the analgesic and anti-inflammatory properties.

Key words: *Kigelia africana*, central and peripheral analgesic, anti-inflammatory.

INTRODUCTION

Kigelia fricana (Lam). Benth. (Family: Bignoniaceae) also called *Kigelia pinnata* is a plant that is widely distributed in the south, central and West Africa. Locally known as the Cucumber or Sausage tree because of the huge fruits (average 0.6 m in length and 4 kg in weight), which hangs from long fibrous stalks. The tree can grow to more than 20 m tall. It is found mostly in riverine areas. Its distribution is restricted to the wetter areas. Different parts of this plant have been claimed to serve various purposes in different parts of the world (Burkill, 1985).The fruits pods are very fibrous with numerous seeds and tend to be inedible to humans as well as being poisonous when unripe. However in Malawi during famine, the seeds are roasted and eaten. The Tonga applies powdered fruit as a dressing to wound. Unripe fruit is used in central Africa as a dressing for wounds, and in the treatment of haemorrhage and rheumatism (Pooley, 1993).

Venereal diseases are commonly treated with the extracts of *K. fricana* usually in palm wine as oral medication. The fruits and barks, ground and boiled in water Are also taken orally or used in treating stomach ailments. The Shona people tend to use the bark as powder or infusion for application to ulcers, or applied in treatment of pneumonia (Pooley, 1993).

Most commonly, traditional healers have used the sausage tree to treat a wide range of skin ailments from relatively mild complaints such as fungal infections, boils, psoriasis and eczema, through to the more serious disease like leprosy, syphilis and skin cancer. Previous studies of the fruits of *K. africana* showed some antibacterial activity (Grace et al., 2002). However there is no report on the anti-inflammatory and analgesic properties of the bark of this plant. This report therefore presents studies on the above mentioned properties of the ethanolic extract of the bark.

MATERIALS AND METHODS

Plant material

The stem barks were collected based on ethno pharmacological information. The barks were collected in Okomu Forest Reserve, Udo in Benin City, Edo State. The botanical identity of the plant and its bark was by Alhaji Alasa Abubakar of the Department of Pharmacogonosy, University of Benin, while it was authenticated at

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Forest Research Institute of Nigeria (F.R.I.N., Ibadan) where a herbarium specimen No FHI 107654 was deposited for future reference.

Immediately after collection, the barks were cut into small pieces and dried under sunlight. The dried barks were pulverized into a smooth powder using impact mill, weighed and kept for further analysis.

Phytochemical screening

Qualitative tests for the presence of plant secondary metabolites such as carbohydrates, alkaloids, tannins, flavonoids, saponins and glycosides were carried out on the bark powdered using standard procedures (Sofowora, 1984).

Extraction

500 g of the powdered material was mixed with absolute alcohol (2.5 l) and left for 72 h. The mixture was stirred at 6 h intervals using a sterile glass rod, the extract were passed through a filter paper. The filtrates were concentrated with a vacuum pump at 40°C, giving a yield of 3.78%, which was stored in universal bottles and refrigerated at 4°C prior to use.

Drugs and chemicals

Acetic acid (BDH Chemicals), indomethacin (Chemiron Ltd), morphine, aspirin (BDH Chemicals) and carrageenan (Sigma Aldrich) were used in the experiments.

Animals

Swiss mice (20 – 25 g) of both sexes and guinea pigs (280 – 350 g) of either sex kept at the Pharmacology Animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria were used. The animals maintained under standard environmental condition had free access to standard diet (Ladokun feeds Ibadan, Oyo State) and water.

Mouse writhing assay

The method of Koster et al. (1959) was used. The extract (100, 200 and 500 mg/kg body weight orally) and aspirin (100 mg/kg body weight intraperitoneally) were administrated to mice 60 and 30 min, respectively, before intraperitoneal injection of acetic acid (0.6% in normal saline, at a dose of 10 m1/kg body weight). Distilled water was used as the control. The number of writhes was counted for 30 min. The mean values were recorded.

Hot plate test

Mice were divided into five groups of five each after initial screening. Distilled water served as the control and was given to the first group at a dose of 10 ml/kg, the extract at 100, 200 and 500 mg/kg body weight were given to the second, third and fourth groups, respectively. The fifth group received the reference drug, morphine at 10 mg/kg body weight intraperitoneally.

The animals were dropped gently on the hot plate maintained at 53 ± 0.5°C (Turner, 1965). This was done 5 min before the administration of distilled water, the extracts and morphine and at 30, 90 and 150 min following administration. The time in seconds for the mouse to either jump or lick its hind paw was taken as the reaction time. The mean values were recorded.

Anti- inflammatory activity

The anti-inflammatory activity was evaluated in guinea pigs in groups of five (5) animals for each dose according to the carrageenan-induced paw oedema described by Winter et al. (1963). The extract at doses of 100, 200 and 500 mg/kg body weight were administered orally an hour before the subcutaneous injection of 0.1 ml of sterile saline solution of carrageenan (1%) in the right hind paw. The control group received distilled water while the reference drug indomethacin 10 mg/kg was also given to another group prior to induction of oedema. Paw sizes were measured with a venier caliper (Tricle brand) 30 min before administration of carrageenan, then thereafter at 30 min, 1 2, 3, 4, 5, and 6 h after the injection of the inflammatory agent. The average size of the paw measured in millimetres was calculated from 3 or 4 measurements which did not differ from more than 4%. These individual records allowed to determine the average size for each group (st) and then the percentages of variation or percentages of oedema by comparison with the average size obtained for each group before any treatment (so).

Percentages of inhibition were obtained for each group and at each record using the following ratio: [(st – so) control – (st – so) treated] x100/ (st – so) control. Where “st” is the mean paw size for each group after carrageenan treatment and “so” is the mean paw size obtained for each group before carrageenan treatment.

Statistical analysis

All data were expressed as mean ± SEM and in bar and line graphs and where applicable was analyzed by student’s t-test using graph pad instant version 2.05a. The level of significance was from $p<0.05$.

RESULTS

Phytochemistry

The stem bark was observed to contain saponins, carbohydrates, glycosides and reducing sugars with no traces of alkaloids, tannins and anthracene derivatives.

Anti-inflammatory

The extract inhibited inflammation induced by carrageenan as shown in Figure 1. The inhibitory effect produced by the ethanolic extract was greatest at the 3rd hour and lasted till the 5th hour. The inhibitory effect of the extract and indomethacin expressed in percentages is shown in Table 1. From this table it is clear that the highest percentage inhibition of oedema was produced by the 500 mg/kg dose of the extract.

Analgesic

In the mouse writhing assay, the extract caused a significant ($p<0.0001$) inhibition of the number of writhes (Table 2). The effect of the extract on hot plate reaction time is shown in Figure 2. The extract failed to increase mice reaction time on hot plate.

Table 1. Inhibitory effects of the extract and indomethacin on carrageenan-induced paw oedema.

Treatments (mg/kg)	Percentage inhibition						
	30 min	1 h	2 h	3 h	4 h	5 h	6 h
100	10.4[a]	16.4[a]	49.5[b]	47.9[b]	29.2[b]	42.9[a]	68.18
200	26.0[a]	32.7[a]	50.5[b]	51.0[b]	26.4	42.9[a]	90.9
500	45.8[a]	49.1[a]	60.0[b]	57.3[c]	52.8[a]	82.5[b]	90.9
Indomethacin (10)	29.2	43.6[a]	61.0[b]	60.4[b]	50.0[a]	96.8[b]	95.5

Values are mean percentage inhibitions of oedema in both the extract and indomethacin treated groups (n = 5 per group). [a]P<0.05, [b]P<0.001, [c]P<0.0001 significantly different from the control group.

Table 2. The effect of the extract and aspirin on acetic acid-induced writhing test.

Treatment (mg/kg)	No of writhes (per 30 min)	Percentage inhibition
Control	85.0 ± 1.34	-
100	36.8 ± 3.10 [a]	56.7
200	26.4 ± 1.75[a]	68.9
500	29.6 ± 7.31[a]	65.2
Aspirin (100).	36.8 ± 5.8[a]	56.7

Values are mean number of writhes ± SEM. (n = 5 per group). [a]P<0.0001 significantly different from control group.

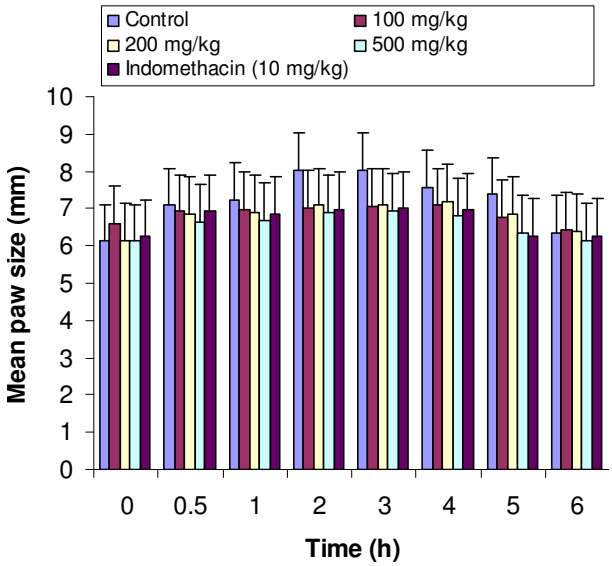

Figure 1. Effect of the extract on carrageenan induced paw oedema compared to the control and indomethacin treated groups. Values are mean paw sizes in mm ± SEM (n = 5 per group). 10 mg/kg body weight of indomethacin was administered. 0h: indicates 30 min before any treatment and 0.5 h is 30 min after treatment.

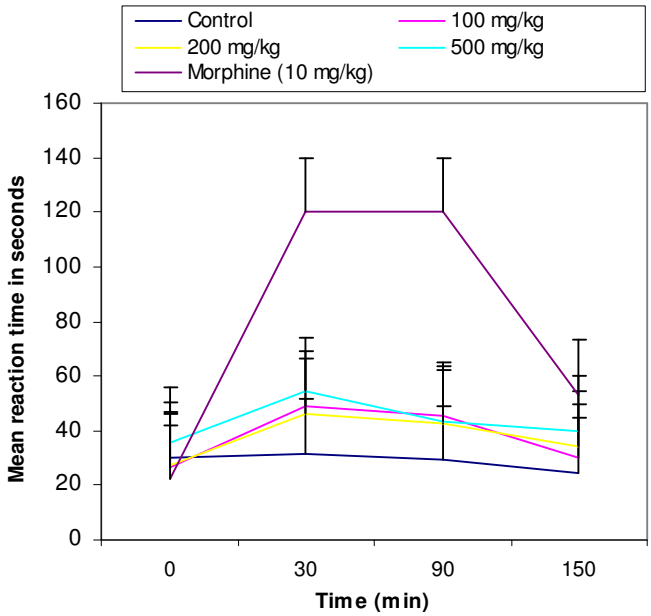

Figure 2. Effect of the extract on hot plate reaction time compared to the control and morphine treated groups. Values are mean reaction time in seconds ± SEM (n = 5 per group). The 0 min indicates the mean reaction time on the hot plate prior to any administration

DISCUSSION

Induction of inflammation by carrageenan involves 3 distinct phases of mediator release. The first phase involves the release of histamine and serotonin and last between the first to the second hour, the second phase is the release of kinins lasting from the second to the third hour while the third phase involves the release of prostaglandins and lasts from the third to the fifth hour (Surender and Mafumdar, 1995). Thus it can be inferred that the mechanism through which the extract elicits its effects is via the inhibition of the synthesis of kinins and prostaglandins, since the extract was effective at these phases of mediator release.

The extract's anti-inflammatory effect was dose-dependent with 500 m/kg body weight dose giving the highest percentage inhibition. When compared with the standard reference drug, indomethacin (10 mg/kg) the percentage inhibition produced by the extract was 98% at the second hour, 94.8% at the third hour and 85% of that

of indomethacin at the fifth hour (results not shown). Its effects were, however, higher at the 30th min, first and fourth hour as the percentage inhibition produced by the extract was 156.8, 112.6 and 105.6% of indomethacin, respectively (result not shown). This data thus provides pharmacological basis for the use of this plant in the treatment of rheumatism and other ailments in which inflammation is implicated (Watt and Breyer-Bradwijk, 1962).

Inhibition of acetic acid-induced writhing in mice suggests that the analgesic effect of the extracts may be peripherally mediated via the inhibition of the synthesis and release of prostaglandins (Koster et al., 1959). Writhes can be described as a wave of constriction and elongation passing caudally along the abdominal wall with twisting of the trunk and extension of the hind limbs in mice. This is due to the nociceptive property of acetic acid (Surender and Mafumdar, 1995). The percentage of inhibition, clearly shown in Table 2, also indicates that the extract at 200 and 500 mg/kg produced a higher inhibition when compared to aspirin (100 mg/kg) a known standard analgesic drug. The inhibitory effect was 139 and 124% of the effect produced by aspirin at 200 and 500 mg/kg doses, respectively (result not shown).

The extract failed to increase mice reaction time on hot plate. The difference between the mean reaction time of the *K. africana* treated groups and the control group was not statistically significant at all doses tested. Its effect was not comparable to morphine which had a mean reaction time of above 2 min which was the cut off point ($p<0.0001$).

The analgesic effect of the stem bark of *K. africana* has not been previously reported and the mechanism by which it occurs is mostly likely via the inhibition of prostaglandin synthesis as indicated by its' inhibition of acetic acid-induced mouse writhing. Also, it is known that centrally acting analgesic drugs elevate the pain threshold of mice towards heat and pressure (Adeyemi et al., 2003). From the above findings, the extract failed to raise the pain threshold on the hot plate which indicates that it might not be centrally acting. The extract thus seems to possess analgesic properties, which are mediated via peripheral inhibitory mechanisms. In conclusion, this work provides a rational for the use of this plant in pain and inflammatory disorders in folk medicine.

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REFERENCES

Adeyemi OO, Okpo SO, Okpaka O (2004). The analgesic effect of the methanolic extract of *Acanthus motanus*. J. Ethnopharmacol. 90: 45-48.

Burkill HM.(1985). The useful plants of west Tropical Africa. (Use P.I WT Afr) 1: 254-257.

Grace OM, Light ME, Lindsey KL, Moholland DA, Staden JV, Jager AK (2002). Antibacterial activity and isolation of antibacterial compounds from the fruit of the traditional African medicinal plant *Kigelia africana*. S. Afr. Bot. 68: 220-222**.**

Koster R, Anderson M, De Beer EJ (1959). Acetic acid for analgesic screening. Fed. Proc. 18: 418-420.

Lorke D (1983). A new approach to practical acute toxicity testing: Arch. Toxicol. 54: 275-287.

Pooley E (1993). The complete guide to trees of Natal, Zululand and Transkei, Natal Flora Publications Trust. pp. 22.

Surender S, Mafumdar DK (1995). Analgesic activity of *Ocinum sanctum* and its possible mechanism of action. Int. J. Pharmacog. 33(3): 188-192.

Sofowora A (1984). Medicinal plants and traditional medicine in Africa. John Wiley, Chichester. pp. 142-145.

Tjolsen A, Berge OG, Hunskaar S, Rosland JH, Hole K (1992). The formalin test: an evaluation of the method. Pain 51: 5-17.

Tuner RA (1965). Screening methods in Pharmacology. Acad. press New york, p. 158.

Walt JM, Breyer-Bradwijk MG (1962). The medicinal and poisonous plants of Southern and Eastern Africa. Livingstone, London. pp. 52.

Winter CA, Risley EA, Nuss GW (1962). Carragenan-induced oedema in hind paw of the rat as an assay for anti inflammatory drugs. Proc. Soc. Exp. Boil. Med. 3: 544-547.