



ANTIULCER ACTIVITY OF HYDROALCOHOLIC SEED EXTRACT OF *MORINGA OLEIFERA* LAM. AND *SYZYGIUM CUMINI* (L.) SKEELS IN PYLORUS LIGATION INDUCED AND ETHANOL INDUCED GASTRIC ULCER IN RATS

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ABSTRACT

The aim of present investigation is to evaluate antiulcer activity of the hydroalcoholic seed extract of *Moringa oleifera* Lam. and *Syzygium cumini* (L.) Skeels in Pylorus ligation induced and Ethanol induced Gastric ulcer in rats. The dose dependent anti-secretory and antiulcer effect of HAEMOS and HAESCS has been observed in pylorus ligation model which was evident by significant decrease in volume of gastric juice and ulcer score and increase in pH of gastric juice. In ethanol induced ulcer model, HAEMOS and HAESCS has exhibited significant decrease in ulcerogenic effect as compared to control group. The results showed that the results of HAEMOS are more than HAESCS in both the models.

Keywords: Anti-ulcer, Hydroalcoholic extract, *Moringa oleifera* Lam. *Syzygium cumini* (L.) Skeels.

1. INTRODUCTION

Ulcer is most common gastrointestinal disorder characterized with break in lining of stomach, initial part of small intestine or infrequently the lower oesophagus. The ulcer mainly associated with symptoms like burning abdominal pain which extends from novel to chest, loss of appetite, nausea, blood and dark stools, unexplained weight loss, indigestions and vomiting. Ulcers in stomach called gastric ulcer and ulcer formed in duodenum call duodenal ulcer, together is called as peptic ulcer [1]. These are examined under endoscopy (gastroscopy) and upper gastrointestinal series. The curative treatment for peptic ulcers involves H2 antihistaminic, proton pump inhibitor, ulcer protectants and anti *H. pylori* therapy [2]. The conventional antiulcer drugs used in management of peptic ulcer may cause undesirable side effect or drug interaction in body upon their prolonged use. In traditional medicine, various herbal preparations are used to cure the gastrointestinal disease with the aim to relieve symptoms and delay its recurrence. Till date, no drugs meet all these goals therapy. Therefore the search for potent, safe and economically effective antiulcer agents from herbal origin has become most desirable area of research.

Moringa oleifera Lam., Family: Moriaceae is commonly cultivated throughout India up to 1500 m. All the parts

of the plant are used medicinally. The leaves and fruits are rich in iron, proteins and vitamins. The plant possess analgesic, antiinflammatory activity and used extensively for the treatment of ulcer in traditional system of medicine. *Syzygium cumini* (L.) Skeels, Family: Myrtaceae is commonly cultivated throughout India up to 1800 m. Bark is used in nonspecific acute diarrhoea and in topical therapy for mild inflammation of the oral-pharyngeal mucosa; externally in mild, superficial inflammation of the skin. The seed is used in hyperglycaemia and polyuria. Seed extract exhibited potent anti-inflammatory action against both exudative and proliferative and chronic phases of inflammation, besides exhibiting significant anti-arthritis, antipyretic and analgesic activities [3].

2. MATERIAL AND METHODS

2.1. Selection, Collection and Authentication of the plant materials

The seeds of *Moringa oleifera* Lam. (Sarjan) and *Syzygium cumini* (L.) Skeels (Jamun) were extensively used in the treatment of ulcer as mentioned in folklore; several tribal people of India are using these plants for the treatment of ulcer, therefore these plants were selected. The seeds of the plant were collected from local area of Bhopal in the month July-Sep. 2019 and were authenticated by Dr. S. N. Dwivedi, Retd. Professor & Head, Department of

Botany, Janata PG College, Rewa, (M. P.) and was deposited in our laboratory, Voucher No. J/Bot/2019-117MOS & J/Bot/2019-118SCS were allotted to the selected plant on the date 13/09/2019.

2.2. Preparation of extract

The seeds of *Moringa oleifera* and *Syzygium cumini* were shade dried and reduced to coarse powder in a mechanical grinder and passed through sieve No. 40. The powdered bark was defatted with petroleum ether (40°-60°C) for about 09 hrs and complete defatting was ensured by placing a drop from the thimble on a filter paper which did not exhibit any oily spot. The defatted material was removed from the soxhlet apparatus and air dried to remove last traces of petroleum ether. The defatted material was subjected to extraction by hydroalcohol as solvent. The extracts were collected in a tarred conical flask. The solvent was removed by distillation. Last traces of solvent were removed under vacuum. The extract obtained with each solvent was weighed to a constant weight and percentage w/w basis was calculated. The obtained crude extract was stored in dark glass bottles for further processing. The extracts thus obtained were subjected to phytochemical analysis [4-5].

2.3. Acute toxicity study of extract

The acute toxicity of extract was determined as per the OECD guideline no. 423 (Acute toxic class method) [6]. The 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 [6].

2.4. Procurement of experimental animals

The mice were used for acute toxicity study as per OECD guidelines 423. The animals were fed with standard pellet diet (Hindustan lever Ltd. Bangalore) and water *ad libitum*. All the animals were housed in polypropylene cages. The animals were kept under alternate cycle of 12 hours of darkness and light. The animals were acclimatized to the laboratory condition for 1 week before starting the experiment. The experimental protocols were approved by Institutional Animal Ethics Committee of Oriental University Indore after scrutinization (IAEC approval no IAEC/2019-20/RP-19 Dated-03-03-20).

2.5. Test compounds

The HAEMOS and HAESCS and standard drug ranitidine (50 mg/kg body weight) were used.

2.6. Experimental animal

Albino rats (100-120 g) used in the present studies were procured from suppliers and the animals were fed with standard pellet diet (Hindustan lever Ltd. Bangalore) and water *ad libitum*. All the animals were acclimatized for a week before use.

2.7. Pylorus ligation induced gastric ulcer in rats

Rats weighing (150-200 g) of either sex were allocated into 4 groups of six animal in each group. Animals were fasted for 18hr prior to drug treatment but had free access to water.

Group 1- Control (Received vehicle- Normal saline 5ml/kg)

Group 2- Standard (Ranitidine 150 mg/kg) orally

Group 3- HAEMOS (100 mg/kg)

Group 4- HAESCS (100 mg/kg)

Pylorus ligation was carried out in all groups of rats for the induction of gastric ulcers and followed by the respective treatments orally. After 6 hrs of ligation, all the animals were sacrificed, the abdomen was opened by using a small incision. The stomachs were dissected out and contents were drained into tubes and centrifuged for 10 minutes at 1000 rpm. Supernatants were subjected to investigation of gastric volume and pH of gastric juice. The stomachs were then cut along the greater curvature and examined for ulceration and the ulcer index (UI) was calculated [7-8].

2.8. Ethanol induced gastric ulcer in rats

Rats weighing (150-200g) of either sex were allocated into 5 groups of six animal in each group. Animals were fasted for 18hr prior to drug treatment but had free access to water. Group 1- Control (Received vehicle- Normal saline 5ml/kg)

Group 2- Standard (Ranitidine 150 mg/kg) orally

Group 3- HAEMOS (100 mg/kg)

Group 4- HAESCS (100 mg/kg)

Animals were given test extract or standard drug. 1 hr later 1ml/200g of 99.80% alcohol was given orally to every animal. After 1 hr of treatment, animals were sacrificed and stomach was incised along the greater curvature and ulceration was scored. The number of ulcers and the ulceration area were measured. Ulcer index was calculated using following formula [7-8]:

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

Where, UI = Ulcer Index, UN = Average of number of ulcer per animal, US = Average of severity score, UP = Percentage of animal with ulcer

2.9. Statistical analysis

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

3. RESULTS AND DISCUSSION

The HAEMOS and HAESCS were screened for acute toxicity study by OECD guideline no. 423 for determination of LD_{50} . The results showed that the extracts were belonging to category-5 (unclassified). Hence, LD_{50} was 2000 mg/kg, therefore, ED_{50} was 200 mg/kg. Therefore doses of 200 mg were selected for present investigation. The results were presented in table 1.

In Pylorus ligation method, there was significant increase in volume of gastric juice and ulcer index of the stomach and increase pH of gastric juice seen in control, untreated

pylorus ligated rats. The dose dependent anti-secretory and antiulcer effect of HAEMOS and HAESCS has been observed in pylorus ligation model which was evident by significant decrease in volume of gastric juice and ulcer score and increase in pH of gastric juice. The percentage protection at doses of 100 mg/kg was found to be 31.86%, 44.95% and 31.89% respectively of Standard, HAEMOS & HAESCS. The result of the test extract found to be lesser potent than ranitidine whereas the result indicate that HAEMOS was found to be more than that of HAESCS. The results emphasizes in table 2.

In ethanol induced ulcer model, HAEMOS and HAESCS has exhibited significant decrease in ulcerogenic effect as compared to control group. The % inhibition in extract treated group of animal at doses of 100 mg/kg was found to be 56.35 %, 45.45 % and 28.39 % respectively to that of standard, HAEMOS and HAESCS, the results showed that the results of HAEMOS are more than HAESCS. Results are shown in Table 3.

Table 1: Determination of LD_{50} and ED_{50} of Extract of *Moringa oleifera* Lam. and *Syzygium cumini* (L.) Skeels

S. No.	No. of Animals	Extract Dose (mg/kg)	No. of death of animals	
			HAEMOS	HAESCS
1.	3	5	0	0
2.	3	50	0	0
3.	3	300	0	0
4.	3	2000	0	0

Table 2: Effect of HAEMOS and HAESCS on Pylorus ligation induced gastric ulcer in rats

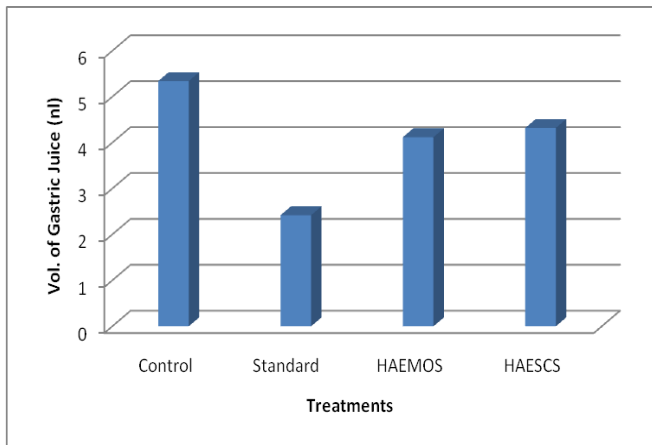
Treatments	Dose (mg/kg)	Vol. of gastric juice (ml)	pH	Ulcer index	% Inhibition
Control	-	5.34±0.12	1.96±0.12	3.84±0.18	-
Standard (Ranitidine)	150	2.42±0.11*	3.98±0.11**	1.10±0.12**	70.9
HAEMOS	100	4.12±0.14**	2.48±0.14*	1.81±0.11**	44.95
HAESCS	100	4.32±0.10*	2.80±0.13**	2.58±0.12*	31.89

Values are expressed as mean ± SEM, n= 6. (One way ANOVA Followed by Dunnette multiple Comparisons test). Statistically significance of * $P < 0.01$, ** $P < 0.001$, when compared with respective control.

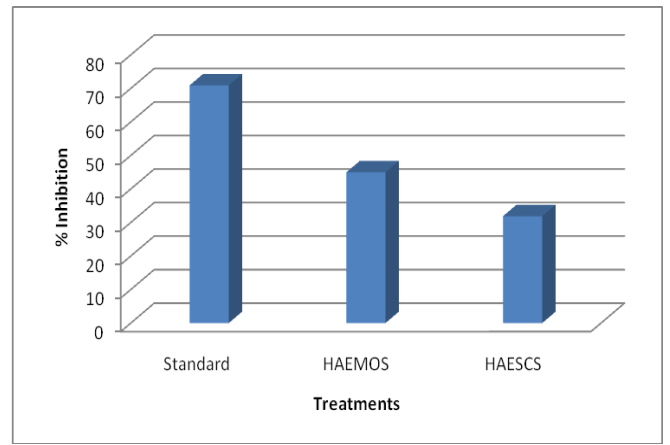
Table 3: Effect of HAEMOS and HAESCS on Ethanol induced gastric ulcer in rats

Treatments	Dose (mg/kg)	Gastric Ulcer index	% Inhibition
Control	-	5.45±0.25	-
Standard (Ranitidine)	150	2.41±0.15**	56.35**
HAEMOS	100	3.01±0.11**	45.45*
HAESCS	100	4.18±0.12*	28.39**

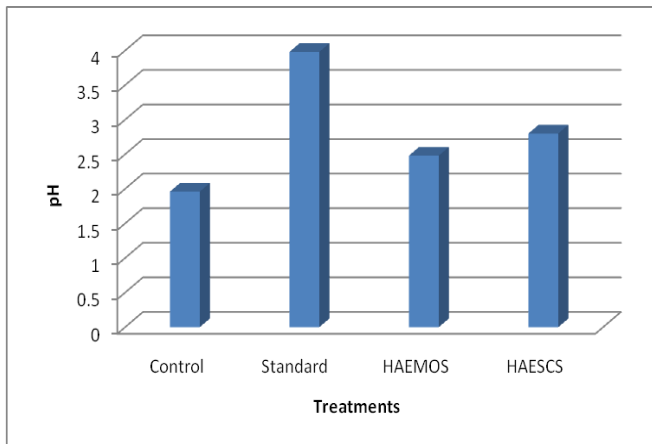
Values are expressed as mean ± SEM, n= 6. (One way ANOVA Followed by Dunnette multiple Comparisons test). Statistically significance of * $P < 0.01$, ** $P < 0.001$, when compared with respective control.



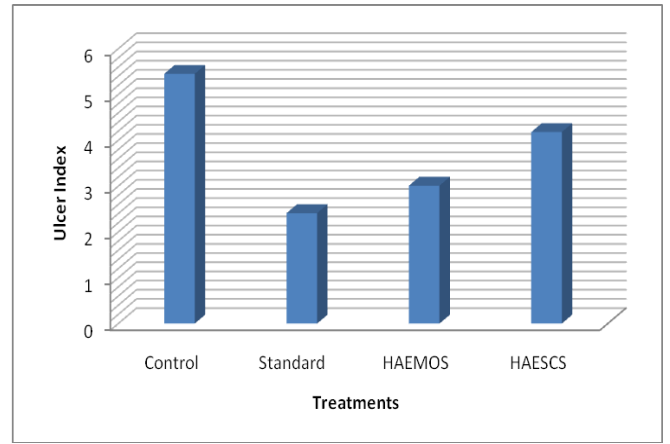
Graph 1: Volume of Gastric Juice of hydroalcoholic extract of *Moringa oleifera* Lam. and *Syzygium cumini* (L.) Skeels. on Pylorus ligation induced gastric ulcers



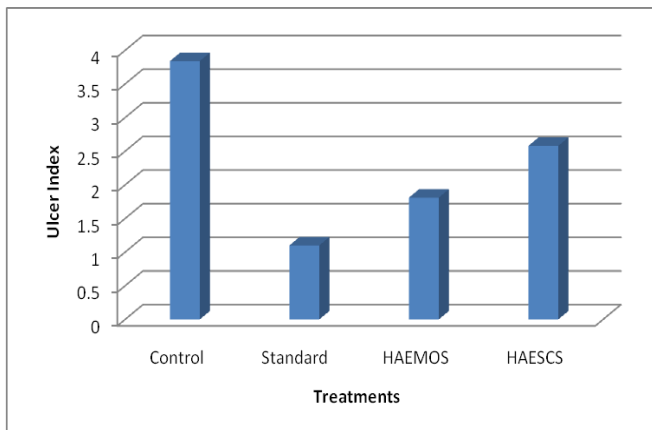
Graph 4: % Inhibition of hydroalcoholic extract of *Moringa oleifera* Lam. and *Syzygium cumini* (L.) Skeels. on Pylorus ligation induced gastric ulcers



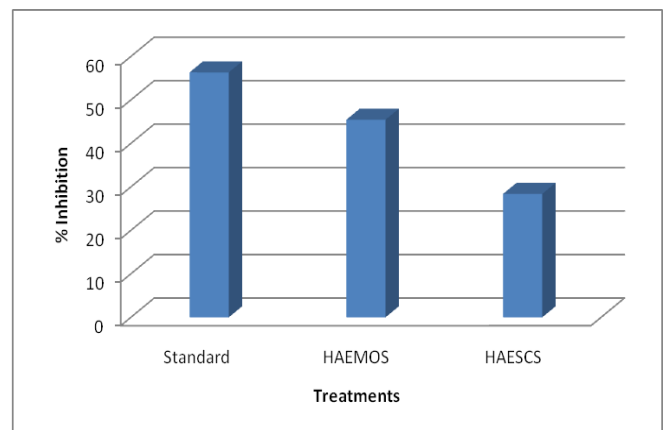
Graph 2: pH hydroalcoholic extract of *Moringa oleifera* Lam. and *Syzygium cumini* (L.) Skeels. on Pylorus ligation induced gastric ulcers



Graph 5: Ulcer Index of hydroalcoholic extract of *Moringa oleifera* Lam. and *Syzygium cumini* (L.) Skeels. on ethanol induced gastric ulcers



Graph 3: Ulcer Index of hydroalcoholic extract of *Moringa oleifera* Lam. and *Syzygium cumini* (L.) Skeels. on Pylorus ligation induced gastric ulcers



Graph 6: % Inhibition of hydroalcoholic extract of *Moringa oleifera* Lam. and *Syzygium cumini* (L.) Skeels. on ethanol induced gastric ulcers

4. CONCLUSION

The results of hydroalcoholic seed extract of *Moringa oleifera* Lam. and *Syzygium cumini* (L.) Skeels in Pylorus ligation induced and Ethanol induced Gastric ulcer in rats indicate that dose dependent antiulcer effect of HAEMOS and HAESCS has been observed in pylorus ligation model which was evident by significant decrease in volume of gastric juice and ulcer score and increase in pH of gastric juice. In ethanol induced ulcer model, HAEMOS and HAESCS has exhibited significant decrease in ulcerogenic effect as compared to control group. The results showed that the results of HAEMOS are more than HAESCS in both the models.

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