

ANTIULCER EFFECT OF THE METHANOLIC EXTRACT OF KIGELIA AFRICANA.LAM, BENTH (BIGNONIACEAE).

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Summary

The anti-ulcer activity of the methanolic extract of *Kigelia africana* was evaluated in Wistar albino rats. This was done to verify its ethno medicinal use in the treatment of ulcer. Ulcer was induced by administering 1 ml of absolute histamine acid phosphate solution (50 mg base) I.P. orally to rats an hour after the administration of drug and extract. Group I (normal saline 10 ml/kg), groups II, III and IV (250, 500 and 1000 mg/kg respectively) while group 5 ranitidine (100 mg/kg). The effect of the extract (1000mg/kg) after induction of ulcer with histamine (curative effect) was also investigated.

A second model involved the oral administration of aspirin 1% carboxymethyl cellulose (20 mg/kg) to rats, an hour after the administration of drug and extract. The first III groups received the methanolic extract (100, 200 and 400 mg/kg), the fourth, ranitidine (100 mg/kg) and the control, 10 ml/kg of normal saline.

In another set of experiment (curative study), the effect of the extract (400mg/kg) administered after induction of ulcer with aspirin (20mg/kg) was investigated. *Kigelia africana* at oral doses of 100, 200 and 400 mg/kg caused a marked inhibition of the ulcerations induced by aspirin ($p < .01$, $p < .0001$) respectively.

The extract in the curative study, produced complete protection against ulcerations. However in the histamine induced ulcers, only the 500 and 1000 mg/kg caused a marked inhibition of the ulcerations ($p < 0.01$), in both the preventive and curative study. The results points to the fact that the crude extract possesses gastro cytoprotective properties.

Key Words: Wistar albino rats, Histamine, Ranitidine, Methanolic extract, Aspirin.

Introduction

The origin of medicinal plants and their uses dates back to ancient times. The use of plants for medicinal purpose is almost universal among non-industrialized societies. World Health Organization described a medicinal plant as one in which some parts can be used directly in the management of a disease. The different parts of the plant used include the stem bark, leaves, root, shoot and flowers.

The plant *Kigelia africana* also called *Kigelia pinnata* is a tropical African genus of large trees and shrubs. The sausage tree as popularly known is widespread in Nigeria and is well distributed in the eastern, mid-west and western parts of Nigeria. Traditional uses of *K. africana* include the use of the leaves for the treatment of malaria, rheumatism, wounds, ulcers, retained placenta, venereal diseases, and diarrhoea and to combat infections.

An ulcer is any break in the skin or in a mucous membrane. The mucous membrane can be described as a thin tissue that lies the interior surface of body openings. Ulcer is used most commonly to refer to ulcers that occur in the upper part of the digestive system, such as peptic ulcers.

In previous studies carried out on the plant, the analgesic and anti-inflammatory activities of the leaves ^[1], antibacterial activity of the leaves ^[2], antibacterial activity of the fruits, central nervous stimulant effect of the leaves, smooth muscle relaxant activity of the leaves and verification of its antipyretic properties were done.

In traditional medicine, many plants are claimed to have an antiulcer effect without any scientific basis. In this study, the biological activity of the methanolic extract of the plant material was evaluated for its effect on histamine and aspirin induced ulcers to examine the claims made of its effects in ulcer therapy in folk medicine.

Materials and Methods

Collection and Identification of Plant Material:

The leaves of *Kigelia africana* were collected in Tirupathi hills, Local Government Area of Andhra Pradesh State. The botanical identity of the plant and its leaves were authenticated by Mr. Madhav Chetti, a herbarium curator of the Department of Botany, Faculty of Master of Science.

Extraction and Preparation of the Extract:

Immediately after collection, leaves were cut into small pieces and air dried. The dried leaves were pulverized into a smooth powder using impact mill, weighed and kept for further analysis. Extraction was done by continuous hot percolation process. The resultant mixture was filtered and the filtrate concentrated with the aid of a vacuum

pump and rotavapour at 40 ° C, giving a yield of 3.78%. The concentrated extract was stored in air tight containers, labelled and refrigerated at -4° C prior to use.

Animals:

Albino wistar rats weighing between 100-150 g of either sex were obtained from the Animal house, Department of Pharmacology and Toxicology, Teegala Ram Reddy College of Pharmacy. The animals were maintained on a standard diet and had access to food and water *ad libitum*. All animals were acclimatized for two weeks and fasted from food, 24 hours prior to the experiment and water withdrawn two hours before the commencement of the experiments.

Animals were exposed to natural lighting conditions and were handled according to standard experimental protocols approved by Animal Ethics Committee.

Pharmacological screening

Histamine induced ulcer:

The rats were randomly divided into 5 groups of 5 animals each and starved for 24 hours but had free access to water. Water was however withdrawn 2 hours before experiments. Group I served as control and received normal saline, Groups II, III and IV received 250, 500 and 1000 mg/kg of the extract respectively by oral intubations, while group V received 100 mg/kg ranitidine orally.

One hour later, 1 ml of absolute histamine acid phosphate solution (50 mg base) I.P. was administered orally to all the groups. An hour following histamine acid phosphate solution (50 mg base) I.P. administration, the animals were sacrificed under anaesthesia. The stomach was isolated, opened along the greater curvature and washed.

The effect of the extract (1000 mg/kg) administered orally 15 minutes after induction of ulcer with histamine (curative effect) was also investigated according to the procedure already described above.

Macroscopic examination of the stomachs of the animals in all the groups was done. The presence of ulcers was counted using a magnifying glass. The diameter of the ulcers was measured using a vernier caliper and scored on scale of 0-10^[3]. The ulcer index (UI) was calculated thus:

$$UI = UN + US + UP \times 10^{-1}.$$

Where, UN = Average number of ulcer per animal

US = Average of severity score

UP = Percentage of animals with ulcers.

Aspirin induced ulcer:

Rats were randomly allotted to 5 groups of 5 animals each and starved for 24 hours with access to water *ad libitum*. Water was however withdrawn 2 hours prior to the experiment. Group I served as the control and received normal saline (10 ml/kg) orally. Group II, III and IV were administered by oral intubations the extract at doses of 100, 200 and 400mg/kg respectively. Group V received ranitidine (100mg/kg orally). This was done one hour before oral administration of aspirin 20 mg/kg in 2 % sodium carbonate solution.

Six hours later, each rat was sacrificed under anesthesia and the stomach removed. 2 ml of formol saline was injected into the totally ligated stomach for overnight storage. The next day, the stomach was opened along the greater curvature and washed. In another set of experiment (curative study), the effect of the extract (400 mg/kg) administered orally 2 hours after induction of ulcer with aspirin (20 mg/kg) was investigated. The procedure used is as already described above. Macroscopic examination of the stomachs of the animals in the groups was done. The presence of ulcer was noted and scoring done according to the method of [3]. Buffer capacity of the methanolic extract of *Kigelia Africana*. The buffer capacity of the extract was evaluated as the ability to resist PH change upon the addition of 1 and 2ml, 0.1N HCL or 0.1 N NaOH .

Statistical analysis:

All data were expressed as mean \pm SEM. Where applicable, the data were analyzed statistically by Student's t-test. The level of significance was $P < 0.05$. n represents five per group.

Results

Intense and widespread thickened gastric lesions of the mucosa were evident in control rats that received 1 ml of absolute histamine acid phosphate solution (50 mg base) I.P. Pre-treatment with *Kigelia africana* (500 and 1000 mg/kg) caused a significant dose related reductions in both number and severity of the gastric lesions, ($P < 0.01$) reductions in the ulcer index in both the preventive and curative studies, and these effects were also found to be better than the effect of ranitidine (Table 1).

Table 2 shows the effect of the extract on aspirin induced ulcers. The control group had an ulcer index of 12.78. Pretreatment with the extract produced a dose dependent reduction in both the number and severity of ulcers. This was 11.20, 5.55 and 5.10 for the 100, 200 and 400 mg/kg doses respectively. While for ranitidine it was 5.13.

All the doses levels of the extract and ranitidine caused a significant ($p < 0.01$, $p < 0.0001$ and $p < 0.0001$) for 100, 200 and 400 mg/kg doses respectively reduction in the ulcer index.

The extract administered 2 hours after aspirin administration in the curative study, produced complete protection against ulcerations. No ulcers was noted, which makes the extract significantly ($p < 0.0001$) better than ranitidine.

The extract did not show buffer capacity (Table 3). The addition of 1 and 2 ml 0.1 N HCL and 0.1 N NaOH produced a PH variation in 1 ml of the extract (equivalent to 100 mg of the extract).

Table 1: Antiulcer activity of the methanolic extract of the leaves of K.A, normal saline and ranitidine on histamine – induced ulcer in rats.

Treatment (mg/kg)	Ulcer index (UI)	Percentage inhibition
Control(10ml/kg normal saline)	11.55±0.26	-
K.A (250)	11.45±0.32	0.86
K.A (500)	10.85±0.231 ^a	6.06
K.A (1000)	10.75±0.09 ^a	6.93
Ranitidine(100)	11.28±0.46	2.34
Curative:(1000)	10.70±0.23 ^a	7.35

Values are expressed as mean ulcer index ± SEM, (n=5 rats per group). a $P < 0.01$, significantly different from the control.

K.A: methanolic extract of *Kigelia Africana*

Table 2: Antiulcer activity of the methanolic extract of the leaves of *K. africana*, normal saline and ranitidine on aspirin e-induced ulcer in rats

Treatment (mg/kg)	Ulcer index (UI).	Percentage inhibition
Control(10ml/kg normal saline)	12.78±0.43	—
<i>K.A</i> (100)	11.20±0.32 ^a	12.4
<i>K.A</i> (200)	5.55±0.42 ^b	56.6
<i>K.A</i> (400)	5.10±0.07 ^b	60.1
Ranitidine(100)	5.13±0.08 ^b	59.9
Curative <i>K.A</i>: (500)	0	100

Values are expressed as mean ulcer index ± SEM, (n = 5 rats per group). a P<0.01 and b P<0.0001, significantly different from the control group.

K.A*: methanolic extract of *Kigelia africana

Table 3: Buffer capacity of the methanolic extract of the leaves of *K. africana*. 1 ml extract is equivalent to 100 mg/ml

Concentrations	PH Values.
1 ml NaOH 0.1N	11.10
1ml HCL 0.1N	2.70
1 ml extract	4.50
1 ml extract + 1 ml NaOH 0.1N	10.30
1 ml extract + 2 ml NaOH 0.1N	10.72
1 ml extract + 1 ml HCL 0.1N	3.36
1 ml extract + 2 ml HCL 0.1N	2.98

Discussion

The anti-ulcer property of the methanolic extract of *Kigelia africana* was evaluated against gastric lesions induced by aspirin and histamine. Although the etiology of ulcer in most cases is unknown, it is generally accepted that they result from an imbalance between aggressive factors and the maintenance of mucosal integrity through the endogenous defensive mechanisms.

To regain the balance, there are different therapeutic agents including medicinal plant extracts that are used to inhibit gastric acid secretion. *Kigelia africana* extract is one

of such medicinal drugs used in the present study primarily to evaluate the anti - ulcerogenic or ulcer preventive potency.

The results show a dose dependent gastro-protective effect in the two models of ulcer. In the aspirin model of ulceration, the extract showed better significant protection ($P<0.0001$) at all doses tested, when compared to the histamine-induced model ($P<0.01$).

The extract (400 mg/kg) curative, administered, after the ulcerogen produced complete protection against ulceration which made the extract significantly better than ranitidine.

Prostaglandins are known to protect gastric mucosal cells against injury caused by aspirin thus it is possible that the extract stimulates the production of endogenous prostaglandins, which gives protection.

In the histamine -induced ulceration, the highest dose (1000mg/kg) showed significant protection ($P<0.01$), better than that of ranitidine. The reduction in ulcer index in both models (preventive and curative) shows the ability of the extract to protect the gastric mucosal against ulceration as well as suppression of already established ulcers.

The extract did not show buffering capacity and so may neither be acting by neutralization of stomach acidity, like antacids nor dilution of the ulcerogen in the gastro-intestinal tract.

Conclusion

The results suggest that the methanolic extract of *Kigelia africana* has a potential anti ulcer effect. Its mechanism might be via release of prostaglandins and possibly free radical scavengers which protect the gastric mucosa.

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