EVALUATION OF ANTIULCER ACTIVITY OF *CASSIA TORA* LEAF EXTRACT USING ETHANOL INDUCED ULCER MODEL IN RATS

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Summary

The antiulcer activity of hydroalcoholic extract of *Cassia tora* leaves (HECT) was evaluated in albino rats using ethanol induced gastric ulcer model. The parameters taken to assess the antiulcer activity were ulcer score, ulcer index, gastric juice volume, pH, free and total acidity. The extract of leaves of *Cassia tora* showed dose dependent antiulcer activity with maximum activity at 500 mg/kg body weight. The effect at this dose was found to be comparable with that of reference standard, Omeprazole 20 mg/kg. The mechanism of this gastroprotective activity may be attributed to free radical scavenging effect along with presence of tannins which can suppress the gastric secretion.

Keywords: Antiulcer, *Cassia tora*, ethanol, Omeprazole.

Introduction

Peptic ulcer is defined as disruption of the mucosal integrity of the stomach and/or duodenum leading to a local defect or excavation due to active inflammation. If it is located in the stomach it is called Gastric ulcer. It has been shown that patients with gastric ulcers tend to have a lower gastric acid output compared to normal subjects suggesting poor mucosal defense component as a reason, accompanied by decreased mucosal blood flow, delayed gastric emptying or impaired epithelial restitution. However, gastric ulcers occurring near the pylorus may be associated with combined *H. pylori* infection plus hyperacidity. Peptic ulcers occur because of an imbalance between aggressive factors (gastric acid and pepsin) and defensive factors (gastric mucus, bicarbonate, PGs)[1-3]. Free radicals have been implicated in the pathogenesis of peptic ulcer and a wide variety of clinical disorders and physical, chemical and psychological factors also contribute in this regards[4].

Cassia tora is a medicinally important plant used in traditional medicine for the treatment of various ailments. It belongs to the family Caesalpinaceae. In India it is found as a rainy season weed[5,6]. The leaves, roots and even the whole plant are employed in the treatment of impetigo, ulcers, helminthiasis and as a purgative. The powdered leaves are applied to ulcers and to parasitic skin conditions. A fresh leaf decoction may be used as lotion for the same purpose[7].

Pharmacological evaluation regarding antiulcer activity of *Cassia tora* Linn. leaves is not yet carried out and there is a need to establish the scientific basis for which these claims are made. So, the basic aim and objective of the present study is to test the efficacy of the leaves of *Cassia tora* in healing gastric ulcers with a view to produce an anti-ulcer drug from natural origin.

Materials and Methods

Collection of Plant Material

Plant samples were collected from roadsides of Bahadurgarh-Jhajjar Road, Haryana in the month of August, 2010. It was identified and authenticated for identification and were then positively identified by Dr. H.B. Singh, 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).

Extraction

Leaves were separated, shade dried and were coarsely powdered. It was then passed through 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. %age yield was calculated. It was then stored in an air tight container in refrigerator for further experimental studies.

Phytochemical Screening

The extract obtained was subjected to Preliminary Phytochemical screening for detection of various plant constituents[8].

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 fed with commercially available rat feed 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).

Anti-ulcer Studies

HECT was evaluated for anti-ulcer activity using ethanol induced gastric ulcer model in albino rats.

Ethanol-induced gastric ulcer model

This method was performed according to the method of Robert *et al*[9]. 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 per 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 will be made as follows[10]

Normal coloured stomach..... (0)
Red coloration..... (0.5)
Spot ulcer.....…… (1)
Hemorrhagic streak..... (1.5)
Ulcers ≥3≤ 5……. (2.0)
Ulcer > 5…… (3.0)
Mean ulcer score for each animal will be expressed as ulcer index. The percentage of ulcer protection was determined as follows:

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:
% 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 microcentrifuge 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:

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

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 ANOVA followed by Dunnett's test. The values of $p<0.05$ and $p<0.01$ were considered to indicate a significant difference between the groups.

Results

Extraction

74.5 g of dried extract was obtained from 735 g of dried leaf powder. Therefore, % yield of HECT was found to be 10.13%.

Preliminary Phytochemical Screening

In the present study, the preliminary phytochemical investigation of the extract revealed the presence of tannins, carbohydrates, triterpenoids and saponins.

Effect of HECT on Ethanol induced gastric ulcers

In the present study, HECT was evaluated for anti-ulcer activity. Effect of HECT on gastric volume, pH, free acidity and total acidity is shown in table 1.

Table 1. Effect of HECT on gastric volume, pH, free acidity and total acidity in ethanol induced gastric ulcers.

Sr. No.	Groups	Volume of gastric juice (ml)	pH	Free Acidity (mEq/l)	Total Acidity (mEq/l)
1.	Control	2.16±0.44	2.84±0.25	70.83±2.38	151.67±7.03
2.	Omeprazole 20 mg/kg	0.71 ±0.16**	3.86±0.48	36.50±1.36**	79.16±3.96**
3.	HECT 125 mg/kg	1.65±0.47	2.65±0.32	55.60±2.42*	110.00±3.41**
4.	HECT 250 mg/kg	0.83±0.16*	3.16±0.44	27.20±2.47**	58.16±2.08**
5.	HECT 500 mg/kg	0.75±0.21**	3.31±0.28	21.40±0.67**	46.66±1.30**

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

HECT significantly reduced the volume of gastric juice, free and total acidity as compared to the control group. 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 (Table 2). Treatment with plant extract suppressed the formation of ulcers with increasing doses i.e. 125 mg/kg, 250 mg/kg and 500 mg/kg with % protection of 35.70, 38.69 and 54.28, 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%. Of these, HECT at a dose of 250 mg/kg and 500 mg/kg, significantly ($p<0.05$) and very significantly ($p<0.01$), respectively, reduced the formation of gastric ulcers. Thus, HECT showed dose dependent cytoprotective effect.

Table 2. Effect of HECT on ulcer score, ulcer index and percent protection in ethanol induced gastric ulcers.

Sr. No.	Groups	Mean ulcer score	Ulcer Index	% Ulcer inhibition
1.	Control	5.50±0.22	11.68	-
2.	Omeprazole 20 mg/kg	1.08±0.71**	3.54	69.69
3.	HECT 125 mg/kg	2.83±0.90	7.51	35.70
4.	HECT 250 mg/kg	2.33±0.73*	7.16	38.69
5.	HECT 500 mg/kg	1.91±0.90**	5.34	54.28

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

Discussion

Antiulcer activity of *Cassia tora* was evaluated by using ethanol induced gastric ulcer model. 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 contributes to the development of the haemorrhage and necrotic aspects of tissue injury. Alcohol rapidly penetrates the gastric mucosa apparently causing cell and plasma membrane damage leading to increased intra cellular membrane permeability to sodium and water. The massive intracellular accumulation of calcium represents a major step in the pathogenesis of gastric mucosal injury. This leads to cell death and exfoliation. Studies suggest 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[11-13]. These effects are secondary to ethanol induced slowing or cessation of gastric mucosal flow[14]. Ethanol also produces a marked contraction of the circular muscles of rat fundic strip. Such a contraction can lead to mucosal compression at the site of the greatest mechanical stress, at the crests of mucosal folds leading to necrosis and ulceration[15].

This reduces the secretion of bicarbonates and production of mucus and also leads to increased neutrophil infilteration into the gastric mucosa. These neutrophils adheres to endothelial cells, thereby blocking capillaries and induce damage to the endothelial cells through the release of proteases, leukotriene (LTC_4) and oxygen free radicals[16-21].

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[22]. In addition, ethanol elicits an increase in the cytosolic concentration of low molecular weight chelatable iron derivatives which further aggravates oxidative stress[23,24].

It is possible that the antioxidant effect of the *Cassia tora* might also play a role in the mechanism of the antiulcer activity. The phytochemical screening of HECT leaves extract showed the presence of tannins, saponins and triterpenoids. The above effects of HECT may also be due to presence of tannins. These compounds have astringent action, precipitating proteins of mucosal membranes and skin. Some tannins suppresses the gastric secretion, having a local action of the protection of the gastric mucosa[25]. In conclusion, HECT possess significant gastroprotective effect against ethanol induced gastric ulcers. This gastroprotective effect is attributed to both antisecretory and cytoprotective mechanism of action.

Conclusion

In conclusion the results obtained from the present study demonstrated that HECT *e*xhibit antiulcer effect and possess cytoprotective property. The present study supports the traditional claims of the use of *Cassia tora* leaves in treatment of ulcer. The mechanisms of its gastro protective activity may be attributed to free radical scavenging effect along with presence of tannins which can suppress the gastric secretion. Further study is needed to identify the phytoconstituents responsible for these pharmacological actions of *Cassia tora*.

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