

ANTIINFLAMMATORY ACTIVITY OF *TEPHROSIA PURPUREA* LEAVES

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Summary

The aim of the present study was to explore the probable antiinflammatory activity of different extracts of *Tephrosia purpurea* leaves using carrageenan induced inflammation in the rat. Male Wistar rats were treated orally with normal saline (as control group) and *Tephrosia purpurea* extracts (100, 200, 400, and 600 mg/kg), 60 min before 0.1 mL 1% carrageenan injection. Paw volume was measured before and 1, 2, and 3 h after the injection of carrageenan. The results were expressed as the Mean \pm SEM and the statistical significance of differences between groups was analyzed by One Way Analysis of Variance (ANOVA) followed by Dunnett's test. The subplantar injection of carrageenan caused a time-dependent paw edema in the rat. Oral administration of *Tephrosia purpurea* extracts (100, 200, 400, and 600 mg/kg) inhibited paw swelling dose-dependently at 1, 2, and 3, h after carrageenan injection. We can conclude from the outcome of the present work that *Tephrosia purpurea* extracts exert an excellent antiinflammatory effect in rats.

Introduction

A large number of herbal drugs are reputed to have excellent medicinal value, and are in use for the treatment of several ailments. In folk medicine, various indigenous drugs are used, in single and/or in combined forms, for treating different types of inflammatory and arthritic conditions, with considerable success. Although the use of these drugs has a sound tradition, and their medicinal uses and general safety are well known to native peoples, their place has yet to be rationalized in therapeutics, using the current methodology. Scientific studies are therefore required to judge their efficacy and some of the medicinal properties popularly claimed, as well as other limitations to widen the scope of these drugs.

Chronic inflammatory diseases remain one of the world's major health problems (1-2). Inflammation is the response of living tissues to injury. It involves a complex array of enzyme activation, mediator release, and extravasations of fluid, cell migration, tissue breakdown and repair (3-4). Inflammation has become the focus of global scientific research because of its implication in virtually all human and animal diseases.

Tephrosia purpurea L. (Leguminosae), commonly known in Sanskrit as Sharapunkha, is a copiously branched, sub-erect, herbaceous perennial which occurs through out the Indian. (5) whole plant has been used to cure tumours, ulcers, leprosy, allergic and inflammatory conditions such as rheumatism, asthma and bronchitis. (6).

The aqueous extract of seeds has shown significant in vivo hypoglycaemic activity in diabetic rabbits (7). The ethanolic extracts of *Tephrosia purpurea* possessed potential antibacterial activity. The flavanoids were found to have antimicrobial activity (8). It has been reported to possess hepatoprotective, mast cell stabilizing and erythrocyte membrane integrity enhancing effect in various experimental models (9-10). Phytochemical investigations on *T. purpurea* have revealed the presence of glycosides, rotenoids, isoflavones, flavanones, chalcones, flavanols, flavones and sterols (11)

However very little pharmacological investigations of the leaves of *Tephrosia purpurea* have been conducted. Based on this, an attempt has been made to evaluate inflammatory potency of *Tephrosia purpurea*

Materials and Methods

Plant Material:

The leaves of the plant *Tephrosia purpurea* were collected from local region in July 2008. The plant material was identified and authenticated by P.G.Diwakar Botanical survey of India, Pune (Voucher No. BSI/WC/Tech/08/340)

Preparation of Extract:

The leaves were cleaned, dried under shade and powdered by a mechanical grinder. 500g of the powder was extracted with Petroleum ether, chloroform, methanol and water in order of their increasing polarity using soxhlet apparatus. The yield of extracts was 2.96%, 7.52%, 11.26% and 2.7% respectively. Methanolic and aqueous extract were dissolved in normal saline whereas Petroleum ether and chloroform extracts were prepared in 2% gum acacia prior to oral administration.

Phytochemical studies:

Freshly prepared *Tephrosia purpurea* extract was subjected to phytochemical screening tests for the detection of various constituents using conventional protocol (12-13.)

Animals:

Albino rats of Wistar strain (150-200 g) and Swiss albino mice (25-30 g) of either sex were procured from National toxicology centre, Pune. They were housed in standard polypropylene cages and kept under controlled room temperature ($24 \pm 20^{\circ}\text{C}$; relative humidity 60-70%) in a 12 h light-dark cycle. The rats were given a standard laboratory diet and water ad libitum. Food was withdrawn 12 h before and during the experimental hours. All experimental protocols were approved by the institutional animal ethics committee (IAEC).

Drugs:

The following chemicals and drugs were used: carrageenan (Sigma-Aldrich), Methanol (Qualigens, Mumbai), Petroleum ether (60-80°C, Qualigens, Mumbai), Chloroform (Qualigens, Mumbai), and Ibuprofen (Vikash Pharma, Mumbai) were used during experimental protocol

Anti-Inflammatory Activity:**Carrageenan induced hind paw edema**

The effect of oral administration of 100, 200, 400 and 600 mg/kg of all the extract of *Tephrosia purpurea*, 40 mg/kg ibuprofen or vehicle (Saline, 10ml/kg) on the hind-paw oedema induced by sub plantar injection of 0.1ml carrageenan (1% w/v) was evaluated according to the method described by Winter *et al.*, (1962) (14). In short, 0.1 mL of 1 % w/v carrageenan was injected into the sub plantar tissue of left hind paw of each rat. Swelling of carrageenan injected foot was measured at 0, 1, 2, 3 h using Plethysmometer (UGO Basile, Italy). Animals were treated with test extract 1hour before the carrageenan injection. Measurement was carried out immediately before and 3hrs following carrageenan injection. Percent inhibition of test drugs was calculated in comparison with vehicle control (100%).

Statistical analysis

Results were analyzed using One way analysis of variance (ANOVA) and expressed as Mean \pm SEM. Data was further subjected to Dunnett's test and differences between means were regarded significant at $P < 0.01$ and $P < 0.05$.

Results**Phytochemical screening**

Preliminary phytochemical analysis of the extracts revealed the presence of flavonoids, glycosides, steroids, tannins and saponins.

Carrageenan induced hind paw edema

In the present study four different types of extracts were evaluated for antiinflammatory activity using carrageenan-induced rat paw edema method and data compared with control.

In vehicle treated rats maximum increase of paw volume at 3 h was 2.45 ± 0.009 mL. The corresponding mean paw volume in the group treated with Ibuprofen (40 mg/kg p.o.) was 1.62 ± 0.001 mL. The *Tephrosia purpurea* extracts (100, 200, 400, and 600 mg/kg, p.o.) showed a significant inhibition of paw volume after 1h, 2 h, and 3 h. ($p < 0.01$). The observations are given in table 1

The Petroleum ether extract of *Tephrosia purpurea* (100, 200, 400, and 600 mg/kg, p.o.) significantly ($p < 0.01$) inhibited carrageenan induced rat paw edema. Maximum inhibition of paw edema was observed in the animals dosed at 600 mg/kg after three hours when compared to the control group. The observations are given in table 1

Incase of chloroform extract of *Tephrosia purpurea* (100, 200, 400, and 600 mg/kg, p.o.) showed a significant inhibition of paw volume after 1h, 2 h, and 3 h ($p < 0.01$). The observations are given in table 1

The aqueous extract of *Tephrosia purpurea* (100, 200, 400, and 600 mg/kg, p.o.) significantly ($p < 0.01$) inhibited carrageenan induced rat paw edema. Maximum inhibition of paw edema was observed in the animals dosed at 100 mg/kg at three hours when compared to the control group. The observations are given in table 1.

Table: 1 Effect of Different extracts of *Tephrosia purpurea* in carrageenan induced rat paw edema

Treatment (mg/kg)	Mean increase in paw volume (mL)				% Decrease in paw volume at 3 h
	0 h	1 h	2 h	3 h	
Control	0.96 ±0.01	1.52 ±0.008	1.91 ± 0.004	2.45 ± 0.009	-
IBU (40)	0.91 ±0.008	1.00 ±0.01**	1.30 ± 0.003**	1.62 ± 0.001**	52.34
PE(100)	0.94 ±0.001	1.30 ±0.001**	1.59 ± 0.001**	2.31 ± 0.007**	8.05
PE (200)	0.93 ±0.001	1.12 ±0.01**	1.40 ± 0.002**	2.12 ± 0.01**	20.13
PE (400)	0.95 ±0.002	1.09 ±0.003**	1.30 ± 0.004**	2.05 ± 0.01**	26.17
PE (600)	0.92± 0.009	1.0± 0.009 **	1.16 ± 0.02**	1.82 ± 0.01**	39.59
CH (100)	0.99 ± 0.01	1.52 ± 0.01	1.92 ± 0.006	2.40 ± 0.008	5.36
CH (200)	0.95± 0.005	1.40 ± 0.005**	1.82 ± 0.02**	2.32 ± 0.003	8.05
CH (400)	0.94± 0.004	1.41 ± 0.008**	1.72 ± 0.009**	2.25 ± 0.04**	12
CH (600)	0.93± 0.01	1.34 ± 0.01**	1.52 ± 0.01**	2.15 ± 0.05**	18.12
ME (100)	0.93± 0.01	1.41 ± 0.01**	1.60 ± 0.003**	2.19 ± 0.04**	15.43
ME (200)	0.91± 0.02	1.36 ± 0.006**	1.51 ± 0.01**	2.00 ± 0.07**	26.84
ME (400)	0.96± 0.003	1.54 ± 0.01**	1.31 ± 0.001**	1.89 ± 0.05**	37.58
ME (600)	0.94± 0.01	1.29 ± 0.008**	1.3 ± 0.01**	1.74 ± 0.06**	46.30
AQ (100)	0.94± 0.007	1.46 ± 0.006*	2.21 ± 0.005**	2.40 ± 0.04**	2.01
AQ (200)	0.91± 0.02	1.41 ± 0.01**	2.16 ± 0.01**	2.31 ± 0.01**	6.04
AQ (400)	0.97± 0.006	1.48 ± 0.02**	1.73 ± 0.01**	2.32 ± 0.03**	9.39
AQ (600)	0.94± 0.01	1.42 ± 0.01**	1.65 ± 0.01**	2.27 ± 0.04**	10.73

N= 5, treatment, mg/kg, data were analyzed using ANOVA and expressed as Mean ± SEM followed by Dunnett's test and differences between means were regarded significant at * (P<0.05), ** (P<0.01), PE: Petroleum ether, CH: chloroform, ME: methanol and AQ: aqueous extracts.

Discussion

In the present study the most interesting extract was the methanolic one. Since the inhibition percentage of carrageenan induced hind paw edema was nearer to that shown by the reference drug in our study, this indicates an interesting anti-inflammatory activity.

The petroleum ether, chloroform, Methanolic and aqueous extracts (600 mg/kg, p.o., each) exhibited 39.59%, 18.12%, 46.30% and 10.73% edema inhibition, respectively. The activity possessed by the methanolic and petroleum ether extracts was comparable to that shown by the reference drug, ibuprofen (40 mg/kg, orally). It was also observed that the percentage edema inhibition increased with increase in time interval and was found to be maximal at 3 h after carrageenan injection as shown in Table 1.

Phytochemical analysis of *Tephrosia purpurea* revealed the presence of terpenes and Sterols, glycosides, flavonoids and triterpenes have been found to be active anti-inflammatory agents at lower doses (15).

The presence of compounds extractable in nonpolar solvents may account for the pronounced anti-inflammatory activity of the petroleum ether extract. Significant activity shown by the chloroform, methanol and aqueous extracts may be due to the presence of certain polar constituents such as flavonoids, glycosides etc.

It is therefore possible that the maximum inhibitory effects on inflammation observed in the methanolic extract may be attributed in part to its flavonoid content. Flavonoids also inhibit the phosphodiesterases involved in cell activation. Much of this effect is upon the biosynthesis of protein cytokines that mediates adhesion of circulating leukocytes to sites of injury. Flavonoids inhibit biosynthesis of prostaglandins, which are involved in various immunologic responses and are the end products of the cyclooxygenase and lipoxygenase pathways (16). Protein Kinases are another class of regulatory enzymes affected by flavonoids. Inhibition of these enzymes provides the mechanism by which flavonoids inhibit inflammatory processes (17).

However, the study of the plant regarding the principal phytoconstituents responsible for the activity is under progress. It was further observed that extracts were well-tolerated in rats after oral administration, as these did not cause any death at the given dose.

Conclusion

Considerable activity has been found with petroleum ether, chloroform, methanolic and aqueous extracts. Thus from the above studies it can be concluded that *Tephrosia purpurea* leaves possess a potential anti-inflammatory property without any toxic effects.

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