Research Article

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GASTROPROTECTIVE ACTIVITY OF *TEPHROSIA PURPUREA*

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ABSTRACT
The antiulcer activity of methanolic extract of *Tephrosia purpurea* (Fabaceae) aerial parts was investigated in pylorus ligation and ethanol induced ulcer model in wistar rats. Toxicity study showed 250 mg/kg as the therapeutic dose and produced significant inhibition of the gastric lesions induced by pylorus ligation and ethanol induced gastric ulcers. Ulcer index, total acidity, acid volume, pH and glutathione peroxidase was determined. The methanolic extract showed significant ($p < 0.01$) reduction in gastric volume, free acidity and ulcer index as compared to the control and the standard drugs treated group. Also shown improvement in glutathione and pH level when compared to the control. The results suggested that methanolic extract was found to posses antiulcerogenic as well as ulcer healing properties and can be used as a potent antiulcer agent.
Keywords: *Tephrosia purpurea*, pylorus ligation, ethanol induced ulcer, free acidity, total acidity, glutathione peroxidise.

INTRODUCTION

Peptic ulcers are caused by an imbalance between protective factors and damaging factors in the gastro intestinal mucosa. Peptic ulcer refers to a group of ulcerative disorders of the upper GIT which appears to have the common participation of acid pepsin in their pathogenesis[1]. Hyperacidity is influenced by nervous system factors by anxiety, other emotional states and stress. This compromise of mucosal integrity can cause pain, bleeding, obstruction, perforation and even death. There are also some other contributing causes or factors as well that may aggravate or encourage the development of ulcers, but these are fairly low risk by comparison to *H. pylori* and NSAID's[2]. Herbal medicines deals with plant and plant extracts in treating diseases. These medicines are considered safer because of natural ingredients with lesser side effects[3]. *Tephrosia purpurea* is a small shrub with stems covered with hairs, leaves alternate, with 11-17 leaflets, narrow elliptic, flower pedicel with hairs, fruit (immature) flattened, slightly curved, with several seeds[4]. *Tephrosia* is widely cultivated throughout India, especially in southern India. It has been used since ancient days for curing various diseases like hepatic disorders, kidney disorders, spleen enlargement, skin infections, dyspnoea, asthma and bleeding disorders. This plant is reported to have potent free radical scavenging property and free radical production may attenuate the reason for various diseases and disorders[5]. Hence the present study is designed to evaluate antiulcer activity of methanolic extract of *Tephrosia purpurea* aerial parts in experimental animal models.

MATERIALS AND METHODS

Dried powdered material of aerial parts of *Tephrosia purpurea* were collected from Madhava Chetty, Botanist, Sri Venkateshwara University, Tirupathi, Andhra Pradesh, India. The powdered drug was subjected to extraction with petroleum ether and methanol in a Soxhlet extractor, temperature was maintained on an electric heating mantel with thermostat control. The extracts were then concentrated to 3/4th of their original mass using rotary vapour apparatus. The concentrated extract were then transferred to a porcelain dish and evaporated on a thermostat controlled water bath till it formed a thick paste. The thick mass was vacuum dried in a dessicator till free form moisture. The alcohol extracts were administered to animals by orally as suspension by triturating with 5 % Tween 80.

Animals

Normal healthy male wistar albino rats and mice (180 – 240 g) were housed under standard environmental conditions at temperature (25 ± 2ºC) and light and dark (12: 12 h). They were fed with standard pellet diet and water *ad libitum*.

Phytochemical Test

Phytochemical tests on the extract and fractions were performed using standard procedures.[6]

Acute Toxicity Studies

All the pharmacological studies were carried out at Teegala Ram Reddy College of Pharmacy (Reg. No. 1447/PO/a/11/CPCSEA). The form B was approved by IAEC members for the title, method selected, animal species and parameters to be evaluated. The acute toxicity studies were performed to study the acute toxic effects and to determine the minimum lethal dose of the drug extracts as per the guideline OECD 423. Swiss albino mice of either sex weighing between 18-25 g were used for the study. The methanolic extracts of *Tephrosia purpurea* were administered orally to different groups of overnight fasted mice at the dose 30, 100, 300, 1000, 2000 and 2500 mg/kg body weight. After the administration of the extracts, animals were observed continuously for the first 8 h for any toxic manifestation.

Thereafter observations were made at regular intervals for 24 h. Further the animals were under investigation up to a period of one week.[7]

Pharmacological Screening

Screening of antiulcer activity by ethanol induced gastric ulcers

All the animals of ethanol treated group were fasted for 36 h before administration of ethanol. The animals in the standard drug group and *Tephrosia purpurea* (test drug) group, animals were pre-treated with respective drugs for 9 days. Later, food and water were withdrawn for 36 hours and respective drugs were administered 1 hour before ethanol administration. Ethanol (90 %) was administered to all animals at a dose of 1 ml/200 g and after 1 hour, the animals were sacrificed, stomach was removed slightly inflated by injecting 15 % formalin solution for 10 minutes. Subsequently, the stomachs were cut opened along the greater curvature and ulcer scoring was done using the dissecting microscope with a square grid eye piece. Average number of ulcers, Ulcer scoring, Percentage of ulcers and Ulcer index (UI) was calculated by using formula.[8]

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

U_N - Average No. of ulcer per animal, Us - Average of severity score, Up - % of ulcers with ulcer

The inhibition percentage was calculated by the following formula. Inhibition % = [(UI control - UI treated) /UI control] x 100. The isolated stomachs were kept in formalin solution (15 %) and then sent to the pathologist for histopathological observation and comments.

Group I: Control group, received distilled water (1 ml/kg, p.o), Group II: Pantaprazole (3.6 ml/kg, p.o) for 9 days followed by ethanol (5 ml/kg, p.o) on 11th day, Group III: Group received *Tephrosia purpurea* (250 mg/kg, p.o) for 9 days followed by ethanol (5 ml/kg, p.o) on 11th day. (Figure 1 and 2)

Screening of gastroprotective effect by pylorus ligation (Shay *et al*) method

Male albino rats weighing 180 – 210 g were housed in individual cages and fasted for 48 h allowing free access to water, care being taken to avoid coprophagy[9]. The animals were divided into 4 groups of 6 animals each. The abdomen of the animals was opened under light ether anaesthesia by a small midline incision below the xiphoid process; pyloric portion of the stomach was slightly lifted out and ligated avoiding traction to the pylorus or damage to its blood supply. The stomach was replaced carefully and the abdominal wall closed by interrupted sutures. The drugs were administered orally once daily for two days and 45minutes prior to pyloric ligation. They were deprived of both food and water during the postoperative period. The animals were sacrificed at the end of 12 hours after ligation. The stomach was dissected out tying the oesophageal end. The stomach was cut open along the greater curvature and the contents drained into the test tube, centrifuged, then subjected to various investigations like pH, free and total acidity[10]. The opened stomach was then pinned and examined using a magnifying lens for the signs of ulcer, haemorrhage and perforation. The scoring was done as described by Harrison *et al* i.e. the mean degree of damage was determined by arbitrary standards and definite values are assigned to various grades of gastrointestinal damage. Group I: Control group, received distilled water (1 ml/kg, p.o), Group II: Group received *Tephrosia purpurea* (250 g/kg, p.o), Group III: Ranitidine group (13.5 mg/kg, p.o). Ulcer scoring was assigned as 0–normal, 1–alteration in the normal rugal pattern, 2–focal hemorrhagic lesions, 4–severe hemorrhagic lesions, 6–mucosal damage, 8–well defined ulcer and 10–perforations. (Figure 3)

Statistical Analysis

Data were expressed as mean ± SEM and analysed by One way ANOVA followed by Tukey's multiple comparison tests. The significance of difference was accepted at $P < 0.01$.

Table 1: Extractive values of *Tephrosea purpurea* leaves

Solvents	Percentage yield (w/w)	Colour	Consistency
Petroleum ether	12 %	Greenish- Black	Dry mass
Methanol	50 %	Greenish- Black	Dry mass

Table 2: Screening of Antiulcer Activity by ethanol induced gastric ulcers values of Biochemical Parameters

Group	Ulcer index	Total acidity	Acid volume	pH	Glutathione
Control	13.5 ± 0.33	100.8 ± 2.4	3.13 ± 0.13	1.98 ± 0.06	4.08 ± 0.09
Ethanol + Pantaprazole (3.6 mg/kg, p.o)	7.15 ± 0.27**	36.0 ± 1.18**	2.10 ± 0.03**	4.87 ± 0.28**	6.73 ± 0.15**
Ethanol + TP (250 mg/kg)	7.6 ± 0.2*	41.5 ± 0.48*	2.41 ± 0.11*	2.98 ± 0.16	4.95 ± 0.25*

Values expressed as Mean ± SED; Number of animals in each group = 6, **P < 0.001, *P < 0.01

Table 3: Results of Ulcer Index

S. No	Groups	Ulcer Index
1	Control	13.5 ± 0.33
2	Ethanol + Pantaprazole	7.15 ± 0.27
3	Ethanol + TP	7.6 ± 0.2

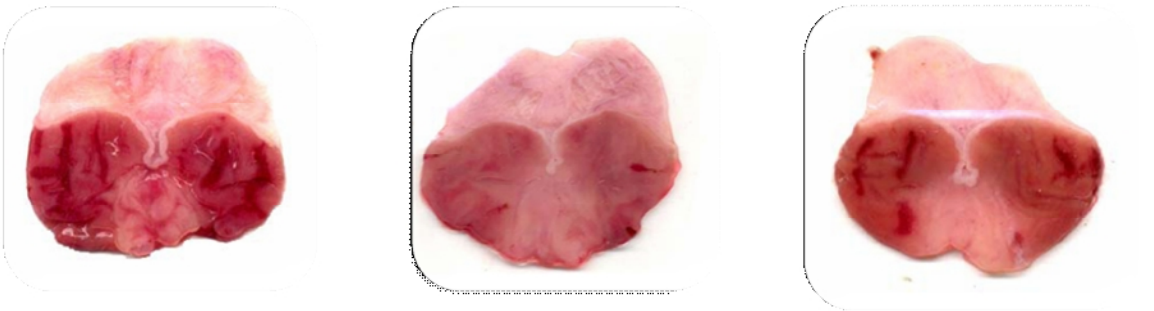
Table 4: Screening of Gastroprotective Effect by Pylorus Ligation Method

Group	Ulcer index	Total acidity	Acid volume	pH
Control	40.2±0.42	97.1±0.29	4.93±0.10	2.2±0.07
Pylorus ligation + Ranitidine	9.21±0.35**	56.8±0.4**	2.17±0.12**	4.18±0.13**
Pylorus ligation + TP	13.9±0.26*	44.1±0.86*	2.41±0.16*	2.18±0.07*

Values of biochemical parameters, Values expressed as Mean ± SED; Number of animals in each group = 6. **P < 0.001, *P < 0.01

Table 5: Results of Ulcer Index

S. No.	Groups	Ulcer Index
1	Control	40.2 ± 0.42
2	Pylorus ligation + Ranitidine	13.9 ± 0.26
3	Pylorus ligation +TP	17.2 ± 0.2

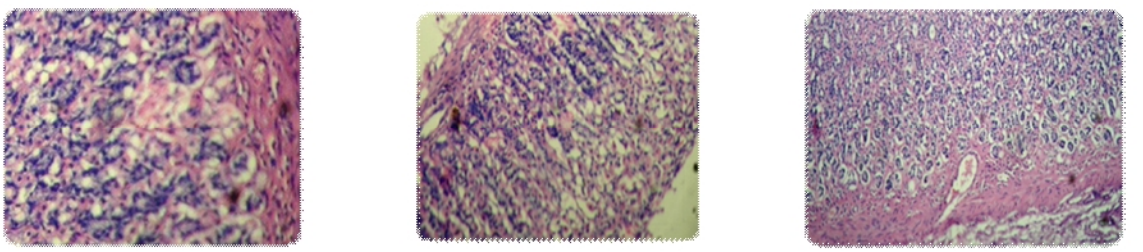


Stomach of control animals received distilled water

Stomach of animals treated with pantaprazole

Stomach of animal treated with *Tephrosia purpurea*

Figure 5: Screening of Antiulcer Activity by ethanol induced Gastric Ulcers

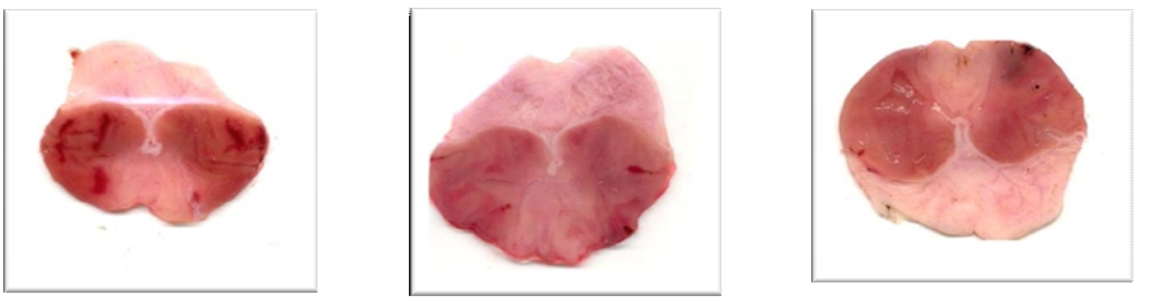


Section of rat stomach received distilled water

Section of rat stomach treated with pantaprazole

Section of rat stomach treated with *Tephrosia purpurea* extract

Figure 2: Screening of antiulcer activity by ethanol induced gastric ulcers: Histopathology of rat stomach

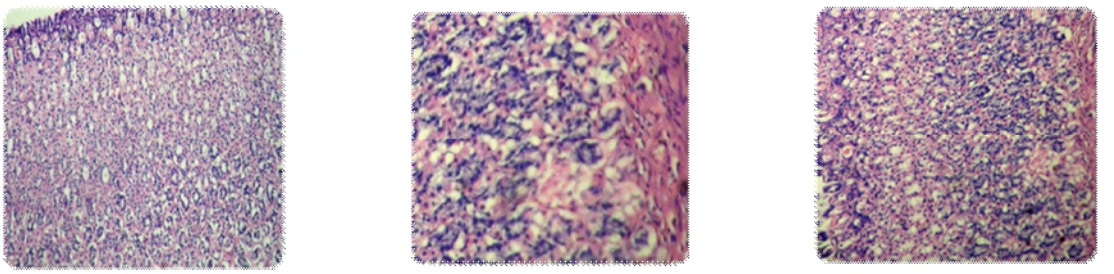


Stomach of control animal

Stomach of animal treated with ranitidine

Stomach of animal treated with *Tephrosia purpurea* extract

Figure 3: Screening of Gastroprotective effect by Pylorus Ligation Method



Section of rat stomach received distilled water | Section of rat stomach treated with ranitidine | Section of rat stomach treated with *Tephrosia purpurea* extract

Figure 4: Screening of Gastroprotective effect by Pylorus Ligation Method: Histopathology of rat stomach

RESULTS

The extractive value indicates the amount of phytoconstituents soluble in the particular solvents used and the percentage yield is given Table 1. Phytochemical screening showed the presence of phytosterols, saponins, alkaloids, flavonoids, phenolic compounds, gums and mucilage in the extract. In toxicity study at the dose of 2500 mg/kg the extract produced mortality and that dose was taken as the lethal dose. The therapeutic dose was fixed from the $1/10^{th}$ of the lethal dose. Hence, 250 mg/kg was fixed as the therapeutic dose. The methanolic extract produced significant ($p < 0.01$) antiulcer activity in the ethanol induced ulcer model when compared with the standard drug pantaprazole ($p < 0.001$). Also the extract produced antisecretory action in the pylorus ligation model ($p < 0.01$) compared with ranitidine the standard used ($p < 0.001$).

DISCUSSION

Peptic ulcer and gastritis have been associated with multipathogenic factors and could be due to disturbances in natural balances between the aggressive factors (e.g. acid, bicarbonate, pepsin) and maintenance of the mucosal integrity through the endogenous defense mechanism (e.g. defensive mechanisms of mucus, mucosal turnover and blood supply (mucosal barrier)[11]. Generally various non-specific methods were used to restore these imbalances including regular food intake, adequate rest and avoidance of ulcerogenic agents (e.g. tobacco, alcohol and coffee)[12]. Their aims are to attenuate and possibly block the gastric acid secretion or to enhance the mucosal defense mechanisms. The latter can be achieved through increasing mucus production, stabilizing the surface epithelial cells, or interfering with the prostaglandin synthesis. In addition, there are also drugs, such as proton pump inhibitors, histamine (H_2)-antagonists, anti-cholinergics and antacids, used in the treatment of ulcer[13]. Despite the availability of many pharmaceutical products for the treatment of gastric ulcers in the market as mentioned above, their successes were limited by presence of several adverse effects (e.g. anaphylaxis reactions, gynecomastia, hematopoietic changes, thrombocytopenia, acute interstitial nephritis, nephrotoxicity and hepatotoxicity)[14]. Due to the reported side effects of available antiulcer drugs, focused have been shifted towards natural products as the new sources of antiulcer agents. With the increasingly growing interest in natural medicine, various plants have been studied based on the traditional knowledge for their pharmacological properties and confirmed to be useful in treating and managing ulcer. Furthermore, medicinal plants have been known to be amongst the most attractive sources of new drugs, and have been shown to give promising results in treatment of various diseases including gastric and duodenal ulcers. *Tephrosia purpurea* reported to exert several pharmacological properties. It has been documented to be effective in uncomplicated NSAID's induced ulcers but it does not cure ulcers. Ethanol has been shown to increase the risk of ulcer in humans but produces potent ulceration in rats. It is believed to produce reactive species responsible for mucosal injury and lipid peroxidation, a free radical mediated process that ultimately destroys lipids membrane. The extract produced a relatively potent antiulcer activity against ethanol induced ulcer which may suggest that the plant possesses some cytoprotective actions against ethanol induced ulcer. The ulcer inhibition of *Tephrosia purpurea* further emphasises its possible cytoprotective action in this model. The effect was more pronounced that those of pantaprazole which may suggest a different mechanism of action or probably pronounced cytoprotection at higher doses. The secondary metabolites identified may also have been responsible for the antiulcer activity of this plant as flavonoids have been reported to possess antiulcer action. The reduced gastric ulceration as indicated by the reduction in the ulcer index in the pylorus ligation model. The results suggested that *Tephrosia purpurea* possesses anti-secretory potency as well as acid neutralizing effect. These effects suggest one of the mechanism through which the extract was able to protect the stomach mucosa from pylorus ligation induced damage. Oxidative stress resulting from the production of oxygen derived free radicals e.g. superoxide anion, hydrogen peroxide and hydroxyl radicals has been known to take part in the pathogenesis of various diseases including gastric ulcer and antioxidants help to protect cells from damage elicited by oxidative stress while enhancing the body's defense systems against degenerative diseases[15]. The methanolic extract of *Tephrosia purpurea* have antioxidant activity and contain various types of phytocompounds such as flavonoids, polyphenolic compounds, saponins and tannins. The gastroprotective effect exhibited was speculated to be attributed to its antioxidant property, which inturn could

be linked to the presence to the presence of flavonoids and polyphenolic compounds, saponins and tannins. These compounds most likely inhibit gastric mucosal injury by scavenging the ligation induced oxygen metabolites.

Histopathology

Specimens of the gastric walls from each rat were fixed in 10 % buffered formalin and processed in a paraffin tissue. Sections of the stomach were made at a thickness of 5 µ and stained with hematoxylin and eosin for histological evaluation. Histological section of gastric mucosa in rats treated with pantaprazole and ranitidine showed mild disruption to the surface epithelium with mild edema and leucocytes infiltration of the sub mucosal layer. No disruption to the surface epithelium with no edema and no leucocytes in the extract treated group were observed.

CONCLUSION

The present study provided preliminary data for *Tephrosia purpurea* which possesses significant antiulcer activity in animal models. It has gastric anti-secretory and acid neutralizing effects that are comparable to reference drug ranitidine and pantaprazole. The antiulcer activity is probably due to the presence of bioactive compounds like flavonoids, saponins and tannins. Further studies are required to confirm the exact mechanism underlying the ulcer healing and protecting property of the extract and to identify the chemical constituents responsible for it.

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