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Anti-inflammatory and analgesic activities of *Tephrosia purpurea* Linn. aerial and root extracts

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ABSTRACT

An ethanolic extract of the aerial (EETPA) and root parts of *Tephrosia purpurea* Linn. (EETPR) was investigated for anti-inflammatory and analgesic activities. The extract (250, 500 mg/kg, b.w) produced dose-related inhibition of carrageenan-induced paw edema and cotton pellet-induced granuloma in rats. At the same doses, analgesic activity was also observed by tail immersion method maintained at 55°C. The results of the present study further confirms that the use of *Tephrosia purpurea* Linn. Traditionally used for the treatment of painful inflammatory pathological conditions like fracture and dislocation.

Key words: Anti-inflammatory; carrageenan induced paw, cotton pellet granulama; edema method; tail-immersion method, *Tephrosia purpurea*

INTRODUCTION

Inflammation is an important causative agent of human morbidity and mortality, such as Systemic Inflammatory Response Syndrome, Multiple Organ Dysfunction Syndrome and Multiple Organ Failure[10]. *Tephrosia purpurea* is a shrub, found throughout India. *Tephrosia purpurea* Linn. is a wild plant known as "Sarapunkha" in Sanskrit, 'Purple Tephrosia' or 'Wild indigo' in English and "Kolanji" in Tamil. *T. purpurea* used for mediated chain reaction has been implicated in the centuries of Indian traditional medicine for the treatment various inflammatory disorders. It is considered beneficial for liver, spleen and kidney disorders and also it has property to cure all types of wounds[13]. Experimental studies suggest that *Tephrosia purpurea* Linn. exerts anti-ulcer[3], anti-oxidant[18], hepatoproductive[12] and hypoglycemic activities[15].

The literature survey reveals that there is no literature related to anti-inflammatory and analgesic activities of this plant. Hence, in the present study, ethanol extracts of *Tephrosia purpurea* Linn**.** root (EETPR) and aerial parts (EETPA) are evaluated for anti-inflammatory activity in acute and sub-acute as well as analgesic activity by tail immersion method are determined. Furthermore, the activities of the plant extracts were compared with the standard indomethacin (20mg/kg, i.p).

MATERIALS AND METHODS

Plant

The plant *Tephrosia purpurea* Linn. is widely found throughout India, especially in South India. In the present work, this plant was collected in the month of March, 2008 at Chennai-600 060 and it was identified and confirmed as *Tephrosia purpurea* by Prof. P. Jayaraman, PARC, Chennai- 600 045.

Extraction of plant material

Dried finely powdered *Tephrosia purpurea* aerial/root parts (500 g) of were soaked with 1500 mL of 95% ethanol overnight[14]. The residue obtained was again resuspended in equal volume of 95% ethanol for 48 h and filtered again. The above two filtrate was mixed and the solvents was evaporated in a roto vapour at 40-50°C under reduced pressure, dark semisolid material obtained was stored at -4°C, until use. For experimental studies, known volume of the extract was suspended in distilled water[8] .

Animals

Colony in breed strains of male wistar rats weighing 150-250 g were used for the pharmacological studies. The animals were kept under standard conditions (day/night rhythm) 8.00 am to 8.00 pm at 22±1°C room temperature in polypropylene cages. The animals were feed on standard pelleted diet and tap water *ad libitum.* The animals were housed for one week in polypropylene cages prior to the experiments to accommodate to laboratory conditions. It is randomly distributed into six different groups with six animals in each group under identical conditions throughout the experiments. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC).

Anti-inflammatory activity

Carrageenan-induced paw edema

Edema was induced by injecting 0.1 ml of 1% (w/v) carrageenan suspension into the subplantar region of the right hind paw of the rats according to the method described by Winter et al[21]. The test

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Table 1. Anti-inflammatory activities of EETPR and EETPA on Carrageenan induced paw oedema in rats.

S.No	Groups	Paw volume in ml (% Inhibition Treatment of Paw Oedema) 0 hr	½ hr	1 hr	2 hr	4 hr	8 hr
1	**Group A** (control)	1.098±0.1189	1.405±0.1037	1.552±0.06814	1.663±0.4271	1.757±0.02848	1.965±0.04064
2	**Group B** (EETPR- 250)	1.083±0.02985	1.282±0.01833 **(68)**	1.330±0.02049* **(58)**	1.077±0.03029*** **(38.4)**	1.077±0.02894*** **(29.7)**	1.070±0.03044*** **(9.8)**
3	**Group C** (EETPR- 500)	1.033±0.04551	1.347±0.04551 **(69.9)**	1.3820±0.02315* **(60)**	1.457±0.01406* **(54.4)**	1.437±0.01745*** **(47.3)**	1.312±0.02613*** **(26.4)**
4	**Group D** (EETPA- 250)	1.027±0.01978	1.210±0.03296 (66.5)	1.403±0.04631*** (60.6)	1.250±0.03531*** (46.9)	1.193±0.02237*** (36.5)	1.180±0.02309*** (18.2)
5	**Group E** (EETPA- 500)	1.087±0.01978	1.325±0.03575 **(69.4)**	1.4670±0.05258* **(62)**	1.490±0.05241* **(55.5)**	1.657±0.04716*** **(54.3)**	1.667±0.02860*** **(41.9)**
6	**Group F** Indomethacin (20 mg/kg, i.p)	1.043±0.01667	1.147±0.3297* **(64.6)**	1.097±0.02155*** **(49.6)**	1.083±0.02028 **(38.7)**	1.073±0.01520* **(29.4)**	1.067±0.02459*** **(9.8)**

*Data Represent mean ± SEM of 6 animals.*P<0.05 and ***P<0.001 compared to control by Dunnett's method The number in the parenthesis indicates the percentage of anti-inflammatory activity*

Table 2. Anti-inflammatory activity of EETPR and EETPA on Cotton pellet induced granuloma

S.No	Groups	Weight of dried cotton pellet (% inhibition of granuloma)
1	**Group A** (control)	182.2±3.260
2	**Group B** (EETPR- 250)	147.3±3.490*** (23.69%)
3	**Group C** (EETPR- 500)	120.8±4.175*** **(50.82%)**
4	**Group D** (EETPA- 250)	174.8±2.688***** (4.23%)
5	**Group E** (EETPA- 500)	155.0±3.386*** (17.55%)
6	**Group F(**Indomethacin (20 mg/kg, i.p)	122.3±3.373*** (48.97%)

*Data represents mean ± SEM of 6 animals, Statistically significant from vehicle control *P<0.05 and *** P<0.001 compared to control by Dunnett's method The number in the parenthesis indicates the percentage of anti-inflammatory activity*

Figure 1. Effect of EETPR, EETPA and Indomethacin on Carrageenan induced paw edema in rats.

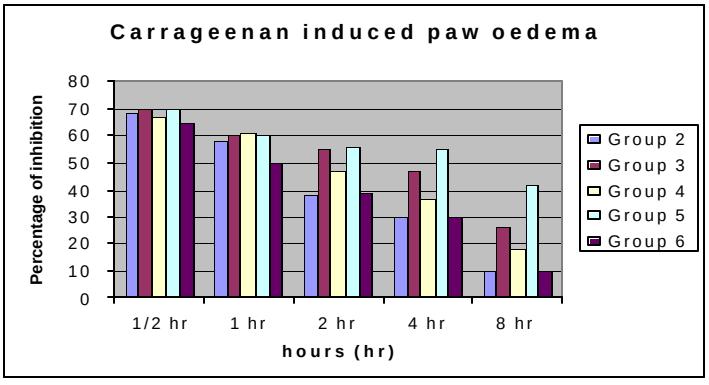


Figure 2. Effect of EETPR, EETPA and Indomethacin on Cotton pellet induced granuloma in rats.

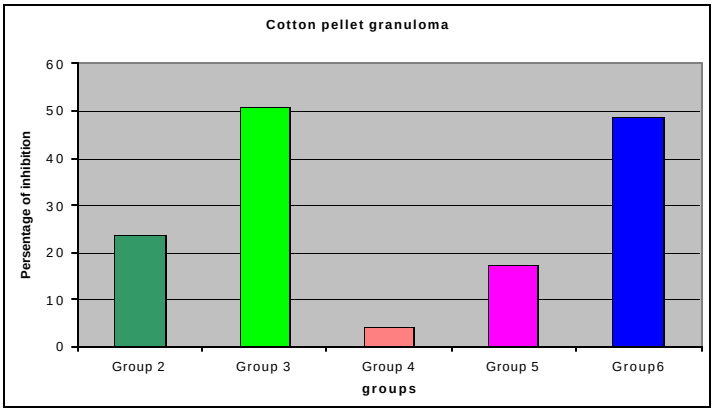
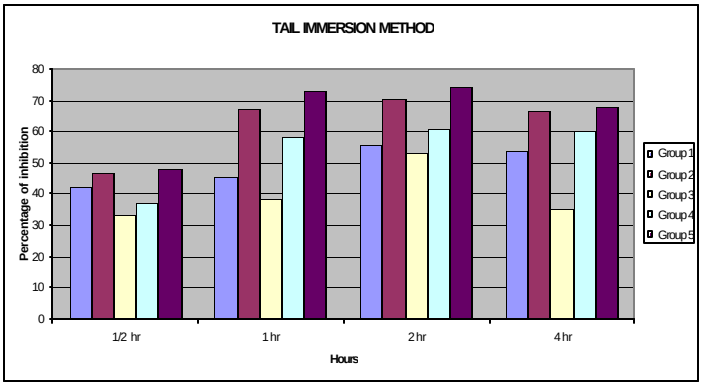


Figure 3. Effect of EETPR, EETPA and Indomethacin on thermally induced nociception in rats



groups (group B and group C) of rats were treated orally with 250, 500 mg/kg of the EETPR and the group D and group E received, 250, 500 mg/kg of the EETPA respectively, 1 h before carrageenan injection. The control group A received 10 ml/kg saline and the reference group F received 20 mg/kg Indomethacin i.p. Measurement of paw size was carried out by Mercuric Displacement method at the time intervals of 0, ½ , 1, 2, 4 and 8 h after the administration of the test drug.

Cotton pellet granulama in rats

A sterilized cotton pellet weighing 100 mg was introduced subcutaneously into the groin region of rats according to the method of Mossa et al[11] The test groups of animals (groups B and group C) were treated orally with 250, 500 mg/kg of the ethanol extract of root part and the test groups (group D and group E) were treated orally with 250, 500 mg/kg of the ethanol extract of aerial part, respectively. Animals in the control and reference (group A and group F) received 0.9% saline (10 ml/kg, p.o.) and indomethacin (20 mg/kg, i.p.), respectively daily for 7 days. The animals were sacrificed on the eighth day after an overdose of chloroform. The pellets surrounded by granuloma tissue (six animals per group) were dissected out carefully and dried at 60°C to a constant weight. Mean weight of the granuloma tissue formed in each group was obtained and the percentage inhibition was expressed by comparing the mean weight in the test groups with the mean weight in the control group.

Table 3. Analgesic activity of EETPR and EETPA on thermally induced analgesic in rats

S.no	Groups	Basal Reaction Time(s)	Percentage Inhibition 0 hr	1/2 hr	1 hr	2 hr	4 hr
1	**Group A** (EETPR- 250)	2	3.250±0.1708	3.500±0.2582* (42.0%)	3.667±0.2472* (45.45%)	4.500±0.2582* (55.55%)	4.317±0.2386* (53.67%)
2	**Group B** (EETPR- 500)	2	3.417±0.2007*	3.750±0.2141* (46.66%)	6.083±0.3516 *** (67.12%)	**6.750±0.2141*** (70.37%)**	6.161±0.2108*** (66.76%)
3	**Group C** (EETPA- 250)	2	3.083±0.1537	3.000±0.1291* (33.33%)	3.250±0.2141* (38.46%)	4.250±0.2141* (52.94%)	3.000±0.2236* (35.04%)
4	**Group D** (EETPA- 500)	2	3.167±0.2789	3.167±0.2108* (36.84%)	4.750±0.3354* (57.89%)	5.083±0.2386* **(60.65%)**	5.003±0.2316* (60.02%)
5	**Group E (**Indomethacin (20 mg/kg, i.p)	2	3.167±0.2789	3.833±0.2789* (47.82%)	7.500±0.2582*** (73.33%)	**7.750±0.1708*** (74.19%)**	6.161±0.2108*** (67.54%)

*Data represents mean ± SEM of 6 animals, Statistically significant from vehicle control *P < 0.05 and *** P < 0.001 compared to control by Dunnett's method.The number in the parenthesis indicates the percentage of analgesic activity*

Analgesic activity

Tail immersion method

Colony in breed stains of male wistar rats of 150-250 g were divided into five groups. The basal reaction time was taken by observing tail flick of rat, when the tip of the tail is kept in water, which is maintained at temperature of 55 ± 0.5°C. The animals must show response within 2-4 sec. After finding basal reaction time, the test groups of animals (group A and group B) were treated orally with 250, 500 mg/kg of the ethanol extract of root part (EETPR) and the test groups (group C and group D) were treated orally with 250, 500 mg/kg of the ethanol extract of aerial part (EETPA). Animals in the reference groups E received indomethacin (20 mg/kg, i.p.).The drug was administered through oral route and tail flick response was taken at 0, ½, 1, 2, 4 and 8 h after the administration of the test drug. A cut off period of 10 sec is considered as maximum analgesic and the tail is removed from source of hot water to avoid the damage. The percentage inhibition was expressed by comparing the mean seconds in the test groups with the mean seconds in the control group.

Statistical analysis

Data are expressed as mean ± SEM. Statistical analyses was performed by one-way ANOVA followed by Dunnett's method. P values < 0.05 were considered significant.

RESULTS

In the Carrageenan-induced paw edema model using the oral doses of *Tephrosia purpurea* Linn.The results show a dose-dependent decrease in the size of the edema from 1.405 ± 0.1037 to 1.147 ± 0.3297. This effect corresponded with the maximum effect of *Tephrosia purpurea* Linn. at ½ h. The responses at 0, ½, 1, 2, 4 and 8 h are shown in Table 1 and graphically represented in Figure 1.

In the Cotton pellet granuloma method, the oral doses of *Tephrosia purpurea* Linn. extract show a dose dependent reduction in the granuloma tissue formation from 182.2 ± 3.260 mg to 120.8 ± 4.175 mg The responses are shown in Table 2 and graphically represented in Figure 2.

Tail immersion method, the oral doses of *Tephrosia purpurea* Linn. extract shows a dose dependent significantly delayed in the time of tail withdrawal response by thermal induction of pain from 6.750 ± 0.2141 to 4.250 ± 0.2151 at 2 h. The responses at 0, ½, 1, 2, and 4 h are shown in Table 3 and graphically represented in Figure 3.

DISCUSSION AND CONCLUSION

Inflammatory events involve micro-vascular changes with increased vascular permeability, flow of exudation, including plasmatic protein and amplification of endogenous chemical mediators[13]. Non-steroidal anti-inflammatory drugs (NSAIDs) are the common drugs against superficial nociception and inflammation. NSAIDs alleviate the hyperalgesic symptoms associated with inflammation by inhibiting the COX enzyme and the resultant inhibition of prostaglandins synthesis from arachidonic acid[6]

The anti-inflammatory effect and analgesic properties of *Tephrosia purpurea* Linn. extracts were investigated in the present study. For the anti-inflammatory effect, it is important to estimate the activities of the extracts in the acute phase of inflammation as well as in the chronic phase of inflammation. Accordingly the carrageenan test was selected because of its sensitivity in detecting orally active anti-inflammatory agents particularly in the acute phase of inflammation[4,5, 7]. The cotton pellet granuloma on the other hand, is a model of chronic inflammation[2,16,19] and the dry weight has been shown to correlate with the amount of granulomatous tissue formed[9,20]

The extracts were evaluated for their anti-inflammatory activity by Carrageenan method. Among the six groups, third group (Group C) which is treated with 500 mg/kg body weight of EETPR showed significant anti inflammatory activity which was maximum, when compared to the standard drug, Idomethacin which is given intra peritoneal (20 mg/kg body weight), Then 5th group (Group E) which is treated with 500 mg/kg body weight of EETPA show less significant activity when compared to Group C and Group F.Group B and Group D which is treated with 250 mg/kg body weight of EETPA and EETPR respectively show the anti-inflammatory activity but it is not much effective. The responses at 0, ½, 1, 2, 4 and 8 h of drug administration were expressed as mean ± S.E.M and were given in Table 1.

The cotton pellet granuloma method has been widely employed to assess the transudative, exudative and proliferative components of sub-acute inflammation. In sub-acute studies, the inflammatory granuloma is the typical features .The fluid absorbed by the pellet greatly influences wet weight by the pellet greatly influences wet weight of granuloma and dry weight correlates well with the amount of granuloma tissue formed. The Group B and Group C, which is treated with EETPR at the dose of 250 and 500 mg/kg body weight show maximum significant

activity when compared to standard drug (Group F). The Group D and Group E, which is treated with EETPA at the dose of 250 and 500 mg/kg body weight show less significant activity when compared to standard drug (Group F). The response was expressed as mean± S.E.M and were given in Table 2.

The results obtained from using the two models show that *Tephrosia purpurea* Linn. ethanol extracts can effectively reduce inflammation in both the acute and chronic phases (Tables 1 and 2). This result provides a scientific basis for the practice of using *Tephrosia purpurea* Linn. extracts in the treatment of wounds.

Analgesic properties were also studied using sensitive model that could provide different grades of noxious stimuli (in thermal stimulus). In the present study, the tail immersion method[9] was selected because of several advantages including the sensitivity to strong analgesics and limited tissue damage. Hot water was used to induce pain in rats to study the analgesic activity of EETPR and EETPA, all the five groups showed marked decrease in response to thermal pain stimulus. Group B which was treated with 500 mg/kg body weight of EETPR showed analgesic activity at 2 h which was maximum and also has significant analgesic activity at 0, ½, 1 and 4 h of drug administration. Group D which was treated with 500 mg/kg body weight of EETPA showed analgesic activity at 2 h which was maximum and also has significant analgesic activity at 0, ½, 1, and 4 h of drug administration. Group A and Group C also show significant analgesic activity. The responses at 0, ½, 1, 2, and 4 h of drug administration were expressed as mean ± S.E.M and were given in Table 3. The observed effect in this study (Table 3) has shown that *Tephrosia purpurea* Linn. can significantly inhibit the responses to thermal stimulus. Therefore, it is concluded that the *Tephrosia purpurea* Linn. extract is capable of inhibiting inflammatory reactions as well as inflammatory pain.

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