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RESEARCH ARTICLE

Gastro Protective effect of Standardized Ethanolic leaf extract of *Indigofera tinctoriao* on experimental Gastric Ulcers in Rats

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ABSTRACT:

Objective:The anti-ulcer activity of ethanolic extract of *Indigofera tinctoria* (Fabaceae) leaf (EEIT) was investigated in Indomethacin and ethanol induced ulcer models in wistar rats. **Methods:** The preliminary Phytochemical evaluation was carried out by standard methods. Indomethacin and ethanol induced ulcer models the common parameter determined was ulcer index. Animals are divided in to four groups. First groups serves as control, second group treated with standard drug third and fourth group treated with EEIT at doses of 200 and 400 mg/kg p.o produced significant inhibition of the gastric lesions induced by Indomethocin induced ulcer and Ethanol induced gastric ulcer. **Results**: Phytochemical evaluation shows that alkaloids and flavonoids are present in extract.The extract (200 mg/kg and 400 mg/kg) showed significant ($P<0.01$) reduction in gastric volume, free acidity and ulcer index as compared to control. This present study indicates that *Indigofera tinctoria* extract have potential anti ulcer activity in the both models.Cellular level changes was reveled with histopathological studies. **Conclusion:**These results may further suggest that ethanolic extract was found to possess antiulcerogenic as well as ulcer healing properties, which might be due to its antisecretory activity.

KEYWORDS: *Indigofera tinctoria*, Indomethacin, Ethanol induced ulcer model, ulcer index.

INTRODUCTION:

Gastric and duodenal ulcers are illnesses that affect a considerable number of people in the world and they are induced by several factors for example: stress, smoking, nutritional deficiencies and ingestion of non steroidal-anti-inflammatory drugs[1]. The etiliology of gastro duodenal ulcers are influenced by various aggressive and defensive factors, such as acid-pepsin secretion, parietal cell, mucosal barrier, mucus secretion, blood flow, cellular regeneration and endogenous protective agents (prostaglandins and epidermic growth factor).

The bacterium Helicobacter pylori is an etiological agent for chronic active gastritis and plays a major role in effecting peptic ulcers[2] .Antacids and/or antibiotic agents against Helicobacter pylori bacterium and active substances with cytoprotective mechanism are very important in the treatment of peptic ulcer. Pharmacological management of gastric ulceration was mainly directed at the reduction and/or neutralization of gastric acid. Secretion despite evidence, According to clinical respect, the patients who suffered from this diseases were found to be either normal or below normal secretions of hydrochloric acid and pepsin. Several alternate hypotheses concerning the pathogenesis of gastric ulcer disease indicate the possible involvement of alternate targets apart from acid secretion thereby necessitating the detail pharmacological investigation. In the traditional systems of the Indian medicine extract of this plant used for the management of several hepatic and nervous disorders. indirubin, component responsible

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for the anticancer activity of *Indigofera tinctoria* yield marked inhibition of Lewis lung cancer carcinoma and walker carcinoma there by suggesting that the plant possess significant anti neoplastic activity *Indigofera tinctoria* are plays a necessary role as a protective mechanism against carcinogenesis and eliminating damaged cells or abnormal excess cell proliferated owing to various chemical agents induction[3]. Indirubin active constituent from the leaves of this *Indigofera tinctoria* having anti proliferate activity. *Indigofera tinctoria* Linn. Were collected separately at different growth stages and analyzed for their rotenoid content found in eight week old tissue after fresh sub culturing and minimum at two weeks. The toxicological studies of in vivo and in vitro extract against the pulse beetle (Callosobruchus chinensis) and mosquito (Anopheles stephensi) larvae, showed that rotenoids were more effective against mosquito larvae than Callosobruchus chinensis. In India, *Indigofera tinctoria* is the source of an important blue dye stuff. The plant contains alkaloids, glycosides and oleoresin. Its action is anti-microbial. Wild Indigo is an herb to be considered wherever there is a focus of infection externally the ointment will help infected ulcers and sore nipples. A douche of the decoction will help leucorrhoea. Mummified remains from Egyptian tombs show that a mixture of Henna and Indigo (*Indigofera tinctoria*) shoots called Henna reng, was used to co lour hair and false beards to a youthful blue-black, a practice that is still found in India today (M. Stuart et al., 1986). It is the leaves that yield the dye, and they are soaked in vats filled with water for several weeks, the solution turning deep yellow. This oxidizes to form an insoluble precipitate of indigo, used to intensify the black hair of Indian women who soak the dried (or fresh) leaves in water and usually apply it mixed with henna as a paste.

In the limelight of its traditional use, the present investigation was carried out to evaluate the Anti-ulcer activity of ethanolic extract of *Indigofera tinctoriao*(EEIT). For this purpose, the anti-ulcer activity of was evaluated using Indomethacin (NSAID) induced Ulcer; the most commonly used experimental models for the evaluation of anti-ulcer activity in animal models. Also, the effect of EEITon gastric secretion was evaluated using the ethanol induced ulcer model. However there are no reports on the antiulcer activity of the plant hence the present study was designed to verify the claims of the native practioners.

MATERIALS AND METHODS:

Plant collection:

Fresh leaves of *Indigofera tinctoria* Linn. was collected during the month of September-December nearby the area of Thanjavur, Tamilnadu, India. India. It was identified and authentificated by Prof. P.Jayaraman Ph.D.,Director-Plant Anatomy Research Centre (PARC) Tambaram. The voucher specimen Number is PARC/ 2009/ 466 and it was submitted to institute of PRC College of pharmacy, for further reference.

Preparation of Extract:

Leaves were dried under shade, pulverized by an electric blender and passed through 40 mesh sieve. It was stored in an air tight container, away from sunlight at room temperature. The coarse powdered leaves were extracted in Soxhlet apparatus for 24h with Ethanol then concentrated and dried under reduced pressure. The extract was weighed and the yield was 15% (w/w). The semisolid mass (black colour) was obtained.

Preliminary phytochemical screening:

The phytochemical examination of the methanoloic extract of *Indigofera tinctoria* was performed by the standard methods, Phytochemical methods, a guide to modern technique of plant analysis Alkaloids were tested by the method of Dragendroff's test, carbohydrates and glycosides was tested using Borntragers test. Salkowski test was used for the identification of steroids. Proteins and flavonoids are tested by the method of biutet and Shinoda test respectively.

Pharmacological evaluation:

1. Ethanol-induced gastric ulcer:

The ulcer was induced by administering ethanol. All the animals were fasted for 36 hours before administration of ethanol[6] The rats were divided into four groups (n = 6). Group 1 served as a vehicle control, which received 5% suspension of Tween 80, whereas animals in group 2 received the reference drug omeprazole (30 mg/kg p.o.). Rats in groups 3 and 4 received ethanolic extracts 200, 400 mg/kg p.o., respectively. Thirty minutes after administer ethanolic extracts of Indigofera tinctoria, each rat was given orally 1.0 ml of a 0.3 M solution of HCl in 60% (v/v) ethanol. The animals were sacrificed one hour later. The stomach was then excised and cut along the greater curvature, washed carefully with 5.0 ml of 0.9% NaCl and the ulceration area (cm2) determined. (L. Guaraldo et al., 2001). The number and gravity of erosions were scored according to the arbitrary scale of Adami et al. (1964). The scales were as follows: (0) no ulcer; (1) hemorrhagic and slight ulcer, length <2 mm; (2) one hemorrhagic and slight ulcer, length <5 mm; (3) more than one ulcer grade 2; (4) one ulcer length <5mm and diameter <2 mm; (5) from one to three ulcers of grade 4; (6) from four to five ulcers of grade 4; (7) more than six ulcers of grade 4; (8) complete lesion of the mucosa with hemorrhage.

The following parameters were determined: (i) Ulcerative Lesion Index (ULI) as 1×(number of ulcers level I) + 2×(number of ulcers level II) + 3×(number of

ulcers level III); (ii) curative ratio, which was determined as follow: %C= 100−(IUtreated ×100/IUcontrol); (iii) total area of lesion; (iv) percentage of lesion area in relation to the total stomach area.

Mean scores for each group were calculated and expressed as ulcer index (UI). From this data, the percentage inhibition of ulceration was determined for each group and also mucus weight calculated.

2. NSAID -induced gastric ulcers in rats:[7]

Swiss albino rats were fasted for 18 h and deprived of water for 12 h. They were divided into four groups (n = 6). The animals in group 1 served as a vehicle control which received 5% Tween 80 Group 2was administered with the reference drug Misoprostol (0.012 mg/kg p.o.). Animals in groups 3 and 4 were administered with ethanolic extracts of *Indigofera tinctoria* at the doses of 200 and 400 mg/kg p.o., respectively, 1 h before the Indomethacin administration (20 mg/kg p.o.). The animals were sacrificed after 1 h. Each stomach was then opened along the greater curvature, rinsed with normal saline and examined grossly.

Scoring of ulcer will be made as follows:

Normal stomach	(0)
Red coloration	(0.5)
Spot ulcer	(1)
Hemorrhagic streak	(1.5)
Ulcers	(2)
Perforation	(3)

Mean scores for each group were calculated and expressed as ulcer index (UI). From this data, the percentage inhibition of ulceration was determined for each group and also mucus weight calculated.

Statistical analysis:

The values are represented as mean ± S.E.M, and statistical significance between treated and control groups was analyzed using of One way ANOVA, followed by Dunnett's test where $P<0.05$ was considered statistically significant.

RESULT AND DISCUSSION:

Phytochemical screening:

Phytochemical investigation of Ethanolic extract of *Indigofera tinctoria* showed the presence of alkaloids, flavonoids, tannins, proteins, saponin, carbohydrate and glycosides. These compounds scavenge the free radical and play an important role in the prevention and therapy of diseases. The details of qualitative chemical tests and phyto constituents present in the extracts are shown in Table-I.

Table:1 Phytochemical investigation of Ethanolic extract of *Indigofera tinctoria*

S.no	Phyto chemicals	EEIT
1	Alkaloids	+
2	Carbohydrates	+
3	Flavanoids	+
4	Glycosides	+
5	Proteins	+
6	Saponin	+
7	Steroids	—
8	Tannin	+

+ Indicates presence - Indicates Absence.

High dose of ethanol induced gastric mucosa of rats shows gastric lesions similar to those occurring in gastric ulcer; hence, for the evaluation of gastro protectiveactivity ethanol-induced gastric ulcers model have been widely used[12]. According to that, it was observed that ethanol administration to rats caused macroscopic lesions to gastric tissue, such as loss of normal color and mucus along with presence of petechiae, hemorrhage and edema (Figs. 2B and 3) These lesions are most likely related to mucus depletion and a constrictive effect on veins and arteries of the gastric mucosal, producing congestion, inflammation and tissue injury[8]. The reduction of gastric mucosal blood flow can result in hemorrhage and necrosis in damaged tissue[5]

In order to confirm the results of antiulcer experiment, the stomachs were also evaluated by histopathological examination (Fig. 2). In histological observation, the stomach of control animals showed no damage (Fig. 2A). However, rats 1 h after of exposure to ethanol presented damage to gastric tissue at a microscopic level. Histopathological injury caused by ethanol administration is characterized by edema and congestion of mucosal, as well as inflammatory process characterized by neutrofils infiltration (Fig. 2B). However, EEIT was able to reverse the damage caused by ethanol, probably exert potent anti inflammatory effect in gastric mucosa. This activity can be confirmed by microscopic evidence obtained in our analysis, decreasing the infiltration of inflammatory cells (neutrophils) (Fig. 2 C and D) in relation to samples from stomachs of rats that received ethanol only. Furthermore, the EEIT at 200 and 400 mg/kg was able to protect the histological structure of the gastric mucosa, preventing swelling and preventing the infiltration of inflammatory cells (neutrophils) at 100, 200 and 400 mg/kg .

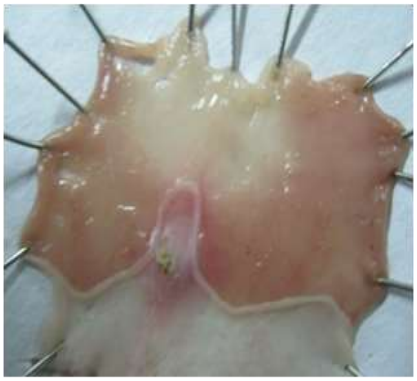
In conclusion, EEIT (200 and 400 mg/kg) were demonstrated gastric mucosal protection against oxidative injuries caused by ethanol and this protection is most likely due to ROS scavenging activity of *Indigofera tinctoria*. In addition, the presence of phenolic acids and flavonoides in EEIT certainly contribute to the antiulcerogenic activity .

Table: 2 Effect of *Indigofera tinctoria* leaf extracts on various parameters in Ethanol induced gastric ulcer.

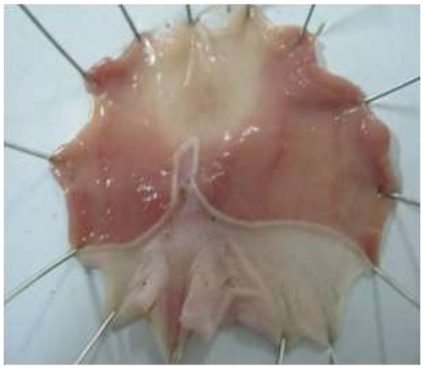
Method	Treatment	Dose (mg/kg)	Total area of lesion (mm^2)	Ulcer index	% inhibition
Ethanol/HCl	control	5% suspension of Tween 80	123.833±6.364	46.000±1.732	—
	Std	Omeperazole 30mg/kg p.o	16.500±0.428 ***	10.000±0.577 ***	78.26 %
	Test I	Ethanolic extracts 200mg/kg	57.333±3.774 ***	20.167±1.851*	56.15 %
	Test II	Ethanolic extracts 400mg/kg	29.167±1.493 ***	10.667±0.715 ***	76.81 %

Table: 3 Effect of *Indigofera tinctoria* leaf extracts on various parameters in Indomethacin induced gastric ulcer.

Method	Treatment	Dose (mg/kg)	Total area of lesion (mm^2)	Ulcer index	% inhibition
Indomethacin	control	5% suspension of Tween 80	141.500±4.924	70.833±3.664	—
	Std	Misoprostol 0.012 mg/kg	18.500±0.764 ***	16.167±1.973 ***	77.17 %
	Test I	Ethanolic extracts 200mg/kg	74.000±1.549 ***	34.000±2.394 ***	51.99 %
	Test II	Ethanolic extracts 400mg/kg	47.667±2.011 ***	21.333±1.926 ***	69.88 %

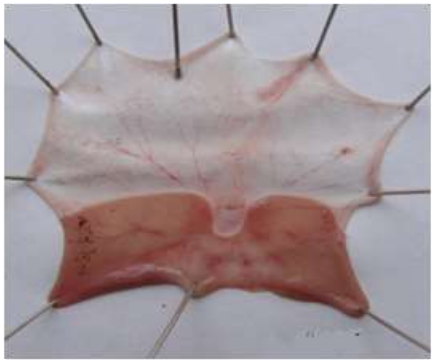


A: Section of glandular stomach of control group showing normal histology. HandE x 5.

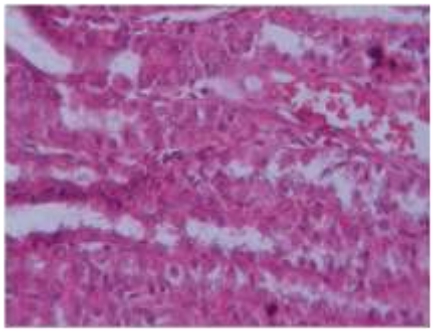


D: Gastric mucosa of standard drug treated group showing no ulceration in the mucosa of glandular stomach. HandE x 5.

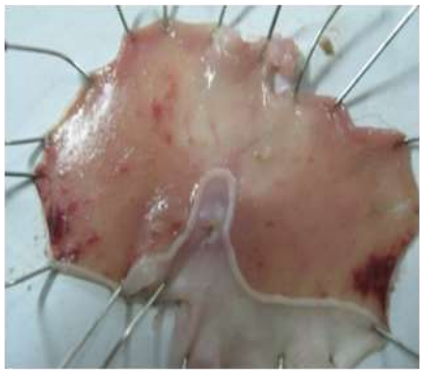
FIG: 1 TOPOGRAPHICAL VIEW OF GLANDULAR PORTION OF THE STOMACH



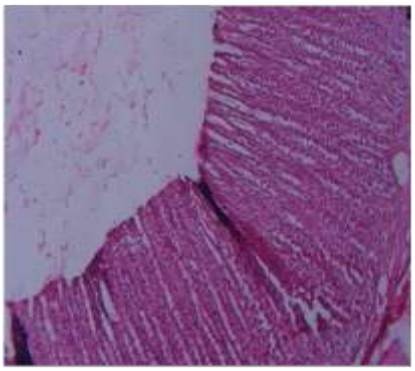
B: Ulcerated gastric mucosa of the diseased control group with frank microscopic ulcer.HandE x 5.



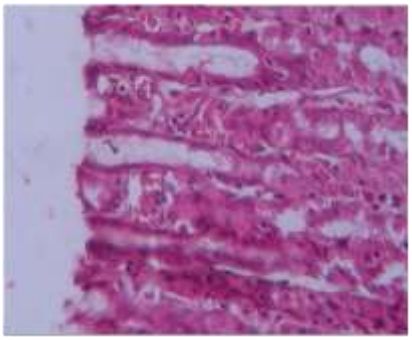
A: Section of glandular stomach of control group showing normal histology. HandE x 5.



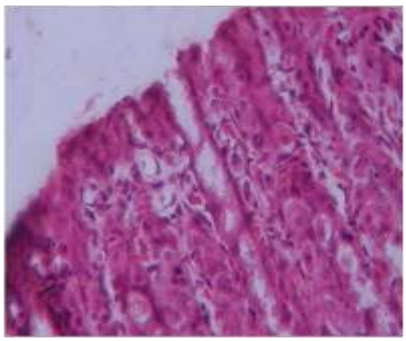
C: Higher magnification of the ulcerated mucosa of glandular stomach of diseased control. HandE x 10.



B. Ulcerated gastric mucosa of the diseased control group with frank microscopic ulcer.HandE x 5.



C: Higher magnification of the ulcerated mucosa of glandular stomach of diseased control. HandE x 10



D: Gastric mucosa of standard drug treated group showing no ulceration in the mucosa of glandular stomach. HandE x 5.

Fig.2: HISTOPATHOLOGICAL VIEW OF GLANDULAR PORTION OF STOMAC.

The results appended in the table 2 and 3 shows that the ulcer surface area and the mean ulcer index were significantly reduced in ethanol and Indomethacin induced model. Therefore, it can be thought that *Indigofera tinctoria* extracts may stimulate the secretion of prostaglandins or possess prostaglandins like-substances. Although extracts could significantly reduce the gastric acidity, the volume of gastric juice and increase the gastric juice pH, the stomach acidity was sufficiently high to be able to induce ulcer. It can then be thought that the antisecretory activity might not be the main mechanism of action of these extracts. Thus, the obvious explanation of extracts protective action is the increase of mucosal barrier defence against damaging factors. The cytoprotective action may results from strengthen the mucosal barrier through the increase of the mucus production. These results support the ethno medical uses of *Indigofera tinctoria* in the treatment of gastric ulcer.

In conclusion, EEIT (200 and 400 mg/kg) were demonstrated gastric mucosal protection against oxidative injuries caused by ethanol and this protection is most likely due to antioxidant properties of *Indigofera tinctoria* . In addition, the presence of phenolic acids and flavonoides in ceSb certainly contribute to the antiulcerogenic activity described here.

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