Pharmaceutical and Pharmacological Letters

Pharm Pharmacol Lett 7 (1997) 2/3: 67 – 70

Protective role of Kigelia africana fruits against benzo(a)pyrene-induced fore-stomach tumourigenesis in mice and against albumen-induced inflammation in rats

Professor H. Kolodziej Institut fuer Pharmazie II Freie Universitaet Berlin, Germany

Abstract

Ethanolic extract of ***Kigelia africana*** fruits was tested for pharmacological activities in vitro and in vivo. The extract showed moderate cytotoxic activity in the brine shrimp (Artemia salina) nauplii bioassay with an LC_{50} of 7500 µg/ml.
It did not induce any DNA damage in the *Salmonella typhimurium* strains TA 98 and TA 100 disc spot mutagenicity assay. The acute toxicity test showed an LD_{50} of 1.3 g/kg i.p. in female Swiss mice.
Oral administration of extract to mice resulted in a significant inhibition in the tumour incidence and burden by 67% and 76% respectively in the benzo(a)pyrene-induced for-estomach tumourigenesis model.
The extract evinced marked anti-inflammatory effects in female Wistar rats as reflected by a significant inhibition of the increase in rat paw circumference of 72% (100mg/kg) and 54% (200mg/kg) which was caused by subplantar injection of fresh egg albumen.
These results add credence to the folclore use of the fruits of ***Kigelia africana*** for the treatment of cancer and oedema in the traditional system of medicine in Nigeria.

Introduction

Kigelia africana (syn. Kigelia pinnata) is a tropical tree, which is one of the African medicinal plants of great value for the treatment of a wide variety of diseases. For example, water extract of the stem bark of this plant is used for the treatment of gynaecological conditions, veneral diseases, dysentery, epilepsy, wounds, sores, abscesses, diarrhoea and oedema.
In Ivory Coast, the fruit decoction is a remedy for elephantiasis and oedema of the legs.
In Sierra Leone heated bark is applied to women's breast to hasten their return to normal after a suckling child has been weaned, while the Sambaa in Tanzania use the bark for the treatment of swelling of the breasts.
In Malawi, the dried root bark is used to treat cancer of the uterus and alimentary canal, while in Nigeria the bark and fruits are used in the treatment of malignant tumours of the breast.
The aim of the present study was to provide evidence for the traditional use of the fruits as an anti-cancer and an anti-oedemateous drug.

Materials and Methods

Animals: All experiments performed on laboratory animals in this study followed the "Principles of laboratory animal care". We obtained inbred Swiss mice and Wistar rats both female and about 6-8 weeks old. They have been living in plastic cages under standard laboratory conditions and acclimatized for one week before use. The animals were fed ad libitum with standard laboratory diet and water.

Chemicals: Benzo(a)pyrene, emetine, streptozotocin and indomethacin were obtained from Sigma Chemical Company, St. Louis, USA. Biotin was purchased from Calbiochem, San Diego, California, USA. Histidine was procured from Riedel-De-Haen, Hannover, Germany. Nutrient broth was obtained from Merck, Germany, while agar was purchased from Aldrich, Germany.
All chemicals were highest purity and commercially available.

Bacterial strains: Histidine auxotrophs of *Salmonella typhimurium* TA 98 and TA 100 were a generous gift from Professor B.N. Amos University of California, Berkeley, USA. The procedures for the maintenance of the stock cultures of each bacterial strain were as reported.

Collection and indentification of plant material: Mature fruits were collected from Abuja, Nigeria. The fruits were indentified as ***Kigelia africana*** fruits by the Institute's taxonomist, Mr. A.O. Ohneri.
A NIPRD voucher herbarium specimen No. 3669 has been preserved in the herbarium of the National Institute for Pharmaceutical Research and Development, Abuja, Nigeria.

Preparation of the extract: The fruits of ***Kigelia africana*** were cut into small pieces, sun dried and coarsely powdered. The powder (200g) was extracted by maceration overnight with 95% ethanol (1:10 ratio) with intermittent vigorous shaking. The extract was filtered and concentrated to give a residue of 41.7g (i.e. 20.8%). This residue is referred to hereafter as KAFE.

Cytotoxicity assay: The method of Meyer et al was followed with some modifications for use in the 96-well microplate. To about 150 ml solution of ocean water in a beaker supplemented with 6 mg/ml dried yeast, about 50 mg of *Artemia salina* (Brine shrimp) eggs was added. After 48h incubation in a warm room (22-29ºC), nauplii were collected with a pasteur pipette after attracting the organisms to one side of the vessel with a light source. Nauplii were seperated from the eggs by pipetting them in a small beakers, containing sea water.
40 mg of KAFE were seperately dissolved in 1 ml of water. 100, 50, 25 and 12.5 µl of each solution was transferred into vials corresponding to 10'000, 5000, 2500 and 1250 µg/ml of KAFE, respectively. The volume was adjusted to 100 µl with the water. Each dosage was tested in triplicate.
A supension of nauplii containing 10-15 organisms (100µl) was added to each well and the final volume made up to 400 µl with sea water. 24 h later, the number of surviving shrimps at each dosage was estimated.
The cytotoxicity data were plotted as dose-effect curves from which the 50% cytotoxicity concentration (LC_{50}) was calculated for each chemical. Emetine was used as positive control.

Genotoxicity study: The bacterial mutagenicity tests were performed using *Salmonella typhimurium* TA 98 and TA 100. KAFE was dissolved in distilled water and applied on sterile filter paper disc (6 mm diameter, Whatman No. 3). They were allowed to air dry in the Laminar flow hood under a diffused light.
A maximum of 500 µg of the extract was applied on each disc. Four discs were used per chemical per tester strain for each experiment.
Briefly, 0.1 ml of overnight bacterial culture were added to 2 ml molten soft agar at 45ºC, gently mixed and poured over minimal glucose agar medium plates. The discs were then placed over the plates, incubated at 37ºC for 48 h and then examined for revertants. The plates were also examined under UV (365 nm) to find out whether or not the compounds did in fact diffuse from the discs. Plates treated with standard mutagen alone (Streptozotocin 5 µg/disc) were kept as positive controls.
Each experiment was repeated twice.

Acute toxicity study: KAFE was subjected to acute toxicity testing in female Swiss albino mice by intraperitoneal (i.p.) route according to Lorke D.

Mouse fore-stomach carcinogenesis study: The effect of KAFE on B(a)P-induced fore-stomach tumours in Swiss mice was studied. Six – eight weeks old female Swiss mice were adminstered with the extract (2 mg/animal/day) for two weeks. From the third week, the mice received 8 doses of 1 mg of B(a)P (twice a week for 4 weeks) in 0.1 ml of peanut oil by gavage. KAFE treatment was continued for 2 weeks after the cessation of the carcinogen treatment. The animals were divided into four treatment groups (15 animals/group).

Group 1	B(a)P-treated positive controls:	Mice received B(a)P twice a week for 4 weeks.
Group 2	B(a)P + KAFE:	Mice received KAFE (2 mg/animal/day) through drinking water two weeks before, during and after the carcinogen treatment.
Group 3	KAFE control:	Mice received KAFE (2 mg/animal/day) through drinking water for 8 weeks.
Group 4	Peanut oil treated controls:	Mice received 0.1 ml of peanut oil twice a week for 4 weeks.

After completion of the experiment, the animals were kept under observation and killed by cervical dislocation at the age of 180 days. The stomach was fixed in an expanded state by intragastric injection of 10% formalin. After 24 h the stomach was cut open longitudinally and papillomas that were more than 1mm in diameter were counted under a dissecting microscope.

Anti-inflammatory study: Adult Wistar rats (180 ± 20g) fasted for 12 h and deprived of water only during the experiment were used. Inflammation of the hind paw was induced 0.05 ml of 50% solution of fresh egg albumen into the subplantar surface of the right hind paw. This treatment was found to cause swelling of the paw which reaches a peak in about 90 min and retained about the same degree of oedema for several hours. Depreviation of water was to ensure uniform hydration and to minimize variability in oedematous response. Indomethacin (10 mg) was used as a positive control, while untreated control rats received an equivalent amount of distilled water. Four experimental groups of five animals each were set up in this study.

Group 1	received intraperitoneal (i.p.) injection of distilled water and fresh egg albumen solution.
Group 2 – 4	received i.p. injections of indomethacin (10 mg/kg), KAFE 100 mg/kg respectively 200 mg/kg dissolved in distilled water.

After one hour, inflammation of the hind paw was induced by injecting the solution of the fresh egg albumen into the subplantar surface of the right hind paw. Oedema was assessed in terms of the difference between zero time linear circumference of the injected paw and its circumference at 30 min intervals after injecting the egg albumen. Average oedema percent inflammation and percent inhibition of oedema was calculated for each dose. Percent inflammation was derived from the formula:

$$\% \text{ inflammation} = \frac{\text{Average inflammation at time t}}{\text{Average inflammation of control at same time}} \times 100$$

Percent inhibition of oedema was calculated from the formula:

$$\% \text{ inhibition} = 100 - \% \text{ inflammation}$$

Statistical analysis: Student's *t*-test was used to determine the statistical differences in:

a) the number of tumours/animal between the control and treated group and
b) the average inflammations between the treated and control groups.

The X^2 test was used to analyse the difference in the number of tumour bearing animals.

Results

Cytotoxicity bioassay of Kigelia africana fruit (KAFE)

Results from the Artemia salina cytotoxicity bioassy showed that the amount of KAFE exerting 50% cytotoxicity (LC_{50}) was 7500 µg/ml. In contrast the emetine which was used as the positive control showed a LC_{50} of 255 µg/ml in the assay.

Bacterial mutagenicity assay of KAFE

Table 1 shows that KAFE was not found to be mutagenic in *Salmonella typhimurium* strains TA 98 and TA 100 tested without metabolic activation. The number of revertants were not different as compared to the negative control, while the positive control plates showed high amount of revertants colonies.
Under the ultraviolet light, the compounds were found to diffuse from the discs.

Table 1 Non-mutagenicity of an ethanolic extract of ***Kigelia africana*** fruit (KAFE) in *Salmonella typhimurium* TA 98 and TA 100 strains without metabolic activation.

	Salmonella typhimurium strains	
Treatment	TA 98	TA 100
KAFE	-	-
Streptozotocin	+	+
Std. revertants	21 ± 3	134 ± 30

Results are mean = S : E, KAFE - 500 µg/disc, streptozotocin = 5 µg/disc
(+) = positive mutagenicity (-) = negative mutagenicity

Acute toxicity assay KAFE was found to be relatively save as no lethalithy was observed at even 1000 mg/kg i.p. in mice. The first death was recorded in the group treated with 1600 mg/kg. The LD_{50} was calculated to be 1.3 g/kg i.p. in female Swiss mice.

Table 2 Inhibition of Benzo(a)pyrene (B(a)P)-induced fore-stomach tumours in female Swiss mice

Group	Wt. Gain (g)	Gross tumour incidence (%)	Inhibition (%)	No. of papillomas/ mouse	Inhibition (%)
B(a)P	3.6 ± 0.5	15 / 15 (100)	5.8 ± 0.4	-	-
B(a)P + KAFE	4.4 ± 0.8	5 / 1576	*0.2 ± 1.4	67	(33) *
KAFE	5.1 ± 0.8	0 / 15 (0)	-	-	-
Control	4.1 ± 0.3	0 / 15 (0)	-	-	-

15 animals/group, values are mean ± S.E., KAFE (2 mg/animal/day), * P : 0.001 compared to B(a)P alone

Inhibition of B(a)P-induced fore-stomach tumours

Table 2 shows the anti-cancer effect of KAFE on the B(a)P-induced fore-stomach tumours in female Swiss mice. Pretreatment with KAFE significantly inhibited the development of the fore-stomach tumours. The incidence of fore-stomach tumours in the group treated with benzo(a)pyrene alone was 100%. Concomitant treatment with KAFE decreased the tumour incidence by 67%. When compared to the group treated with benzo(a)pyrene alone that had 5.8 ± 0.4 papillomas/mouse, KAFE decreased the average number of papillomas/animal by 76%. Animals that received only KAFE or peanut oil alone did not develop any fore-stomach tumours.
The mean body weight gain was increased in groups that received KAFE alone, otherwise they were not significantly different in all the other groups.

Table 3 Inhibition of fresh egg albumen-induced inflammation in Wistar rats

Treatment group	Inflammation (%)					
	30 min	60 min	90 min	120 min	150 min	180 min
Indomethacin (10 mg/kg)	61.7 (38.3)	51.2 (48.8)	61.1 (38.9)	53.5 (46.5)	47.6 (52.4)	38.5 (61.5)
KAFE (100 mg/kg)	93.8 (6.2)	48.9 (31.1)	62.2 (37.8)	55.5 (44.5)	28.6 (71.4)	28.2 (71.8)
KAFE (200 mg/kg)	72.8 (27.2)	26.1 (73.9)	35.5 (64.5)	38.4 (61.6)	40.5 (59.5)	46.1 (53.9)

No of animals - 5/group, values in parenthesis represent % inhibition of oedema

Inhibition of fresh egg albumen-induced inflammation

Table 3 shows the average size of oedema and the percent inhibition by the ethanolic extract of ***Kigelia africana*** (KAFE) in rats. In the control group, fresh egg albumen induced a progressive increase in rat paw circumference with a maximum swelling occuring at 90 – 120 min.
KAFE evinced a good anti-inflammatory activity against the acute inflammation surpressing the rat paw oedema. The reduction in oedema of 71.8% (100 mg/kg) and 53.9% (200 mg/kg) in the extract treated groups respectively at 180 min were significantly potent ($p < 0.001$) and comparable to the indomethacine (10 mg/kg) elicited inhibition of paw oedema of 61.5%.

Discussion

Our study showed that the ethanolic extract of ***Kigelia africana*** fruits (KAFE) has significant anti-cancer and anti-inflammatory properties in these test models.
It decreased both the gross tumour incidence and tumour burden in the benzo(a)pyrene induced fore-stomach cancer model in Swiss mice and inhibited the increase in rat paw circumference caused by fresh egg albumen in Wistar rats.

Also, KAFE proved to be non-genotoxic and non-toxic. The validity and interpretation of the data gleaned from any chemotherapeutic and / or chemopreventive study are significant negative responses (e.g. reduced weight gain) to predicated upon observations made in the absence of any the treatment regimen.
In this study, the gross behaviour and weight of animals treated with high doses of the extract was indistinguishable from that of controls indicating its low acute toxicity.
This suggests that a clinical application of the extract as a single and/or adjuvant agent may be safe.
The effective dose in the anti-tumour study (2 mg/mouse, approx. 100 mg/kg) or the most effective anti-oedema dose (200 mg/kg) was about 1/3 of the LD50 and 1/16 of the maximum tolerated dose.
The success of discovering new potential anti-tumour agents from higher plants strongly benefits from screening of plants which have proved to be efficacious in traditional medicine.

The present results show that the extract of Kigelia africana fruits used in ethnomedicine for the treatment of breast, gastrointestinal and skin cancer significantly inhibited both appearances and development of tumours induced by chemical carcinogens in mice.

Thus, the demonstrated anti-tumour activity not only provides evidence for the acclaimed ethnomedicinal use, but is also consistent with the observed anti-neoplastic activity by other workers

- see attached research of Professor P.J. Houghton, King's College London.

Further, the extract of ***Kigelia africana*** has been reported to be non-cytotoxic in the CA-9KB tumour cell culture which is in agreement with the moderate cytotoxic effect observed in this study, and to be inactive in the mouse L-1210 and P-388 cell lines.
Rheumatic diseas is a progressive inflammatory condition and the traditional therapy is directed primarily at the inflammatory processes. Many anti-inflammatory agents modify the inflammatory responses by accelerating the destruction or antagonizing the action of inflammatory mediators like prostaglandins as reported for *Diodia sarmentosa* and *Palisota hirsuta*.
In the present study, our positive control, indomethacin, is a strong non-steriodal anti-inflammatory drug and a potent inhibitor of prostaglandin synthesis.
Thus, it may appear, that KAFE acts in a similar manner, eliciting anti-inflammatory activity through the inhibition of prostaglandin synthesis.
Looking at the possible correlation between the observed anti-carcinogenic and anti-inflammatory activity of KAFE, it may be that inhibition of prostaglandin synthesis is benificial in cancer conditions.

In summary, this study clearly demonstrates that the ethanolic extract of Kigelia africana fruits possesses significant anti-neoplastic and anti-inflammatory activities, thus giving credence to the traditional use of these plant extract in the treatment of cancer and oedema.