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Dhanasree Basipogu
Asst. Professor, Dept. of
Biochemistry, K.V.R Govt.
Degree College (W)(A),
Kurnool, A.P. India.

Nizamuddin Basha Syed
Dept. of Biochemistry,
S.K. University, Anantapur,
A.P. India.

Gastro protective activity of *Lawsonia inermis* (Henna). A well-known traditional medicinal plant

Dhanasree Basipogu, Nizamuddin Basha Syed

Abstract:

The aim of our present study is to evaluate the pharmacological action relevant to ulcer healing activity of *Lawsonia inermis* (Henna) leaf, a folk and ethno medicine availing in the treatment of Gastric Ulcers. After Serial extraction of *Lawsonia inermis* we have chosen the Methanol extract for further experimental study. MELI- Methanol extract of *Lawsonia inermis* was done by using soxhlet apparatus and was further subjected to analysis of phyto chemicals. Then the Methanolic extract of leaf was tested against ulcer induced rats. The methanol extract of leaf (200mg/Kg) has shown remarkable protection against ulcer damage to the tissue (53.55 compared to 59.24 % of RD).

Hence, it setup a fact that the Methanol extract of antiulcer activity of *Lawsonia inermis* (Henna) is offering an ineffable mark to the folk medicine, which is a folklore as well as traditional medicine for the treatment of ulcers in India.

Keywords: Gastric ulcers, Anti ulcer, folklore, Folk Medicine, Henna, *Lawsonia inermis*.

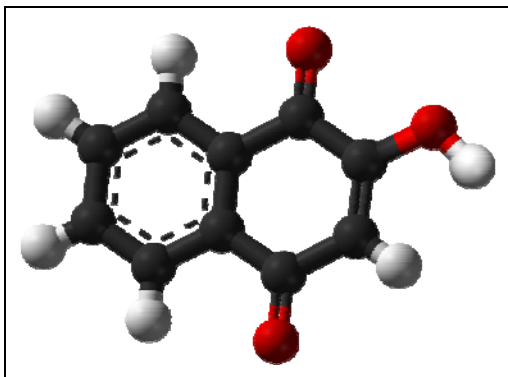
1. Introduction

Peptic ulcer is a conglomerate of heterogenous disorders which manifests itself as a break in the lining of the gastrointestinal mucosa bathed by acid and/or pepsin. NSAIDS ingestion is associated with erosions, petechiae type C gastritis, ulceration, interference with ulcer healing, Ulcer complications and injury to the small and large intestine [1].

Although a number of antiulcer drugs such as H₂ receptor antagonists, proton pump inhibitors and cyto protectants are available for ulceration, but all these drugs have side effects and limitations [2]. Herbal medicine considered safer because of the natural ingredients with no side effects [3].

In India, Henna *Lawsonia inermis*, also known as hina, the henna tree, the mignonette tree, and the Egyptian privet [4, 5]. Henna has been used since antiquity to dye skin, hair and fingernails, as well as fabrics including silk, wool and leather. The name is used in other skin and hair dyes, such as *black henna* and *neutral henna*, neither of which is derived from the henna plant [6, 7]. Historically, henna was used for cosmetic purposes primarily in Ancient India the henna plant is native to northern Africa, western and southern Asia, and northern Australasia, in semi-arid zones and tropical areas [8]. Henna is known as a traditional Ayurveda medicine. It shows various health benefits such as hypoglycemic and hypolipidemic activities, inhibits the tuberculosis bacteria, and useful in skin diseases. Henna is widely known as to be an excellent cooling agent and hence it is applied to scrapes and burns. Henna is also used as a home remedy to lower the body temperature while suffering from a high fever or to treat heat exhaustion. Historically, henna has been used for severe diarrhea caused by a parasite, enlarged spleen, headache, jaundice, and skin conditions. These days, people take henna for stomach and intestinal ulcers. Henna is sometimes applied directly to the affected area for dandruff, eczema, scabies, fungal infections, and wounds. People also use henna on the skin as temporary "tattoos." Moreover, henna extract prevents the liver damage occurred from exposure of carbon tetrachloride [9] Lawsone (2-hydroxy-1, 4-naphthoquinone), also known as hennotannic acid, is a red-orange dye present in the leaves of the henna plant (*Lawsonia inermis*) its Formula is C₁₀H₆O₃ [10].

Correspondence
Dhanasree Basipogu
Asst. Professor,
Dept. of Biochemistry, K.V.R
Govt. Degree College (W)(A),
Kurnool, A.P. India.



Structure of Lawsone

In preliminary phytochemical investigations it has found that the leaves of *Lawsonia inermis* contain lipids, carotenoids, flavonoids, alkaloids and reducing and non-reducing sugars. Another Phytochemical compound Quercetin, of this plant is found to be shown ant malarial and antioxidant activity^[11]. Some recent reports have indicated that many flavonoids possess antiulcerogenic activity. Oral treatment with the ether fraction of the flavonoid extract demonstrated a good level of gastric protection. Mucous content was increased and accompanied by proportionate increase in proteins and hexosamines^[2]. Quercetin, kaempferol, morin, myricetin and rutin when tested were found to inhibit the mucosal content of platelet activating factor (PAF) in a dose dependent manner suggesting that the protective role of these substances may be mediated by endogenous PAF^[12]. Flavonoids exhibit several biological effects such as anti-inflammatory, anti-hepatotoxic and antiulcer actions^[13]. In this present study we have evaluated the gastro protective activity of MELI leaf in ulcer induced experimental rats by taking the consideration of Phytochemical compounds of *Lawsonia inermis* (Henna)

2. Materials and Methods

2.1. Plant collection:

The whole plant of *Lawsonia inermis* (Henna) was collected from our local area, identified and authenticated.

2.2. Preparation of extract

The plant of *Lawsonia inermis* (Henna) were shade dried and the leaves reduced to coarse powder in a mortar and pestle. The powdered material obtained was then subjected to successive extraction by hot percolation method using petroleum ether, chloroform and methanol solvents in a soxhlet apparatus. The different extracts obtained were evaporated at 47^o C to get a semisolid mass. The extracts thus obtained were subjected to phyto-chemical analysis. The percentage yield alcoholic extract was found to be 55.5%w/w and the Methanolic extract was taken for further *Phytochemical Screening* Qualitative tests for the presence of plant secondary metabolites such as carbohydrates, alkaloids, tannins, tannic acids, flavonoids, proteins, saponins and glycosides were carried out on extracts using standard procedure studies.

2.3. Animals used

Male albino Wister rats between 1 to 2 months of age and weighing 125-150g were procured and were maintained as per the guidelines of National Institute of Nutrition (NIN) Animal User's Manual. Animals were acclimatized for 7

days to our animal house, maintained at temperature of 20-24 °C. The light source in the animal room was regulated with 12 h light period followed by 12 h dark schedule. Two to three animals were housed per cage sized (41 × 28 × 14 cm). Paddy husk was used for bedding and on every alternative day bedding was changed and washed thoroughly with water along with Domex, a disinfectant and a detergent. The rats were fed on a standard pellet diet purchased from Sai Durga Feeds and Foods Bangalore, and water ad libitem.

2.4. Experimental design

The animals were divided into four groups, each consisting of six rats. Group I represented the Normal control group, which received distilled water orally. Groups II represented the Control group, which received Ethanol 1ml/100g.b.w^[14]. Groups III received Methanol extract of *Lawsonia inermis* 200 mg/kg and, Ranitidine, in the dose of 20 mg/kg were administered orally for group IV as reference standard drug. The gastric ulcers were induced in rats by administering absolute ethanol (95%) (1 ml/100 g b.w.) Orally¹⁴. After 45 min of Methanol extract and Ranitidine treatment. They were kept in specially constructed cages to prevent coprophagia during and after the experiment. The animals were anaesthetized 1hr latter with anesthetic ether and stomach was incised along the greater curvature and ulceration was scored.

3. Ulcer Index / Scoring Of Ulcer

3.1. Measurement Of Ulcer Index

Stomach mucosa was flushed with saline and lesions in glandular portion were then exposed and examined under a 10x magnifying glass^[15]. Ulcer index of each animal was calculated by adding the values and their mean values were determined by the following scoring system^[15].

For the macroscopic observations, the number, lengths and severity of ulcers were noted and scored. The ulcer index (U.I.) of each stomach was the sum of its scores. The ulcer index was reported as arithmetic means ± S.E. The significance of differences between means was evaluated by Student's *t* - test for unpaired data. *P* < 0.05, versus control, was taken as significant.

- (i) Normal coloured stomach – 0,
- (ii) red colouration - 0.5,
- (iii) spot ulceration – 1,
- (iv) Haemorrhagic streak – 1.5,
- (v) Ulcers (< 2mm) – 2,
- (vi) Ulcers (>2 < 4 mm) perforations – 3.
- (vii) 3 Ulcers (< 4mm) : -4

Percentage inhibition was calculated using the following formula:

$$\% \text{ Inhibition} = \frac{\text{UI}_{\text{ulcer control}} - \text{UI}_{\text{treated}}}{\text{UI}_{\text{ulcer control}}} \times 100$$

4. Sample collection and preparation for biochemical estimations and assays

4.1. Measurement of gastric secretion and P^H^[16].

The stomach of aspirin induced ulcer rats was carefully excised keeping oesophagus closed and opened along greater curvature and luminal contents were removed. The gastric juice thus collected was centrifuged at 3000 rpm for 10 min and expressed in terms of ml/100 g of body weight. The pH of the supernatant was measured using digital pH meter^[16].

4.2. Measurement of free and total acidity [17]

Free and total acidity were determined by titrating with 0.01N NaOH using Topfer’s reagent and phenolphthalein respectively as indicators and were expressed as meq/l per 100 g [17].

4.3. Measurement of gastric juice and pH [16]

The gastric juice was collected was centrifuged at 3000 rpm for 10 min and expressed in terms of ml/100 g of body weight. The pH of the supernatant was measured using digital pH meter.

4.4. Determination of free acidity and total acidity [18]

The gastric contents were centrifuged at 1000rpm for 10min. 1ml of supernatant was diluted with 9ml of distilled water. A volume of 2ml diluted gastric juice was titrated with 0.1N Sodium hydroxide run from a micro burette using 3-4 drops of Topfer’s reagent as indicator until canary yellow colour was observed. Volume of NaOH required was noted. This corresponds to free acidity. Further 2-3 drops of phenolphthalein was added and titrated with NaOH until pink colour was restored. This gives total acidity. Free acidity and total acidity is expressed in terms of ml of 0.1N HCl per 100 gms of gastric contents. This is the same as mEq/lit. To obtain this figure multiply the burette reading obtained from titration by 10.

Acidity was calculated by using the formula:

$$\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Normality of NaOH} \times 100}{0.1} \text{ meq/L/100g}$$

Table B: Anova Test For Anti Ulcer Activity of Pylorus Ligated Ulcer Induced Model

S.NO.	Treatment	Volume of Gastric juice(ml)	pH	Free Acidity meq/l/100gm	Total Acidity meq/l/100gm
1.	Control	3.024 ± 0.1414	1.544 ± 0.03985	21.492 ± 0.8815	78.39 ± 1.296
2.	Ranitidine	1.223 ± 0.05213	2.856 ± 0.1235	10.756 ± 0.5986	38.493 ± 1.135
3.	MELI	1.423 ± 0.04233	2.599 ± 0.1297	12.788 ± 0.6003	48.792 ± 1.066
F, d, f Value		104.21 (2/15)	36.211 (2/15)	53.547 (2/15)	299.28 (2/15)
P Value		P < 0.0001	P < 0.0001	P < 0.0001	P < 0.0001

* P < 0.01 when compared with Control

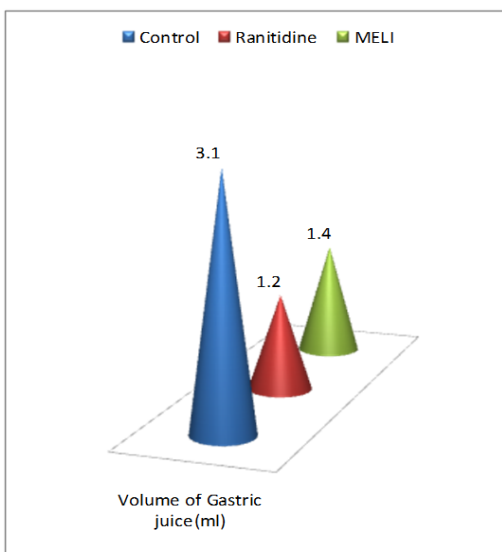


Fig B: Anova Test for Gastric Juice Volume

5. Result

Anti Ulcer Effect of MEAF in Ethanol Induced Model

Table A: Anova Test For Anti Ulcer Activity of Ethanol Induced Model

S.No	Treatment	Ulcer Index
1.	Control	6.849 ± 0.2286
2.	Ranitidine	3.258 ± 0.3172
3.	MELI	2.714 ± 0.3025
F, d, f Value		53.557 (2/15)
P Value		P < 0.0001

* P < 0.01 when compared with Control

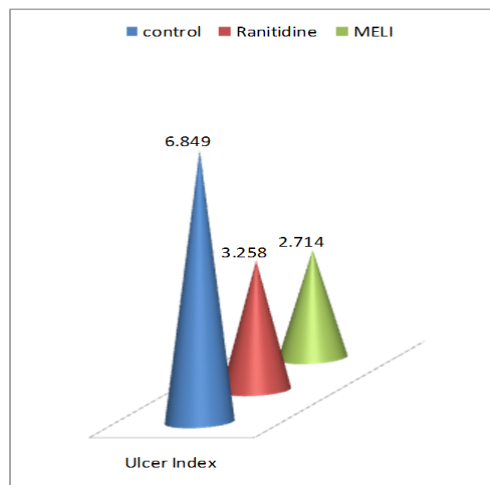


Fig A: Graphical Representation of Ulcer Index

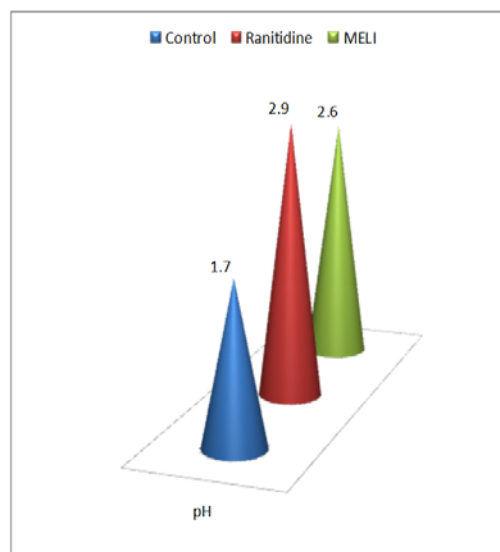


Fig C: Anova Test for Ph

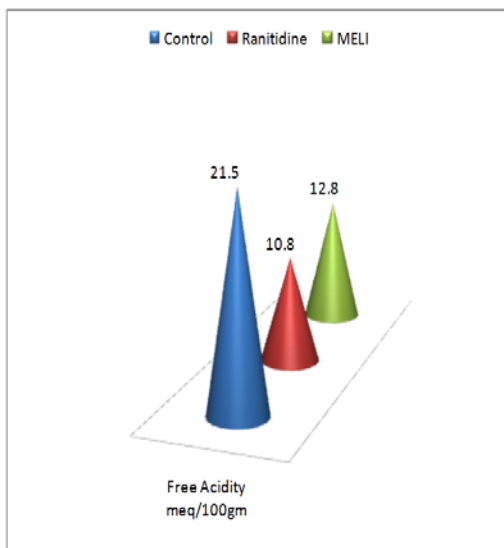


Fig D: Anova Test for Free Acidity

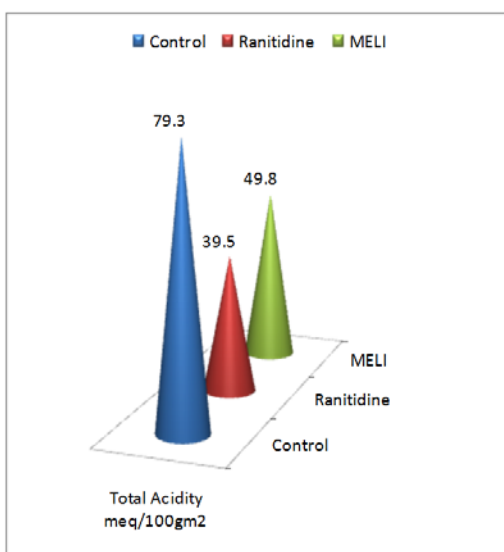


Fig E: Anova Test for Total Acidity

6. Discussion

The Oral administration of methanol extract of *Lawsonia inermis* (Henna) leaves at a dose of 200 mg/kg has shown a dose dependent inhibition Percentage of 53.55 compared to that of ulcer control, proving the anti-ulcer activity of extract whereas ranitidine (20 mg/kg) produced 59.24 % inhibition of ulcer index against Ethanol induced ulcer. MELI significantly protected the mucosa against the damage induced by ethanol and Curative ratio of the MELI of 200 mg/kg was found to be 91.12% Oral administration of MELI 1 hr before an induction of stress reduced the cold restrained stress induced ulcers. The MELI exhibited a dose dependent inhibition percentage of 53.55 at doses of 200 mg/kg dose, where as the standard drug Ranitidine showed an inhibition percentage of 59.24.

Ethanol administration (20mg/kg) results in the production of gastric mucosal damage. The ulcer index in control animals was (6.849). Methanol extract (2.714) significantly reduced the ulcer index ($p < 0.01$) as compared to control. Ranitidine, a standard anti-ulcer drug showed ulcer index (3.258). The results are tabulated in Table A.

Pre treatment of rats with *Lawsonia inermis* (Henna) extracts produced a dose dependent protection in the ethanol induced ulceration model as compared to control group. However the protection was statistically significant reduced the severity of ulcer and caused a significant reduction of ulcer index in this model. Ranitidine produced significant gastric ulcer protection as compared to control group. Pre treatment with MELI has delivered a remarkable anti-ulcer effect which can observe by the effect on gastric secretion in Ethanol induced ulcer. Gastric juice volume, total and free acidity significantly increased and pH decreased in ulcer control animal. MELI (200 mg/kg) produced dose dependent effect and decreased gastric juice volume, total and free acidity and increased pH significantly.

Hence it establishes the fact that the Methanol extract of *Lawsonia inermis* (Henna) has shown the protection against the ulcers which was induced by the ethanol and it is further recommended to perform in vivo bio analytical such as enzymatic studies.

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