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Research Article

EVALUATION OF ANTIULCER ACTIVITY OF ETHANOLIC EXTRACT ON FLOWERS OF *LAWSONIA INERMIS*

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A B S T R A C T

Ulcer can be developed inside the inner lining of the stomach (gastric ulcer) or the small intestine (duodenal ulcer). Both the ulcers are also cumulatively referred as peptic ulcers. It affects nearly 10% of world population. To investigate the antiulcer activity of ethanolic extract of Lawsonia inermis (EELI) on albino rats, the present study was carried by pylorus ligation, ethanol induced ulcer models in albino rats. The antiulcer activity of EELI (200, 400 mg/kg p.o. for 7 days) was compared with standard drugs (Ranitidine 50 mg/kg). In pyloric ligation induced ulcer model, the studied parameters were gastric volume, pH, total acidity, free acidity, and ulcer index, whereas in ethanol induced ulcer model, the ulcer index was determined for severity of ulcers. The parameters studied were ulcer index, gastric juice volume, pH, free acidity and total acidity. In pyloric ligation model; the volume of gastric content, total/free acidity and pepsin activity was significantly decreased at $p<0.05$ and $p<0.01$ and pH of the gastric juice was significantly increased at $p<0.05$ and $p<0.01$ in EELI treated groups as compared to control group. All the doses of EELI showed dose dependent antiulcer effect as well as significant ($p<0.05$ and $p<0.01$) reduction in the ulcer index as compared to control group in all the experimental models. The results of the study indicate that the EELI have better potential against ulcer which supports the traditional claims in folklore medicine.

INTRODUCTION

Ulcer is a major disease of gastrointestinal system which affects 10% of the world population with different aetiologies. Chronic alcohol intake, smoking, excessive stress, chronic usage of non-steroidal anti-inflammatory drugs and *H.pylori* bacterial infection are the crucial causes of peptic ulcer characterized by inflammation, mucosal bleeding and abdominal pain in patients [1,2]. These ulcers can develop when the imbalance occurs between the gastro protectives (mucus, bicarbonate and prostaglandins) and aggressives (acid, pepsin, bile salts and *Helicobacter pylori* bacteria) [3]. The recent approach to peptic ulcer is managed by inhibition of gastric acid secretion, promotion of gastro-protection, blocking apoptosis and stimulation of epithelial cell proliferation for effective healing. The conventional drugs used in the treatment of ulcer include histamine receptor antagonists, prostaglandins analogues, proton pump inhibitors, cytoprotective agents, antacids and anticholinergics, but most of these drugs produce undesirable side effects or drug interactions and may even alter biochemical mechanisms of the body upon chronic usage.

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Hence, herbal medicines are generally used in such chronic cases, wherein drugs are required to be used for long periods [4]. Common names English : Henna, Samphire, Cypress shrub Sanskrit : Dviranta, Mendika, Medhini Arabic : hina or henna, French : Alcana d'orient Greek : Kypros Gujrat : Medi Hindi : Hena, Mehindi Marthi : Mendhi, Mendi Tamil : Maruthoni, Aivani Telugu : Maruvam [5, 6].

Lawsonia inermis is commonly known as 'Maruthoni' in Tamil, belonging to Lythraceae family. It is native of North Africa and south-West Asia and widely cultivated as an ornamental and dye plant through India [7].

MATERIALS AND METHODS

The fresh flowers of *Lawsonia inermis* were collected from the surrounding area of our college and dried under room temperature for 15 days. In-vivo antiulcer study was performed from April 2018 to May 2018 and the ethical approval was permitted from our college vadakkankulam Thirunelveli district, Tamil nadu, India.

Drugs and Chemicals

Ethanol (Merck, Mumbai, India), and Ranitidine (Kopran Pharma Ltd., Mumbai, India) were obtained. All other chemicals and solvents used in this study were of analytical grade.

Plant material

The fresh flowers of *L. inermis* were collected in the month of February 2018 from the local areas of vadakkankulam, Thirunelveli Dist, Tamil nadu, India. The plant was identified and authenticated by Mr. Chelladurai, Rtd Professor from CCRAS, Thirunelveli, where the voucher specimen was deposited for further reference in pharmacognosy department of SA Raja Pharmacy College.

Preparation of Plant Extract

The flowers of *L. inermis* were washed thoroughly in water to remove foreign matter and allowed to shade dry with a relative humidity of 40–45%. Then, the flowers were powdered in roller grinder and passed through a sieve (No. 40). Then, the fine powder (Approx. 1000 gm) was directly extracted with 2 litre of 70% ethanol at room temperature by using Soxhlet apparatus for 72 hour. The resultant extract was filtered and concentrated in a rotary evaporator under reduced pressure to obtain a thick semi solid brown paste, which was stored at −20°C until required. The yield of the extract was found to be 09.58 %w/w.

Phytochemical Screening

The preliminary phytochemical screening was performed with the ethanolic extract of *L. inermis* flower (EELI) for the detection of various phytochemicals [8].

Experimental Animals

Adult albino rats (40 numbers) of 150 - 200g of body weight of either sex were procured from the animal house of Sri Sivani College of Pharmacy, Srikakulam, Andhra Pradesh, India. The study protocol was approved from the Institutional Animal Ethics Committee (IAEC) under the reference no. 1427/PO/a/11/CPCSEA and CPCSEA guidelines were adhered during the maintenance and experiment. All animals were maintained under standard husbandry conditions with food and water ad libitum.

Acute Oral Toxicity Study

Healthy adult male *albino* rats were fasted overnight prior to the experiment. Different doses (50-2000 mg/kg, P.O) of the ethanolic extract of *L.inermis* were administered to each group of rats (Each group carries 8 rats) and they were observed continuously for1 hour and then at half-hourly intervals for 4 hour, for any gross behavioural changes and further up to 72 hour, followed 14 days for any mortality as per the OECD (Organization for Economic Co-operation and Development) Guideline 425 [9]. The leaf extract of *L.inermis* was found to be non-toxic up to the maximum dose of 2000 mg/kg body weight. Dose selected for antiulcer evaluation was 200 and 400 mg/kg respectively [9].

Animal Grouping and Antiulcer Treatment Schedule

In all the experimental models, male *albino* rats were selected and divided into four groups of six animals each. Animals were fasted for 24 hour before the study, but had free access to water. Group I treated as vehicle control, received only distilled water; group II and III treated as treatment groups, received the graded dose of ethanol extract of *L. inermis* (EELI) at 200 and 400 mg/kg, (P.O.) for 7 days (once in a day) respectively and group IV as standard group, received ranitidine 50 mg/kg (P.O.).

Pyloric Ligation Induced Gastric Ulceration

In this method, albino rats were fasted in individual cages for 24 hour EELI, reference drug and control vehicle was administered 1 hour prior to pyloric ligation. Then the pre-treated animals were anaesthetised by anaesthetic ether; the abdomen was opened by a small midline incision below the xiphoid process. The pyloric portion of the stomach was ligated without causing any damage to its blood vessels. The stomach was isolated carefully and the abdominal wall was sealed by interrupted sutures. The animals were deprived of water during the postoperative period. Four hours after ligation, the stomach was dissected out and contents were collected into clean tubes. The volume, pH and total acid content of gastric juice were determined. The contents were centrifuged, filtered and subjected to titration for estimation of total acidity. From the supernatant, aliquots (1 ml each) were taken for the determination of pH, total or free acidity and pepsin activity [10]. Each stomach was examined for lesions in the fore stomach portion and indexed according to severity [11].

The numbers of ulcers were counted and scoring of ulcer was made as follows: Normal colored stomach (0), Red coloration (0.5), Spot ulcer (1), Haemorrhagic streak (1.5), Deep ulcers (2) and Perforation (3). Mean ulcer score for each animal was expressed as ulcer index [12]. Ulcer index (U) was measured by using following formula: U= U + U + U× 10−1 Where, U (Ulcer Index); U (Average number of ulcers per animal); U (Average number of severity score); U (Percentage of animals with ulcers). The percentage inhibition of ulceration was calculated and compared with control.

Ethanol Induced Mucosal Damage in Rats

The rats were fasted for 24 hours before the experiment. After 1 hour of administration of EELI, ranitidine and vehicle control treatment, 1ml of absolute ethanol (0.5 ml/100g) was orally administered to each rat of every group. After 1 hour, the animals were sacrificed with excess of anaesthetic ether and stomach was opened along the greater curvature, cleared of residual matter with saline and the inner surface was examined for severity of ulceration [13]. Ulcer index and % ulcer protection were calculated.

Statistical Analysis

The results were expressed as the Mean±SD for each group. Statistical differences were evaluated using a one-way analysis of variance (ANOVA) followed by Dunnet's t-test. Results were considered to be statistically significant at $p<0.05$.

RESULTS

Preliminary Phytochemical Screening

The EELI showed the presence of Glycosides, Proteins, Amino acids, Steroids, Flavonoids, Phenols and Saponins.

Effect of the EELI on Pylorus Ligation Induced Gastric Ulceration

In pylorus ligated rats, the volume of gastric content, pH, pepsin activity, total and free acidity are shown in [Table/Fig-2]. In EELI treated groups, the volume of acid secretion, total acidity and pepsin activity was decreased and pH of the gastric juice was increased compared to ulcer control group. The effects of ethanolic extract of *L. inermis* on acid parameters showed significant ($p<0.01$and $p<0.05$) effect at 200 and 400

mg/kg doses compared to ulcer control animals. In addition, *L.inermis* showed a dose dependent curative ratio compared to ulcer control groups. The extracts exhibited an inhibition percentage of 18.56, 38.42 at doses of 200, 400 mg/kg doses respectively; and, the standard drug, ranitidine, exhibited an inhibition percentage of 54.87. EELI at 400 mg/kg was shown equipotent antiulcer activity with ranitidine.

Effect of the EELI on Ethanol Induced Gastric Ulceration

The animals pre-treated with EELI groups showed significant ($p<0.01$) as well as dose dependent inhibition of ulcer index as compared to control group [Table/Fig-3]. EELI showed 18.56, 38.42 % at doses of ulceration inhibition at 200 and 400 mg/kg respectively whereas ranitidine showed 54.87% ulceration inhibition.

The cause of gastric ulcer is due to stress induced increase in gastric acid (HCl) secretion and these acid secretions promote ulceration due to exposure of the unprotected lumen of the stomach to the accumulating acid [14-16] Pylorus ligation induced ulcers are shown by auto digestion of the gastric mucosa and breakdown of the gastric mucosal barrier which resulted as upper gastrointestinal damage including lesions, ulcers and life threatening perforation and haemorrhage. The pyloric ligation of the stomach causes accumulation of gastric acid which leads to development of ulceration in stomach. The agents who decrease gastric acid secretion and increase mucus secretion are effective in preventing the ulcers induced by this method. Like ranitidine, omeprazole acts as anti-ulcer agent by antisecretory mechanism via inhibition of gastric secretion and pepsin activity [10]. In the present study, EELI prevents the ulcer may be by antisecretory and cytoprotective property. Ethanol is responsible for disturbances in gastric secretion, damage to the mucosa, alterations in the permeability, gastric mucus depletion and free radical production. The generation of free radicals was produced by continuous release of superoxide anion and hydroperoxy free radicals during metabolism of ethanol [17]. Ethanol induced gastric ulceration may be occurred due to stasis in gastric blood flow which contributes to the development of the haemorrhage and necrotic tissue injuries. Alcohol has ability to penetrate the gastric mucosa and causing the cellular damage which increases the permeability to sodium and water. In other hand, the accumulation of intracellular calcium causes the pathogenesis of gastric injury that leads to cell death and exfoliation of surface epithelium [18]. The present study observed that the EELI significantly reduced ethanol induced ulcer by cytoprotective action via antioxidant effect. The EELI extract showed cytoprotection against the ethanol induced ulceration by reducing the gastric acid secretion. The results of this study found that EELI established a cytoprotective action against ethanol induced cellular damage in the gastric mucosa of rats. Cytoprotection of anti-ulcer drugs has been recognised due to the generation of prostaglandins [19]. It has also been observed that EELI significantly and dose dependently reduced the extent of gastric ulceration in pylorus ligated rats without affecting the gastric secretion or pepsin activity. The defence potential of mucus perimeter of gastric mucosa depends upon a delicate balance between the processes affecting the synthesis and secretion of mucin constituents. EELI prevented the mucosal lesions induced by alcohol [20]. The modern approach towards a potent antiulcer agent involves a delicate balance of controlling the synthesis, secretion and metabolism of proteins, glycoproteins and lipids, so as to strengthen the mucosal integrity [21]. Several scientific studies revealed that the phytoconstituents like flavonoids, tannins, terpenoids and saponin were responsible for gastro protective agents [22]. Tannins possess as an antiulcer agent by its astringency property and vasoconstriction effects. Due to precipitation of micro proteins on the ulcer site, a protective layer was formed which hinders gut secretions and protects the mucosa from toxins and other irritants. Previous studies have recommended that these above active compounds had ability to stimulate mucus, bicarbonate and prostaglandin secretion and neutralize with the deteriorating effects of reactive oxidants in gastrointestinal lumen [23]. Therefore, EELI possess antiulcer activity, may be due to presence of tannins, flavonoids and terpenoids.

CONCLUSION

The present study concluded that the antiulcer activity of EELI may be attributed to antisecretory, cytoprotective and antioxidant properties. The bioactivity-guided phytochemical screening of EELI revealed the presence of flavonoids, glycosides and saponins which may be responsible for the anti-ulcer effect and can be further fractionated and investigated for their role and utility in any of the anti-ulcer mechanisms.

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RESULTS

In pyloric ligation model; the volume of gastric content, total/free acidity and pepsin activity was significantly decreased at $p<0.05$ and $p<0.01$ and pH of the gastric juice was significantly increased at $p<0.05$ and $p<0.01$ in EELI treated groups as compared to control group. All the doses of EELI showed dose dependent antiulcer effect as well as significant ($p<0.05$ and $p<0.01$) reduction in the ulcer index as compared to control group in all the experimental models.

CONCLUSION

The results of the study indicate that the EELI have better potential against ulcer which supports the traditional claims in folklore medicine on *Albino* Rats.

Table 1 Effect of EELI on percentage of ulcer inhibition by pyloric ligation, ethanol induced ulcers in rats.

Group	Dose	Ulcer index mean ± SEM	% of ulcer protection
Group I	Normal control	2.75 ± 0.52	00.00
Group II	Low dose 200mg/kg test drug	2.15 ± 0.26	18.56
Group III	High dose 400mg/kg test drug	1.75 ± 0.38**	38.42
Group IV	Ranitidine 50mg/kg	1.52 ± 0.26**	54.87

Table 2 Effect of EELI on gastric parameters by pyloric ligation induced ulceration in rats.

Group	Dose	Gastric juice volume(ml/4h)	Total acid output(mEq/L)	Pepsin activity (m/h)
Group I	Normal control	8.25 ± 1.22	196 ± 8.72	2.95 ± 0.18
Group II	Low dose 200mg/kg test drug	9.77 ± 1.65	198 ± 6.30	2.82 ± 0.45
Group III	High dose 400mg/kg test drug	9.24 ± 1.10	185 ± 5.55	1.90 ± 0.22
Group IV	Ranitidine 50mg/kg	7.15 ± 0.32	115 ± 2.53	1.74 ± 0.78

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