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Effect of Leaves and Fruits of *Moringa Oleifera Lam* on Gastric and Duodenal Ulcers In Rats

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ABSTRACT

Plant *Moringa oliefera* (family –*Moringaceae*) is a exceptionally nutritious vegetable tree with a variety of potential uses. In the present study, Effect of leaves and fruits of *Moringa oleifera* plant were studied on gastric and duodenal ulcers using methanol and acetone extraction. The antiulcer activity was performed by using two models Ethanol induced gastric ulcers and Cysteamine induced ulcers. The fruit extracts of the plant did not show any significant effect on the healing of gastric ulcers, Whereas the methanol and acetone leaf extracts increased the healing effect . The healing of ulcers in ethanol–induced gastric ulcers may be due to decreased acid secretion, increased or decreased GI motility incase of methanol and acetone extracts, and the ulcer healing effect is mainly due to reduction in gastric motility.

Keywords: Moringa Oleifera, Gastric ulcer, Duodenal ulcer etc.

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INTRODUCTION

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Ulcers are a gastrointestinal disorder occurred due the formation of sores and erosions in the lining of the stomach and the duodenum. Stomach ulcers are also known as gastric ulcers. This gastric and duodenum ulcers together is called as "Peptic ulcers[1]."

A peptic ulcer is an open sore or raw area in the lining of the stomach (gastric) or the upper part of the small intestine[2] (duodenal). Gastric and duodenal ulcers are breaks in the gastric and duodenal mucosa. Both gastric and duodenal ulcers relate to the corrosive action of pepsin and hydrochloric acid on the mucosa of the upper gastrointestinal tract. Ulcers generally range between 3 mm and several centimeters in diameter.

As many as 70–90% of such ulcers are associated with *Helicobacter pylori*, a spiral-shaped bacterium that lives in the acidic environment of the stomach; however, only 40% of those cases go to a doctor. Ulcers can also be caused or worsened by drugs such as aspirin, ibuprofen, and other NSAIDs. Four times as many peptic ulcers arise in the duodenum the first part of the small intestine, just after the stomach—as in the stomach itself[3].

Ulcers can develop in the esophagus, stomach or duodenum, at the margin of a gastroenterostomy, in the jejunum, in Zollinger-Ellison syndrome, and in association with a Meckel's diverticulum containing ectopic gastric mucosa. Peptic ulcer disease is one of several disorders of the upper gastrointestinal tract that is caused, at least partially, by gastric acid. Patients with peptic ulcer disease may present with a range of symptoms, from mild abdominal discomfort to catastrophic perforation and bleeding[4].

Moringa oleifera is the most widely cultivated species of a monogeneric family, the *Moringa*ceae, that is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan. This rapidly-growing tree (also known as the horseradish tree, drumstick tree, benzolive tree, kelor, marango, mlonge, moonga, mulangay, nébéday, saijhan, sajna or Ben oil tree), was utilized by the ancient Romans, Greeks and Egyptians; it is now widely cultivated and has become naturalized in many locations in the tropics[5].

It is an exceptionally nutritious vegetable tree with a variety of potential uses. The tree itself is rather slender, with drooping branches that grow to approximately 10 m in height. In cultivation, it is often cut back annually to 1 meter or less and allowed to regrow so that pods and leaves remain within arm's reach[6].

The aim of the present study was to investigate and compare the gastro protective effect of different extracts of *Moringa Oleifera* (500mg/kg of body weight) for antiulcer activity.

Objective:

To investigate and to compare the gastric ulcer and duodenal ulcer activity of methanol and

acetone extracts of *Moringa Oleifera* leaves and fruits and to compared with the positive control and standard drug- Ranitidine.

Specific Objectives

- To perform extraction of the plant using methanol and acetone.
- To investigate the effects of the methanol and acetone extract of *Moringa* on the antiulcer activity by using ethanol induced gastric ulcer and cysteamine induced duodenal ulcer method with the standard drug -Ranitidine.
- The aim of present study is therefore to assess activity of select indigenous medicinal plants in ulcers by using suitable animal models. Various studies have been carried out for determining gastric ulcer activity whereas less studies are carried out to determine the duodenal ulcer activity.
- So, our objective is mainly to carry out the studies on the effect on duodenal ulcers in rats.

MATERIALS AND METHOD

Collection of plant material and extraction procedure:

The leaves and fruits of *Moringa Oleifera* were collected from the SV University , Tirupati and authentified from Dr. Madhav Chetty.

Extraction of plant materials

The fresh leaves and fruits were collected, dried in the sun for 7days and finally in an oven below 60^0 c. The dried plant material was ground into fine coarse powder and extracted with methanol in cold condition. In cold extraction, the coarse powder is submerged in a suitable solvent (Methanol and Acetone separately) or solvent mixture in a flat bottom flask at room temperature and allowed to stand for several days with occasional shaking. When the solvent become concentrated, the content is then filtered with cotton and filter paper. Evaporation of solvent in vacuum rotary evaporator affords a crude extract of the soluble components and the extract was given to the animals. Two extracts are prepared from methanol and acetone for the leaves and fruits.

Animals:

Sixty Albino rats, Wister strain weighing 160±180g, were obtained from Nizam Institute Of Pharmacy Animal Laboratory.

Drugs and chemicals:

Anti-ulcer agent (Zantac ™ (Ranitidine) was obtained in the form of ampoules from pharmacy, Cysteamine hydrochloride , Methanol ,Acetone, Ethanol, distilled water ,chloroform ,sodium hydroxide ,phentholin, topper's reagent, normal saline.

Acute toxicity study

The acute oral toxicity study was performed according to the OPPTS (Office of Prevention, Pesticide and Toxic Substance. The different extracts were suspended ZX using 0.5% sodium carboxyl methylcellulose and were administered orally. All the extracts of leaves and fruits were safe at a dose of 5000 mg=kg, p.o., and one-tenth of this dose was selected for evaluation of antiulcer activity[7].

EXPERIMENTAL MODELS:

Ethanol induced gastric ulcer in rats:

- ANIMALS: Albino rats. (4 groups of 5 animals each)
- Group I : Positive control – Ethanol (0.5/100g)
- Group II: Treatment group - Ranitidine(6mg/100g)
- Group III : Methanol extacts of *Moringa* leaves (500mg/kg)
- Group IV : Acetone extacts of *Moringa* leaves (500mg/kg)
- Group V: Methanol extacts of *Moringa* fruits (500mg/kg)
- Group VI: Acetone extacts of *Moringa* fruits (500mg/kg)

EXPERIMENTAL DESIGN:

Ethanol-induced ulcers:

All Animals were fed on the basal diet and water *ad libitum* and they were maintained under healthy conditions of humidity, temperature (20-25°C) and light (12-h light: 12-h dark cycle) for one week before starting the experimental to acclimatization. After acclimatization period rats were divided into six groups. Group I, Group II, Group III, Group IV, Group V and Group VI,

Rats were fasted for 36 h before administration of 90% ethanol (1mL=200 g).The leaf extracts(500 mg=kg, p.o.) were administered 1 h before ethanol antiulcer activity of *Moringa oleifera* Lam administrated. One hour after ethanol is administration ,the animals were sacrificed, stomach was isolated, and ulcer index was determined[8].

Group I: kept as positive group with toxicant-ethanol.

Group II: Treatment group - Ranitidine(6mg/100g)

Group III and Group IV : Methanol extacts of *Moringa* leaves (500mg/kg) and Acetone extracts of *Moringa* leaves (500mg/kg)

Group V and Group VI: Methanol extracts of *Moringa* fruits(500mg/kg) and Acetone extracts of *Moringa* fruits (500mg/kg)

Moringa extracts were administered by tube feeding with of standard dose of 500 mg/kg respectively. At the end of the day all rats were sacrificed, stomach was isolated from the rat and the gastric contents are collected in a tube

Macroscopic evaluation of stomach:

The stomachs were opened along the greater curvature, rinsed with saline to remove gastric contents and blood clots and examined by a ×5 magnifier lens to assess the formation of ulcer. The number of ulcers was counted. Ulcer scoring was undertaken according to Vogel[8] . The scores were: 0 = no ulcer, 1= superficial ulcer, 2= deep ulcer, 3= perforation. Ulcer area was assessed by using 3 M scaled surgical transpore tapes, which was fixed on a light and transparent sheet. Each cell on the tape was 1mm2 in area, so the number of cells was counted and the ulcer area was measured for each stomach. Ulcer index was measured by using following formula according to Vogel. The stomach

samples were scanned using a computer scanner, and the total mucosal area and total ulcerated area were measured using public domain image processing and analysis program.

$UI = UN + US + UP \times 10^{-1}$

- UI = ulcer index,
- UN = mean of ulcer number,
- US =mean of ulcer score,
- UP = ulcer probability (incidence %) for each group.

EXPERIMENTAL DESIGN:

Cysteamine-induced duodenal ulcers:

Group -I: Positive control – Ethanol (0.5/100g)

Group II: Treatment group - Ranitidine(6mg/100g)

Group III: Methanol extacts of *Moringa* leaves (500mg/kg)

Group IV : Acetone extacts of *Moringa* leaves (500mg/kg)

Group V : Methanol extacts of *Moringa* fruits (500mg/kg)

Group VI : Acetone extacts of *Moringa* fruits (500mg/kg)

Experimental design:

Duodenal ulcers were induced by administering cysteamine hydrochloride (400 mg=kg, p.o.) twice at an interval of 4 h. Leaf extracts (500 mg=kg, p.o.) were administered 30 min prior to each dose of cysteamine hydrochloride. After 24 h of the first dose of cysteamine, animals were sacrificed and the duodenum was excised carefully and cut opened along the antimesenteric side. The duodenal ulcer area, ulcer score, and ulcer index were determined[8,13].

The ulcers were given scores based on their intensity as follows: 0 ¼ no ulcer, 1 ¼ superficial mucosal erosion, 2 ¼ deep ulcer or transmural necrosis, 3 ¼ perforated or penetrated ulcer.

The ulcer index (UI) was calculated using the following equation :

$$UI = \text{Arithmetic mean of intensity in a group} + \frac{\text{Number of ulcer positive animals}}{\text{Total number of animals}} \times 2$$

STATISTICAL ANALYSIS

The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Dunnet's comparison test. For comparing nonparametric ulcer scores, ANOVA followed by non-parametric Dunn posttest was used. The values are expressed as mean SEM and $p < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Invivo results have been shown in tables 1 – 8

Ethanol-induced gastric ulcers

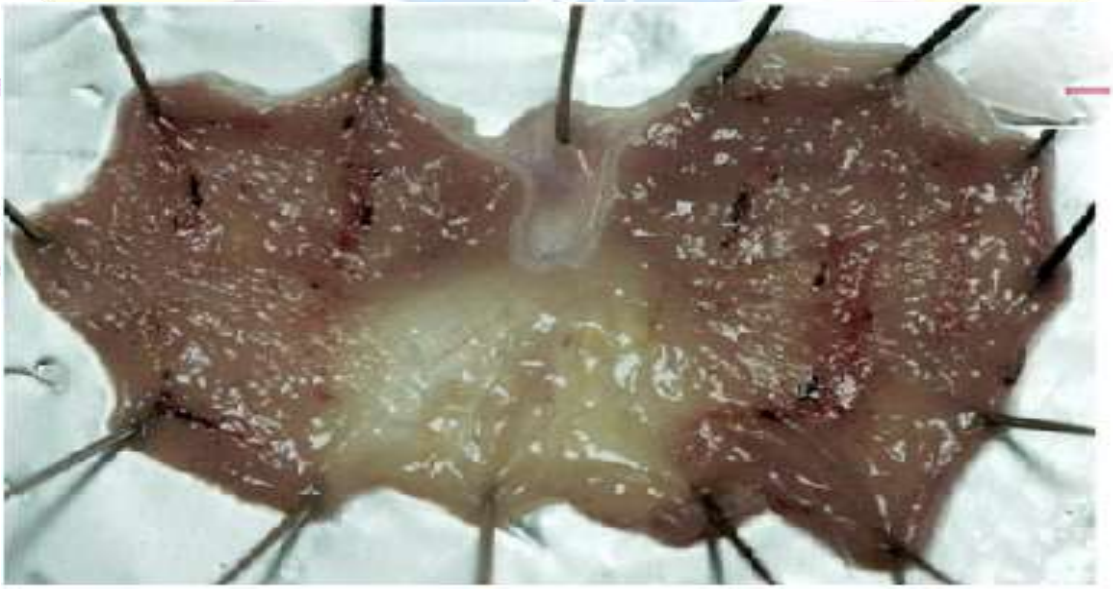
The acetone and methanol leaf extracts of *Moringa oleifera* showed a significant reduction in ulcer index when compared with control ($p < 0.05$) in Table-1 and no significant results were found with the fruits of *Moringa oleifera* in Table-2.

Table-1: Effect of *Moringa oleifera* leaf extracts in ethanol-induced gastric ulcers compared with positive control- Ranitidine

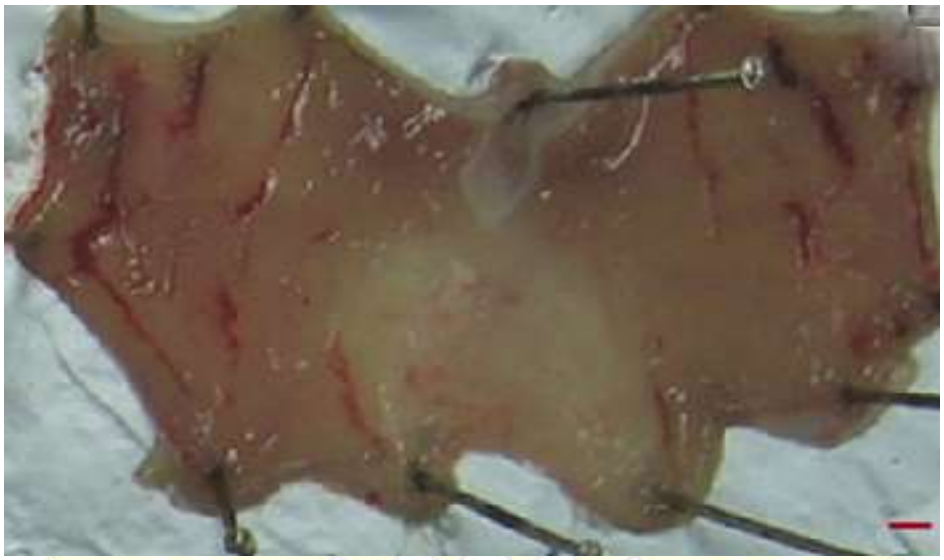
Treatment	Dose	Ulcer index	Ulcer score
Positive Control	1ml/200g	0.496 ± 0.057	2.0 ± 0.3651
Standard Ranitidine	6mg/100g	0.133 ± 0.044	1.3± 0.2108
Methanol leaf extract	500 mg/kg	0.142 ± 0.045	1.3± 0.2108
Acetone leaf extract	500mg/kg	0. 130 ± 0.825	1.2± 0.1108

Table 2: Effect of *Moringa oleifera* fruit extracts in ethanol-induced gastric ulcers compared with positive control and standard-Ranitidine

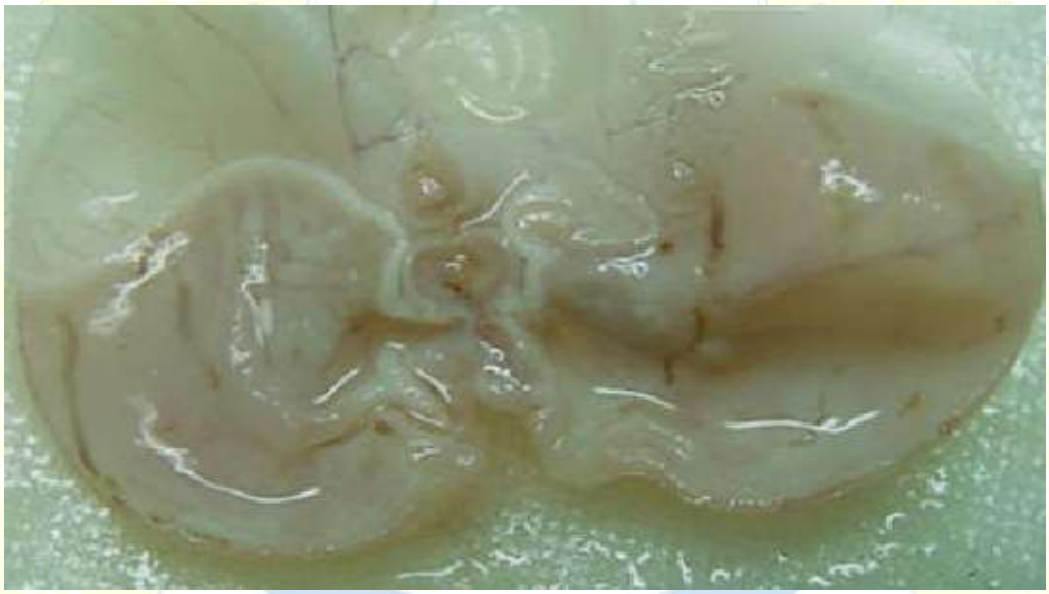
Treatment	Dose	Ulcer index	Ulcer score
Positive Control	1ml/200g	0.100 ± 0.037	0.01± 0.3651
Standard Ranitidine	6mg/100g	0.001 ± 0.038	0.1± 0.2651
Methanol fruit extract	500 mg/kg	0.002 ± 0.004	0.1± 0.1651
Acetone fruit extract	500 mg/kg	0.0 04 ± 0.002	0.1± 0.1651



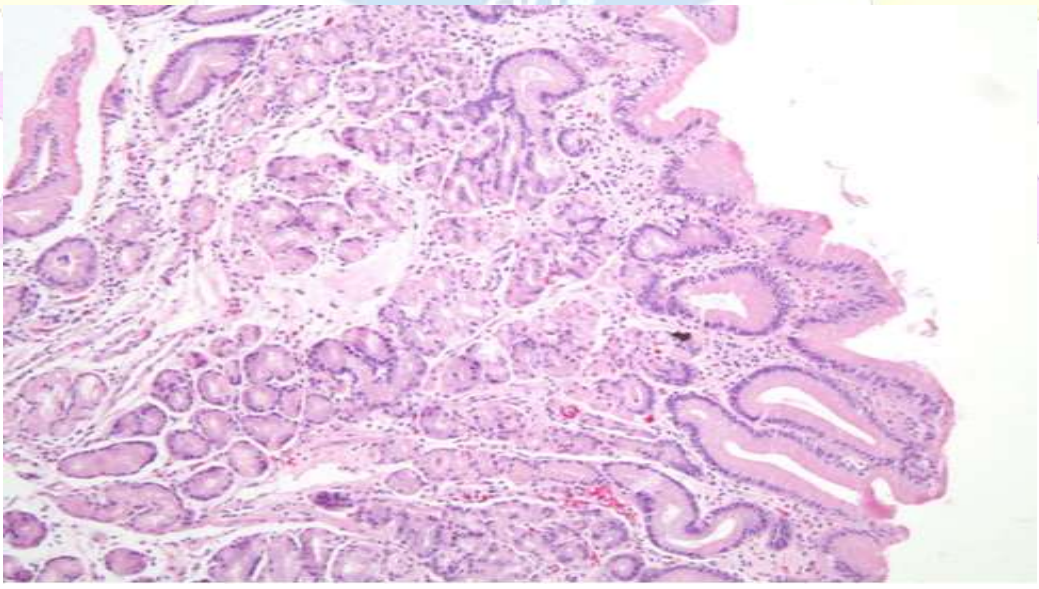
Positive Control with ethanol (toxicant)



Ethanol induced model with leaf extract



Ethanol induced model with fruit extract



Cysteamine induced ulcer with positive control

Total acidity of gastric juice

An aliquot of 1ml of gastric juice was taken in to a 50 ml conical flask and two drops of phenolphthalein indicator was added and titrated with 0.01N NaOH until a permanent pink color was established. The volume of 0.01N NaOH consumed was noted.

The total acidity was expressed as meq/l by the following formula[8]:

$$N \times 0.01 \times 36.45 \times 1000;$$

N is volume of NaOH consumed, 40.0 is molecular weight of NaOH, 0.01 is normality of NaOH and 1000 is the factor to be respected in liter

Table 3: Effect of *Moringa oleifera* leaf extracts on gastric ulcers volume, free acidity, total acidity and pH

Treatment	Dose	volume	Free acidity	Total acidity	pH
Positive Control	1ml/200g	4.9 ±0.4	6.74± 0.3581	13.78 ± 0.765	1.14 ±. 0.01
Standard Ranitidine	6mg/kg	7.2 ± 0.9	4.04 ± 0.454	8.83 ± 1.765	1.05±. 0.01
Methanol leaf extract	500 mg/kg	6.5 ±0.4	3.75 ± 0.430	10.23 ± 0.742	1.08. ±0.02
Acetone leaf extract	500 mg/kg	5.1± 0.6	1.75 ± 0.230	08.23 ±0.742	1.00 ± 0.02

Table 4 : Effect of *Moringa oleifera* fruit extracts on gastric ulcers volume, free acidity, total acidity and p^H

Treatment	Dose	volume	Free acidity	Total acidity	P^H
Positive Control	1ml/200g	5.4 ±0.2	2.74± 0.2881	1.78 ± 0.665	1.14 ±. 0.01
Standard Ranitidine	6mg/kg	4.2 ± 0.1	1.04 ± 0.254	8.63 ± 1.565	0.05±. 0.01
Methanol fruit extract	500 mg/kg	4.5 ±0.4	1.75 ± 0.230	8.23 ± 0.642	0.08 . ±0.02
Acetone fruit extract	500 mg/kg	5.1± 0.6	0.75 ± 0.130	8.13 ±0.342	0.01 ± 0.02

Cysteamine-induced duodenal ulcers

The acetone leaf extract of *Moringa oleifera* showed ahighly significant reduction in ulcer area when compared with control ($p < 0.01$). The methanol leaf extracts of*Moringa oleifera* showed a significant reduction in ulcer area when compared with that of control ($p < 0.05$).

Table 5: Effect of *Moringa oleifera* leaf extracts in cysteamine -induced duodenal ulcers compared with positive control and standard-Ranitidine

Treatment	Dose	Ulcer index	Ulcer score
Positive Control	1ml/200g	7.2	2.0 ± 0.3651
Standard Ranitidine	6mg/kg	4.0	1.3± 0.2108
Methanol leaf extract	500 mg/kg	4.2	1.3± 0.2108
Acetone leaf extract	500 mg/kg	2.2	1.2± 0.1108

Table 6: Effect of *Moringa oleifera* fruit extracts in cysteamine -induced duodenal ulcers compared with positive control and standard-Ranitidine

Treatment	Dose	Ulcer index	Ulcer score
Positive Control	1ml/200g	3.5	2.0 ± 0.3651
Standard Ranitidine	6mg/kg	2.0	1.3± 0.2108
Methanol leaf extract	500 mg/kg	2.9	1.3± 0.2108
Acetone leaf extract	500 mg/kg	2.9	1.2± 0.1108

Table 7: Effect of *Moringa oleifera* leaf extracts on duodenal ulcers volume, free acidity, total acidity and p^H

Treatment	Dose	volume	Free acidity	Total acidity	p^H
Control	1ml/200g	10.9 ± 0.12	5.74± 0.3581	11.78 ± 0.665	5.00 ±. 0.04
Standard Ranitidine	6mg/100g	13.1± 0.2	3.04 ± 0.454	7.83 ± 1.565	6.01.± 0.11
Methanol leaf extract	500mg/kg	14.5± 0.4	2.85 ± 0.430	08.23 ± 0.642	6.05± 0.04
Acetone leaf extract	500mg/kg	15.1± 0.6	2..75 ± 0.230	07.23 ±0.642	7.00 ± 0.32

Table 8: Effect of *Moringa oleifera* leaf extracts on duodenal ulcers volume, free acidity, total acidity and p^H

Treatment	Dose	volume	Free acidity	Total acidity	p^H
Control	1ml/200g	10.9 ± 0.2	5.74± 0.381	15.78 ± 0.665	5.04 ±. 0.01
Standard Ranitidine	6mg/100g	15.5± 0.12	3.04 ± 0.154	6.83 ± 1.465	6.05± 0.01
Methanol leaf extract	500mg/kg	12.5± 0.12	2.75 ± 0.130	07.23 ± 0.442	5.02 ± 0.02
Acetone leaf extract	500mg/kg	12.1± 0.3	0.15 ± 0.130	05.23 ±0.642	5.00 ± 0.02

The following mentioned tables deals with the all significant results except the extract of fruits of *Moringa oleifera* , which does not show any significant results.

DISCUSSION:

The current study dealt with the effect of different extracts of *Moringa Oleifera* leaves and fruits on the gastric and duodenal ulcers in rats. The extracts that showed ulcer-healing effect in ethanol -induced gastric ulcers were screened further to determine their effect on antiulcer activity and gastric secretion.

The fruit extracts of the plant did not show any significant effect on the healing of gastric ulcers induced by ethanol. Whereas the methanol and acetone leaf extracts of *Moringa Oleifera* increased healing of the ethanol –induced gastric ulcers and cysteamine induced duodenal ulcers.

In control groups, the ulcer parameters were evident and indicate that the method was effective enough to produce gastric ulcers. Volume of gastric contents (ml) was the first parameter noted.

Total acidity and pH were two other factors which were measured[6]. The results showed that no significant changes were observed in acidity (or pH) in extract treated groups compared to respected control groups. However, the reference drug ranitidine (i.p.) resulted in a significant reduction in acidity(Tables 1 and 2). Number and scoring were other two parameters compared to respected control groups and the differences were not significant for all of the treatment groups

Ethanol-induced gastric ulcer was employed to study the Cytoprotective effect of the extracts. The methanol and acetone extracts of *Moringa Oleifera* leaves were effective in reducing ulcer index in both these model[12].

Cysteamine-induced duodenal ulcer in rat is a widely used model of peptic ulcer disease. Cysteamine hydrochloride inhibits the alkaline mucus secretion from the Brunner glands in the proximal duodenum and stimulates gastric acid secretion rate.

Gastric emptying is also delayed, and serum gastrin concentration is increased. The methanol and acetone leaf extracts of *Moringa Oleifera* were effective in reducing the ulcer area in cysteamine-induced duodenal ulcers[10,14].

The acetone and methanol extracts of the leaves were effective in all the tested models of peptic ulcer disease, whereas the fruits does not show significant results with the different extracts of *Moringa oleifera*[9,15]. Hence, the healing of gastric ulcers in ethanol–induced gastric ulcers may be due to decreased acid secretion, increased, or decreased GI motility in case of methanol and acetone extracts, and the ulcer healing effect mainly due to reduction in gastric motility.

CONCLUSION:

Two models were used to study the antiulcer activity of *Moringa* leaves and fruits. Both showed significant results except the fruits of *Moringa* which has no significant role to play in results The two models ethanol induced gastric ulcers and cysteamine induced gastric ulcers results were significant when compared with the control and the standard and is effective in showing antiulcer activity. *Moringa Oleifera* contains a number of flavonoids, triterpines, steroids, alkaloids, and many other chemical constituents. The flavonoid quercetin present in the leaves is a well-known antiulcer agent. Further, the leaves contain rutina flavonoid that is reported to have gastric Cytoprotective effect which explains potent ulcer healing effect of methanol and acetone extracts of the leaves. Apart from flavonoids, the leaves of the plant contain steroids such as b-sitosterol and b-carotene, and both of these are known to reduce the development of gastric ulcers .The results of the current study suggest that consumption of the leaves of *Moringa Oleifera* may be beneficial in healing of ulcers in patients suffering from peptic ulcer disease. No significant results were found with fruits may be because of less flavonoids content or other specific chemical constituents to produce antiulcer activity. Hence, many herbal drug can be used for antiulcer activity to eradicate the adverse effects which are caused by the synthetic drugs.

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