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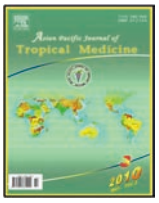

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Evaluation of antiinflammatory activity of *Tephrosia purpurea* in rats

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ABSTRACT

Objective: To evaluate the antiinflammatory activity of orally administered ethanolic extract of *Tephrosia purpurea* in acute and subacute inflammation in rats. **Methods:** An ethanolic extract of *Tephrosia purpurea* was prepared. Carrageenan induced paw edema and cotton pellet granuloma were the models for acute and subacute inflammation respectively. Four groups of rats in each model were treated orally with 2% gum acacia, 100 mg /kg of aspirin, 500 mg/kg and 1 000 mg/kg of ethanolic extract of *Tephrosia purpurea* respectively. In carrageenan induced paw edema model, subplantar injection of 1% carrageenan was made into the hind paw of the rats sixty minutes after the administration of the respective drugs. The paw volume was measured immediately after injection of carrageenan, at 3 hours and at 6 hours. Then percentage inhibition of edema was calculated. In the cotton pellet granuloma model, animals were administered drugs for six days after placing cotton pellets in the axilla on each side. On the 7th day, dry weight of granuloma was calculated. **Results:** The rats treated with *Tephrosia purpurea* did not exhibit any significant decrease in paw volume and serum ceruloplasmin levels as compared to the control and aspirin treated groups in the acute inflammation model; while, there was a significant ($P < 0.01$) decrease in the weight of granuloma in *Tephrosia purpurea* and aspirin treated groups as compared to control in subacute inflammation. **Conclusions:** The ethanolic extract of orally administered *Tephrosia purpurea* shows significant antiinflammatory effect in subacute inflammation but not in acute inflammation in rats.

1. Introduction

Tephrosia purpurea (English - purple Tephrosia, Sanskrit -sarapunkha) has been used in the traditional system of Indian medicine to treat inflammation, boils and pimples[1]. It has been shown to have antiulcer[2] and antioxidant[3] activities. There has been no study to evaluate the antiinflammatory activity of orally administered ethanolic extract of *Tephrosia purpurea* in both acute and subacute inflammation. Hence, the present study was done to assess the antiinflammatory activity of orally administered ethanolic (using 95% ethanol) extract of *Tephrosia purpurea* in acute and subacute inflammation in rats.

2. Materials and methods

2.1. Collection and preparation of ethanolic extract of *Tephrosia purpurea*[4]

The plant, *Tephrosia purpurea*, was obtained from a local shop in Udupi and verification was done by the Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal. The plant parts were cleaned, dried under shade and powdered by a mechanical grinder. The powder was loaded into soxhlet extractor (Tensil glass works, Bangalore, India) in eight batches of 250 g each and was subjected to extraction for about 30 - 40 hours with 95% ethanol. After extraction, the solvent was distilled off and the extract was concentrated under reduced pressure at a water bath temperature below 50 ℃ to a syrupy consistency. Then it was dried in the desiccator, (Quality traders, Ernakulum, Kerala) finally yielding 73 g from 2 kg of dried plant.

2.2. Animal care and handling

Healthy, albino Wistar rats (150–200) g, 12 weeks old, of either sex, bred locally in the animal house of Kasturba Medical College, Manipal were used for the study. They were housed under controlled conditions of temperature (23±2) ℃, humidity (50±5)% and 10–14 hour light and dark cycles. The animals were housed individually in

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polypropylene cages (U.N. Shah manufacturers, Mumbai, India) containing sterile paddy husk (produced locally) as bedding and were maintained on normal diet (Amrut lab animal feed, Pranav Agro Industries Ltd, Sangli, Maharashtra) and water *ad libitum*. Animals were fasted for 12 hours before the start of experiment. The study was undertaken after obtaining approval of Institutional Animal Ethics Committee.

2.3. Study design

Two models-carrageenan induced paw edema and cotton pellet granuloma - were used for acute and subacute inflammation respectively.

Four groups of animals (control, standard and two test groups) were used for each model. There were six animals in each group in each model. The drug treatment was as follows:

Group I (control) - 2 mL of 2 % gum acacia orally; Group II (standard drug) - aspirin 100 mg/kg body weight of the rat orally[5]; Group III (test drug) - ethanolic extract of *Tephrosia purpurea* 500 mg/kg body weight of the rat orally; Group IV (test drug) - ethanolic extract of *Tephrosia purpurea* 1000 mg/kg body weight of the rat orally.

The test and standard drugs were administered after dissolving them in 2% gum acacia. The dose of the test drug was selected based on previous studies[6, 7].

2.3.1. Drugs and chemicals

Gum acacia (Nice Chemicals, Cochin, India), aspirin (Sigma Chemicals, USA), carrageenan (Sigma Chemicals, USA), ethanol (Qualigens Fine Chemicals, Mumbai, India), normal saline (Fresenius Kabi India Pvt. Ltd).

2.3.2. Acute inflammation model: carrageenan induced paw oedema

Four groups of animals were treated orally with 2 mL of 2% gum acacia, 100 mg/kg of aspirin, 500 mg/kg and 1 000 mg/kg of ethanolic extract of *Tephrosia purpurea* respectively. Sixty minutes later, an injection of 0.1 mL of 1% carrageenan in normal saline was made into the subplantar region of the right hind paw of each rat in each group. The paw volume was measured immediately after injection at 0 hours, again at 3 and 6 hours by a digital plethysmometer (UGO Basille, Italy)[8,9]. The edema was expressed as an increase in paw volume. The difference in the initial volume and volume at 3 hrs indicates paw volume at 3 hrs. Accordingly, paw volume at 6 hrs was calculated. Then percentage of inhibition of edema was calculated for each group with respect to the control group as follows:

$$\text{Percentage of inhibition of paw edema} = (1 - Vt/Vc) \times 100$$

where Vc and Vt represent average paw volume of control and drug treated animals respectively.

Blood (2 mL) was collected by retro orbital puncture at 0 hrs and at 6 hrs in each rat for estimation of serum ceruloplasmin, an acute phase reactant [10].

2.3.3. Subacute inflammation model: cotton pellet granuloma

Four groups of animals were used in this model. Subacute inflammation was produced using cotton pellets[11,12]. Under aseptic precautions, an incision was made on the back of each rat in each group and sterile cotton pellets (50±1) mg were implanted subcutaneously bilaterally in the axilla under ether anesthesia. Four groups of animals were treated orally with 2 mL of 2% gum acacia, 100 mg/kg of aspirin, 500 mg/kg and 1000 mg/kg of *Tephrosia purpurea* respectively for six days. On the seventh day, animals were sacrificed; pellets were removed along with the granulation tissue and dried at 60 ℃ for 24 hrs. The net dry weight of the granuloma was determined[11].

2.4. Statistical analysis

Data of paw volume and weight of cotton pellet granuloma was analyzed by One Way Analysis of Variance (ANOVA) followed by Scheffe's test. Repeated measure ANOVA was used to analyze serum ceruloplasmin values. $P< 0.05$ was considered significant.

3. Results

3.1. Carrageenan induced rat paw edema model

In the acute inflammation model, there was no significant decrease in paw volume and serum ceruloplasmin levels of *Tephrosia purpurea* treated groups as compared to control and aspirin treated group. There was a significant ($P<0.01$) decrease in paw volume in aspirin treated group as compared to control and groups treated with *Tephrosia purpurea* (Table 1). There was a decrease in serum ceruloplasmin levels in aspirin treated group (29.21±4.41) mg/dL which was significant ($P < 0.003$) as compared to control (49.99±5.09) mg/dL and *Tephrosia purpurea* treated groups (48.09±3.32) mg/dL and (45.20±3.18) mg/dL (Table 2).

3.2. Cotton pellet granuloma model

The dry weight of cotton pellet granuloma in control, aspirin and two *Tephrosia purpurea* treated groups was (88.34±12.20) mg, (38.25±8.05) mg, (49.43±5.80) mg and (41.72±9.29) mg respectively (Table 3). There was a significant ($P<0.01$) decrease in the dry weight of granuloma in aspirin and *Tephrosia purpurea* treated groups as compared to control.

4. Discussion

The study was done to evaluate the antiinflammatory activity of orally administered ethanolic extract of *Tephrosia purpurea* in acute and subacute inflammation in rats.

Carrageenan, an irritant, is used to induce paw edema. Carrageenan induced paw edema in rats is a commonly used model to study the antiinflammatory effects of a compound in acute inflammation. The ethanolic extract of *Tephrosia purpurea* administered orally did not show any significant decrease in paw volume. Aspirin, an antiinflammatory drug, decreased the paw volume in rats.

Serum ceruloplasmin has been reported to increase in carrageenan induced paw edema in rats[13]. It was significantly decreased in rats treated with aspirin but not in *Tephrosia purpurea* treated rats. Thus, the ethanolic extract of *Tephrosia purpurea* administered orally did not exert antiinflammatory effect in acute inflammation.

The cotton pellet granuloma model for subacute

Table 1
Effect of ethanolic extract of *Tephrosia purpurea* in carrageenan induced rat paw edema.

Group/Drug	Dose	Mean increase in paw volume in mL at		Percent inhibition at	
		3h	6 h	3 h	6 h
Group I/Gum acacia	2 mL	0. 43±0.02	0. 41±0.08	–	–
Group II/ Aspirin	100 mg/kg	0.21±0.01[a]	0.18±0.02[a]	51.16	56.09
Group III/ *T. purpurea*	500 mg/kg	0.42±0.04	0.38±0.06	2.32	6.97
Group IV/*T. purpurea*	1 000 mg/kg	0.39±0.01	0.36±0. 02	9.30	12.19

T. purpurea – *Tephrosia purpurea*, values are expressed as Mean ± SEM, [a]$P < 0.01$ vs control, *Tephrosia purpurea* treated groups.

Table 2
Effect of ethanolic extract of *Tephrosia purpurea* on serum ceruloplasmin in acute inflammation.

Group/Drug	Dose	Serum ceruloplasmin (mg/dL)	
		0 h	6 h
Group I/Gum acacia	2 mL	24.95±2.90	49.99±5.09
Group II/Aspirin	100 mg/kg	23.27±4.74	29.21±4.41[a]
Group III/*Tephrosia purpurea*	500 mg/kg	28.04±6.72	48.09±3.32
Group IV/*Tephrosia purpurea*	1 000 mg/kg	31.99±6.98	45.20±3.18

Values are expressed as Mean ± SD, [a]$P < 0.003$ vs control, *Tephrosia purpurea* treated groups.

Table 3
Effect of ethanolic extract of *Tephrosia purpurea* on cotton pellet granuloma in rats.

Group/ Drug	Dose	Dry weight (mg)of granuloma	% inhibition of granuloma formation
Group I /Gum acacia	2 mL	88.34±12.20	–
Group II / Aspirin	100 mg/kg	38.25±8.05[a]	56.70
Group III /*Tephrosia purpurea*	500 mg/kg	49.43±5.80[a]	44.04
Group IV / *Tephrosia purpurea*	1 000 mg/kg	41.72±9.29[a]	52.77

Values expressed as Mean ± SEM, [a]$P < 0.01$ vs control.

inflammation represents the proliferative phase of inflammation which takes place over days. The inhibition of proliferative phase by the ethanolic extract of *Tephrosia purpurea* and aspirin could have resulted in a decrease in the weight of granuloma formation.

Therefore, the ethanolic extract of orally administered *Tephrosia purpurea* has significant antiinflammatory effect in subacute inflammation. Its antiulcerogenic properties could be an advantage. Further studies need to be conducted to compare it with antiinflammatory agents like corticosteroids which commonly cause peptic ulcer as a side effect.

Conflict of interest statement

We declare that we have no conflict of interest.

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