

Antiulcer Activity of Leaves Extract of *Murraya Koenigii* In Experimentally Induced Ulcer In Rats.

Praveen Sharma^{1*}, Gali Vidyasagar², Anil Bhandari³, Sunder Singh⁴, Santosh Ghule¹, Ashish Agrawal⁵, Swapnil Goyal⁵, Mangal S. Panwar⁵

1. College of Pharmacy, IPS Academy, Rajendra Nagar A.B. Road, Indore-452012 (M.P.).

2. Veerayatan Institute of Pharmacy, Jakhania, Bhuj-Mandvi Road, Kutch-370460, Gujarat, India

3. Jodhpur Pharmacy College, Jhanwar road, Boranada Jodhpur- 342006

4. Vinayaka College of pharmacy, Kullu, Himachal Pradesh .

5. Mandsaur Institute of Pharmacy, Mandsaur-458001, India

***Author for Correspondence**

E-mail: praveen81_2006@yahoo.com

College of Pharmacy,

IPS Academy Ragendra nagar A.B. road

Indore- 452012 (M.P.), INDIA.

Tel: 09303869279

Summary

The anti-ulcer activity of Aqueous extract of the leaves of *Murraya koenigii* was evaluated by using models of acute gastric lesions induced by ethanol induced, aspirin induced, cold restraint stress and pylorus ligation in rats. Animals pretreated with doses of 200 mg/kg and 400 mg/kg of Aqueous extract showed significant reduction in lesion index, total affected area and percentage of lesion in comparison with control group in the ethanol induced, aspirin induced, cold restraint stress-induced ulcer and pylorus ligation models. These findings indicate that aqueous extract of the leaves of *Murraya koenigii* displays good antiulcer activity, corroborating the folk use of *Murraya koenigii* preparations, and contributing for its pharmacological validation.

Key words: *Murraya Koenigii*, Gastric ulcer, Pylorus ligation

Introduction

Gastric hyperacidity and gastroduodenal ulcer is a very common global problem today. It is now generally agreed that gastric lesions develop when the delicate balance between some gastro- protective and aggressive factors are lost. Major aggressive factors are acid, pepsin, *Helicobacter pylori* and bile salts. Defensive factors mainly involve mucus bicarbonate secretion and prostaglandins. Hypersecretion of gastric acid is a pathological condition, which occurs due to uncontrolled secretion of hydrochloric acid from the parietal cells of the gastric mucosa through the proton pumping H⁺K⁺ATPase. Even the normal rate of acid secretion may cause ulceration in the breached mucosa when some gastroprotective factors are lost. The modern approach to control gastric ulceration is to inhibit gastric acid secretion, to promote gastroprotection, block apoptosis and stimulate epithelial cell

proliferation for effective healing. Most of the antisecretory drugs such as proton pump inhibitors (omeprazole lansoprazole, etc.) and histamine H₂-receptor blocker (ranitidine, famotidine, etc.) are extensively used to control increased acid secretion and acid related disorders caused by stress, NSAID's and *H. pylori*; but there are reports of adverse effects and relapse in the long run. On the contrary most of the herbal drugs reduces the offensive factors and are proved to be safe clinically effective, having better patient tolerance, relatively less expensive and globally competitive. Plant extracts, however, are some of the most attractive sources of new drugs and have been shown to produce promising results in the treatment of ulcers¹.

An aqueous extract of the leaves of *Murraya koenigii* possesses alexeteric, antihelminthic, analgesic, dysentery, purgative and blood disorders. Also they are reported to be useful in inflammation, healing of wounds, injuries, antioxidative activity²⁻³. In folklore practice, the decoction of *Murraya koenigii* leaves has been reported to be useful in gastric ulcer. There is no scientific report on the effect of *Murraya koenigii* on the ulcer. The present investigation was undertaken to evaluate the effect of *Murraya koenigii* on experimentally induced ulcer in rats.

Materials and Methods

Plant Material

Fresh leaves of *Murraya koenigii* (5 kg) were collected locally from the Indore district of Madhya Pradesh and got identified by Department of Botany, Saifia college of science and education, Bhopal. Specimen voucher no. is 168/Bio/saifia/10. The leaves were shade dried and were crushed to moderately coarse powder.

Preparation of extract

The powder was extracted with distilled water using soxhelt at boiling temperature (100 °C) up to 10 h. A dark brown colour extract is obtained. This dark brown extract was cooled and filtered to remove the residue. The extract was concentrated on rotavapour under reduced pressure and then lyophilized to get a powder weighing about 7.5 g⁴.

Preliminary Phytochemical Screening

The preliminary phytochemical screening was carried out on the aqueous extract of the leaves of *Murraya Koenigii* for qualitative identification^{5,6}.

Experimental Animals

Albino Wistar rats of both sex weighing between 150-250 g were used. The experimental protocol was approved from Institutional Animal Ethics Committee. Animals were housed under standard conditions of temperature (24 ± 2°C) and relative humidity (30-70%) with a 12:12 light: dark cycle. The animals were given standard diet and water *ad libitum*.

Drugs and Chemicals

Aqueous solution of *Murraya koenigii* was prepared in distilled water and was administered orally. Omeprazole (OMZ) was procured from Medley Pharmaceuticals Ltd, Daman, India. Rats were divided in four group containing six rats. Group I was control and given distilled water as vehicle. Group II and III were given *Murraya koenigii* (200 and 400 mg/kg, p.o). Group IV received Omeprazole as standard (20 mg/kg, p.o.).

Acute Toxicity Study

Four groups of rats of both sex (six animals per group) were administered orally a single dose of either 5, 10, or 15 times of effective dose of aqueous extract of *Murraya koenigii* leaves. The rats were observed for gross behavioral, neurologic, autonomic, and toxic effect continuously. Food consumption, faeces and urine were also examined at 2 h and then at 6 h intervals for 24 h^{7,8}.

Cold Restraint Stress Induced Ulcers⁹

Animals of different group were subjected to cold stress after 45 min of the extract and OMZ treatment. Rats were deprived of food, but not water, for about 18 h before the experiment. Rats were immobilized by strapping the fore and hind limbs in restraint cage and kept for 2 hr, at a temperature of 4°C. After 2 hr, animals were sacrificed, the stomach was incised along the greater curvature and ulcer was scored as: Red coloration (0.5), Spot ulcer (1), Haemorrhagic streak (1.5), Ulcers (2), Perforation (3). Mean ulcer score for each, animal was expressed as ulcer index. The percentage of ulcer protection was calculated as mean ulcer index of control-mean ulcer index of test / mean ulcer index of control x 100.

Pylorus Ligation Induced Ulcers¹⁰

After 1 hr of treatment to different groups, the animals were anaesthetized using thiopentone sodium (35 mg/kg, i.p.), the abdomen was opened and pylorus ligation was done without causing any damage in its blood supply. After 4 hr their stomachs were dissected and its contents were collected into tubes for analysis of volume of gastric juice, pH, total and free acidity. The ulcers were scored as described under cold stress induced ulcers. The gastric juice was collected after 4 hr of Pylorus ligation induced ulcers and centrifuged for 5 min at 2000 rpm. The supernatant was collected and the volume of gastric juice was expressed as ml/100 g body weight. Total acidity was determined in the supernatant by titrating against 0.1 N NaOH, using 2-3 drops of toppers reagent as indicator until canary yellow color was observed. Volume of NaOH required was noted and this corresponds to free acidity. Further 2-3 drops of phenolphthalein was added and titrated with 0.1 N NaOH until pink color was restored and this gives total acidity. Free acidity and total acidity is expressed in terms of 0.1 N HCL per 100 g of gastric contents and titrated with 0.1 N NaOH until pink color was restored and this gives total acidity.

Ethanol induced ulcers¹¹

The animals were divided into four groups, each consisting of six rats. Group I represented the control group, which received distilled water orally. Groups II and III received aqueous extract of *Murraya Koenigii* 200 and 400 mg/kg and, Omeprazole, in the dose of 20 mg/kg were administered orally for group IV as reference standard drug. The gastric ulcers were induced in rats by administering absolute ethanol (90%) (1 ml/200 g b.w.) orally, after 45 min of aqueous extract and Omeprazole treatment. They were kept in specially constructed cages to prevent coprophagia during and after the experiment. The animals were anaesthetized 1hr latter with anesthetic ether and stomach was incised along the greater curvature and ulceration was scored. A score for the ulcer was study similar to cold restraint stress induced ulcer model.

Aspirin induced ulcer¹²

The animals were divided into four groups, each consisting of six rats. Group I represented the control group, which received distilled water orally. Groups II and III received aqueous extract of *Murraya Koenigii* 200 and 400 mg/kg. Omeprazole (20 mg/kg) were administered orally for group IV as reference drug. Aspirin in dose of 500 mg/kg was administration to the animals after 45 min of extract and omeprazole treatment. The animals were sacrificed after 4 hr and the stomach was then excised and cut along the greater curvature, washed carefully with 5.0 ml of 0.9% NaCl and ulcers were scored by a person unaware of the experimental protocol in the glandular portion of the stomach. Ulcer index was then calculated by adding the total number of ulcers per stomach and the total severity of ulcers per stomach.

Statistical Analysis

The data are represented as mean \pm S.E.M, and statistical significance was carried out employing one way analysis of variance (ANOVA) followed by Dunnett t-test where $p < 0.05$ was considered statistically significant.

Results

Preliminary phytochemical investigation of aqueous extracts of leaves of *Murraya koenigii* revealed the presence of phenols, carbazole, alkaloids, flavonoids and tannins.

In acute toxicity study, Four groups of rats of both sex (six animals per group) were administered orally a single dose of either 5, 10, or 15 times of effective dose of aqueous extract of *Murraya koenigii* leaves. The rats were observed for gross behavioral, neurologic, autonomic, and toxic effect continuously. Food consumption, faeces and urine were also examined at 2 hr and then at 6 hr intervals for 24 hr..

The cold restraint stress-induced ulcer model observed a significant reduction in lesion index, total lesion area and in the percentage of lesion in animals treated with extract of *Murraya koenigii* and OMZ in comparison with the control group. The percentages of inhibition of ulcers were 76.92 % and 84.61 % for the groups treated with 200 mg/kg and 400 mg/kg of *Murraya koenigii* and 86.15 % for standard group (OMZ), respectively. Ulcer index score of extract treated and standard group are summarized in **Table 1**.

Similarly, the gastric secretion determination model used ligated pylorus, treatment with *Murraya koenigii* extract (200 and 400 mg/kg) and OMZ (20 mg/kg) respectively, reduced the volume of gastric juice, free acidity, total acidity and raised gastric pH significantly in comparison with the control group (**Table 2**). The extract also significantly reduced gastric mucosa lesion compared with the control group in the ethanol-induced ulcer. The percentage of inhibition of mucosa lesion was 70% and 73% for group treated with (200 and 400 mg/ kg of extract) compared to 81% caused by OMZ (20 mg/ kg) (Table 3). In the aspirin-induced model, *Murraya koenigii* extract and OMZ significantly reduced gastric mucosa lesion, compared with the control group (Table 4). The percentage inhibition of ulcers was 67% and 72% (200mg/ kg and 400 mg/ kg of extract) compared to 86% caused by OMZ (20 mg/ kg)

Table 1: Effect of aqueous extract of *Murraya koenigii* on ulcer index score against Cold restraint stress (CRS) induced gastric ulcer in rats.

Group	Treatment	Dose mg/ kg p.o.	Ulcer index
I	Control	1 ml/ 100ml	6.5 ± 0.83
II	<i>Murraya Koenigii</i>	200	1.5 ± 0.35 ^a
III	<i>Murraya Koenigii</i>	400	1.0 ± 0.38 ^a
IV	Omeprazole (Standard)	20	0.9 ± 0.41 ^a

All values are mean ± S.E.M. (n = 6) per group. Statistical comparison was performed by using ANOVA followed by Dunnett t-test, ^ap<0.01 were consider statistically significant when compared to control group.

Table 2: Gastroprotective activity of aqueous extract of *Murraya koenigii* on pylorus ligated ulcer

Treatment	Dose	Mean ulcer index	% protection	Gastric Juice ml	pH of gastric juice	Free acidity meq/ltr	Total acidity meq/lt
Vehicle	1ml/100g	11.05±3.40	--	4.5± 0.22	1.4 ± 0.09	25.35 ± 2.13	42.75 ± 0.21
Omeprazole	20 mg/kg	2.00±0.76**	82.60%	1.98 ± 0.12	4.8 ± 0.07	9.0 ± 0.04	28.57 ± 0.14
Aqueous extract of <i>Murraya Koenigii</i>	200 mg/kg	3.08 ± 1.06**	66.95 %	3.15 ± 0.10	2.5 ± 0.17	17.5 ± 0.12	35.23 ± 0.10
Aqueous extract of <i>Murraya Koenigii</i>	400 mg/kg	3.01 ± 0.67**	73.04 %	2.5 ± 0.18	3.68 ± .8	11.15 ± 0.11	30.25 ± 0.06

Results are expressed as Mean ± SEM (n = 6). Significant at **p<0.01 compared to control group.

Table 3: Effect of aqueous extract of *Murraya koenigii* on ulcer index score against Ethanol induced gastric ulcer in rats.

Group	Treatment	Dose mg/ kg p.o.	Ulcer index
I	Control	1 ml/ 100ml	24.22 ± 0.83
II	<i>Murraya Koenigii</i>	200	7.22 ± 0.35 ^a
III	<i>Murraya Koenigii</i>	400	6.55 ± 0.38 ^a
IV	Omeprazole (Standard)	20	4.58 ± 0.41 ^a

Results are expressed as Mean ± SEM (n = 6). Significant at **p<0.01 compared to control group.

Table 4: Effect of aqueous extract of *Murraya Koenigii* on ulcer index score against aspirin induced gastric ulcer in rats.

Group	Treatment	Dose mg/ kg p.o.	Ulcer index
I	Control	1 ml/ 100ml	16.80 ± 0.56
II	<i>Murraya Koenigii</i>	200	5.50 ± 0.35 ^a
III	<i>Murraya Koenigii</i>	400	4.70 ± 0.38 ^a
IV	Omeprazole (Standard)	20	2.20 ± 0.41 ^a

Results are expressed as Mean ± SEM (n = 6). Significant at **p<0.01 compared to control group.

Discussion

Ulcers are caused due to imbalance between aggressive and defensive factors of the gastric mucosa. Pepsin and gastric acid make up the offensive factors, whose proteolytic effect is buffered by mucin secretion, mucosal glycoprotein, cell shedding, cell proliferation and prostaglandins¹³. Different therapeutic agents including plant extracts are used to inhibit the gastric acid secretion, or to stimulate the mucosal defense mechanism by increasing the mucus production protecting the surface epithelial cells or interfering with the PG synthesis¹⁴. Stress-induced ulcers are probably mediated by histamine release with enhancement in acid secretion, a reduction in mucus production and generation of free radicals etc., mast cell activation, alterations in prostaglandin generation, cytokine liberation and breakdown of normal cytoprotective mechanism. Ulcers due to cold stress are both due to physiological and psychological factors¹⁵⁻¹⁶. The gastro protective action of aqueous extract of *Murraya koenigii* against stress-induced ulceration could be due to its histamine antagonistic, anticholinergic and/ or antisecretory effects.

After 45 min of treatment with an aqueous extract of *Murraya koenigii*, pylorus ligation of rats for 4 hr resulted in accumulation of gastric secretory volume and increase in titrable acidity and ulceration. The cause of gastric ulcer after pyloric ligation is believed to be due to stress-induced increase in gastric hydrochloric acid secretion and/ or stasis of acid. Pylorus ligation-induced gastric ulcers occur because of an imbalance between aggressive factors and the maintenance of mucosal integrity through the endogenous defence mechanisms¹⁷. Ulcers caused by pyloric ligation are due to increased accumulation of gastric acid and pepsin leading to auto digestion of gastric mucosa and break down of the gastric mucosal barrier¹⁸. In the present study, aqueous extract of *Murraya koenigii* reduced the free acidity, total acidity and ulcer index.

Conclusion

In conclusion, aqueous extract of *Murraya Koenigii* possessed significant antiulcer activity against cold restraint stress induced ulcers, pylorus ligation ulcer model, ethanol induced ulcer and aspirin induced ulcer when compared to control group. These findings indicate that aqueous extract of *Murraya Koenigii* display potential antiulcerogenic activity. This activity thus lends pharmacological credence to the suggested use of plant as a natural remedy in the treatment of management of ulcer.

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