



ANTI ULCER POTENTIAL OF *LAWSONIA INERMIS* LEAF

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ABSTRACT

The effect of different extracts of *Lawsonia innermis* was assessed in different acute and chronic gastric ulcer models in rats. Gastric ulcers induced in swiss albino rats (200g) by oral administration of ethanol and induced the stress. The phytochemical investigation showed, In alcoholic extract and aqueous extract carbohydrate, glycosides, tannins, phenolic compounds and gums and mucilage were present in good quantity and saponins, alkaloids, phytosterols, fixed oils, fats, proteins, amino acids, volatile oils were absent and in case of chloroform extract, phenols and sterols present. The anti ulcer activity was assessed by determining and comparing the ulcer index in the test drug groups with that of the vehicle control and standard (sucralfate). *Lawsonia innermis* leaf extracts 200-400 mg/kg administered orally, twice daily for 5 days showed dose-dependent ulcer protective effect in Ethanol induced ulcers (81% at 200 mg/kg % 74% at 400 mg/kg in aqueous extract, 92% at 200 mg/kg & 94% at 400 mg/kg in ethanolic extract and 83% at 200 mg/kg & 88% at 400 mg/kg in chloroform extract at $p < 0.001$), Cold-restraint stress (CRS)-induced ulcers (56% at 200 mg/kg % 38% at 400 mg/kg in aqueous extract, 30% at 200 mg/kg & 23% at 400 mg/kg in ethanolic extract and 52% at 200 mg/kg & 56% at 400 mg/kg in chloroform extract at $p < 0.001$). So ethanolic extract of *Lawsonia innermis* leaf have more anti ulcer activity in a dose dependent manner, when compare to other extracts in ethanol induced model.

Keywords: *Lawsonia innermis*, Ethanol, Cold-restraint stress, Ulcer index.

INTRODUCTION

India, the richest floristic regions of the world, has got a source of plants and their products since antiquity. Man uses them as food and medicine as per his desires. Among the entire flora, estimated 2,500,000 higher plant species on earth, only 35,000 to 70,000 species (less than 1 %) have been used for medicinal purpose¹. There are plenty of chances to find out a new compound derived from plant². Medicinal plants are part and parcel of human society to combat diseases, from the dawn of civilization. There exists a plethora of knowledge, information and benefits of herbal drugs in our ancient literature of Ayurvedic (Traditional Indian Medicine), Siddha, Unani and Chinese medicine. According to the World Health Organization, 2003 about 80% of the population of developing countries being unable to afford pharmaceutical drugs rely on traditional medicines, mainly plant based, to sustain their primary health care needs³. *Lawsonia innermis* L. (Henna) more commonly called as *Mehendi* which belongs to the family *Lythraceae*, grows as a glabrous, much branched shrub or small tree. It is cultivated in tropical and warm temperate regions as a hedge plant. Henna leaves have long been used in India and Middle East countries for colouring palms of hands, sole of feet. Leaves contain important cosmetic dye. The principal coloring matter is a lawsone, which is used as a tropical sunscreen and as a prophylactic against skin diseases. They have astringent property. They have been used in the form of paste or decoction against boils, bruises and skin inflammation⁴

have reported analgesic, anti-inflammatory and anti pyretic effects of henna in rats & antimicrobial property⁵.

Gastric hyperacidity and ulcer are very common causing human suffering today. It is an imbalance between damaging factors within the lumen and protective mechanisms within the gastro duodenal mucosa. Although prolonged anxiety, emotional stress, hemorrhagic surgical shock, burns and trauma are known to cause severe gastric irritation, the mechanism is still very poorly understood⁶. Recently the involvement of neural mechanism in the regulation of stress responsiveness and complex neurotransmitter interactions were reported causing gastric ulceration⁷. To the best of our knowledge there were no scientific reports available in support of its traditional claims. Therefore, present study was designed to demonstrate the effect of *L. innermis* extract (LIE) on physical and chemical factors induced gastric ulceration in rats.

MATERIALS AND METHODS

Collection of plant material

The leaves plant of *L. innermis* was collected from Botanical Garden of N.B.R.I (National Botanical Research Institute), Lucknow, India in month of September 2010. The plant materials were authenticated by Dr. Sayeeda Khatoon, chemo taxonomist at National Botanical Research Institute, Lucknow and voucher specimens were deposited in the departmental herbarium of National Botanical Research Institute, Lucknow, India for future reference.



Phytochemical investigation

The extracts after preliminary phytochemical investigation was shown the presence of active principle⁸.

Extraction of *L. inermis*

The leaves were left to dry at room temperature for 24 hours. The dried leaves were ground to a powder and were kept in dry containers. Two types of extract were prepared in the present study: alcoholic and water-based extracts. The alcoholic extract was prepared by mixing 25 gm of henna powder with 250 ml of 70% ethanol for 12 hours. This mixture was cooled and filtered by Buchner funnel and filter paper. The solvent was dried and concentrated using Rotary evaporator at 50°C. Water based and chloroformic henna extract was prepared in the same way except that distilled water was used instead of alcohol⁹.

Animals

Swiss albino rats weighing (150-200 gm) were procured from National Botanical Research Institute (Lucknow). They were housed in the departmental animal house under standard conditions (26 ± 2°C and relative humidity 30-35%) in 12 hours light and 12 hours dark cycle respectively for 1 week before and during the experiments. Animals were provided with standard rodent pellet diet and had free excess to water. The composition of diet is 10% protein, 4% arachis oil, 1% fibers, 1% calcium, 1000 IU/gm vitamin A and 500 IU/gm vitamin D¹⁰.

Experimental Procedure

Animals were divided into eight groups (n=6). Group-I received 2% gum acacia that served as control, group-II

received Sucralfate orally (250 mg/kg), group-III,IV received aqueous extract (200mg/kg, 400mg/kg), group-V, VI received ethanolic extracts (200mg/kg, 400mg/kg) and group-VII, VIII received chloroform extract (200mg/kg, 400mg/kg) respectively.

Ethanol (EtOH)-induced ulcers

The gastric ulcers were induced in rats by administrating 100% EtOH (1 ml/200 g, 1 h) and the animals were sacrificed by cervical dislocation and stomach was incised along the greater curvature and examined for ulcers. The ulcer index was scored, based upon the product of length and width of the ulcers present in the glandular portion of the stomach (square millimeters per rat). After one hour the treated animals were sacrificed with high dose of ether anesthesia, stomach was removed from body, cut the stomach with greater curvature to measure the ulcer index¹¹.

Cold-restraint stress (CRS)-induced ulcers

Rats were deprived of food, but not water, for about 18 h before the experiment. On day six, the experimental rats were immobilized by strapping the fore and hind limbs on a wooden plank and kept for 2 h, at temperature of 4 – 6°C. Two hours later, the animals were sacrificed by cervical dislocation and ulcers were examined on the dissected stomachs as described above¹².

Statistical analysis

All results were expressed as mean ± SEM for 6 rats. The difference among means been analysed by unpaired student's t-test (Newman-keuls multiple comparison test).

Table 1: Effect of different leaf extract of *L. inermis* on ulcer index in Ethanol induced gastric ulcers

Group	Treatment	Dose (mg/kg)	Ulcer index (mm ² /rat)	% Protection
I.	Control	Gum acacia (2%)	12±0.28	-
II.	Sucralfate	250	1.3±0.21*	89
III.	Aqueous extract	200	2.3±0.08*	81
IV.	Aqueous extract	400	3.1±0.09	74
V.	Ethanol extract	200	1.0±0.15*	92
VI.	Ethanol extract	400	0.76±0.06*	94
VII.	Chloroform extract	200	2.03±0.08*	83
VIII.	Chloroform extract	400	1.45±0.19*	88

Values are mean ± SEM (n=6) one way ANOVA followed by Student- Newman-keuls test.
Where * represents very significant at p<0.001 when compared to control group.

Table 2: Effect of different leaf extract of *L. inermis* on ulcer index on Cold restraint induced gastric ulcers.

Group	Treatment	Dose (mg/kg)	Ulcer index (mm ² /rat)	% Protection
I.	Control	Gum acacia (2%)	11.9±0.36	-
II.	Sucralfate	250	1.4±0.12*	88
III.	Aqueous extract	200	5.2±0.13*	56
IV.	Aqueous extract	400	7.4±0.11	38
V.	Ethanol extract	200	8.3±0.13	30
VI.	Ethanol extract	400	9.2±0.16	23
VII.	Chloroform Extract	200	5.7±0.12*	52
VIII.	Chloroform Extract	400	5.2±0.11*	56

Values are mean ± SEM (n=6) one way ANOVA followed by Student- Newman-keuls test
* represents very significant at p<0.001 when compared to control group.



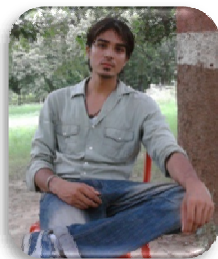
RESULTS AND DISCUSSION

In alcoholic extract and aqueous extract carbohydrate, glycosides, tannins, phenolic compounds and gums and mucilage were present in good quantity and saponins, alkaloids, phytosterols, fixed oils, fats, proteins, amino acids, volatile oils were absent and in case of chloroform extract, phenols and sterols present. We evaluated effects of aqueous, chloroform and ethanol extracts obtained from henna leaves in animals using the different standard experimental models of induced gastric ulcers. Ulcers were frequently observed in the stomach of all the control animals. Administration of henna extracts resulted in a significant reduction in ulcer index in dose dependent manner with compared to control. Henna prevented the mucosal lesions induced by Ethanol & CRS (Table 1 & 2). This suggests that the components present in the extract must be suppressing gastric damage. The efficacy of henna extract against gastric ulcers led us to perform yet another model i.e. Ethanol induced & CRS. This model too resulted in a significant percentage protection against gastric ulcers. In case of ethanol induced model ethanolic extract more significant result when compare to others extracts (92% and 94%). In case of CRS, Chloroform extract showed significant result in a dose dependent manner. So we can say henna have an anti ulcer property in a dose dependent manner. The etiology of ulcer is not clear. It results probably due to an imbalance between the aggressive and the defensive factors¹³. In the stomach, mucus and bicarbonate, stimulated by the local generation of prostaglandins, protect the gastric mucosa. If these defenses are disrupted, a gastric or duodenal ulcer may form. The treatment and prevention of these acid-related disorders are accomplished either by decreasing the level of gastric acidity or by enhancing mucosal protection¹⁴.

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