

EVALUATION OF ANTI ULCER ACTIVITY OF MADHUCA INDICA EXTRACT IN EXPERIMENTAL ANIMAL MODEL

Dr R. Nirmala¹, T. Sravanthi²

¹Associate Professor, Department of Pharmacology, Surabhi Dayakar Rao College of Pharmacy, Gajwel, Siddipet, Telangana, India 502312

²Student, Surabhi Dayakar Rao College of Pharmacy, Gajwel, Siddipet, Telangana, India 502312

ABSTRACT

In the present study evaluation of the anti-ulcer activity of *Madhuca Indica* Study was carried out, by using three methods i.e., alcohol, paracetamol and stress induced ulcers in rats pretreated with the doses of 250 mg/kg AQSMI and ALMI, 10mg/kg Omeprazole and 50mg/kg Ranitidine. To evaluate the anti ulcer activity of aqueous and alcoholic extracts of *Madhuca Indica* leaves (AQSMI and ALMI) at 250 doses using different experimentally induced gastric ulcer models in rats. In alcohol-induced ulcers, AQSMI and ALMI were effective in reducing lesion index and increasing the gastric mucus content. It was also effective in decreasing ulcer index in paracetamol induced ulcers. All the results obtained with *Madhuca Indica* were dose dependent. The results suggest that AQSMI and ALMI possesses significant and dose dependent anti ulcer activity. The anti ulcer activity of AQSMI and ALMI can be attributed to its cyto protective and anti secretory action.

Keywords: *Madhuca Indica*, antisecretory, cytoprotective, gastric ulcer, alcohol induced ulcers, paracetamol-induced ulcers and stress induced ulcers.

1. INTRODUCTION

Peptic ulcer is one of the most common gastrointestinal diseases. In recent years, a widespread search has been launched to identify new antiulcer drugs from natural sources. Peptic ulcer disease (PUD) is caused by disruption of gastric mucosal defense and repair system. The recurrence rates of PUD are high and have been associated with several factors, including persistent *Helicobacter pylori* infection, sustained presence of mucosal damaging factors (e.g. use of non-steroidal antiinflammatory drugs) and diminished mucosal defense ability (Hawkey et al., 2000; Crespo and Suh, 2001). Reactive oxygen species (ROS) which include superoxide anions and hydroxyl radicals have been implicated in several degenerative diseases including hypercholesterolemia, atherosclerosis, carcinogenesis, diabetes mellitus, ischemic reperfusion cardiac injury and digestive system disorders such as hypersecretion and gastric mucosal damage (Dhuley, 1999). It has been shown that there is alteration in the antioxidant status following ulceration, indicating that free radicals seem to be associated with the pylorus-ligation induced (Rastogi et al., 1998) and ethanol induced (Pihan et al., 1987) ulceration in rats. The plant of *M. indica* is known to possess various therapeutic properties and has been one of the noteworthy plants mentioned in various medicinal systems. It is a good laxative and is used in treating habitual constipation, piles and hemorrhoids. The leaves of *M. indica* have been reported to contain myricetin rhamnoside (Subramanian and Nair, 1972) and the tannin content in the leaves was found to be 4.86% (Daniel et al., 1978). Myricetin showed antioxidant property in neuro blastoma cell model of rotenone neurotoxicity (Molina-Jimenez et al., 2005), β -sitosterol modulates antioxidant enzyme response in Raw 264.7 macrophages (Moreno and Vivancos, 2005).

2. MATERIAL AND METHODS

2.1. Experimental animals

Wistar rats (150-200 g) and were procured from Animals were housed in appropriate cages in uniform hygienic conditions and fed with standard pellet diet (Amrul Laboratory Animal Diet) and water ad libitum. All the animals were maintained under standard conditions, that is room temperature $26 \pm 1^\circ\text{C}$, relative humidity 45 - 55% and 12:12 h light - dark cycle. The animals were housed in large spacious hygienic cages during the course of the experimental period. Animal studies had approval of IAEC.

2.2. Plant Material Collection

The leaves of *Madhuca Indica* were collected from the Botanical garden and was identified and authenticated from Department.

The plant material was cleaned, reduced to small fragments, air dried under shade at room temperature and coarsely powdered in a mixer. The powdered material was stored or taken up for extraction process.

2.3. Preparation of plant extracts

Fresh leaves of *Madhuca Indica* were collected and washed under tap water. The leaves extract used was prepared by taking 20gms of finely cut leaves into 250ml beaker containing 200ml of water. The contents were mixed well and then the mixture was boiled up to 80-100°C for 4-5hrs. Further the extract was filtered with whatmann filter paper. The filtrate was boiled until the concentrated residue is formed. The concentrated product was sealed in sample covers and stored under room temperature and used for further experiment to check the activities.

2.4. Selection of dose for animal study

The dose considered for the experiment on rats was obtained from conversion of human dose of *Madhuca Indica* (3-5g/kg). The conversion factor of human dose (per 200g body weight) is 0.018 for rats (Ghosh1984). Hence the calculated dose for the rats (considering human dose 3 and 5g/kg) is 200mg/kg. Acute toxicity was done at dose of 2000mg/kg bodyweight.

2.5. Acute oral toxicity:

The acute oral toxicity of aqueous and alcoholic extracts of *Madhuca Indica* was determined by using rats which were maintained under standard conditions. The animals were fasted 12 hour prior to the experiment, up and down procedure OECD guideline no. 425 were adopted for toxicity studies. Animals were administered with single dose of individual extract up to 2000mg/kg and observed for its mortality during 7days and 21days study period (long term) toxicity and observed upto 7days for their mortality, behavioral and neurological profiles.

2.6. Acute stress-induced ulcer

The rats were deprived of food for 24 h, although water was allowed. Albino rats weighing between 160 - 180 g were divided into 12 groups consisting of six animals each. Experimental design and dosing schedule was as follows.

Animals were divided into four (I-V) groups.

Group I - Control group received distilled water (1ml, p.o).

Group II- Ulcer control

Group III - Standard group received Cimetidine (32mg/kg i.p).

Group IV - Test group received aqueous extract of *Madhuca Indica* (250mg/kg p.o).

Group V - Test group received alcoholic extract of *Madhuca Indica* (250mg/kg p.o).

2.7. Alcohol Induced Ulcers in Rats

Alcohol induced ulcer model, in rats was studied for all extractives of both plants to determine the ulcer index and ulcer inhibition. Albino rats weighing between 160 - 180 g were divided into 12 groups consisting of six animals each. Experimental design and dosing schedule was as follows.

Animals were divided into four (I-V) groups.

Group I - Control group received distilled water (1ml, p.o).

Group II- Ulcer control

Group III - Standard group received Omeprazole for seven days (2mg/kg i.p).

Group IV - Test group received aqueous extract of *Madhuca Indica* (250mg/kg p.o) for seven days.

Group V - Test group received alcoholic extract of *Madhuca Indica* (250mg/kg p.o) for seven days.

2.8. Paracetamol Induced Modified Pylorus Ligated Model

The selected extractives of both plants were subjected to anti ulcer studies using Paracetamol induced model. Adult Wistar albino rats of either sex weighing 180-250 g were fasted for 48h with free access to water and divided into six groups of six animals each. They were placed in cages with grating floor to avoid coprophagy. The experimental design and dosing schedule was carried out as follows.

Group I: Normal control

Group II: Ulcer control (Solvent) (10 ml/kg) + Paracetamol (200 mg/kg)

Group III: Ranitidine (50 mg/kg)

Group IV: AQMI (250 mg/kg)

Group V: ALMI (250 mg/kg)

Statistical analysis

The values were expressed as mean \pm SEM data was analyzed using one-way ANOVA followed by T-test. Two sets of comparison had made. i.e. Normal control Vs All treated groups. Differences between groups were considered significant at $P < 0.001$ and $P < 0.05$ levels.

3. RESULTS AND DISCUSSION

3.1. Acute toxicity study

Administration of the *Madhuca Indica* extracts in rats at doses of 250mg/kg by oral gavage did not reveal any adverse effects or signs of toxicity. Observations twice daily for fourteen days also did not reveal any drug related observable changes or mortality. Accordingly, the acute oral LD50 of the extractives was concluded to exceed 2000 mg/kg body weight, the highest dose tested in the study.

3.2 Effect on alcohol induced gastric ulcers

Oral administration of 80% alcohol produced haemorrhagic gastric lesions in glandular portion of stomach. Pretreatment with AQMI and ALMI at the dose of 250 mg/kg and Omeprazole (10 mg/ kg) significantly ($p < 0.001$) protected the gastric mucosa as shown by reduced values of lesion index (19.3 ± 0.35 and 27.47 ± 0.75 respectively) against alcohol challenge as compared to solvent control (26.14 ± 0.24).

Table 1: Effect of *Madhuca Indica* at various doses on alcohol induced gastric ulcer in rats.

Treatment (n=6)	Dose mg/kg (p.o.)	Lesion index	% Inhibition of ulcer	Mucus content (μg Alcian blue/g wet tissue)
1% CMC	-	26.14 ± 0.24	-	0.50 ± 0.01
Ulcer control	-	35.94 ± 0.36	-	0.57 ± 0.02
Omeprazole	10	27.47 ± 0.75	20.12	0.66 ± 0.01
AQMI	250	30.21 ± 0.43	7.63	0.51 ± 0.02
ALMI	250	19.3 ± 0.35	45.01	0.86 ± 0.01

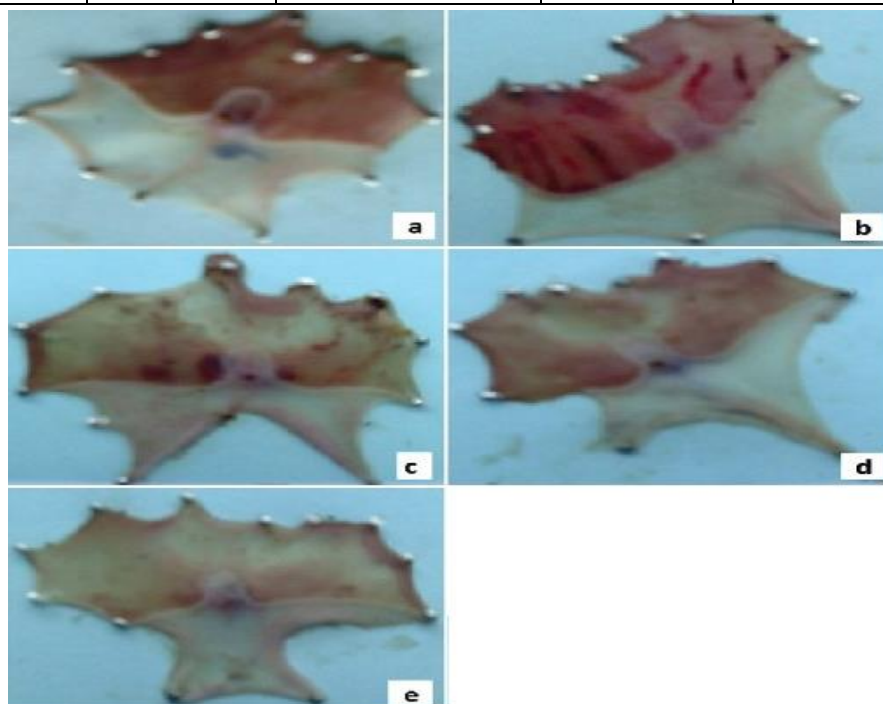


Fig-1: Effect of *Madhuca Indica* on alcohol induced ulcers in the rats in the study (a) Normal Control (b) Ulcer Control (c) AQMI (250 mg/kg) treated (d) ALMI (250 mg/kg) treated (e) Omeprazole (10 mg/kg) treated

3.3. Effect on Paracetamol induced gastric ulcers

In *Madhuca Indica* treated groups (250 mg/kg), the ulcer index values (0.43 ± 0.02 respectively) were significantly reduced ($p < 0.001$) when compared to solvent control (0.70 ± 0.02), while the ulcer index for ranitidine treated group was 0.25 ± 0.02 ($p < 0.001$). The %inhibition of ulcer showed by AQMI and ALMI (250mg/kg) and ranitidine was 51.3%, 37.2% and 53.2% respectively.

Table 2. Effect of *Madhuca Indica* at various dose levels on paracetamol induced gastric ulcer in rats.

Treatment (n=6)	Dose mg/kg (p.o.)	Ulcer index	% Inhibition of ulcer
1% CMC	-	0.70 ± 0.02	-
Ulcer control	-	0.84±0.01	--
Ranitidine	50	0.25 ± 0.02	51.3
AQMI	250	0.43 ± 0.02	37.2
ALMI	250	0.30 ± 0.02	53.2

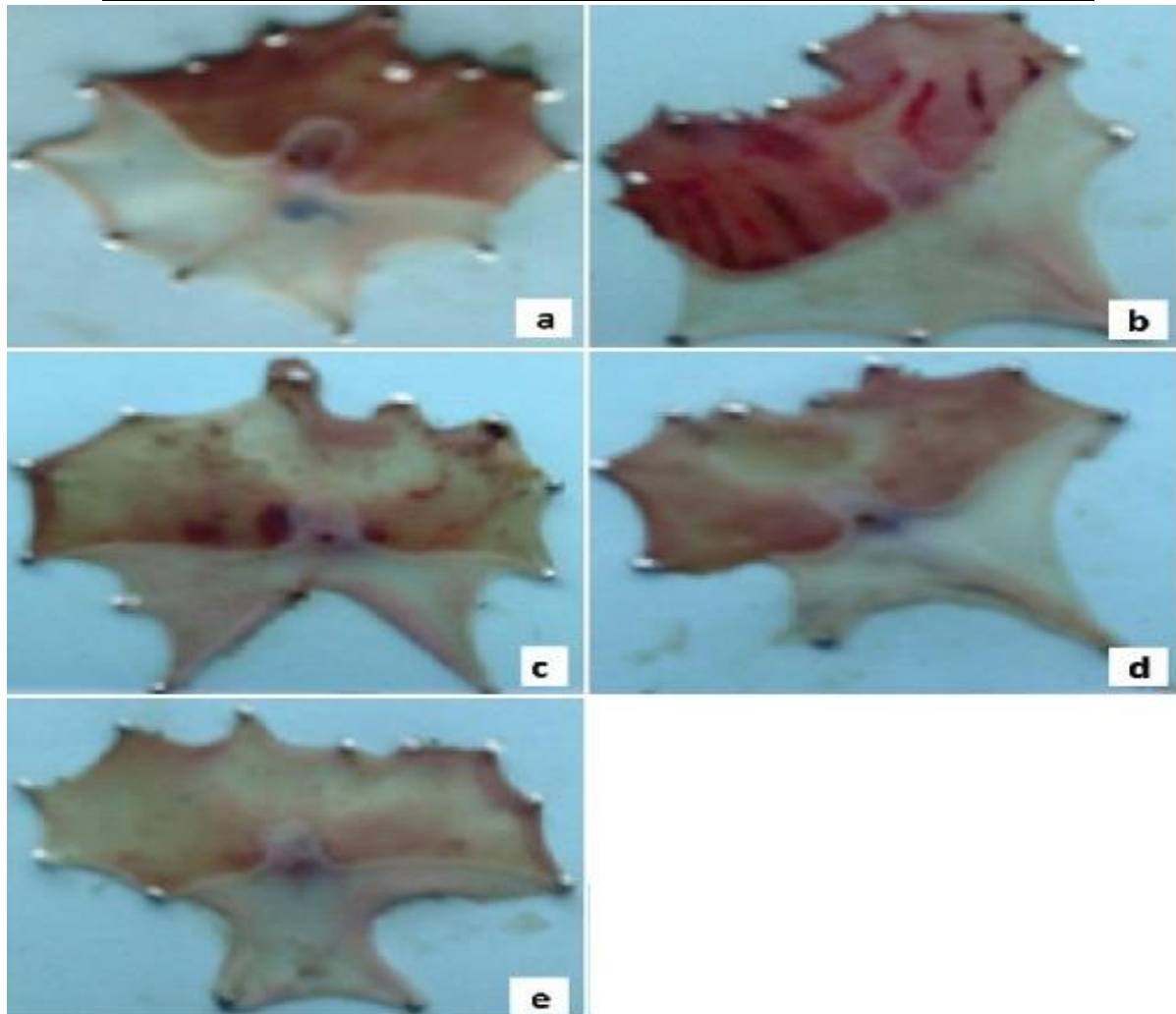


Fig-2: Effect of *Madhuca Indica* on paracetamol induced ulcers in the rats in the study (a) Normal Control (b) Ulcer Control (c) AQMI (250 mg/kg) treated (d) ALMI (250 mg/kg) treated (e) Ranitidine (50 mg/kg) treated

3.4. Stress-induced ulcers:

In water immersion stress induced ulcers, the mean score value of ulcer inhibition was found to be significant ($P<0.001$) for 250 mg/kg of the extract.

The percentage ulcer inhibition was 75.29 and 84.55 for 250 mg/kg for both aqueous and alcoholic extracts, and that of the standard was found to be 91.42.

Table 5. Effect of *Madhuca Indica* at various dose levels on Stress induced gastric ulcer in rats.

Group	Ulcer index	Percentage inhibition
Normal Control	00.00±0.00	-----
Ulcer control	22.73±4.31	-----
Standard	2.86±0.13	91.42
AQMI	6.90±3.02	75.29
ALMI	4.34±2.87	84.55

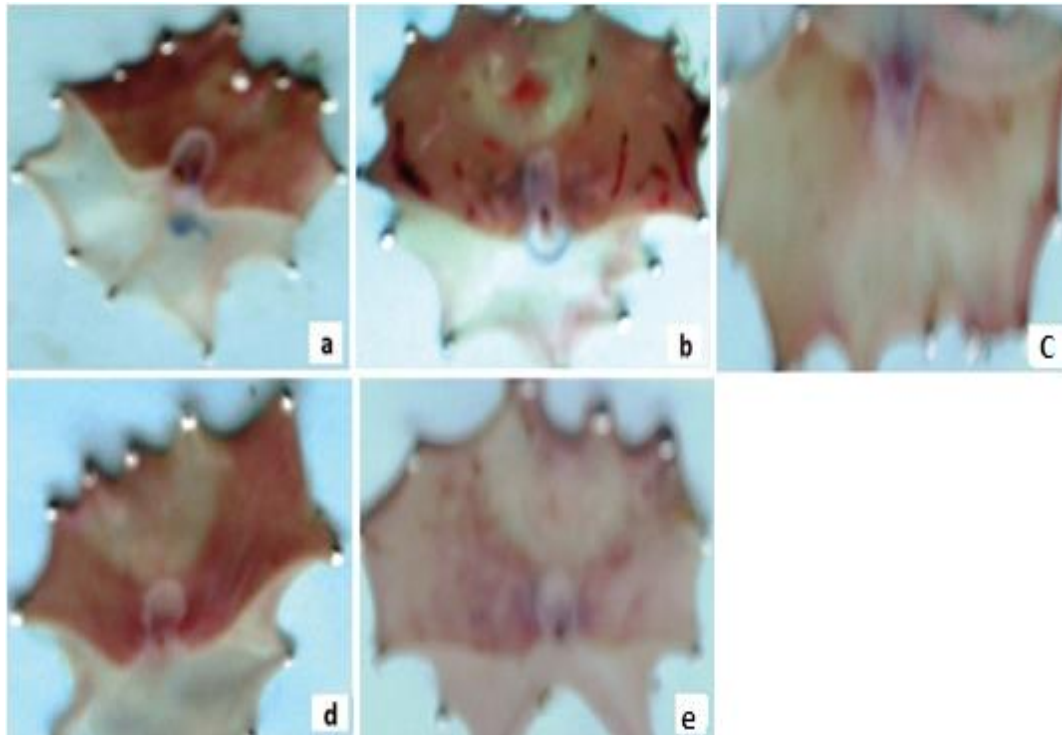


Fig-3: Effect of *Madhuca Indica* on stress induced ulcers in the rats in the study (a) Normal Control (b) Ulcer Control (c) AQMI (250 mg/kg) treated (d) ALMI (250 mg/kg) treated (e) Omeprazole (10 mg/kg) treated).

4. CONCLUSION

The antiulcer activity of *Madhuca Indica* extracts in stress induced model is evident from its significant reduction in gastric volume, ulcer index and increase in pH of gastric juice. Because of animals treated with *Madhuca Indica* extracts significantly inhibited the formation of ulcer in the stomach and also decreased both acid concentration, gastric volume and increased the pH values. It is suggested that *Madhuca Indica* extracts can suppress gastric damage induced by aggressive factors. It is generally accepted that gastric ulcers result from an imbalance between aggressive factors and the maintenance of the mucosal integrity through endogenous defence mechanisms. The excess gastric acid formation by prostaglandin (PG) includes both increases in mucosal resistance as well as a decrease in aggressive factors, mainly acid and pepsin. Inhibitions of PG synthesis by aspirin coincide with the earlier stages of damage to the cell membrane of mucosal, parietal and endothelial cells.

5. REFERENCES

- [1] Allison MC, Howatson AG, Caroline MG. Gastrointestinal damage associated with the use of nonsteroidal anti-inflammatory drugs. *N Engl J Med* 1992; 327: 749–754.
- [2] Lenz HJ, Ferrari-Taylor J, Isenberg JI. Wine and five percent ethanol are potent stimulants of gastric acid secretion in humans. *Gastroenterology* 1983; 85: 1082–1087.
- [3] Cohen S, Booth GH Jr. Gastric acid secretion and lower-esophageal - sphincter pressure in response to coffee and caffeine. *N Engl J Med* 1975; 293: 897–899.
- [4] Feldman EJ, Isenberg JI, Grossman MI. Gastric acid and gastrin response to decaffeinated coffee and a peptone meal. *JAMA* 1981; 246: 248–250.
- [5] Dubey P, Sundram KR, Nundy S. Effect of tea on gastric acid secretion. *Dig Dis Sci* 1984; 29: 202–6.
- [6] Korman MG, Hansky J, Eaves ER, Schmidt GT. Influence of cigarette smoking on healing and relapse in duodenal ulcer disease. *Gastroenterology* 1983; 85: 871–874.