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Research Article

ANTIULCER ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *CASSIA TORA* LINN USING ETHANOL INDUCED ULCER

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ABSTRACT

Background and Objective: *Cassia tora* Linn. (Caesalpinaceae) is a well known oriental herb which has been used in traditional medicine for the treatment of ulcer and various skin diseases. Therefore, the present study was planned to evaluate the antiulcer activity of hydroalcoholic extract of *Cassia tora* seeds.

Methods: Pharmacological evaluation of *Cassia tora* was done using ethanol induced gastric ulcer model in wistar albino rats. Hydroalcoholic extract at doses 125 mg/kg, 250 mg/kg and 500 mg/kg was administered orally. Parameters evaluated were gastric volume, pH, free acidity, total acidity, mean ulcer score and ulcer index.

Results: Pre-treatment of the extract showed ulcer protection in a dose dependent manner (125 mg/kg, 250 mg/kg and 500 mg/kg). Formation of ulcers decreased significantly ($p<0.05$) at 125 mg/kg and very significantly ($p<0.01$) at 250 mg/kg and 500 mg/kg dose. Volume of gastric juice decreased significantly ($p<0.05$) at 250 mg/kg and 500 mg/kg dose, while free acidity and total acidity decreased very significantly ($p<0.01$) at all the three doses.

Conclusion: The results obtained from the present study demonstrated that hydroalcoholic extract exhibit both antisecretory and antiulcer properties. This supports the traditional use of *Cassia tora* in the treatment of ulcer.

Keywords: *Cassia tora*, Antiulcer, Ethanol, Hydroalcoholic extract.

INTRODUCTION

Peptic ulcer disease (PUD) encompassing gastric and duodenal ulcer is the most prevalent gastrointestinal disorder[1]. Three out of 1000 individuals have peptic ulcer every year and an estimated 15,000 deaths occur each year as a result of PUD[2]. Pathophysiology of PUD involves an imbalance between the aggressive (acid, pepsin and *Helicobacter pylori*) and the defensive (gastric mucus and bicarbonate secretion, prostaglandins, innate resistance of the mucosal cells) factors[3].

Agents that are presently available for the treatment of gastric ulcers act by either reducing gastric acid secretion (H_2 Blockers, Proton Pump Inhibitors, Antimuscarinic Agents), acting as physical barriers (Sucralfate, Colloidal bismuth subcitrate) or increasing the mucous and bicarbonate secretion (prostaglandin analogues, carbenoxolone). Even though these agents are effective in healing of gastric ulcers, continued use of these agents can in turn lead to a plethora of side effects ranging from dryness of mouth to achlorydria, atrophic gastritis, osteodystrophy and encephalopathy, arrhythmias, impotence, gynaecomastia, enterochromaffin like cell (ECL) hyperplasia and haematopoietic changes[4,5]. Reports on clinical evaluation of these drugs showed development of tolerance, incidences of relapses and danger of drug interactions during ulcer therapy. This has been the rationale to search an indigenous drug with fewer side effects and lower incidence of relapse to have a better and safer alternative for the treatment of peptic ulcer.

Therefore in the past decades, extensive studies and research has been undertaken which mainly focuses on search of antiulcer agents of plant origin because there is a wide spread belief that traditional drugs claims the therapeutic efficacy and are less toxic compared to synthetic drugs[6,7].

Cassia tora Linn. (Caesalpinaceae) is a well known oriental herb used in traditional medicine which grows up to 1-2 m in height and is found as a rainy season weed throughout India. It constitutes an ayurvedic preparation "Dadrughan-vati" which is used for ringworm, leucoderma, etc. Chakramardha tailamu, a compound ayurvedic oil of this herb is beneficial in eczema, ringworm and other skin diseases[8,9]. Whole plant is employed in the treatment of impetigo, ulcers, helmenthiasis and as a purgative[10]. Traditional Chinese healers use this herb to treat blindness, xerophthalmia, and conjunctivitis. The seeds are reputed in Chinese medicine as vision improving, antiasthenic, aperient, diuretic and an effective agent in lowering cholesterol and reducing blood pressure[11].

It has been reported that seeds of *Cassia tora* have antioxidant effect. Antioxidant potential of the seeds of *Cassia tora* and the traditional use of this plant in the treatment of ulcers has been the rationale for this study. Biological and Pharmacological activities previously reported on seeds of *Cassia tora* are antibacterial, anthelmintic, antidiabetic, anticancer, antiestrogenic, antigenotoxic, antimutagenic, antioxidant, antishigellosis, immunostimulatory, hypolipidemic, hepatoprotective, hypotensive[12]. However, pharmacological evaluation regarding antiulcer activity of hydroalcoholic extract of *Cassia tora* Linn. seeds (HECT) is not yet carried out. So, the basic aim and objective of the present study is to test the efficacy of the herb *Cassia tora* in healing gastric ulcers with a view to produce an anti-ulcer drug from natural origin.

MATERIAL AND METHODS

Plant material

Plant samples were collected from roadsides of Bahadurgarh-Jhajjar Road, Haryana in the month of August, 2010. They were positively identified by Dr. H.B. Singh, Head, Raw Materials Herbarium and Museum, National Institute of Science Communication and Information Resources (NISCAIR), New Delhi where a voucher specimen of the plant has been deposited for future identification (Ref. No. NISCAIR/RHMD/Consult/2010-11/1497/95). Pods (Seeds) were collected later in bulk in the month of October-November.

Preparation of plant extract

Seeds were crushed in a grinder and then passed through mesh sieve no. 40 to obtain a fine powder. Drug powder was packed in a soxhlet apparatus and was defatted with petroleum ether for 72 hr. Defatted material was completely freed of petroleum ether and the marc was extracted with hydroalcohol (30% water + 70% alcohol) in soxhlet apparatus. Extract obtained was concentrated in a rotary evaporator and finally complete solvent was removed from the marc. The yield of extract was calculated. It was then stored in an air tight container in refrigerator for further experimental studies.

Animals

Healthy albino wistar rats of either sex (150-250g) were procured from diseased free animal house of Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana. They were maintained in a controlled environmental condition of temperature 25±5°C, Relative humidity 55±10% under 12 h light 12 h dark cycle and were fed with standard pellet diet (Aashirwad Industries, Chandigarh) and water *ad libitum*. After 1 week of acclimatization they were used for further experimental studies. All the experimental procedures and protocols used in the study were reviewed by the Institutional Animal Ethics Committee and were in accordance with the guidelines of the CPCSEA, Ministry of Forest and Environment, Government of India (Reg. No. 562/02/a/CPCSEA).

Preliminary phytochemical screening

The extract obtained was subjected to phytochemical screening for detection of various plant constituents[13].

Assessment of Anti-ulcer activity

Ethanol induced gastric ulceration

This method was performed according to the method of Robert *et al*[14]. The albino rats of either sex were divided into 5 groups of 6 animals each and fasted for 24 hrs with water *ad libitum* prior to experiment. Control group received only distilled water 1 ml/100 g body weight through oral route. HECT at 125, 250 and 500 mg/kg, p.o. were given to the animals in the treatment groups. Omeprazole (20 mg/kg) was used as standard. Absolute Ethanol 1 ml/200 g body weight was administered per oral to all the animals of respective groups 30 min. after the respective treatments. The animals were sacrificed after 1 hour of ethanol administration using overdose of chloroform anaesthesia and stomach was incised along greater curvature and examined for ulcers.

Macroscopic evaluation of stomach

The stomachs were opened along the greater curvature, rinsed with saline to remove gastric contents and blood clots and examined by a 10X magnifier lens to assess the formation of ulcers. The numbers of ulcers were counted.

Scoring of ulcer was done as follows:

Normal coloured stomach..... (0)

Red coloration..... (0.5)

Spot ulcer..... (1)

Hemorrhagic streak..... (1.5)

Ulcers ≥3≤ 5..... (2.0)

Ulcer > 5..... (3.0)

Calculation of Ulcer Index

$U_I = U_N + U_S + U_P \times 10^{-1}$

Where,

U_I= Ulcer Index; U_N = Average number of ulcers per animal; U_S = Average number of severity score; U_P = Percentage of animals with ulcers

Percentage inhibition of ulceration was calculated as below:

$$\% \text{ Inhibition of Ulceration} = \frac{(\text{Ulcer index}_{\text{Control}} - \text{Ulcer index}_{\text{Test}}) \times 100}{\text{Ulcer index}_{\text{Control}}}$$

Measurement of volume of gastric juice

After dissection, stomach was put on a watch glass, and cut along the greater curvature. With the help of a syringe, gastric secretion was collected into graduated micro centrifuge tubes.

Determination of pH

Gastric juice was centrifuged at 2000 rpm for 10 min. From the supernatant, aliquots of 1 ml gastric juice were diluted with 1 ml distilled water and pH of the solution was measured using pH meter.

Determination of free acidity

An aliquot of 1ml gastric juice diluted with 1ml of distilled water was taken into a 50 ml conical flask and 2-3 drops of topfer's reagent as indicator was added to it and titrated with 0.01N NaOH until a canary yellow colour was observed.

The volume of 0.01N NaOH consumed was noted. The free acidity was calculated by using the following formula:

$$\text{Acidity} = \frac{\text{Vol. of NaOH} \times \text{Normality} \times 100 \text{ mEq/L}}{0.1}$$

Determination of total acidity

Titration was further continued using against 0.01N NaOH phenolphthalein as indicator, until a permanent pink colour was observed. The volume of 0.01N NaOH consumed was noted. Total acidity was also calculated by using the same formula as that of free acidity.

Statistical Analysis

All the data are expressed as Mean±SEM. The values obtained for the above parameters in extracts were compared with control group using one way analysis of variance (ANOVA) followed by Dunnett's 't' test. The values of $p<0.05$ and $p<0.01$ were considered to indicate a significant difference between the groups.

RESULTS

Extraction

124.45 g of dried extract was obtained from 960 g of dried seed powder. Therefore, % yield of HECT was found to be 12.9%.

Preliminary Phytochemical Screening

In the present study, the preliminary phytochemical investigation of the extract revealed the presence of flavonoids, anthraquinone glycosides, tannins and saponins.

Ethanol induced gastric ulceration

Effect of HECT on gastric content, pH, free acidity and total acidity is shown in Table 1. Ethanol caused the accumulation of gastric secretions of 2.16±0.44 ml with pH 2.84±0.25 in Control group. Free acidity and Total acidity in the control group were found to be 70.83±2.38 mEq/l and 151.67±7.03 mEq/l, respectively. Significant reduction in mean ulcer score was observed as compared to rats pretreated with distilled water (Control) which clearly produced linear haemorrhagic streaks in the glandular portion of stomach mucosa (Figure 1). Pretreatment with HECT, significantly ($p<0.05$) reduced the volume 0.93±0.16 and 0.83±0.31 ml and raised the pH to 3.35±0.32 and 3.68±0.27 at 250 mg/kg and 500 mg/kg dose, respectively. Free acidity and Total acidity reduced very significantly ($p<0.01$) at all the 3 doses of extract. Significant reduction in mean ulcer score was observed as compared to rats pre-treated with distilled water (Control) which produced linear haemorrhagic streaks in the glandular portion of stomach mucosa. Pre-treatment with HECT suppressed the formation of ulcers with increasing doses i.e. 125 mg/kg, 250 mg/kg and 500 mg/kg with % protection of 23.45, 54.62 and 56.33%, respectively. The effect of HECT was found to be comparable to that of Omeprazole 20 mg/kg, used as reference standard which showed % protection of 69.69%. HECT at a dose of 125 mg/kg significantly ($p<0.05$), while at doses 250 mg/kg and 500 mg/kg, very significantly ($p<0.01$), reduced the formation of gastric ulcers (Table 2, Figure 2-5). Thus, HECT showed dose dependent cytoprotective effect.

DISCUSSION

In the present study, *Cassia tora* antiulcer activity was evaluated by employing ethanol induced gastric ulcer models in albino rats. HECT pre-treatment showed significant antiulcer activity against gastric ulcers in increasing order of doses.

Ethanol induced gastric ulcer model was employed to study the cytoprotective effect of the extract. Ethanol induced gastric lesion formation may be due to stasis in gastric blood flow which leads to the development of the haemorrhage and necrotic aspects of tissue injury. Alcohol immediately penetrates the gastric mucosa

apparently causing cell and plasma membrane damage leading to increased intra cellular membrane permeability to sodium and water. The large intracellular accumulation of calcium represents a major step in the pathogenesis of gastric mucosal injury, which leads to cell death and exfoliation. There is much evidence that the ethanol damage to the gastrointestinal mucosa starts with micro-vascular injury, namely disruption of the vascular endothelium resulting in increased vascular permeability, oedema formation and epithelial lifting. These effects are secondary to ethanol induced slowing or cessation of gastric mucosal flow. Ethanol also induces a marked contraction of the circular muscles of rat fundic strip. Such a contraction may lead to mucosal compression at the site of the greatest mechanical stress, at the crests of mucosal folds leading to necrosis and ulceration.

This reduces the secretion of bicarbonates and production of mucus and increases the neutrophil infiltration into the gastric mucosa. These neutrophils adhere to endothelial cells, causing blockage of capillaries and induce damage to the endothelial cells through the release of proteases, leukotriene (LTC_4) and oxygen free radicals[15-17]. These oxygen free radicals also cause increased lipid peroxidation which causes damage to cell and cell membranes, thereby playing a major role in pathogenesis of acute mucosal injury induced by ethanol[18]. Ethanol enhances the oxygen radical attack on proteins at the lipophilic side chain of amino acids[19]. In addition, it exhibits an increase in the cytosolic concentration of low molecular weight chelatable iron derivatives which further aggravates oxidative stress[20,21]. Free radical scavenging activity of *Cassia tora* may be responsible for protection against ethanol induced gastric ulcers.

Table 1: Effect of Hydroalcoholic extract of *Cassia tora* seeds (HECT) on volume of gastric juice, pH, free acidity and total acidity in ethanol induced gastric ulcer

Group	Volume of gastric juice (ml)	pH	Free acidity (mEq/l)	Total acidity (mEq/l)
Control	2.16±0.44	2.84±0.25	70.83±2.38	151.67±7.03
Omeprazole 20 mg/kg	0.71±0.16**	3.86±0.48	36.50±1.36**	79.16±3.96**
HECT 125 mg/kg	1.10±0.19	3.00±0.33	57.66±0.91**	112.50±3.81**
HECT 250 mg/kg	0.93±0.16*	3.35±0.32	44.83±1.79**	91.50±2.43**
HECT 500 mg/kg	0.83±0.31*	3.68±0.27	41.16±2.24**	85.83±1.53**

Values are expressed as (Mean ± SEM), n=6, *p<0.05 **p<0.01 when compared with control group.

Table 2: Effect of Hydroalcoholic extract of *Cassia tora* seeds (HECT) on mean ulcer score, ulcer index and percent protection in ethanol induced gastric ulcer

Group	Mean ulcer score	Ulcer Index	% Protection
Control	5.50±0.22	11.68	-
Omeprazole 20 mg/kg	1.08±0.71**	3.54	69.69
HECT 125 mg/kg	2.66±1.03*	8.94	23.45
HECT 250 mg/kg	1.50±0.89**	5.3	54.62
HECT 500 mg/kg	0.41±0.23**	5.1	56.33

Values are expressed as (Mean ± SEM), n=6, *p<0.05 **p<0.01 when compared with control group.

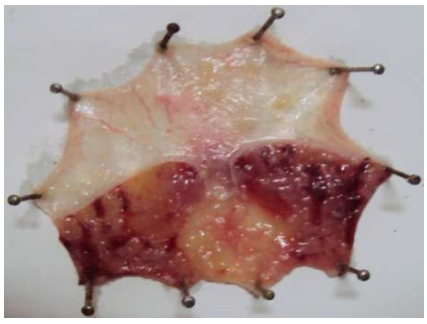

Fig. 1: Control group

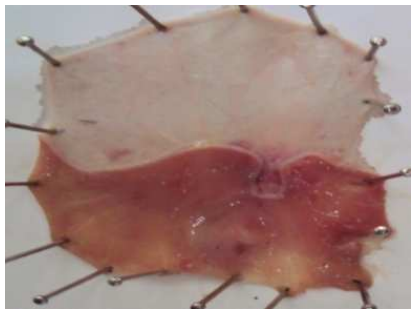

Fig. 2: Omeprazole 20 mg/kg treated group

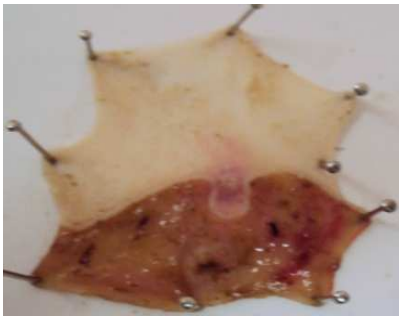

Fig. 3: HECT 125 mg/kg treated group

Fig. 4: HECT 250 mg/kg treated group

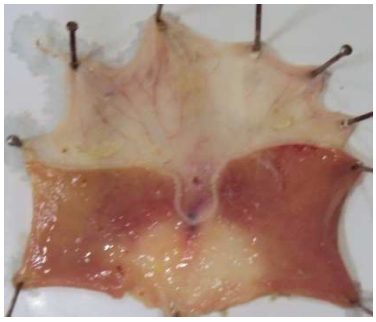

Fig. 5: HECT 500 mg/kg treated group

CONCLUSION

In conclusion the results obtained from the present study demonstrated that HECT exhibit considerable antiulcer, antisecretory and cytoprotective activity. This supports the traditional use of *Cassia tora* in the treatment of ulcer. *Cassia tora* may be a new alternative remedy for clinical management of gastric ulcer diseases. Though, we have not studied the active principles of *Cassia tora* responsible for its gastro protective activity, it is likely that flavonoids or tannins might be involved in this action as both of these have been reported to be involved in gastro protective activity. Tannins have astringent action, precipitating proteins of mucosal membranes and skin and some of them suppress the gastric secretion, having a local action of protection of the gastric mucosa[22,23]. However, it is difficult to explain the exact mechanism of action underlying. Therefore, further study is needed to explain the exact mechanism of anti-ulcerogenic activity of *Cassia tora*.

REFERENCES

1. Valle DL. Peptic ulcer diseases and related disorders. In: Braunwald E, Fauci AS, Kasper DL, Hauser SL, Longo DL, Jameson JL, editors. Harrison's Principles of Internal Medicine, 16th ed. New York: McGraw-Hill; 2005. p. 1746-62.
2. Dharmani P, Palit G. Exploring Indian medicinal plants for antiulcer activity. Indian J Pharmacol 2006; 38(2):95-99.
3. Sachin SS, Archana RJ. Antiulcer activity of methanol extract of *Erythrina indica* Lam. leaves in experimental animals. Phcog Res 2009; 1(6):396-401.
4. Nair V, Arjuman A, Gopalakrishna HN, Dorababu P, Mirshad PV, Bhargavan D, *et al*. Evaluation of the anti-ulcer activity of NR-ANX-C (a polyherbal formulation) in aspirin & pyloric ligature induced gastric ulcers in albino rats. Indian J Med Res 2010; 132:218-23.
5. Mahajan N, Sakarkar D, Sanghai D. Evaluation of Anti-ulcer potential of leaves of *Jasminum grandiflorum* L. Int J Ph Sci 2009; 1(2):247-49.
6. Goel RK, Sairam K. Antiulcer drugs from indigenous sources with emphasis on *Musa sapientum*, Tamrabhasma, *Asparagus racemosus* and *Zingiber officinale*. Indian J Pharmacol 2002; 34(2):100-110.
7. Singh R, Madan J, Rao HS. Antiulcer activity of black pepper against absolute ethanol induced gastric mucosal damage in mice. Phcog Mag 2008; 4(15):232-36.
8. Chauhan NS. Medicinal and aromatic plants of Himachal Pradesh. Indus Pub. Co. New Delhi; 1999. p. 151-52.
9. Anonymous. The Wealth of India A dictionary of Indian raw materials & Industrial products, Publications & Information Directorate, C.S.I.R., Vol.3, p. 368-70.
10. Manojlovic I, Bogdanovic-Dusanovic G, Gritsanapan W, Manojlovic N. Isolation and Identification of anthraquinones of *Caloplaca cerina* and *Cassia tora*. Chemical Pap 2006; 60(6):466-68.
11. Foster S, Chongxi Y. Major Chinese medicinal herbs-weeds, In: Herbal Emissaries, Bringing Chinese herbal to the west. Healing arts Press, Vermont; 1992. p. 311-17.
12. Jain S, Patil UK. Phytochemical and pharmacological profile of *Cassia tora* Linn.- An Overview. Indian J Nat Prod Res 2010; 1(4):430-37.
13. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. 31st ed. Nirali Prakashan; 2005, p. 593-97.
14. Robert A, Nezamis JE, Lancester C, Hanchar AJ. Cytoprotection by prostaglandins in rats. Gastroenterology 1979; 77: 433-43.
15. Bardi DAA, Sarah Khan MA, Sabri SZ, Kadir FA, Mahmood AA, Zahra AA, *et al*. Anti-ulcerogenic activity of *Typhonium flagelliforme* aqueous leaf extract against ethanol-induced gastric mucosal injury in rats. Scientific Research and Essays 2011; 6(15):3232-39.
16. Ohya Y, Guth PH. Ethanol-induced gastric mucosal blood flow and vascular permeability changes in the rat. Dig Dis Sci 1988; 33(7):883-88.
17. Alrdahe SS, Abdulla MA, Razak SA, Kadir FA, Hassandarvish P. Gastroprotective activity of *Swietenia mahagoni* seed extract on ethanol-induced gastric mucosal injury in rats. World Acad Sci Eng Tech 2010; 67:883-87.
18. Shetty R, Vijay Kumar K, Naidu MUR, Ratnakar KS. Effect of *Gingko biloba* extract on ethanol-induced gastric mucosal lesions in rats. Indian J Pharmacol 2000; 32(5):313-17.
19. Remmer H, Kessler W, Einsele H, Hintze TH, Toranzo GDD, Gharaibeh AM, *et al*. Ethanol promotes oxygen-radical attack on proteins but not on lipids. Drug Metab Rev 1989; 20(2-4):219-32.
20. MacDonald RA, Baumslag N. Iron in alcoholic beverages. Possible significance for hemochromatosis. Am J Med Sci 1964; 247(6):649-54.
21. Halliwell B. Free radicals and metal ions in health and disease. Proc Nutr Soc 1987; 46(1):13-26.
22. Parmar NS, Parmar S. Antiulcer potential of flavonoids. Indian J Physiol Pharmacol 1998; 42(3): 343-51.
23. Bhalke RD, Giri MA, Anarthe SJ, Pal SC. Antiulcer activity of ethanol extract of leaves of *Sesbania grandiflora*(Linn.). International Journal of Pharmacy and Pharmaceutical Sciences 2010; 2 Suppl 4:206-208.