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EVALUATION OF ANTI-ULCER ACTIVITY OF TINOSPORA CORDIFOLIA IN ALBINO RATS

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ABSTRACT

This study was conducted to evaluate anti-ulcer activity of *Tinospora cordifolia* in albino rats. Albino rats of wistar variety of either sex weighing 140-165gms were evenly divided into different treatment groups. The aqueous extract of *Tinospora cordifolia* was investigated for its anti- ulcer activity against pylorus ligation, aspirin induced and ethanol induced gastric ulcer in rats at 400mg/kg body weight p.o. In pylorus ligated rats, *Tinospora cordifolia* extract has shown significant ($P<0.01$) reduction in gastric volume, total acidity & ulcer index as compared to control. There was also significant ($P<0.01$) reduction in ulcer index seen among *Tinospora cordifolia* extract treated rats of aspirin and ethanol induced models. The anti-ulcer activity was further confirmed by histopathological examination of rat stomach. Thus the present study concludes *Tinospora cordifolia* extract having potential anti- ulcer activity in the three models tested.

KEY WORDS:*Tinospora cordifolia*, **pylorus ligation, aspirin, ethanol**

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INTRODUCTION

Peptic ulcer disease is one of the common diseases affecting mankind. Man has lived with peptic ulcers since ancient times. Perhaps the first description of this malady is the one inscribed on the pillars of the temple of Aesculapius at Epidarus from around fourth century B.C[1].Peptic ulcer results probably due to an imbalance between the aggressive factors (acid-pepsin) and defensive factors (gastric mucus and bicarbonate secretion)[2].In the past few decades increased knowledge about the pathophysiology of peptic ulcer has paved the path for understanding the possible cause like decreased mucosal defense (cytoprotection) in the presence of normal acid secretion. The recognition of the role of Helicobacter pylori in peptic ulcer provided the therapeutic insight that eliminating this bacterium would be a useful strategy for promoting healing of ulcers and preventing the recurrence[1]. There is lots of evidence from experimental data suggesting the generation of oxygen derived free radicals and lipid peroxidation as one of the mechanism in the pathogenesis of peptic ulcer[3]. Therefore the scope for research into the therapy for peptic ulcer has widened considerably. Even though the objectives of therapy remain same viz. relief of pain, promotion of ulcer healing, prevention of recurrences and complications, there will be a definite role for new drugs.The biological and experimental evaluation of plants based on their use in the traditional system of medicine is a sound and cost effective strategy to develop new drugs from plants.*Tinospora cordifolia*(guduchi) is one such plant advocated in Ayurveda for various clinical conditions. It is said to relieve thirst and is used as an appetizer and digestive agent. It is recommended for gastrointestinal diseases including dyspepsia, flatulence, jaundice, splenomegaly and hemorrhoids[4,5].Hence this study was undertaken to elucidate the anti-gastric ulcer activity of *Tinospora cordifolia* (Guduchi).

MATERIALS AND METHODS

The present study was conducted at Gauhati Medical college & Hospital after obtaining permission from the Institutional animal ethics committee before carrying out experiments on the animals.

(i) Preparation of Tinospora cordifolia extract

Aqueous extract of *Tinospora cordifolia* was prepared by soxhlet extraction. The stem of *Tinospora cordifolia* with its bark was collected and cut into big pieces of about 15-20cm and washed with distilled water, then it was minced and air dried. Then it was powdered mechanically and made ready for extraction. The powder was weighed and inserted into the central tubule of the extractor and aqueous extract of *Tinospora cordifolia* was prepared by soxhlet extraction[6]. The extract was concentrated using a water bath and a yield of 11.4% (w/w) was obtained.

(ii) Animals

Albino rats of wistar variety weighing 140-165gms were used in the experiment. The animals of either sex were evenly divided into different treatment groups. The present study followed three models to evaluate antiulcer activity of *Tinospora cordifolia* extract.

1) Pylorus ligation method
2) Aspirin induced method
3) Ethanol induced method

(iii)Pylorus ligation method[7]

Animals were divided into 3 groups A, B & C with 6 animals in each group. All the animals in group A received normal saline & group B received ranitidine (20mg/kg) as standard & group C received *Tinospora cordifolia* extract (400mg/kg)[8] for 7 days along with standard diet before pylorus ligation. On the seventh day, half an hour after saline or drug treatment in 36 hours fasted rats, pylorus was ligated under light ether anaesthesia as per the method Shay

et al. The Post operative period was deprived of food and water and after 6 hours animals were sacrificed by ether overdosing and stomach was dissected out after ligating its cardiac end and cut open along the greater curvature. Stomach contents were collected and measured for volume, centrifuged and subjected to analysis for total acidity and inner surface was examined for any ulceration both macroscopically and microscopically. The ulcer index was calculated as given below[9].

Ulcer index = 10/X
Where X = Total mucosal area / Total ulcerated area

To measure the mucosal area & ulcerated area dissected stomach was spread on cardboard with the mucus surface upwards avoiding corrugation. Tracing paper was placed over the stomach and the outline of stomach and the areas of erosions & ulceration were traced on it[10].The gastric juice was collected 6 hours after pyloric ligation[11] and the total acidity of the gastric juice was determined as given below[12]:

$$\text{Acidity} = \frac{\text{volume of NaOH} \times \text{Normality} \times 100}{\text{Eq/l.100gm. 0.1}}\ \text{m}$$

(iv) Aspirin induced method

Aspirin is suspended in 1% carboxy methyl cellulose in water (20 mg/ml) and administered orally (gavage) in a dose of 500 mg/kg in 36 hours fasted rats in all the 3 groups. Along with aspirin group A receives normal saline as control, group B receives ranitidine (20mg/kg) as standard & group C receives *Tinospora cordifolia* extract (400mg/kg). Four hours later the animals were sacrificed. The stomachs were removed and opened along the greater curvature to determine the ulcer index as explained before[13]

(v) Ethanol induced method

Group A received normal saline 30min before administration of 1ml ethanol (99.9%) by gavage and was taken as control; Group B received ranitidine 20mg/kg once daily orally for six days and 30min prior to ulcerogen on the seventh day. Group C received *Tinospora cordifolia* extract (400mg/kg) once daily for seven days and 30min prior to ulcerogen on seventh day.The animals in all the groups were fasted for 24hrs prior to the administration of ulcerogen, with water ad libitum. Animals were sacrificed one hour after the administration of ethanol and stomach was dissected out and examined for ulceration[13]. Ulcer indices were calculated as described above[10].

(vi) Statistical analysis

Results were analyzed by one way analysis of variance (ANOVA), followed by Dunnet's multiple comparison test. All the results are expressed as Mean ± SD. Significance was established when the probability value was less than 0.05.

(vii) Histopathological studies

After macroscopic examination, the stomachs were immersed in 10% formalin solution for 24 hours. A strip of gastric wall was cut from the forestomach to the pylorus through the entire glandular mucosa, necessarily including red streaks or sites of ulceration. This sample was subsequently processed for the preparation of sections (4-5mm thick) after embedding in paraffin wax and staining with haematoxylin and eosin using routine techniques[14].

RESULTS

In pylorus ligation induced gastric ulcer the Tinospora cordifolia extract has shown significant ($P<0.01$) reduction in gastric volume, total acidity & ulcer index when compared with control group (Table 1). There is also significant reduction in ulcer index in *Tinospora cordifolia* extract treated rats of aspirin and ethanol induced models when compared with control group (Table 2).

Table 1
Effect of Tinospora cordifolia extract on gastric secretion, total acidity & ulcer index in Pylorus ligated rats:

Groups	Treatment	Mean ulcer index± SD	Mean volume of Gastric secretion ± SD	Total acidity (mEq/l/100gm)
A	Normal saline	0.1308 ± 0.04	2.633 ± 0.62	79.53 ± 3.34
B	Ranitidine	0.0234± 0.004*	1.316 ± 0.360 *	44.25 ± 4.67 *
C	*Tinospora cordifolia* (400mg/kg)	0.0348 ± 0.005*	1.483 ± 0.541 *	47.41 ± 3.87 *

Values are mean ± SD ; (n = 6)
**** P< 0.01 when compared with control***

Table 2
Effect of Tinospora cordifolia extract on ulcer index in aspirin and ethanol induced ulcer models:

Groups	Treatment	Mean ulcer index ± SD (By aspirin induced method)	Mean ulcer index ± SD (Ethanol induced method)
A	Normal saline	0.3305 ± 0.06	0.4366 ± 0.07
B	Ranitidine	0.0536 ± 0.01*	0.1863 ± 0.05*
C	*Tinospora cordifolia* (400mg/kg)	0.0564 ± 0.008*	0.0788 ± 0.01*

Values are mean ± SD ; (n = 6)
*** P < 0.01 when compared with control**

Histopathology of rat stomach

In histopathological examination of stomach specimens of group A from all the models it was seen that there was extensive gastric damage, even involving all the layers of the stomach wall in some regions. The mucosal epithelial cells were completely eroded and there was severe infiltration by inflammatory cells. The submucosal layer was edematous and engorged blood vessels could be seen. The muscular layer was also edematous. (Figure 2) .However the albino rats from groups B and C treated with *Tinospora cordifolia* extract and ranitidine respectively, did not show any such findings of extensive gastric damage in all the models (Figure 3 & 4).

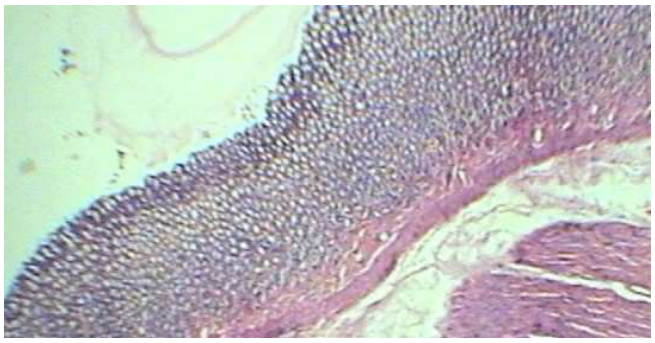

Figure 1
Section showing normal gastric mucosa of the rat (Mag. X100)

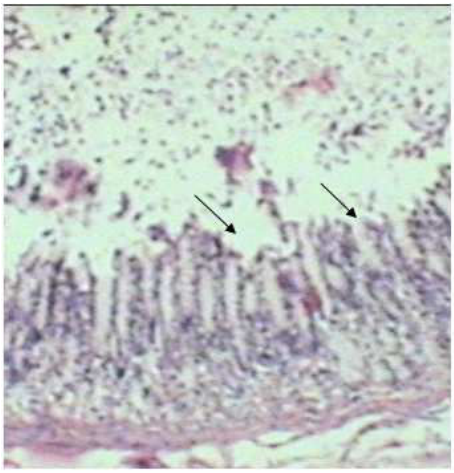

Figure 2
Section showing ulcerated gastric mucosa of rat treated with ethanol (Arrows showing mucosal breach) (Mag. X100)

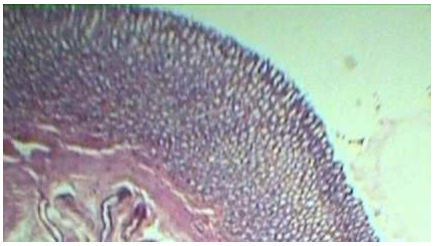

Figure 3
Section through gastric mucosa of rat treated with ranitidine showing no significant changes (Mag. X100)

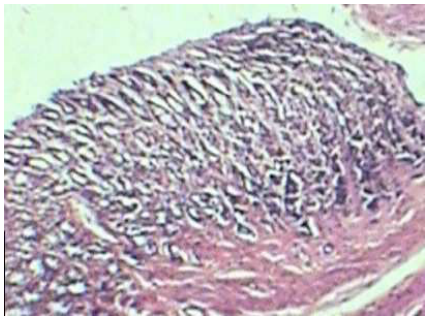

Figure 4
Section through gastric mucosa of rat treated with Tinospora cordifolia extract showing no significant changes (Mag. X100)

DISCUSSION

The cause of gastric ulcer after Pylorus ligation is believed to be due to stress induced increase in gastric hydrochloric acid secretion and or stasis of acid. According to Shay et al, the volume of secretion is also important factor in the formation of ulcer due to exposure of the unprotected lumen of the stomach to the accumulating acid.In the present study, in

pylorus ligation induced gastric ulcer model, *Tinospora cordifolia* extract treated group has shown significant (P<0.01) reduction in gastric volume, total acidity & ulcer index thus indicating anti-secretory mechanism involved in the extract for their anti-ulcerogenic activity.In the second model, gastric mucosal damage by NSAID (Aspirin) is used. This model was chosen because NSAID abuse is the main exogenous cause of refractory peptic ulcer constituting 39% of the cases of peptic ulcer[15]. NSAIDs produce a spectrum of injury to the gastroduodenal mucosa, from hemorrhages and petechiae to erosions and ulcers.Aspirin is known to inhibit Cyclo-oxygenase enzyme leading to reduced production of PGE and Endothelial cell PGI which is required for maintaining mucosal lining by increasing the mucosal blood flow and by stimulation of mucus and bicarbonate secretion in the stomach[16,17]. It can also cause mast cell degranulation resulting in the release of histamine. Tissue damaging free radicals which are produced from the conversion of hydro-peroxy to hydroxy fatty acids further contributes to cell destruction[18].In the present study *Tinospora cordifolia* extract treated group has shown significant (P<0.01) reduction in gastric mucosal damage by aspirin when compared to that of the control group which may be by potentiating the defense factors.*Tinospora cordifolia* extract has also shown protection against ethanol induced gastric mucosal damage where the reason for gastric lesion by ethanol has been attributed to free radical damage which results in lipid peroxidation products[18]. The protective role of *Tinospora cordifolia* may be due to the presence of flavonoids as one of their constituents[19]. Flavonoids are said to possess antioxidant properties[20]. An isoprenyl flavonoid known as sofalcone has been extensively studied for its anti ulcer potential. As mentioned earlier prostaglandins has shown to be involved in its anti ulcer and cytoprotective effects. Sofalcone has been shown to inhibit the activity of PG metabolizing enzyme 15-Hydroxy-PG-Dehydrogenase and thereby increasing the PGE_2 content of the gastric mucosa in rats subjected to absolute ethanol induced gastric mucosal damage and Taurocholate induced gastritis[21]. This may be a possible mechanism for protection against ethanol induced gastric mucosal damage.Oxidative stress is one of the factors in gastric mucosal damage. Preventive anti-oxidants, such as superoxide dismutase (SOD) and catalase (CAT) enzymes are the first line of defense against reactive oxygen species[22]. A study by Bafna et al have shown that administration of a herbomineral formulation (Pepticare) in which *Tinospora cordifolia* is one of the constituent has resulted in a significant increase in the SOD, catalase and reduced glutathione levels as compared to the control animals, which suggests its efficacy in preventing free radical-induced damage[23].Mast cell degranulation with histamine release is one of the factors in gastric mucosal damage[24]. A study by Nayampalli et al have reported that aqueous extract of *Tinospora cordifolia* has afforded protection against histamine induced bronchospasm in guinea pigs[25]. *Tinospora cordifolia* extract might have protected the gastric mucosa from damage probably by interfering with the release of histamine.Thus, from the above discussion it is seen that *Tinospora cordifolia* extract has anti-ulcer property by different mechanisms

CONCLUSION

Thus the present study concludes Tinospora cordifolia having anti-ulcer activity in the models tested. Since *Tinospora cordifolia* is already being used by the Ayurvedic physicians, further clinical studies can be taken up and it may be used as an adjunct to the present drugs in the treatment of peptic ulcer.

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