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Research article

Investigation of stem bark of *Moringa Oleifera* (lam.) for antiulcer activity

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ABSTRACT

The stem bark of *M. oleifera* was used traditionally for the treatment of ulcer sores. The high antioxidant/radical scavenging effects observed for different parts appear to provide justification for their widespread use in traditional medicine. The effect of *Moringa oleifera* (Lam.) aqueous extract was evaluated in albino wistar rats against ethanol induced gastric ulcer model. It was found effective at dose of 200 mg/kg. The percentage inhibition of ulcer in ethanol induced ulcer was found to be 64.30%. All treatment groups showed a marked increase in the amount of PH. The decreased ulcer lesions, gastric volume, free acidity and total acidity confirmed a significant increase in ulcer reduction. *oringa oleifera* was shown to exert mucoprotective and gastric antisecretory activity and the mechanism involved may be due to the presence of flavonoids and terpenoids.

Keywords: Ulcer, H. pylori, Phyto-constituents, Acute toxicity, Animal activity.

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. The modern approach to control gastric ulceration is to inhibit gastric acid secretion, to promote gastro protection, block apoptosis and stimulate epithelial cell proliferation for effective healing. Most of the antisecretory drugs such as proton pump inhibitors (omeprazole, Pantoprazole etc.) and histamine H_2-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. *Moringa oleifera* (Lam.) is a versatile and exceptionally nutritious vegetable tree with a variety of potential uses. It is the most widely cultivated species of Moringaceae family. Commonly it is known as in English – Moringa or Drumstick tree or Horseradish tree, in Hindi - Sahjan, in Latin – Moringa oleifera, in Sanskrit - Surajana, in Nepali - Sajiwan or Swejan etc. It is useful not only for human beings but also for animals and also in various industrial applications. The plant is a rich source of antioxidant agents and stem bark contains phytoconstituents like 4-hydroxymellein, vanillin, ß-sitosterone, octacosanic acid, ß-sitosterol, and4-(a-L-rhamnopyranosyloxy)-benzylglucosinolates. The plant stem bark was used traditionally for the treatment for ulcer sores. The high antioxidant/radical scavenging effects observed for different parts of *M. oleifera* appear to provide justification for their widespread use in traditional medicine in different continents. The acetone and methanol extract of leaves showed antiulcer activity. Antiulcer activity of alkali preparation of root and fresh leaf juice of *Moringa oleifera* Lam. has been reported. The petroleum ether, acetone, and methanol extracts of *Moringa oleifera* were effective in reducing the ulcers induced by stress. *Moringa oleifera* is also reported to possess various pharmacological activities like antihypertensive, diuretic, hepatoprotective, antispasmodic, cholesterol lowering, antioxidant, antimicrobial, antifertility, abortifacient and anti-inflammatory. However detail investigation of antiulcer activity of bark extract of *Moringa oleifera* had not been carried out so far. Hence this leads us study the antiulcer activity of bark extract of *Moringa oleifera* in different ulcer models [1].

MATERIAL AND METHOD

Instruments Used

Simple microscope of magnification 10× was used to visualize the stomach for determining the ulcer score. Centrifuge machine was used to centrifuge the gastric juice collected for getting the supernatant and titrating it for determining the free acidity and total acidity, and pH meter was used to determine the pH of the gastric juice in the pylorus ligation induced model.

Drugs and Chemicals

Aspirin (Cipla pharmaceuticals limited, Pithampur Indore, India), Omeprazole (Torrent Pharmaceutical, Ahmedabad, India), Ranitidine (Abhirami Pharmachem, India), Catechin and chemicals used for phytochemical analysis were of analytical grade and procured from local firms [2].

Plant Material

Stem bark of the plant *Moringa oleifera* has been collected from local garden of Indore, (M.P.). Authentification of plant on basis of pharmacognostic study and organoleptic characteristics was done by Botanical survey of India Pune. A voucher specimen number BSI/WC/Tech./2011/904 of bark of *Moringa oleifera* has been deposited in museum of Dept. of Botany, Botanical survey of India.

Preparation of Extract

The ayurvedic literature reveals the traditional claim for the use of fresh bark of *Moringa oleifera* for the treatment of ulcer (vrad dosh nasak). The bark is powdered and lepa is applied externally and given orally. The decoction of the plant is prepared as shobbhajanakwatha for the treatment of various ailments as in spleen enlargement, since the traditional claim involves its use by administrating the plant as decoction.

The bark was powdered in the form of coarse particles, then decoction was prepared by mixing one part of the plant to 16 times water and it is boiled till the extract remains one eighth of the prior addition of water. A dark brown residue is obtained when concentrated. The shelf life of the decoction is 48 hours.

Preliminary Phytochemical Screening of Extract

Preliminary phytochemical analysis was carried out to check and identify the active constituents of the aqueous extract of *Moringa oleifera* stem bark such as saponins, flavonoids, terpenoids, amino acids, proteins, alkaloids, and carbohydrates by using foam formation test, Dragendroff and Mayer test, lead acetate test, Millons test, Biuret test and Fehling's test, respectively.

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 (Trimurti Feeds, Maharashtra, India) and water *ad libitum.* The present investigations employed albino wistar rat as the same had been used by an earlier investigators for antiulcer studies [3].

Pharmacological activity

Acute toxicity studies

The acute toxicity study was carried out to select the dose, by using up & down method. Rat was given a dose of 175 mg/kg orally and then the dose progression and reduction factor was 3.2 times of the previous dose as according to Organization for Economic Co-operation and Development (O.E.C.D.) guideline 425 different doses ranging from $1/10^{th}$ as lower dose and $1/50^{th}$ as the maximum safe dose were selected [4].

Antiulcer Activity

Ethanol Induced Ulcer

The animals were divided into four groups, each consisting of four rats. Group I represented the control group, which received distilled water orally. Groups II received Catechin, in the dose of (200 mg/kg) as reference standard antioxidant agent. Group III and IV received *Moringa oleifera* extract at dose of (200 mg/kg) and (100 mg/kg) respectively. The gastric ulcers were induced in rats by administrating absolute ethanol (90 %), (1 ml/200gm) orally, after 45 min of aqueous extract and catechin treatment. They were kept in specially constructed cages to prevent coprophagia during and after the experiment. The animals were anaesthetized one hour later with anesthetic ether and stomach was incised along the greater curvature and ulceration was scored. The ulcer was scored as: Red coloration (0.5), Spot ulcer (1), hemorrhagic 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 [5].

RESULTS AND DISCUSSION

Preliminary Screening

Preliminary phytochemical screening of the stem bark extract revealed the presence of alkaloids, flavonoids, terpenoids, carbohydrates, glycosides, proteins and saponins [6].

Acute toxicity study

No mortality was observed after treatment with the highest tested dose (2000 mg/kg *p.o.*) of the aqueous extract. The extract was found to be safe upto the dose of 2 g/kg *p.o* [7].

Ethanol Induced Ulcer

Pretreatment of rats with the aqueous extract of *Moringa oleifera* (200 mg/kg) and (100 mg/kg) produced significant protection of ulcer index ($p < 0.001$) from ethanol induced ulceration as compared to control animals. Catechin 200 mg/kg produced significant gastric ulcer protection ($p < 0.001$) as compared to control group. The aqueous extract at 200mg/kg and 100mg/kg showed protective effect of 64.30% and 46.68% respectively against ethanol induced Ulcerogenesis [8].

Peptic ulcer is a lesion of gastric or duodenal mucosa occurring at a site where the mucosal epithelium is exposed to

aggressive factors. *Moringa oleifera* (Lam.) is one such plant whose bark was used traditionally for treatment of ulcer sores. The effect of different extracts of leaves and fruits of *Moringa oleifera* Lam. on gastric and duodenal ulcers was evaluated in different ulcer models which revealed that leaves contains flavonoid β-sitosterol which are known to reduce gastric ulcers. Ethanol is proved to significantly increase the plasma concentration of gastric hormone, gastrin and an increase in gastric mucosal H^+K^+ATase activity. The H^+K^+ATase is the dimeric enzyme responsible for H^+ ion secretion by gastric parietal cells. The results of the present study revealed that the treatment with *Moringa oleifera* significantly decreased the lesion index and percent of lesion and thus possesses an antiulcerogenic effect related to cytoprotective activity [9].

Statistical Analysis

The data are represented as mean ± S.D., and statistical significance was carried out employing one way analysis of variance (ANOVA) followed by Tukey's test. $P<0.05$ was considered statistically significant [10].

CONCLUSION

The significant results in the model showed that the extract may possess cytoprotective activity. Preliminary phytochemical analysis of extract revealed presence of alkaloids, carbohydrates, proteins. The other secondary metabolites like flavonoids, terpenoids, etc are also present. The nonspecific gastroprotective activities of the extract may be the result of a combined effect of the different phytoconstituents present. The flavonoidal compounds were proved to have antisecretory and cytoprotective properties due to free radical scavenging activity during lipid peroxidation and the action of terpenes includes reduction of mucosal prostaglandin metabolism and gastric vascular permeability. However, based on the published studies, terpenes and flavonoids seem to be the most likely candidates eliciting gastroprotective effect.

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