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Anti-ulcer activity of extract of *Moringa oleifera* Lam. using pylorus ligation induced ulcer

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Abstract
Moringa oleifera Lam commonly known as Moringa, a native plant from Africa and Asia, and the most widely cultivated species in Northwestern India, is the sole genus in the family Moringaceae. MO has been recognized as containing a great number of bioactive compounds. The most used parts of the plant are the leaves, which are rich in vitamins, carotenoids, polyphenols, phenolic acids, flavonoids, alkaloids, glucosinolates, isothiocyanates, tannins and saponins. The present investigation was carried out to evaluate anti-ulcer activity of various extract of the plant using Pylorus ligation induced ulcer. Results indicates that the acetone and methanolic flower extracts of *Moringa oleifera* and ranitidine showed a significant reduction in free acidity and total acidity when compared to control (p<0.05). The petroleum ether extract did not show significant reduction in free acidity and total acidity when compared to control.

Keywords: *Moringa oleifera*, anti-ulcer activity, pylorus ligation induced ulcer

Introduction
Studies have shown that peptic ulcer disease (PUD) occurs because of an imbalance between aggressive injurious (e.g., pepsin, HCl) and defensive mucosa-protective factors (e.g., prostaglandins, mucus and bicarbonate barrier and adequate blood flow) [4]. All ulcers of the upper gastrointestinal tract were originally thought to be caused by the aggressive action of pepsin and gastric acid on mucosa. However, the denomination "peptic ulcer" has lately pointed to Helicobacter pylori infection, where the chronic use of non-steroidal anti-inflammatory drugs (NSAIDs) and acetylsalicylic acid (ASA) are some of the disease-causing factors. Thus, based on the latest advances on this field and stress the fact that PUD is an important cause of morbidity and health care costs, the present report aims to provide a general overview on peptic ulcers, namely considering their epidemiology, main symptoms and clinical features, pathogenesis, where a particular emphasis will be given to H. pylori infection, pharmacological agents used in an effective management and also pointing out the latest challenges and opportunities of using plant phytochemicals as upcoming antiulcerogenic agents. Lastly, a special emphasis was given on plant products safety and security, in order to trigger the interest in deepening skills on this matter and to ensure an effective managing competence for health-related systems [1, 2].
Drumstick tree, also known as horseradish tree and ben tree in English, is a small to medium-sized, evergreen or deciduous tree native to northern India, Pakistan and Nepal. It is developed and has turned out to be naturalized well past its local range, including all through South Asia, and in numerous nations of Southeast Asia, Central America, tropical Africa, the Caribbean, the Arabian Peninsula and tropical South America. The tree more often than not develops to 10 or 12 m in stature, with a spreading, open crown of hanging, weak branches, padded foliage of tripinnate leaves, and thick, corky, profoundly fissured whitish bark. It is esteemed for the most part for its eatable natural products, leaves, blooms, roots, and seed oil, and is utilized widely in conventional prescription all through its local and presented ranges. It is developed and has turned out to be naturalized in different pieces of Pakistan, India, and Nepal, just as in Afghanistan, Bangladesh, Sri Lanka, Southeast Asia, West Asia, the Arabian promontory, East and West Africa, all through the West Indies and southern Florida, in Central and South America from Mexico to Peru, just as in Brazil and Paraguay. *Moringa oleifera* is a little, quickly developing evergreen or deciduous tree that generally grows up to 10 or 12 m in tallness. It has a spreading, open crown of hanging, delicate branches, fluffy foliage of tripinnate leaves, and thick, corky, whitish bark [3-5].
Since the reports about the antiulcer activity of the leaves of *Moringa oleifera* sparsely

documented, it was considered worthwhile to investigate the antiulcer activity of the flower and seeds extract of *Moringa oleifera* and substantiate its ethnopharmacological claim of providing relief in ulcer.

Methodology

Collection of plant material and extraction procedure

The flowers and seeds of *Moringa oleifera* Lam were collected from the botanical garden of RKDF university Bhopal between 28/01/2017 to 25/09/2017. Flowers and seed were authenticated by the Head of Department of botany Dr.Zia Ul Hasan Professor of Safia College of Science Bhopal. Plant authentication no. is 346/Bot/Safia/2017 & 347/Bot/Safia/2017 on the date14/10/2017.

Extraction of leaves and fruits [6]

The flowers and seeds of *Moringa oleifera* Lam were shade dried and reduced to coarse powder in a mechanical grinder and passed through sieve No. 40. The powdered material obtained was then subjected to successive extraction in batches using petroleum ether, chloroform, and acetone and methanol solvents in a Soxhelet extractor. The different extracts obtained were evaporated in rotary evaporator to get a semisolid mass.

Phytochemical estimations of the extracts

The extracts of *Moringa oleifera* Lam were subjected to qualitative analysis for the various phytoconstituents like alkaloids, carbohydrates, glycosides, phytosterols, saponins, tannins, proteins, amino acids and flavonoids [6, 7].

Experimental animals

Male albino Wistar rats weighing between 200-250 gm were used. Institutional Animal Ethics Committee permitted the experimental procedure; animals were maintained under standard conditions in an animal house approved by Committee for the Purpose of Control, and Supervision on Experiments on Animals (CPCSEA).

Acute toxicity study

The intense oral poisonous quality examination was performed by the OPPTS (Office of prevention, pesticide and toxic substance) Up and Down method (Health Effect Test Guideline 2004) The different extracts were suspended using 0.5% sodium carboxy methylcellulose and were administered orally. The concentration was adjusted in such a way that it did not exceed 1ml/kg b/w of the animal [8].

Pylorus ligation induced ulcers [9-11]

The animals were fasted for 36 hours before pylorus ligation with water *ad libitum* by placing them individually in cages to avoid coprophagy and cannibalism. Typical saline (1ml/rodent p.o.) was managed twice day by day to every one of the creatures. Under light ether anesthesia, the midriff was opened by midline entry point underneath the xiphoid procedure. The pyloric part of the stomach was marginally lifted out and ligated, maintaining a strategic distance from harm to its blood supply. The concentrates (500mg/kg, p.o.) or ranitidine (50mg/kg, p.o.) was controlled intraduodenally following pylorus ligation. The stomach was set back cautiously and the stomach divider was shut with sutures. The creatures were denied of nourishment and water amid the postoperative period and the creatures were relinquished six hours after pylorus ligation by over portion of ether anesthesia.

The stomachs were separated and the substance of the stomachs were gathered and centrifuged. The volume of the gastric juice was measured and this was used for estimation of free acidity, total acidity, pepsin content and total proteins. The stomachs were cut open along the greater curvature and the stomach samples were scanned using a computer scanner. The total mucosal area and total ulcerated area was measured using public domain image processing and analysis program developed at National Institute of Health, USA. The PC version of the program was downloaded free from Scion (http://www.scioncorp.com) (Scion Image for Windows, Release Beta 4.0.2). The scale was set at 6.1 pixels per millimeter.

Briefly the process of quantification of gastric lesions using scion image is as follows; Click on the file button in the menu of scion image and open the image file from the document. Select the heart symbol from the tool bar and trace the outline of the stomach. Then click on analyze button, next click on set scale and set the scale as 6.1 pixels/mm [203]. Then again click on the analyze button followed by measure which gives the area of the selected image on the info screen. The ulcerated area in this model was quantified as follows; click on the file button in the menu of scion image and open the image file from the document. Then click on the process button, followed by a click on convert to gray scale then click on arithmetic subtract. Here subtract the unlesioned surface by starting from 100 with an increment or decrement of 5-10 units until the entire unlesioned surface is subtracted. The subtraction unit used was 110. Then click on analyze button in the menu and set the scale as 6.1 pixels/mm. Finally again click on the analyze button and the measure. This displays the area of the lesioned area in the info screen.

Note the area of ulcerated area and total mucosal area and calculate the ulcer index as mentioned below.

The ulcer index was determined using the formula

$$Ulcer\ index = \frac{10}{X}$$

$$where\ X = \frac{\text{Total mucosal area}}{\text{Total ulcerated area}}$$

Statistical analysis

The arithmetical implication was assessed using one-way analysis of variation (ANOVA) followed by Dunnet comparison test. For comparing nonparametric ulcer scores, ANOVA followed by non-parametric Dunn posttest was used. The standards are articulated as mean + SEM and $p<0.05$ was considered significant.

Results and Discussion

The present study deals with the study of effect of the different extracts of flower and seeds of *Moringa oleifera* on gastric and duodenal ulcers. Phytochemical analysis of the flower extracts revealed that petroleum ether extract contains steroids and alkaloids, acetone extract had carbohydrates, proteins, alkaloids, tannins, flavonoids and glycosides while the methanol extract contained saponins along with all the constituents present in acetone extract.

In the acute toxicity study, no mortality was observed after treatment with the highest tested dose (5 g /kg *p*.o) of all the extracts of flower and seeds. Hence, 1/10th of the tested dose (500 mg/kg *p*.o) was selected for evaluation of anti-ulcer

activity. The anti-ulcer effect was evaluated using the Pylorus ligation induced ulcer. The acetone and methanolic flower extracts of *Moringa oleifera* and ranitidine showed a significant reduction in free acidity and total acidity when compared to control (p<0.05). The petroleum ether extract did not show significant reduction in free acidity and total acidity when compared to control. None of the treatments produced any significant effect on ulcer index and total hexoses content (Table: 1).Similarly none of the treatments produced any significant effect on mucin content, pepsin activity and total proteins (Table: 2).

Table 1: Effect of *Moringa oleifera* flower extracts on free acidity, total acidity, ulcer index and total hexoses

Treatment mEq/litre	**Free acidity** mEq/litre	**Total acidity**	**Ulcer Index**	**Total Hexoses**
Control	6.74±0.3581	13.78±0.765	0.098±0.019	1.98±0.231
Ranitidine	3.98±0.732*	8.25±1.838*	0.108±0.033	2.35±0.293
Petroleum ether flower extract	4.90±0.837	14.04±1.233	0.058±0.008	2.12±0.330
Acetone flower extract	4.04±0.454*	8.83±1.765*	0.058±0.010	1.56±0.287
Methanol flower extract	3.75±0.430*	10.23±0.742	0.098±0.025	2.213±0.330

All values are mean ± SEM, n = 5-6. *p<0.05, **p<0.01, ***p<0.001 when compared to control group

Table 2: Effect of *Moringa oleifera* flower extracts on mucin content, pepsin content and total proteins

Treatment	**Mucin content**	**Pepsin content**	**Total proteins**
Control	6.92±0.376	0.163±0.011	54.49±8.01
Ranitidine	7.60±1.092	0.098±0.152	83.20±19.23
Petroleum ether flower extract	7.6±2.240	0.172+0.034	51.32±4.30
Acetone flower extract	7.60±1.154	0.085±0.023	74.85±14.96
Methanol flower extract	7.66±1.630	0.143±0.277	71.78±13.22

All values are mean ± SEM, n = 5-6. *p<0.05, **p<0.01, ***p<0.001 when compared to control group

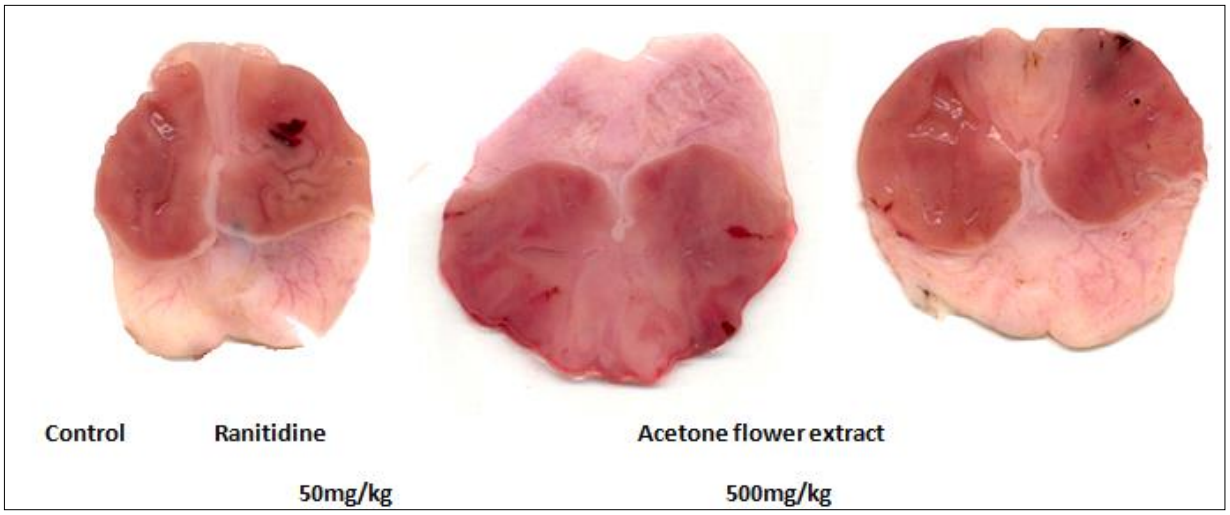

Fig 1: Pylorus ligation induced ulcer

Conclusion

Pylorus ligation actuated ulcer was utilized to think about the impact of concentrates on gastric corrosive emission and bodily fluid discharge. The ligation of the pyloric end of the stomach causes gathering of gastric corrosive in the stomach. This expansion in the gastric corrosive emission causes ulcers in the stomach. The first Shay rodent display includes fasting of rodents for 72 hours pursued by ligation of pyloric end of the stomach. The ulcer record is resolved 19 hours after pylorus ligation. The injuries delivered by this technique are situated in the rumen area of the stomach. Numerous creators have altered the first model. In the present investigation, the Shay rodent show portrayed by Kulkarni was pursued. In contrast to the first model, where ulcers are delivered in the rumen locale of the stomach, in this model, the ulcers created as injury in the glandular bit of the stomach. The specialists that decline gastric corrosive emission and increment bodily fluid discharge are powerful in ensuring the ulcers initiated by this technique. The acetone, methanolic flower extract of *Moringa oleifera* and ranitidine significantly decreased the total acidity and free acidity. Where as petroleum ether flower extract did not show significantly effect on total acidity and free acidity. The acetone and methanolic extracts of *Moringa oleifera* increased the mucus content but the increase in mucus content was not significant when compared with that of control. The petroleum ether, acetone, methanolic flower extracts of *Moringa oleifera* and ranitidine were not effective in increasing the total hexoses and total protein content. This suggests that acetone and methanolic extracts of *Moringa oleifera* are having antisecretory effect.

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