STUDIES ON THE ANTI-INFLAMMATORY AND ANALGESIC ACTIVITY OF THE ETHANOLIC FRACTION OF THE ROOT EXTRACT OF TEPHROSIA PURPUREA (LINN)

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ABSTRACT

Tephrosia Purpurea has been widely used in the traditional medicinal system for the treatment of a variety of diseases. The effect of ethanolic extract of Tephrosia Purpurea was studied in different experimental animal models and it was revealed that the extract possess significant analgesic and anti-inflammatory activities. Anti-inflammatory activity of ethanolic fraction of the root extract of Tephrosia purpurea was tested on carrageenan-induced hind paw oedema and cotton pellet granuloma models in Wistar albino rats. Diclofenac (25 mg/kg p.o.) & Morphine (5 mg/kg p.o) were used as standard drugs for anti-inflamamtory & analgesic activities. The paw diameter was measured at different time intervals and the dry granuloma weight was taken after the treatment. The evaluation of anti-inflammatory & analgesic activities were carried out using Carrageenan induced paw edema volume, Hot plate and Writhing response model which is comparable with standard drug Diclofenac (25 mg/kg p.o.). The ethanolic fraction of the root extract of Tephrosia purpurea (400 mg/kg) showed the maximum inhibition (94.23%) of oedema at the end of 3hr following carrageenin-induced rat paw oedema. In subacute inflammation, the extract showed (76.25%) reduction in granuloma weight. The TPEE at doses of (200 and 400 mg/kg) showed promising effect in reducing the carrageenan induced paw edema volume in rats when compared with vehicle treated group. The TPEE at doses (200, 400 mg/kg) significantly reduced thermal and chemical induced nociception (Hot plate and writhing response) in mice when compared with vehicle treated group. The results prove that the the ethanolic fraction of the root extract of Tephrosia purpurea showed highest anti-inflammatory activity & analgesic activity in acute and subacute inflammation and also support the usage of traditional claims.

Keywords: Tephrosia purpurea, Anti-inflamamtory & Analgesic activity, Diclofenac, Morphin, Carrageenan induced paw edema, Hot plate and Writhing response, Experimental animal, TPEE: Tephrosia purpurea ethanolic extract.

INTRODUCTION

Inflammation (Latin, inflammatio, to set on fire) is the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. It is a protective attempt by the organism to remove the injurious stimuli as well as initiate the healing process for the tissue.

Inflammation is a process by which the body’s white blood cells and chemicals protect us from infection and foreign substances such as bacteria and viruses. When inflammation occurs, chemicals from the body’s white blood cells are released into the blood or affected tissues in an attempt to rid the body of foreign substances. This release of chemicals increases the blood flow to the area and may result in redness and warmth. Some of the chemicals cause leakage of fluid into the tissues, resulting in swelling. The inflammatory process may stimulate nerves and cause pain. Sometimes, however, the white blood cells and their inflammatory chemicals cause damage to the body’s tissues.

(1995-2007 The Cleveland Clinic Foundation)

The International Association for the Study of pain defines, “Pain is an unpleasant sensory or emotional experience associated with actual or potential tissue damage, or described in terms of such damage. Pain is always subjective. Each individual learns the application of the word through experiences related to injury in early life. It is unquestionably a sensation in a part of the body, but it is also unpleasant, and therefore also an emotional experience. Many people report pain in the absence of tissue damage or any likely pathophysiological cause; usually this happens for psychological reasons. There is no way to distinguish their experience from that due to tissue damage, if we take this subjective report.” (IASP Pain 1979(6):249-252,Shipton, 1993).

Anti-inflammatory effects

The primary action of the drugs is to inhibit arachidonate cyclooxygenase and, thus to inhibit the production of prostaglandins and thromboxanes. NSAIDs reduce the components of inflammation that are caused by COX-2 action, which include vasodilation, edema, and pain. These drugs have no effect on the processes that contribute to tissue destruction in RA; they simply reduce the generation of toxic O2 products and inhibit lymphocyte activation.

Analgesic effects

NSAIDs are effective against pain that is caused by prostaglandins acting on nociceptors (i.e. pain associated with inflammation or tissue damage)%. Decreased prostaglandin production leads to less sensitization of nociceptive nerve endings to the inflammatory mediators, bradykinin and 5-hydroxytryptamine (Rang et al, 1995).

Effect of Tephrosia purpurea ethanolic extracts of dose 200 and 400mg/kg were determined for its anti-inflammatory activity & analgesic activity.

MATERIALS AND METHODS

Plant

Tephrosia purpurea (Linn.) Pers. (Fabaceae), commonly known in Sanskrit as Sharapunkha is a highly branched, sub-erect, herbaceous perennial herb. Fresh and green plants were collected from Trichy, Tamil nadu, India and authenticated by G.V.S Murthy, Joint Director, Botanical Survey of India, Coimbatore, Tamil Nadu. After due authentication, fresh plants were collected, cleaned thoroughly with distilled water, cut in to two halves and subsequently dried under shade. The shade dried plants were pulverized in a mechanical grinder to obtain coarse powder. Useful Parts: Root, leaves, seeds, bark, whole plant.

Extraction of plant material

The powder of dried plants of Tephrosia purpurea was extracted separately by continuous hot extraction process using soxlet apparatus.

Tephrosia purpurea aerial/root parts (500 g) of were dried finely powdered soaked with 1500 mL of 95% ethanol overnight. The residue obtained was again resuspended in equal volume of 95%
ethanol for 48 hr and filtered again. The above two filtrate was mixed and the solvents was evaporated in a roto-evapourator at 40-
50°C under reduced pressure, dark semisolid material obtained was
stored at - 4°C, until use15. For experimental studies, known volume
of the extract was suspended in distilled water.

Animals

Experiments were performed on Wistar albino rats of either sex
weighing about 120-
160 g, divided into groups of six each. All the animals were approved
by the ethics committee of the institute.

METHODS

1. Anti-inflammatory activity by Carrageenan induced paw
edema in rats

Wistar albino rats weighing around 180-220 were used for this
study. They were divided into 4 groups consisting of 5 animals each.
Group-I (vehicle 1% CMC, 1ml/kg, p.o.), Group-II (ethanolic extract
of Tephrosia purpurea (200mg/kg, p.o.), Group-III (ethanolic extract
of Tephrosia purpurea (400mg/kg, p.o.), Group-IV (Diclofenac
(25mg/kg p.o.).

Anti inflammatory activity was assessed by the method Carrageenan
induced paw edema [Winter et al., 1968]12,13. The rats were divided into
groups of 5 animals each. The different groups were treated with
ethanolic extract of Tephrosia purpurea, Diclofenac and control
vehicle per oral. After 30 min, the rats were challenged with
subcutaneous injection of 0.1 ml of 1% w/v solution of carrageenan
into the sub plantar region of left paw. The paw was marked with
ink at the level of lateral malleolus and immersed in mercury upto
the mark. The paw volume was measured at 0, 1, 2, 3, 4 and 24 h
after carrageenan injection using a volume transducer (model no.0V-
2723) attached with strain gage coupler of Student Physio-Graph
(model no. PG-02, INCO, Ambala, India)14. The difference between
initial and subsequent reading gave the actual edema volume.

2. Analgesic activity by Eddy’s hot plate method in rats

Swiss albino mice weighing around 20-25 g were used for this study.
They were divided into 4 groups consisting of 5 mice each. Group-I
(vehicle 1% CMC, 1ml/kg, p.o.), Group-II (ethanolic extract
of Tephrosia purpurea 200mg/kg, p.o.), Group-III (ethanolic extract
of Tephrosia purpurea 400mg/kg, p.o.), Group-IV (Morphine sulphate
(5mg/kg, s.c.).15.

Hot plate method (Eddy et al., 1953)

Mice were screened by placing them on a hot plate (Medicraft
analogeimeter Mark III, Medicraft electro medicals (P) Ltd.,
Lucknow, India) maintained at 55 ± 1°C and the reaction time in
seconds for hind paw licking or jumping were recorded. Only mice
which reacted within 5 sec and which did not show large
variation when tested on four separated occasions, each 15 min
apart, were used in this study16. Morphine (5 mg/kg, S.C.)
was used as standard. The latency period for hind paw licking or
jumping on the heated plate of analogeimeter was taken as the
reaction time.

Effect of Tephrosia purpurea ethanolic extracts (200,400mg/kg, p.o)
and morphine on pain inhibition percentage of nociceptive
responses to thermal stimuli21. Both the extracts at doses 200 and
400 mg/kg, p.o. significantly exerted protective effects on heat
induced pain in hot plate method in mice. Ethanol extract at dose
400 mg/kg, p.o. showed maximum analgesic effect in hot plate test
in mice. Morphine at dose 5 mg/kg, significantly increased pain
latency.

Writhe test in mice

The effect of Tephrosia purpurea ethanolic extracts
(200,400mg/kg,p.o) and Diclofenac sodium are evaluated by acetic
acid induced writhing responces in mice.The no of writhings of each
animal with in 25 min after acetic acid injection was cumulatively
counted immediately and the percentage protection was calculated
using the following ratio: percentage of protection=(control mean-
mean treated)/control mean) x 10015.

Statistical analysis

Values are expressed as mean ±SEM of 5 animals in a group, the
significance among the groups were determined by ANOVA followed
by Dunnett’s tests were compared with the control17.

RESULTS AND DISCUSSION

Anti-inflammatory activity by carrageenan induced paw edema
in rats

Effects of ethanolic extract of Tephrosia purpurea and Diclofenac
sodium on carrageenan induced paw edema in rats are shown in
Table-1. Oral administration of the ethanolic extract at doses 200
and 400 mg/kg significantly suppressed the paw edema at 3 and 4 h
after carrageenan injection in rats. Diclofenac sodium at a dose of
25mg/kg, significantly suppressed paw edema at 3 and 4 h after
carrageenan administration (Table 1). In the control group, paw
edema volume was maximum at the fourth hour20.

In the light of the Table 1, the extracts at doses 200 and 400 mg/kg
p.o. seems effective only in the second phase. So these extracts might
block prostaglandin and /or bradykinin release rather than
acetylcholine and /or serotonin21. Diclofenac also has shown similar
effect only at second phase22.

Analgesic activity by Eddy’s hot plate method in rats

Effect of Tephrosia purpurea ethanolic extracts (200,400mg/kg,p,o)
and morphine on pain inhibition percentage of nociceptive
responses to thermal stimuli is summarized in Table 2. Both
the extracts at doses 200 and 400 mg/kg, p.o. significantly
exerted protective effects on heat induced pain in hot plate method
in mice. Ethanol extract at dose 400 mg/kg, p.o. showed maximum
analgesic effect in hot plate test in mice. Morphine at dose 5 mg/kg,
significantly increased pain latency (Table 2)23.

Writhe Test In Mice

The effects of plant extracts and Diclofenac on writhing test are
shown in Table3. Tephrosia purpurea at 400mg/kg dose was
significantly inhibited the writhing response of mice caused by
intraperitoneal injection of acetic acid. The maximal inhibition of
nociceptive response was 82.24% which is very close to that of
Diclofenac sodium 84.78%16. Extract of Tephrosia purpurea
at 200mg/kg was inhibited with 36.95%. So ethanolic extract
of Tephrosia purpurea exerts its pain-relieving effect in a dose
dependent manner. Since the abdominal constriction induced by
acetic acid involves the process or the release of arachidonic acid
metabolite via cyclooxygenase (COX) and prostaglandin biosynthesis
(Elisabetsky et al., 1995)25. So probably the extracts of
Tephrosia purpurea may act by inhibiting the release of arachidonic acid26.

Table 1: Effect of Tephrosia purpurea extracts on carrageenan-induced paw edema in rats

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Dose (mg/kg, p.o)</th>
<th>Paw edema volume at different time interval (in ml)</th>
<th>1h</th>
<th>2h</th>
<th>3h</th>
<th>4h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>-</td>
<td></td>
<td>0.21±0.055</td>
<td>0.324±0.024</td>
<td>0.408±0.034</td>
<td>0.492±0.048</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>25</td>
<td></td>
<td>0.20±0.069</td>
<td>0.084±0.040**</td>
<td>0.060±0.026**</td>
<td>0.288±0.029**</td>
</tr>
<tr>
<td>TPEE</td>
<td>200</td>
<td></td>
<td>0.228±0.048</td>
<td>0.348±0.051</td>
<td>0.036±0.014**</td>
<td>0.384±0.044</td>
</tr>
<tr>
<td>TPEE</td>
<td>400</td>
<td></td>
<td>0.084±0.014</td>
<td>0.132±0.034**</td>
<td>0.048±0.012**</td>
<td>0.336±0.024*</td>
</tr>
</tbody>
</table>

Values are mean ±SEM of 5 animals in a group. *p<0.05, **p<0.01, of ANOVA followed by Dunnett’s test compared with the control.

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Table 2: Effect of Tephrosia purpurea extracts on Eddy’s Hot plate in mice

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Dose (mg/kg, p.o.)</th>
<th>Pain inhibition percentage (Pip)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30min</td>
<td>1 h</td>
</tr>
<tr>
<td>Vehicle</td>
<td>-</td>
<td>18±7.3</td>
</tr>
<tr>
<td>Morphine</td>
<td>5</td>
<td>300±42.5**</td>
</tr>
<tr>
<td>TPEE</td>
<td>200</td>
<td>12±7.3</td>
</tr>
<tr>
<td>TPEE</td>
<td>400</td>
<td>26.4±16.1</td>
</tr>
</tbody>
</table>

Values are mean ±SEM of 5 animals in a group.*p<0.05, **p<0.01, ***p<0.001 of ANOVA followed by Tukey comparison test, compared with the control.

Table 3: Effect of Tephrosia purpurea extracts on writhing test in mice

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Dose (mg/kg, p.o)</th>
<th>No of animals</th>
<th>Writhing times (Mean±SEM)</th>
<th>percentage protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>vehicle</td>
<td>-</td>
<td>5</td>
<td>55.2±4.35</td>
<td>-</td>
</tr>
<tr>
<td>TPEE</td>
<td>200</td>
<td>5</td>
<td>34.8±7.16*</td>
<td>36.95</td>
</tr>
<tr>
<td>TPEE</td>
<td>400</td>
<td>5</td>
<td>9.8±1.2**</td>
<td>82.24</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>25</td>
<td>5</td>
<td>8.4±1.03**</td>
<td>84.78</td>
</tr>
</tbody>
</table>

Values are mean ±SEM of 5 animals in a group.*p<0.05, **p<0.01, ***p<0.001 of ANOVA followed by Dunnett test, compared with the control.

CONCLUSION

The Tephrosia purpurea is a common perennial herb found in all districts throughout India. Extracts of the whole plant with three different solvents such as petroleum ether, chloroform and ethanol were used in present study. These extracts TPEE at various doses (200 and 400 mg/kg, p.o.) have been used to study anti-inflammatory (carrageenan induced paw edema) and analgesic (Hot plate and Writhing response) parameters in experimental animals. The TPEE at doses of (200 and 400 mg/kg) have shown promising effect in reducing the carrageenan induced paw edema volume in rats when compared with vehicle treated group.

The TPEE at doses (200, 400 mg/kg) significantly reduced thermal and chemical induced noiception (Hot plate and Writhing response) in mice when compared with vehicle treated group. Hence it is evident that the ethanolic extracts of Tephrosia Purpurea plant at doses 200 and 400 mg/kg have promising effect in the management of inflammation and pain.

REFERENCES


