Journal of Applied Pharmaceutical Science

Available online at www.japsonline.com

Received: 06-04-2011
Revised on: 10-04-2011
Accepted: 16-04-2011

Anti-ulcer potential of *Lawsonia inermis l*. Leaves against gastric ulcers in rats

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ABSTRACT

To evaluate antiulcer effects of aqueous, chloroform and ethanol extracts prepared from the henna leaves in rats employing the pylorus ligation and aspirin induced models. Gastric ulcers induced in Swiss albino rats (200g, N=6) by oral administration of aspirin suspension and pylorus ligation. The antiulcer activity was assessed by determining and comparing the ulcer index in the test drug groups with that of the vehicle control and standard ranitidine. In case of aspirin induced ulcers, the chloroform extract showed significant reduction of ulcers in a dose dependent manner. The parameters taken to assess antiulcer activity were volume of gastric juice, free acidity, total acidity and ulcer index. The results indicated that aqueous, ethanol and chloroform extract significantly ($p<0.001$) decreased the volume of gastric acid secretions, free acidity and total acidity and ulcer index.

Key words: *Lawsonia inermis, Pylorus ligation, Acidity, Ulcer index.*

INTRODUCTION

Medicinal plants are part and parcel of human society to combat diseases, from the dawn of civilization (Grieve M, 2005). 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 needs (Kirtikar KR et al, 1956). *Lawsonia* is monotypic genus, represented by *L. inermis*, native of North Africa and south-west Asia, widely cultivated as an ornamental and dye plant throughout India (Grieve M, 2005). Henna has different names in all over the world. The plant is grey-white and has spines. The length of the leaves of this plant is 2-3cm. The chemical components of henna are not well- known now but it has a color agent (lawsone), with chemical formula ($C_{10}H_6O_3$), which is the most effective component of henna (Zargari A, 1992, Kathlene Parfitt, 1585). Henna has been reported to have many different healing effects, antibacterial effects specially for gram positive bacteria, anti tumoral effects in rat, antifungal activity against dermatophytes and wound healing (Ayatollahi M et al, 1996, Singh VK et al, 1989). 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 understood (Rao et al, 2000). Recently the involvement of neural mechanism in the regulation of stress responsiveness and complex neurotransmitter interactions were reported causing gastric ulceration (Sairam et al, 2001). To the best of our knowledge there were no scientific reports available in support of its traditional claims. Therefore, present study was

the effect of *L. inermis* extract (LIE) on physical and chemical factors induced gastric ulceration in rats.

MATERIAL AND METHOD

Collection of plant material

The leaves plant of *L. inermis* were 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.

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 alcohol (Abdulmoneim MA et al, 2007).

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 D.

Experimental Procedure

Animals were divided into eight groups (n=6). Group-I received 2% gum acacia that served as control, group-II received ranitidine orally (50 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.

Study of antiulcer activity using pylorus ligation method

Animals were fasted for 24 h and the dose was administrated 30 min prior to pylorus ligation. Animals were sacrificed 4 h later and the stomach was removed. The gastric content was collected and centrifuged. The volume, free acidity, total acidity of gastric fluid was determined. The stomach was then incised along the greater curvature and observed for ulcers. The number of ulcers was counted using a magnifying glass. Mean ulcer score for each animal was expressed as ulcer index. The ulcers were graded using the following scoring system- 0= Normal mucosa; 0.5= Red coloration; 1.0=Spot ulcer; 1.5=Hemorrhagic streaks; 2.0=Ulcere”3 mm but d”5 mm; 3.0=Ulcer e” 5 mm (Shay et al, 1945).

Study of antiulcer activity using aspirin induced ulcers

The animals were treated with respective dose of 8 days as mentioned in previous model. After 8 days of treatment animals were fasted for 24 h. ulcer was induced by administration of aqueous suspension of aspirin (200 mg/kg) 4 h later. The animals were sacrificed and stomach was opened to calculate the ulcer index as given earlier (Goel et al, 1986).

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).

RESULT AND DISCUSSION

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. In case of Pylorus ligation model, the total acid output of the gastric juice and accumulation of gastric secretary volume were increased. Circular and linear lesions 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 (Table 1 & 2).

Table: 1 Effect of different leaf extract of *L. inermis* on ulcer index in pylorus ligation induced ulcer

Group	Treatment	Dose (mg/kg)	Ulcer index (mm^2/rat)	%Protection
I.	Control	Pylorus ligation	2.18±0.12	-
II.	Ranitidine	50	0.72±0.01***	66.97
III.	Aqueous extract	200	1.75±0.05**	19.72
IV.	Aqueous extract	400	1.76±0.04**	19.26
V.	Ethanol extract	200	2.31±0.09*	5.96
VI.	Ethanol extract	400	2.66±0.07***	22.01
VII.	Chloroform extract	200	1.31±0.10***	39.90
VIII.	Chloroform extract	400	1.81±0.10**	16.97

Values are mean ± SEM (n=6) one way ANOVA followed by Student- Newman-keuls test Where * represents significant at p<0.05, ** represents highly significant at p< 0.01, *** represents very significant at p<0.001. when compared to control group.

Table: 2 Effect of different leaf extract of *L. inermis* on volume of gastric juice, Total acidity and Free acidity in pylorus ligation induced ulcer

Group	Treatment	Dose (mg/kg)	Vol. of Gastric juice (ml)	Free acidity (m/Eg/1) 100g	Total acidity (m/Eg/1) 100g
I.	Control	Pylorus ligation	9.5±0.10	20.38±0.09	53.46±1.90
II.	Ranitidine	50	3.5±0.10*	5.45±0.16*	15.15±0.22*
III.	Aqueous extract	200	4.5±0.12*	5.31±0.23*	17.52±0.37*
IV.	Aqueous extract	400	5.5±0.14*	10.2±0.34*	19.40±0.44*
V.	Ethanol extract	200	5.6±0.08*	13.92±0.07 *	36.75±0.8*
VI.	Ethanol extract	400	6.5±0.11*	18.19±0.24 *	43.87±0.43*
VII.	Chloroform Extract	200	3.4±0.12*	8.0±0.32*	18.38±0.34*
VIII.	Chloroform Extract	400	4.4±0.06*	7.11±0.26*	20.25±0.13*

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.

The etiology of ulcer is not clear. It results probably due to an imbalance between the aggressive and the defensive factors (Tripathi KD, 2003). 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 protection (Goodman & gilman's, 2006). Henna prevented the mucosal lesions induced by pylorus ligation. 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. aspirin induced. This model too resulted in a significant percentage protection (85.6 %) against gastric ulcers. The percentage protection observed was very much the same as that of standard drug ranitidine (Table 3). It has been postulated that histamine might be involved in the formation of pylorus ligated ulcers and plays a mediating a role in gastric secretions stimulated by gastrin vagal excitation. The H_2-receptor antagonists inhibit acid production by reversibly competing with histamine for binding to H_2 receptors on the basolateral membrane of parietal cells. Four different H_2-receptor antagonists, which differ mainly in their pharmacokinetics and propensity to cause drug interactions, are available in the United States : cimetidine (TAGAMET), ranitidine (ZANTAC), famotidine (PEPCID), and nizatidine (AXID). These drugs are less potent than proton pump inhibitors but still suppress 24-hour gastric acid secretion by about 70%. The H_2-receptor antagonists predominantly inhibit basal acid secretion, which accounts for their efficacy in suppressing nocturnal acid secretion. Because the most important determinant of duodenal ulcer healing is the level of nocturnal acidity, evening dosing of H_2-receptor antagonists is adequate therapy in most instances. Ranitidine and nizatidine also may stimulate GI motility, but the clinical importance of this effect is unknown. All four H_2-receptor antagonists are available as prescription and over-the-counter formulations for oral administration. Intravenous and intramuscular preparations of cimetidine, ranitidine, and famotidine also are available. When the oral or nasogastric routes are not an option, these drugs can be given in intermittent intravenous boluses or by continuous intravenous infusion (Goodman & gilman's, 2006).

Table: 3 Effect of different leaf extract of *L. inermis* on ulcer index on Aspirin induced gastric ulcers

Group	Treatment	Dose (mg/kg)	Ulcer index (mm^2/rat)	% Protection
I.	Aspirin	200	12.5±0.13	-
II.	Sucralfate	250	2.21±0.11*	82.32
III.	Aqueous extract	200	3.36±0.08*	73.12
IV.	Aqueous extract	400	4.35±0.09*	65.2
V.	Ethanol extract	200	6.25±0.10	50.0
VI.	Ethanol extract	400	7.3±0.12	41.6
VII.	Chloroform Extract	200	1.8±0.09*	85.6
VIII.	Chloroform Extract	400	2.4±0.07*	80.8

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.

CONCLUSION

Here present study was carried out to investigate antiulcer activity of ethanolic, aqueous and chloroformic extract of *L.inermis* leaves in pylorus ligatated and aspirin induced ulceration in the rats. Pylorus ligation induced ulcer is one of the most widely used methods for studying the effect of drug on gastric secretion. Pylorus ligation induced ulcers are due to auto digestion of gastric mucosa and breakdown of the gastric mucosal barrier. These factors are associated with the development of upper gastrointestinal damage including lesions, ulcers and life threatening perforation and hemorrhage. Prostaglandin E2 and I2 are predominantly synthesized by the gastric mucosa and are known to inhibit the secretion of gastric acid and stimulate the secretion of mucus and bicarbonate. Hydrophobic surfactant-like

phospholipids secretion in the gastric epithelial cells is also stimulated by the prostaglandin. Effect of pylorus ligation has caused the accumulation of gastric secretion . The total acidity, free acidity, ulcer index of gastric secretions were increases. Ranitidine and leaves extract of *L.inermis* significantly decreased the gastric volume, total acidity, free acidity, and ulcer index. Similar studies support our results. In case of aspirin & pyrolus ligation induced ulcer, the chloroform extract of *L.inermis* leaves possess significant antiulcer properties in a dose dependent manner.

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