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# EVALUATION OF ANTI – ULCER ACTIVITY OF *TINOSPORA CORDIFOLIA* AGAINST EXPERIMENTALLY INDUCED GASTRIC ULCERS IN RATS

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**ABSTRACT**

The purpose of the study was to investigate the antiulcerogenic property of *Tinospora cordifolia* Miers in aspirin and ethanol induced gastric ulceration models in albino rats and to compare its efficacy with the standard drug ranitidine. 48 albino rats of either sex, weighing 130-200g were selected and divided into groups of 6 animals of either sex randomly. Aspirin, suspended in 1% carboxymethyl cellulose in water was administered in a dose of 500mg/kg orally, ethanol (99.9%) was administered in a dose of 1ml and ranitidine was given in the dose of 20mg/kg. The test compound – *Tinospora cordifolia* was suspended in 1% carboxy methyl cellulose in water (1ml = 100mg) and was given in the doses of 400mg/kg and 600mg/kg. In each group the total score, mean score, standard deviation, standard error of mean, P Value, ulcer index and ulcer incidence were calculated. Statistical analysis was performed using the Student 't' test and Wilcoxan rank test . On evaluation, *Tinospora cordifolia* was found to possess statistically significant ($p<0.05$) antiulcerogenic effect in both aspirin and ethanol induced gastric ulceration models. But, when compared to standard control (ranitidine) statistical significant difference was not seen in case of aspirin induced gastric ulceration. In case of ethanol model, when compared with ranitidine group, *Tinospora cordifolia* in the dose of 400mg/kg did not show any significant difference, but in the dose of 600mg/kg showed a statistically significant ($p<0.05$) better protection. Hence, it can be concluded that *Tinospora cordifolia* pretreatment has provided significant protection against both aspirin and ethanol induced gastric mucosal lesions. At the dose of 600mg/kg, its antiulcerogenic effect was found to be better than ranidine against ethanol induced gastric lesions.

**Key words:** *Tinospora cordifolia*, gastric ulcer, experimental animals, aspirin, ethanol.

## INTRODUCTION

Peptic ulcer is one of the commonest gastrointestinal disorder in clinical practice (Bafna PA and Balaraman R, 2005). Ulceration occurs when there is a disturbance of the normal equilibrium caused by either an increase in the acid secretion, a decrease in the gastric mucosal resistance or protection and due to an induction of oxidative stress. (Bairy KL *et al*., 2001) There are some factors which can predispose to peptic ulceration including smoking, high consumption of alcohol and intake of certain medications such as non-steroidal anti-inflammatory drugs, stress and nutritional deficiencies. Although mortality rates of peptic ulcer are low, the high prevalence of the disease, the accompanying pain and its complications are very troublesome. (Mohamed Morsy and Azza El-Sheikh, 2011). The therapeutic goal of treating peptic ulcer disease is to relieve pain, heal the ulcer and prevent ulcer recurrence. (Lawande YS *et al*., 2011) Usage of medications available for treatment and prevention of peptic ulcer is faced with several drawbacks in the form of limited effectiveness, numerous side effects and the high cost of medications. Therefore a number of

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traditional anti-ulcer drugs are being investigated for the prevention as well as in treatment of peptic ulcer. (Mohamed Morsy and Azza El-Sheikh., 2011) These traditional, herbal compounds are supposed to have the additional advantage of being safer, cheaper and usually having limited, if any, side effects. They also have better patient acceptability and are easily available. (Kaur D *et al*., 2012)

One such herbal remedies, *Tinospora cordifolia* has been used for its antiulcer activity.( Bairy KL *et al* 2001) *Tinospora cordifolia* (Willd.) Hook. f. and Thoms. *(Guduchi)* is a large, glabrous, deciduous climbing shrub belonging to the family Menispermaceae. It is distributed throughout the tropical Indian subcontinent and China, ascending to an altitude of 300 m. 'Guduchi', the Sanskrit name, means one which protects the entire body. The term 'amrita' is attributed to its ability to impart youthfulness, vitality and longevity. It is also used as a 'rasayana' to improve the immune system and body resistance against infections. The whole plant is used medicinally; however, the stem is approved for use in medicine as listed by the Ayurvedic Pharmacopoeia of India. This is due to higher alkaloid content in the stems than in the leaves. (Upadhyay AK *et al*., 2010) The present study was aimed to investigate the antiulcer properties of the starch known as Giloe-ka-sat obtained from the stem of *Tinospora cordifolia* against gastric lesions induced by aspirin and ethanol.

## MATERIALS AND METHODS

### Plant Material

Fresh stems of *Tinospora cordifolia* were collected from locally available resources and were thoroughly washed with tap water to remove adhering impurities. For preparing the satwa, these stems were cut into small pieces and crushed thoroughly. This pounded mass was kept for soaking overnight (12 h) with four times of potable water. Next morning this mass was macerated thoroughly with hands for about 1 hour, filtered slowly through a clean folded cotton cloth. The liquid was kept aside undisturbed for 4 hours, the supernatant liquid was carefully drawn out . White starchy sediment, which had settled at the bottom, was collected, air dried under running fan and stored in airtight glass jars.

*Animals*: Forty eight (48) albino (Wistar) rats of either sex weighing 130-200g bred at the Central Animal House at KIMS, Hubli under standard laboratory conditions were used. The animals were fed with standard pellet diet and had free access to water. The experimental protocol was approved by the Institutional Animal Ethics Committee and conducted according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India

### Dose of drugs and test compounds

Doses of the drugs were calculated for each animal based on the body weight and respective volumes were administered orally. Aspirin (Astra – IDL) was suspended in 1% carboxymethyl cellulose in water and administered in a dose of 500mg/kg orally (gavage), (8) Ethanol (99.9%) was administered in a dose of 1ml, Ranitidine (Astra –IDL) was suspended in 1% carboxymethylcellulose (1ml = 25mg) and was given in the dose of 20mg/kg. (9)

Test Compound, *Tinospora cordifolia* was suspended in 1% carboxy methyl cellulose in water (1ml = 100mg) and was given in the doses of 400mg/kg and 600mg/kg (as estimated by a preliminary study)

### Experimental Design

48 albino rats of either sex, weighing 130-200g were selected. They were fed standard diet and were divided into groups of 6 animals of either sex randomly.

In the present study, animals were divided into following 8 groups

Group I (Control) – Aspirin 500mg/kg.
Group II (Standard control) – Ranitidine 20mg/kg + Aspirin 500mg/kg.
Group III (Test compound A) – T. cordifolia 400mg/kg + Aspirin 500mg/kg.
Group IV (Test compound B) – T. cordifolia 600mg/kg + Aspirin 500mg/kg.
Group V (Control) – Ethanol 1ml (99.9%).
Group VI (Standard control) – Ranitidine 20mg/kg + Ethanol 1ml (99.9%).
Group VII (Test compound A) – T. cordifolia 400mg/kg + Ethanol 1 ml (99.9%).
Group VIII (Test compound B) – T. cordifolia 600mg/kg + Ethanol 1 ml (99.9%).

Group I and Group V were taken as control and received the ulcerogen only.

Group II and group VI were taken as standard control. All other groups received the test compound in the doses specified.

The animals in Group I, II, III and IV were fasted for 36 hours, the compound under investigation were administered orally, 1 hour before aspirin.

In case of Group V, VI, VII and VIII the animals were starved for 24 hours and the compound under investigation were administered by gavage 30 minutes before administration of ethanol. ((Parmar NS and Desai JK, 1993)

During the period of fasting the animals had free access to drinking water. 4 hours after administration of aspirin and 1 hour after ethanol administration. (Samant AR *et al*.,1998) the animals were sacrificed. The anterior abdominal wall was opened and the stomach dissected out.

The stomach was opened along the greater curvature, and mounted on a moist cork board, the mucosal surface was examined and scored according to the method described by Laurence and Bacharach. (Bonny Castle DD, 1964)

0-Normal
1-Scattered haemorrhagic spots
2-Deeper haemorrhagic spots and some ulcers
3-Haemorrhagic spots and ulcers
4-Perforation

Ulcer Index was calculated for each group by the method of Sunita and Devdas. (Jain S and Santani D., 1996)

$$\text{Ulcer Index} = \text{Arithmetic mean of the } 2 + \left( \frac{\text{Ulcer positive No} \times 2}{\text{Total No. of Rats}} \right) \text{ intensity in a group}$$

In each group the total score, mean score, standard deviation, standard error of mean, P Value, ulcer index and ulcer incidence were calculated.

**Statistical analysis**

The significance of the difference between mean values for various treatment were tested using the Student 't' test and Wilcoxan rank test . $P<0.05$* was taken significant and $P<0.01$** as highly significant.

**RESULTS**

In this study aspirin produced gastric lesions in all the six animals, Ulcer Incidence was 83.5% and the Ulcer Index was 3.84 (Table 1). In ranitidine pretreated animals (group II), gastric lesions were produced only in four animals, Ulcer Incidence was 16.7% and the Ulcer Index was 1.33, when compared to group I was statistically significant (*$P<0.05$). In case of animals pretreated with T. cordifolia (group III and group IV, the gastric lesions were seen in four animals of each group, the Ulcer Incidence being 33.3% and 16.7% and the Ulcer Index 1.67 and 1.18 respectively for each group. When compared with group I, the results were statistically significant (*$P<0.05$) but when compared group II (Ranitidine group) statistical significance was not seen.

Ethanol also produced gastric lesions in all the six animals, Ulcer Incidence being 100% and Ulcer Index 4.66 (Table 2). Pretreatment with T. cordifolia at the dose of 400mg/kg produced gastric lesions in all animals, but the Ulcer Incidence was 50% and Ulcer Index was 2.5. At the dose of 600mg/kg, T.cordifolia produced lesions in four animals, Ulcer Incidence was reduced to 16.7% and Ulcer Index to 1.16. When compared with Group V, the results obtained with T. cordifolia 400mg/kg and 600mg/kg were significant. Ranitidine pretreatment also reduced the Ulcer Incidence to 83.3% and Ulcer Index to 3.83 which was statistical significant (*$P<0.05$) when compared to Group V. When compared with Ranitidine group T. cordifolia in the dose of 400mg/kg (Group VII) did not show any significance, but the Group VIII i.e. T. cordifolia in the dose of 600mg/kg showed a statistically significant (*$P<0.05$) reduction.

**Table 1. Effect of Test Drugs on Aspirin Induced Gastric Mucosal Damage**

| Group | Treatment | Total Score | Mean Score | Ulcer Incidence (%) | Ulcer Index |
|---|---|---|---|---|---|
| I | Aspirin 500mg/kg | 13 | 2.17 | 83.5 | 3.84± 0.31 |
| II | Ranitidine 20mg/kg + Aspirin 500mg/kg | 6.0 | 1.0 | 16.7 | 1.33*± 0.26 |
| III | T. cordifolia 400mg/kg + Aspirin 500mg/kg | 6.0 | 1.0 | 33.3 | 1.67*± 0.36 |
| IV | T. cordifolia 600mg/kg + Aspirin 500mg/kg | 5.0 | 0.84 | 16.7 | 1.18*± 0.31 |

*(p<0.05*, p<0.01**)*

**Table 2. Effect of Test Drugs on Ethanol Induced Gastric Mucosal Damage.**

| Group | Treatment | Total Score | Mean Score | Ulcer Incidence (%) | Ulcer Index |
|---|---|---|---|---|---|
| V | Ethanol 1ml | 16 | 2.66 | 100 | 4.66+ 0.21 |
| VI | Ranitidine 20mg/kg + Ethanol 1ml | 11 | 1.83 | 83.3 | 3.83*+ 0.17 |
| VII | T. cordifolia 400mg/kg + Ethanol 1ml | 9.0 | 1.5 | 50 | 2.5*+ 0.22 |
| VIII | T. cordifolia 600mg/kg + Ethanol 1 ml | 5.0 | 0.83 | 16.7 | 1.16** + 0.31 |

*(p<0.05*, p<0.01**)*

## DISCUSSION

In the present study, the protective effect of T. cordifolia was studied in two models of aspirin and ethanol induced gastric mucosal damage in albino rats. Non steroidal anti-inflammatory drugs (NSAID) abuse is an important exogenous cause of refractory peptic ulcer constituting 39% of the cases of peptic ulcer. NSAIDs can cause a spectrum of injury to the gastroduodenal mucosa, ranging from hemorrhages and petechiae to erosions and ulcers. Aspirin, an NSAID, by inhibiting Cyclo-oxygenase enzyme leads to decreased production of PGE and endothelial cell PGI. NSAIDs can also cause mast cell degranulation resulting in the release of histamine. There is a lot 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. (Desai JK *et al*., 1997) In the aspirin induced gastric ulceration model of our study, pretreatment with T. cordifolia gave significant protection. The protective effect may be due to its inhibitory action on PG metabolizing enzyme 15-hydroxy - PG - dehydrogenase resulting in the elevation of the $PGE_2$ content of the gastric mucosa. (Konturek SJ *et al.,* 1986) A study by Nayampalli *et al* have earlier reported that aqueous extract of *Tinospora cordifolia* has afforded protection against histamine induced bronchospasm in guinea pigs (Nayampalli SS *et al* 1986). *Tinospora cordifolia* extract might have protected the gastric mucosa from damage probably by interfering with the release of histamine. The protective role of T. cordifolia may also be due to presence of flavonoids as one of its constituents. Flavonoids are said to possess antioxidant properties. (Parmar NS and Parmar S, 1998).

*Tinospora cordifolia* demonstrated significant anti-ulcer property in aspirin model probably acting by different mechanisms but when compared to the standard drug ranitidine, the protection afforded by T. cordifolia in aspirin induced gastric ulceration did not show statistical significance which means that T. cordifolia is equally or less effective when compared to ranitidine.

Ethanol induced gastric mucosal damage was seen in the glandular portion of the stomach as red streaks or black necrotic areas. Vascular injury is the early pathogenetic factor in the development of ethanol induced gross hemorrhagic erosions, which may be related to direct effect of ethanol and its metabolites on endothelial cells. Increased vascular permeability as a result of endothelial lesions occur very early (within 1 min) in ethanol induced injury. Ethanol induced gastric lesions have been attributed to free radical damage as well.(Desai JK *et al*., 1997) The protective effect of *Tinospora cordifolia* may be due to its prevention of oxidative damage. (Saha S, Ghosh S, 2012) and induction in the levels of various endogenous antioxidant enzymes. (Bafna PA, Balaraman R., 2011) In a study conducted by Nayampalli *et al* it has been reported that the capillary permeability was reduced significantly when T. cordifolia was administered orally to mice.(Nayampalli SS *et al.,* 1986).

Thus in the present study, in case of ethanol induced gastric ulceration, pretreatment with T. cordifolia showed significant results. When compared to ranitidine pretreated group, T. cordifolia in the dose of 600mg/kg showed statistically significant protection, which means that T. cordifolia in the doses of 400mg/kg and 600mg/kg is protective against ethanol induced gastric mucosal damage. At the dose of 600mg/kg it offers more protection than ranitidine.

Hence, in our study T. cordifolia pretreatment has given significant gastric mucosal protection against ethanol induced gastric mucosal lesions than aspirin induced lesions (Table 11, 13). Ranitidine pretreatment has given better protection in aspirin induced ulcers than in ethanol induced ulcers. This difference in protection by T. cordifolia in ethanol and aspirin induced lesions might be because of different mechanisms by which these two agents produce gastric mucosal lesions and it may be that T. cordifolia acts by more than one mechanism.

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