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Antiulcer activity of an isolated compound (MK–1) from *Murraya koenigii* (Linnaeus) Sprengel leaf in rats

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Abstract

An active compound (MK-1) was isolated from *Murrya koenigii* (Linnaeus) Sprengel leaf and its antiulcer activity was studied against ethanol, hydrochloric acid, indomethacin, stress and pyloric ligation induced gastric ulceration in albino rats. A significant antiulcer activity of MK-1 was observed in all the models. MK-1 thus provides a scientific rationale for the use as antiulcer drug.

Key words: *Murraya koenigii*, Gastric ulcer, Isolated MK-1

INTRODUCTION

Murraya koenigii (Linnaeus) Sprengel has been described as a medicinal plant of the family 'Rutaceae'. It has several vernacular names. In Nepali it is called 'meehi saag', in Hindi 'bursunga' and in English it is known as 'curry leaf tree'. *Murrya koenigii* is widely distributed at the foothills of the Himalayas from Kumaon to Sikkim, Bengal, Assam, middle and lower hill forests up to the height of 1500 m. It is a large shrub with dark green bark, often cultivated. February to May is the flowering time of the plant. *Murraya koenigii* has several medicinal uses. Leaves and roots are bitter, acrid, cooling, alexeteric, antihelmintic, analgesic, cure piles, useful in leucoderma and blood disorders. Burk is used to cure eruptions, poisonous animal bites etc. The plant has also stomachic and tonic properties (Chopra & Chopra1958; Gurung, 2002).

Recently we observed the anti-ulcer activity of *Murraya koenigii* leaf against ethanol induced gastric ulcer in albino rats (Mitra *et al* 2010). Tempted by this observation we undertook studies on isolation of the active compound present in *Murraya koenigii* leaf and the antiulcer activity of the isolated compound against ethanol, hydrochloric acid, indomethacin, stress and pyloric ligation induced gastric ulceration in albino rats.

METHODOLOGY

Murrya koenigii (Linnaeus) Sprengel leaf was collected from the Garden of Medicinal Plants, North Bengal University, during September 2009 and was identified in the Taxonomy & Environmental Biology Laboratory of the Department of Botany, University of North Bengal. A voucher specimen has been deposited in the NBU Herbarium [Accession No. 9541] for future reference.

Isolation of active principle (MK-1) from *Murrya koenigii* leaf

Fresh leaves were shade dried at room temperature, ground into fine powder. 50 g of this leaf-powder was then extracted with 500 ml of methanol for 24 hours in a soxhlet apparatus at 60° C. The extract was concentrated to 10 ml under reduced pressure in a rotary evaporator. This was then subjected to column chromatography using silica gel mesh (200 – 400 size) as adsorbent. Elution was done by 50% methanol-chloroform mixture. Eluted material was evaporated to dryness and extracted with 10 ml ethyl acetate. The ethyl acetate extract was further subjected to column chromatography using polyamide as adsorbent. The fraction obtained after elution with ethyl formate : formic acid mixture (100 : 5, v/v) was subjected to repeated crystallization when a compound was crystallized. The compound was given a trivial name MK-1. The compound was preserved for acute toxicity study as well as for anti-gastric ulcer activity.

Experimental animals:

Wistar strain albino rats of both sexes were used for the study. The animals were housed in colony cages (5 rats/ cage) and were kept for at least a week in the experimental wing of the animal house (room temperature 25 – 28° C and humidity 60 – 65% with 12 h light and dark cycle) before experimentation. Animals were fed on laboratory diet with water *ad libitum*. For each set of experiment 8 animals were used. The animal experiment was approved by the ethics committee of the Institute (i.e. NBMC).

Chemicals:

Indomethacin (Torrent Research Centre, Gandhinagar), ethanol (Baroda Chemical industries Ltd., Dabhoi), HCl LR (Thomas baker, Mumbai), omeprazole (Kopran Pharma Ltd., Mumbai).

Test drug:

Isolated compound (MK-1) was used as the test drug.

Production of gastric ulcer:

Ethanol induced gastric ulcer (Sairam *et al*. 2001): Rats were fasted for 18 h when no food but water was supplied ad *libitum*. Gastric ulcers were induced by administering ethanol (95%, 1 mL/ 200 g body weight) orally through a feeding tube. 1h after administration of ethanol, animals were sacrificed by cervical dislocation and the stomach was taken out and incised along the greater curvature. The stomach was then examined for the presence of ulcers.

HCl induced gastric ulcer (Parmar & Desai 1993): 0.6M HCl (1 mL/200 g body weight) was orally administered to all rats. Rest part is same to that of ethanol induced gastric ulcer group.

Indomethacin induced gastric ulcer (Parmar & Desai 1993)

Indomethacin (10 mg/kg) was given orally to rats in two doses at an interval of 15 hour. Rest part is same to that of ethanol induced gastric ulcer group.

Stress induced gastric ulcer (Alder 1984)

Rats were fasted for 24h when no food but water was supplied *ad libitum*. Stress ulcer was induced by forced swimming in the glass cylinder (height 45 cm, diameter 25 cm) containing water to the height of 35 cm maintained at 25 degree centigrade for 3h. Rats were then sacrificed. Rest part was same to that of ethanol induced gastric ulcer group.

Induction of gastric ulcer by pyloric ligation method (Parmar & Desai 1993)

Rats were fasted for 24h when no food but water was supplied *ad libitum.* Under light ether anesthesia, abdomen was opened and the pylorus was ligated. The abdomen was then sutured. After 4h the rats were sacrificed with excess of anesthetic ether and the stomach was dissected out. Rest part was same to that of ethanol induced gastric ulcer group.

Acute oral toxicity study (Ghosh 2005)
Acute toxicity studies were carried out on Swiss albino mice. Isolated compound (MK-1) from *Murrya koenigii* (Linnaeus) Sprengel leaf was given orally at doses of 100, 500, 1000 and 3000 mg/kg to five groups of mice, each group containing six animals. After administration of the compound, the animals were observed for the first three hours for any toxic symptoms followed by observation at regular intervals for 24 hours up to seven days. At the end of the study, the animals were also observed for general organ toxicity, morphological behavior and mortality.

Anti gastric ulcer study
Rats were divided into 5 groups:
Group 1: Control
Group 2: Ulcerogenic drug or Method (Ethanol / HCl / Indomethacin / Stress / Pyloric ligation)
Group 3: Ulcerogenic drug or method + MK-1 (5 mg/kg)
Group 4: Ulcerogenic drug or method + MK-1 (10 mg/kg)
(MK-1 was given orally 30 minutes prior to administration of ulcerogenic drug or method)
Group 5: Ulcerogenic drug or method + Omeprazole (8 mg/kg orally 30 minutes prior to administration of ulcerogenic drug or method). Omeprazole was used as per the method of Malairajan *et al* (2008).

Evaluation of ulcer index (Szelenyi & Thiemer 1978)
Gastric lesions were counted and the mean ulcerative index was calculated as follows :
I - Presence of edema, hyperemia and single sub mucosal punctiform hemorrhage.
II – Presence of sub mucosal hemorrhagic lesions with small erosions.
III – Presence of deep ulcer with erosions and invasive lesions.
Ulcer index = (number of lesion I) x1 + (number of lesion II) x2 + (number of lesion III) x 3.

Statistical analysis
Statistical analysis of the results was done by the method of Das & Bhattacharya (1974). p values less than 0.05 were considered significant.

RESULTS

Acute toxicity studies
Acute toxicity studies revealed that MK-1 did not produce any toxic symptoms when administered orally to mice in doses of 100, 500, 1000 and 3000 mg/kg. Animals were healthy, cheerful and behaved normal throughout the experimental period. No death of animal was recorded during seven days of experiment.

Effect of MK-1 on ethanol induced gastric ulcer
Result is given in Table-1. Ethanol produced massive gastric ulcers in all albino rats. Ulcers were mostly superficial. Bleeding of the stomach was followed by adhesion and dilatation. Ulcer index came 29.9 ± 1.95. Pretreatment of rats with MK-1 produced a dose dependent protection (14.71% and 46.49% for the doses of 5 mg/kg and 10 mg/kg of MK-1 respectively) from ethanol induced ulceration as compared to ethanol group. However, the protection was not statistically significant at 5 mg/kg dose. Omeprazole produced significant gastric ulcer protection (66.22%). Efficacy of MK-1 in the dose of 10 mg/kg was 70.20% when compared with that of omeprazole group.

Effect of MK-1 on hydrochloric acid induced gastric ulcer
0.6M HCl when administered to rats orally produced massive ulcers in stomach of all rats. Adhesion and dilatation of the stomach were seen. Ulcer index was 28.1 ± 1.86. Pretreatment with MK-1 gave dose dependent protection (26.69% and 41.63% for the doses of 5 mg/kg and 10 mg/kg of MK-1 respectively) from HCl induced ulceration. Protection was statistically significant and comparable with omeprazole group where ulcer protection was 63.70% (Table -2).

Table-1. Effect of MK-1 on ethanol induced gastric ulcer

Group	Ulcer index (mean ± SEM)	% Ulcer protection
Control	Nil	--
Ethanol	29.9 ± 1.95	--
Ethanol+ MK-1 (5 mg/kg)	25.5 ± 1.63	14.71
Ethanol+ MK-1 (10 mg/kg)	15.6 ± 1.57**	46.49
Ethanol + Omeprazole (8mg/kg)	10.1 ± 1.11**	66.22

Table-2. Effect of MK-1 on hydrochloric acid induced gastric ulcer

Group	Ulcer index (mean ± SEM)	% Ulcer protection
Control	Nil	--
Ethanol	28.1 ± 1.86	--
Ethanol+ MK-1 (5 mg/kg)	20.6 ± 1.76*	26.69
Ethanol+ MK-1 (10 mg/kg)	16.4 ± 1.55**	41.63
Ethanol + Omeprazole (8mg/kg)	10.2 ± 1.00**	63.70

Table-3. Effect of MK-1 on indomethacin induced gastric ulcer

Group	Ulcer index (mean ± SEM)	% Ulcer protection
Control	Nil	--
Ethanol	29.2 ± 1.81	--
Ethanol+ MK-1 (5 mg/kg)	22.7 ± 1.43*	22.26
Ethanol+ MK-1 (10 mg/kg)	16.5 ± 1.39**	43.49
Ethanol + Omeprazole (8mg/kg)	10.7 ± 1.23**	63.35

Table-4. Effect of MK-1 on stress induced gastric ulcer

Group	Ulcer index (mean ± SEM)	% Ulcer protection
Control	Nil	--
Ethanol	30.4 ± 1.99	--
Ethanol+ MK-1 (5 mg/kg)	22.6 ± 1.56*	25.65
Ethanol+ MK-1 (10 mg/kg)	14.7 ± 1.40**	51.64
Ethanol + Omeprazole (8mg/kg)	10.6 ± 1.21**	65.13

Table-5. Effect of MK-1 on pyloric ligation induced gastric ulcer

Group	Ulcer index (mean ± SEM)	% Ulcer protection
Control	Nil	--
Ethanol	26.3 ± 1.74	--
Ethanol+ MK-1 (5 mg/kg)	12.2 ± 1.55	53.61
Ethanol+ MK-1 (10 mg/kg)	10.7 ± 1.31**	59.31
Ethanol + Omeprazole (8mg/kg)	9.9 ± 1.1**	62.35

Each group had eight rats. *P<0.01, ** p<0.001

Effect of MK-1 on indomethacin induced gastric ulcer

Result is given in Table-3. Indomethacin produced gastric ulcers in all albino rats. Ulcers were superficial in nature.There were adhesion, dilatation and bleeding in the stomach. Ulcer index came 29.2 ± 1.81. Pretreatment of rats with MK-1 produced dose dependent protection (22.26% and 43.49% for the doses of 5 mg/kg and 10 mg/kg of MK-1 respectively) from indomethacin induced ulceration as compared to indomethacin group. Protections were statistically significant. Omeprazole produced significant gastric ulcer protection (63.35%). In this model efficacy of MK-1 in the dose of 10 mg/kg was 68.65% when compared with that of omeprazole group.

Effect of MK-1 on stress induced gastric ulcer

Swimming stress produced massive ulcers in stomach of all rats. Adhesion and dilatation of the stomach were seen. Ulcer index was 30.4 ± 1.99. Pretreatment with MK-1 gave dose dependent protection (25.65% and 51.64% for the doses of 5 mg/kg and 10 mg/kg of MK-1 respectively) from stress induced ulceration. Protection was statistically significant and comparable with omeprazole group. In omeprazole group ulcer protection was 65.13%. Results were shown in Table -4.

Effect of MK-1 on pyloric ligation induced gastric ulcer

Result is given in Table-5. Pyloric ligation produced gastric ulcers in all albino rats. Ulcers were superficial in nature.There were adhesion, dilatation and bleeding in the stomach. Ulcer index came 26.3 ± 1.74. Pretreatment of rats with MK-1 produced dose dependent protection (53.61% and 59.31% for the doses of 5 mg/kg and 10 mg/kg of MK-1 respectively) from pyloric ligation induced ulceration as compared to pyloric ligation group. Protection was only statistically significant at 10 mg/kg dose of MK-1. Omeprazole produced significant gastric ulcer protection (62.35%).

DISCUSSION

The term "Peptic ulcer " refers to an ulcer in the lower oesophagus, stomach or duodenum, in the jejunum after surgical anastomosis to the stomach or, rarely in the ileum adjacent to a Meckel's diverticulum. Ulcer in the stomach (gastric ulcer) may be acute or chronic. Quincke (1963) was probably the first to use the term 'Peptic ulcer'. Because of its frequency and worldwide distribution, peptic ulcer continues to be a subject of numerous investigations, both experimental and clinico pathological. In this respect peptic ulcer occupies a place secondary to carcinoma in the field of gastroenterology.

There is medicine to treat peptic ulcer (Tierrey *et al*. 1978). In case, the ulcer is due to infection of *Helicobacter pylori (H. pylori),* the different medications are usually prescribed. This is known as "Triple therapy". This includes a proton pump inhibitor viz. omeprazole to reduce acid production and two antibiotics to get rid of the organism. Sometimes, instead of one of the antibiotics, bismuth salicylate may be the third medication recommended. This drug, available over the counter, coats and soothes the stomach, protecting it from the damaging effects of acid. Two, rather than three, drug regimens are currently being developed. For non *H. pylori* ulcers number of drugs are now available for treatment. These drugs are broadly classified into two categories :

1. Those that decrease or counter acid – pepsin secretion viz. ranitidine, famotidine etc. (H_2 - blockers), pirenzepine, telenzepine etc. (M1– blockers), omeprazole, lansaprazole etc. (proton pump inhibitors)
2. Those that affect cytoprotection by virtue of their effects in mucosal defense factors like sucralfate, carbenoxolone etc. (Yeomans *et al* 1998)

No doubt the above said drugs have brought about remarkable changes in peptic ulcer therapy, the efficacy of these drugs is still debatable. Reports on clinical evaluation of these drugs show that there are incidences of relapses and adverse effects and danger

of drug interactions during ulcer therapy. Hence, the search for an ideal anti – ulcer drug continues and has also been extended to medicinal plants / herbs in search for new and novel molecules, which afford better protection and decrease the incidence of relapse.

Numerous medicinal plants showed anti gastric ulcer activity. Sanyal *et al.* 1961 found that vegetable banana is efficacious not only for experimentally induced gastric ulcers in albino rats, guinea pigs etc. but also for human being suffering from gastric ulcers. Akah *et al* 1999 demonstrated anti gastric ulcer activity of the herb *Cassampelos mucronata*. Likewise Shetty *et al* 2000 , Sairam *et al*. 2001, Maity *et al.* 1995, 2003 and Dharmani & Palit 2006 confirmed anti gastric ulcer activities of *Ginkgo biloba*, *Convolvulus pluricaulis Chois*, tea root extract and *Vernonia lasiopus* respectively. We also reported anti gastric ulcer activities of few medicinal plants in different experimental ulcer models (Mitra 1980, 1981, 1982, 1985, 2001; Mitra & Mitra 2005, 2008; Mitra *et al.* 2008).

Recently we have noted anti gastric ulcer activity of *Murrya koenigii* leaf against ethanol induced gastric ulcer in albino rats (Mitra *et al*. 2010). Tempted by the observation we had undertaken isolation studies of the active compound from *Murrya koenigii* leaf for which the plant showed anti gastric ulcer activity.

By different solvent extraction processes and chromatographic experiments an active compound was isolated from *Murrya koenigii* leaf. A trivial name of the compound was given as MK-1. Anti gastric ulcer activity of MK-1 was studied against ethanol, hydrochloric acid, indomethacin, stress and pyloric ligation induced gastric ulceration in albino rats. Two doses of MK-1 (5 mg/kg and 10 mg/kg) were used. Results were compared with omeprazole, a known anti gastric ulcer drug.

Significant anti gastric ulcer activity of MK-1 was observed in all the models employed. Results showed that pretreatment of rats with MK-1 produced dose dependent protection. The protections were statistically significant ($p<0.001$) and comparable with that of omeprazole group.

It is known that peptic ulcer is formed either through offensive mechanism (acid – peptic secretion) or through defensive mechanism (mucus secretion). Anti gastric ulcer activity of MK-1 may be related with any one of the two mechanisms. Work in this direction is now under progress.

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