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Research Article

ANTI-ULCER ACTIVITY OF DIFFERENT EXTRACT OF *KIGELIA PINNATA* IN EXPERIMENTAL ANIMALS

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ABSTRACT

Objective: *Kigelia pinnata* (Bignoniaceae) has been extensively used in medicine for the treatment of ulcer. Hence, the present study was intended to evaluate total Chloroform, Diethylether and ethanolic leaf extracts of *Kigelia pinnata* for peptic ulcer activities. Methods: The Peptic ulcer activity was studied by Indomethacin, Alcohol and pyloric ligation models experimentally induced ulcer. Acute toxicity study and preliminary phytochemical screening were also studied to evaluate the toxicity

Results: No toxicity profile was observed in rats after oral administration of the different extracts at the dose of 2g/kg body weight. The different extracts at a dose of 100 mg/kg and 200 mg/kg administered with the extracts of *Kigelia pinnata* there was significant ($P < 0.001$) reduction in volume of gastric acid secretion, PH, free acidity, total acidity and ulcer index with respect to control. Phytochemical screening of the plant extract revealed the presence of tannins, alkaloids, flavonoids and saponins, and terpenoids.

Conclusion: It can be concluded that the anti-ulcer activity elucidated by *Kigelia pinnata* could be mainly due to the modulation of defensive factors through an improvement of gastric cytoprotection and partly due to acid inhibition.

Keywords: *Kigelia pinnata,* Indomethacin, Alcohol, pyloric ligation, anti-ulcerogenic

INTRODUCTION

Peptic ulcer is one of the most common gastrointestinal disease.[1]The exact Causes of peptic ulcer disease is not known but it may be result from an imbalance between acid-pepsin secretion and mucosal defence factors.[2] It is the most common gastrointestinary disorder in clinical practice. Considering the several side effects (arrhythmia's, impotence, funaecomastia and haematopoeitic changes) of modern edicine.[3] Indigenous drugs possessing fewer side effects should be looked for as a better alternative for the treatment of peptic ulcer. There is evidence concerning the participation of reactive oxygen species in the etiology and pathophysiology of human diseases, such as neurodegenerative disorders, inflammation, viral infections,autoimmune pathologies and digestive system disorders such as gastrointestinal inflammation and gastric ulcer. [4]Treatments available for ulcer is generally non-specific and is usually aimed at reducing the production of gastric acid and re-enforcing gastric mucosal protection such as regular food, adequate rest and avoidance of ulcerogenic agents such as coffee, alcohol and tobacco. The drugs used in the treatment of ulcer include receptor blockers, proton pump inhibitors, drugs affecting the mucosal barrier and act on the central nervous system. [5] Even though a range of drugs are available for the treatment of ulcer, many of these do not fulfill all the requirements and have side effects. [6,7] Recently, there has been much interest in natural medicines derived from the traditional knowledge of plant pharmacological properties. Large number of medicinal plants and dietary nutrients have been shown to posses gastro-protective activity. [8] *Kigelia pinnata* (or *K. africana*, Bignoniaceae family), also known as Sausage Tree, or Worsboom, in view to its bulky, characteristically shaped fruits, has a variety of traditional medicinal uses throughout Africa where it grows as an endemic species in different habitats,[9]as well as in India and the Middle East whereby the tree has been widely cultivated.[10] The extensive phytochemical examinations of the plant has resulted in the isolation of different secondary metabolites such as iridoids and naphtoquinoids, considered to convey much of its pharmacological activities. [11] Although flavonoids, lignans, terpenoids, coumarins, phenylethanoids, phenylpropanoids and sterols have been isolated, as well. [12] The literature survey revealed that there is no experimental evidence of anti-ulcer effect of the plant. Therefore, the present study was undertaken to investigate the potential anti-ulcer activity of kigelia pinnata in experimental animals

MATERIALS AND METHODS

Plant Material

The leaves of *Kigelia pinnata* were collected at in the month of march, 2013 from local area of Coimbatore,Tamilnadu,India. and authenticated by the Botanical survey of India, Coimbatore,Tamilnadu,India.where a voucher specimen has been preserved for future identification.

Extract Preparation [13]

The leaves were shade-dried and made into a coarse powder which was passed through a 40-mesh sieve to get a uniform particle size and then used for extraction. A weighed quantity (500 g) of the powder was then subjected to continuous hot extraction in Soxhlet apparatus with Chloroform, Diethyl ether and Ethanol the residual marc was collected. The extract was filtered through a cotton plug, followed by whatman filter paper (no.1). The extract was evaporated underreused pressure using a rotovac evaporator at a low temperature (40-60^{o}C) until all the solvent had been removed to give an extract sample with a yield of 16 %w/w,13% w/w and 18% w/w, in relation to the dried starting material. Preliminary Phytochemical analysis was carried out to identify presence of Phytoconstituents in the crude extract.

Preliminary Phytochemical Screening

The various extracts of *Kigelia pinnata* were then subjected to preliminary phytochemical analysis to assess the presence of various phytoconstituents, it revealed that the presence of alkaloids, steroids, polyphenolic constituents like flavonoids, quercetin, naringin, saponins, glycosides, tannins, gums and mucilages. Preliminary Thin layer chromatography studies also confirmed these constituents. [14]

Animals

Male Wistar rats (175±5gm) were provided with standard rat feed and tap water ad libitum. Theanimals were kept in our animal room with maintenance of room temperature (22± 2ºC) and light: dark exposure of 12:12 h. Institutional Animals Ethical Committee (IAEC) approved the experimental protocol and care of animals was taken as per guidelines of CPCSEA, Department of Animal Welfare and Government of India.

Acute Toxicity Studies

Acute toxicity studies were performed according to organization for economic co-operation and development (OECD) guidelines. [15] Animals were divided in groups (n=5). The animals were fasted for 4 h. with free access to water only. The extracts was administered orally in doses of 1000 and 2000 mg/kg to different groups of mice and observed over 14 days for mortality and physical/behavioral changes.

Indomethacin induced gastric ulcer

In the Indomethacin-induced ulcer experiments[16] five groups of wister rats (150–200 g), with each group consisting of six animals were used. The first group served as a control group, the second group served as standard drug treatment and the remaining group served as the test group. The second group were treated respectively with ranitidine (20 mg/kg) and test groups chloroform, diethyl ether and ethanolic, extracts of *Kigelia pinnata* (100 &200mg/kg), orally for 8 days. Control animals received normal saline (2 ml/kg) for 8 days. After 8 days of treatment, animals were fasted for 24 h. Ulcer was produced by administration of Indomethacin (10 mg/kg) was given orally to rats in two doses at an interval of 6 hour on the day of sacrifice. The animals were sacrificed 4 h later and stomach was opened to calculate the ulcer index by Kunchandy method. [17]

Alcohol-induced gastric ulcer[18]

The male wister rats were randomly divided into five groups and fasted for 24 h with free access to water. Animals were given vehicle(normal) chloroform,diethyl ether and ethanolic, extracts of the *Kigelia pinnata a*t a dose of 100 and 200 mg/kg (test),Ranitidine[20 mg/kg,(std)] orally. four hour later, 1 ml of 80% ethanol was administered orally to each animal Animals were sacrificed by cervical dislocation, one hour after ethanol administration,stomach were isolated and cut open along the greater curvature and pinned on a soft board. The length of each gastric lesion was measured and the lesion index was expressed as sum of the length of the entire lesion in mm.

Pylorus- ligation induced gastric ulcer

Male wister rats were selected for pyloric ligation ulcer model. [19] Rats were divided into five groups, each group consisting of six animals. Animals were fasted for 24 h. One group received normal saline 2 ml/kg (normal control), the second group received Ranitidine 20 mg/kg by oral route (standard) and the remaining groups received Chloroform, diethyl ether and ethanolic extracts *Kigelia pinnata* (100&200 mg/kg) by oral route, 30 min prior to pyloric ligation. Animals were sacrificed 4 h later and the stomach was opened to collect the gastric contents. The total volume of gastric content was measured. The gastric contents were centrifuged at 1000 rpm for 10 min. One ml of the supernatent liquid was pipetted out and diluted to 10 ml with distilled water. The solution was titrated against 0.01N NaOH using Topfer's reagent as indicator, to the endpoint when the solution turned to orange colour. The volume of NaOH needed was taken as corresponding to the free acidity. Titration was further continued till the solution regained pink colour. The volume of NaOH required was noted and was taken as corresponding to the total acidity. Acidity was expressed as:

$$\text{Acidity} = \frac{\text{Volume of NaOH x Normality x 100 mEq/1}}{0.1}$$

Statistical analysis

The values Mean±SEM are calculated for each parameter. For determining the significant inter group difference each parameter was analysed separately and one-way analysis of variance was carried out and the individual comparisons of the group mean values were done using Dunnet's test[20] (Dunnet, 1964).

RESULTS

Preliminary phytochemical screening revealed the presence of Alkaloids, Steroids, polyphenolic constituents like flavonoids, Saponins, glycosides, tannins, gums and mucilages. Acute toxicity studies of the various extracts of the *Kigelia pinnata* did not exhibit any signs of toxicity up to 2 g/kg body weight. Since there was no mortality of the animals found at high dose. Hence 100 and 200 mg/kg dose of the extract selected for evaluation of anti-ulcer activity.

Indomethacin induced ulcer

Table 1 summarizes the results obtained in the experimental model of indomethacin-induced gastric ulceration in rats. The ethanolic extract was found to possess remarkable ulcer-protective properties at 100 and 200 mg/kg when compare to other two extracts. The maximum effect of ulcer protection (57.84%),(46.46%)&(70.46%),were produced at 200 mg/kg for chloroform diethyl ether and ethanolic extracts, and the standard drug (Ranitidine 20 mg/kg) gave 81.53% of ulcer protection (Table 1).

Alcohol induced ulcer

Pretreatment of rats with *Kigelia pinnata* extracts produced a dose dependent protection in the alcohol induced ulceration model as compared to control group. However the protection was statistically significant reduced the severity of ulcer and caused a significant reduction of ulcer index in this model. Ranitidine produced significant gastric ulcer protection as compared to control group (Table 1).

Pylorus ligation induced ulcer

The chloroform, diethyl ether and ethanolic extracts of the *Kigelia pinnata* in the doses of 100 and 200 mg/kg produced a reduction in the ulcer index, gastric volume, free acidity, total acidity and raised gastric pH significantly in comparison with control group. Ranitidine reference drug produced significant reduction gastric ulcer and total acid output as compared to control group (Table 2). The results of the present study indicate that the ethanolic extract of *Kigelia pinnata* significantly reduces the total volume of gastric juice, free and total acidity of gastric secretion and also has activity against gastric ulcers in rats when compare to other two extracts. (Table 2) The animals had ulcers and haemorrhagic streaks, whereas in animals administered with the extracts of *Kigelia pinnata* there was significant reduction in ulcer index ($P < 0.001$) (Figure 2).

Table 1: Effect of various extracts of *Kigelia pinnata* against indomethacin and Alcohol induced gastric ulcer in rats.

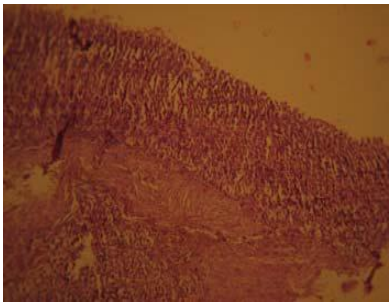
Treatment	Dose (mg/kg) p.o	Indomethacin		Alcohol	
		Ulcer Index	% of ulcer protection	Ulcer Index	% of ulcer protection
Normal control	2ml/kg	6.5±0.50	–	6.5±0.50	–
Standard (Ranitidine)	20mg/kg	1.20±0.24	81.53***	1.20±0.24	81.53***
Chloroform extract	100mg/kg	4.68±0.28	28.00*	4.62± 0.25	28.92*
	200mg/kg	2.74± 0.36	57.84**	2.68± 0.33	58.76**
Diethylether Extract	100mg/kg	4.86±0.29	25.23*	4.82±0.26	25.84*
	200mg/kg	3.48± 0.33	46.46**	3.42±0.29	47.38**
Ethanolic Extract	100mg/kg	4.14± 0.24	36.30*	4.10 ±0.22	36.92*
	200mg/kg	1.92± 0.32	70.46***	1.98± 0.38	66.53***

Results are mean ± S.E.M.(n = 6). Statistical comparison was performed by using ANOVA coupled with student't' test.* P<0.05, ** P<0.01, *** P<0.001 were consider statistically significant when compared to control group.

Table 2: Effect of various extracts of *Kigelia pinnata* against Pylorus ligation induced gastric ulcer in rats

Treatment	Dose (mg/kg) p.o	Volume of gastric juice (ml/4h)	PH	Free Acidity (mEq/L)	Total Acidity (mEq/L)	Ulcer Index	% Inhibiton of ulcer
Normal control	2ml/kg	4.02±0.11	1.84± 0.14	26.84±0.08	70.16±0.30	3.68±0.56	–
Standard (Ranitidine)	20mg/kg	1.94± 0.18	4.960±0.18	10.42±0.02	22.24±0.18	0.71±0.14	80.70***
Chloroform extract	100mg/kg	3.79± 0.16	3.20± 0.14	22.96±0.08	60.48±0.24	2.62±0.36	28.80*
	200mg/kg	3.28± 0.21	3.88± 0.16	13.68±0.02	35.45±0.33	1.52±0.44	58.69**
Diethylether Extract	100mg/kg	3.86 ±0.14	2.86± 0.14	25.54±0.04	64.16±0.19	2.76±0.49	25.00*
	200mg/kg	3.68± 0.12	3.24± 0.16	16.62±0.06	39.52±0.32	1.96±0.56	46.73**
Ethanolic Extract	100mg/kg	3.66± 0.16	3.12± 0.14	21.18±0.05	52.14±0.38	2.34±0.24	36.41*
	200mg/kg	2.40± 0.14	4.56± 0.18	11.76±0.06	30.62±0.26	1.10±0.29	70.10***

Results are mean ± S.E.M.(n = 6). Statistical comparison was performed by using ANOVA coupled with student't' test.* P<0.05, ** P<0.01, *** P<0.001 were consider statistically significant when compared to control group.



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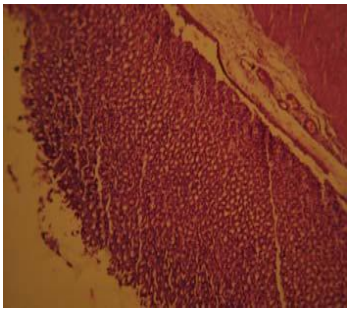

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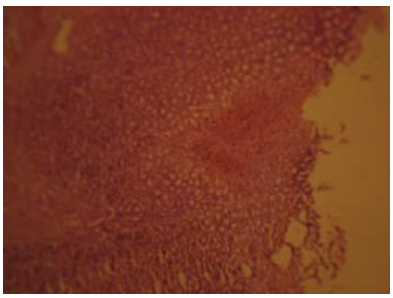

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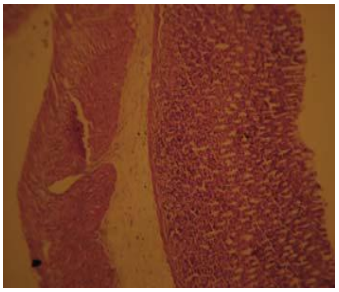

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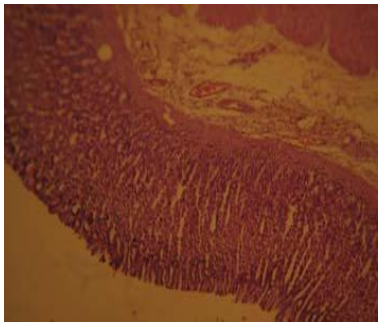

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DISCUSSION

The anti-ulcer activity of the plant of *Kigelia pinnata* was evaluated by employing indomethacin, alcohol and pylorus ligation ulcer models. These models represent some of the most common causes of gastric ulcer in humans. Many factors and mechanisms are implicated in the ulcerogenesis and gastric mucosal damage induced by different models employed in the present study involving, depletion of gastric wall, mucin mucosal damage induced by non-steroidal anti-inflammatory drugs and free radical production. [21] Ethanol extract of the plant of *Kigelia pinnata* was significantly effective in protecting gastric mucosa against indomethacin induced ulcers at all the dose level studied. Ethanol induced gastric injury is associated with significant production of oxygen free radicals leading to increased lipid peroxidation, which causes damage to cell and cell membrane. The extracts of the *Kigelia pinnata* has significantly protected the gastric mucosa against ethanol challenge as shown by reduced values of lesion index as compared to control group suggesting its potent cytoprotective effect. It has been proposed that in pyloric ligation, the digestive effect of accumulated gastric juice and interference of gastric blood circulation are responsible for induction of ulceration. [22] The antiulcer activity of *Kigelia pinnata* extracts in pylorus ligation model is evident from its significant reduction in gastric volume, total acidity, free acidity, ulcer index and increase in pH of gastric juice. Because of animals treated with *Kigelia pinnata* extracts significantly inhibited the formation of pylorus ulcer in the stomach and also decreased both acid concentration, gastric volume and increased the pH values. It is suggested that *Kigelia pinnata* extracts can suppress gastric damage induced by aggressive factors. It is generally accepted that gastric ulcers result from an imbalance between aggressive factors and the maintenance of the mucosal integrity through endogenous defence mechanisms. [23] The excess gastric acid formation by prostaglandin (PG) includes both increase in mucosal resistance as well as a decrease in aggressive factors, mainly acid and pepsin. Inhibitions of PG synthesis by indomethacin coincide with the earlier stages of damage to the cell membrane of mucosal, parietal and endothelial cells. [24] The preliminary phytochemical studies revealed the presence of flavonoids in ethanolic extract of *Kigelia pinnata*; various flavonoids have been reported for its anti-ulcerogenic activity with good level of gastric protection. So the possible mechanism of antiulcer action of *Kigelia pinnata* may be due to its flavonoid content. In this study we observed that *Kigelia pinnata* provides significant anti-ulcer activity against gastric ulcers in rats.

CONCLUSION

On the basis of the present results and available reports, it can be concluded that the anti-ulcer activity elucidated by *Kigelia pinnata* could be mainly due to the modulation of defensive factors through an improvement of gastric cytoprotection and partly due to acid inhibition

REFERENCES

1. P.C.Dandiya, S.K.Kulkarni.Introduction to Pharmacology. Vallabh Prakashan New Delhi, 2005. pp. 247
2. Padmaja Udaykumar.Textbook of medicalPharmacology,CBS publishers,NewDelhi, 2005 pp. 317.
3. Akhtar MS, Khtar AH, Khan MA. Int J Pharmacog 1992;30:97-8.
4. Repetto MG, Liesuy SF. Braz J Med Biol Res. 1994; 35:523-34.
5. Manonmani,S.,V.P.Viswanathan,S.Subramanianand S. Govindasamy, 1995. Biochemical studies on the antiulcerogenic activity of cauvery 100, an ayurvedic formulation in experimental ulcers. Ind. J. Pharmacol.,27: 101-105
6. Anoop,A.and M. Jegadeesan, 2003. Biochemical studies on the anti-ulcerogenic potential of *Hemidesmus indicus* R. Br. Var. indicus. J.Ethnopharmacol., 84: 149-156
7. Dharmani,P.,P.K.,Mishra, R.Maurya,V.S.Chauhan and G. Palit, 2005. *Allophylus serratus*: A plant with potential anti-ulcerogenic activity. J. Ethnopharmacol., 99: 361-366.
8. Borrelli, F. and A.A. Izzo, 2000. The plant kingdom as a source of anti-ulcer remedies. Phytother. Res., 14: 581-591
9. Houghton PJ, The sausage tree (*Kigelia pinnata*): Ethnobotany and recent scientific work. *South Afr J Botany* 2002, 68: 14-20.
10. William Carey M.Rao NV, Kumar BR, Mohan GK. Anti-inflammatory and analgesic activities of methanolic extract of kigelia pinnata DC flower. J Ethnopharmacol 2010;(1);179-82.
11. Picerno P, Autore G, Marzocco S, Meloni M, Sanogo R, Aquino RP: Anti-inflammatory activity of verminoside from Kigelia africana and evaluation of cutaneous irritation in cell cultures and reconstituted human epidermis. *J Nat Prod* 2005; 68;(11.);1610-4.
12. Gouda YG, Abdel-Baky AM, Mohamed KM, Darwish FM, Kasai R, Yamasaki K:Phenylpropanoid and phenylethanoid derivatives from Kigelia pinnata DC. fruits. *Nat Prod Res* 2006; 20;(10.);935-9.
13. Dr CK Kokate, Practical Pharmacognosy, III-Edition1991, Page number 128, Published by Shri Dinesh K.Furia, Nirali Prakasham, Pune.
14. H Wagner, Bladts, EN Zgainski, Plant drug Analysis –A Thin Layer Chromatography Atlas, Springer Yerlay berlin, New York, Page number: 225-227, 234, 235, 244, 245 (1984).
15. Ecobichon DJ. (1999): The basis of toxicology testing 2nd edn, Newyork, CRC press, pp 43-46.
16. Parmar, NS and Desai, JK., (1993). A review of the current methodology for the evaluation of gastric and duodenal antiulcer agents, Indian J Phrmaco 1993; 25 : 120-35.
17. Kunchandy J, Khanna S and Kulkarni SK. Arch Int Pharmacodyn 1985;275:123–138.
18. Robert A. Cytoprotection by prostaglandins. Gastroenterol. 1979; 77:761-767.
19. Shay H. Gastroenterol 1945; 5: 43–61.
20. Dunnet CW. (1964): New tables for multiple comparisions with a control. Biometrics, 20:482 -91.
21. Udaya Bandyopadhyay, Dipak Das, Debashis Bandyopadhyay, Mrinalini Bhattacharjee, Ranajit K, Banerjee. Cur Sci 1999;76(1): 55-6.
22. Gordan MH. The mechanism of the anti-oxidant action in vitro. In B. J. F. Hudson, Food Anti-oxidants. London, Elservier, 1990; p 1-18.
23. Szabo S and Szienji S. Trends Pharma Sci 1987; 8: 149–154.
24. Rainsford KD, Adv Inflamm Res 1984; 6:51–64.