Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Issue 1, 2012

Research Article

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

RAMBABU BATHINI[1], Dr. KOKKULA SATHYANARAYANA[2], KIRAN THADLKALA*[3], RAJAKUMAR DEVERA[4], RAMCHANDER THADKAPALLY[5], KEMISETTI DURGA PRASAD[6]

[1] Department of Pharmacology, [2] Department of Pharmacognosy, [3] Department of Pharmaceutics, [4, 5] Department of Biotechnology, [6] Department of Pharmaceutical chemistry, [1, 2, 3, 4, 5, 6] Mother Teresa College of Pharmacy, Ghatkesar, Hyderabad, Andhra Pradesh, India. *Email: kiran.thadkala@gmail.com

Received: 24 Aug 2011, Revised and Accepted: 13 Nov 2011

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 possesses 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 (25mg/kg p.o.) & Morphine (5 mg/kg p.o) were used as standard drugs for anti-inflammatory & analgesic activities. The paw diameter was measured at different time intervals and the dry granuloma weight was taken after the treatment. 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 (25mg/kg p.o.). The ethanolic fraction of the root extract of *Tephrosia purpurea* (400 mg/kg) showed the maximum inhibition (84.23%) 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) 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 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 animals, 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[1]. It is a protective attempt by the organism to remove the injurious stimuli as well as initiate the healing process for the tissue[2].

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 viruses3. 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 swelling4. 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 tissues5. *(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[6]. 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 cyclo-oxygenase and, thus to inhibit the production of prostaglandins and thromboxanes[7]. NSAIDs reduce the components of inflammation that are caused by COX-2 action, which include vasodilation, edema, and pain[8]. These drugs have no effect on the processes that contribute to tissue destruction in RA; they simply reduce the generation of toxic O_2 products and inhibit lymphocyte activation.

Analgesic effects

NSAIDs are effective against pain that is caused by prostaglandins acting on nociceptors (ie. pain associated with inflammation or tissue damage) [9]. Decreased prostaglandin production leads to less sensitization of nociceptic 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[10].

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[5]. 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 use[11]. 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 weighed 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.vt-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 analgesiometer 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 study[16]. Morphine (5 mg/kg, S.C.) was used as standard. The latency period for hind paw licking or jumping on the heated plate of analgesiometer 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 stimuli[17]. 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.

Writhing 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-treated mean)/(control mean) x100[18].

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 Dunnets tests were compared with the control[19].

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 hour[20].

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 histamine and /or serotonin[21]. Diclofenac also has shown similar effect only at second phase[22].

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].

Writhing 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 intraperitonial injection of acetic acid. The maximal inhibition of nociceptive response was 82.24% which is very close to that of Diclofenac sodium 84.78%[24]. 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 acid[26].

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

Drugs	Dose (mg/kg, p.o)	Paw edema volume at different time interval (in ml)			
		1h	2h	3h	4h
Vehicle	-	0.216±0.055	0.324±0.024	0.408±0.034	0.492±0.048
Diclofenac	25	0.204±0.069	0.084±0.040**	0.060±0.026**	0.288±0.029**
TPEE	200	0.228±0.048	0.348±0.051	0.036±0.014**	0.384±0.044
TPEE	400	0.084±0.014	0.132±0.034**	0.048±0.012**	0.336±0.024*

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

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

Drugs	Dose (mg/kg, p.o.)	Pain inhibition percentage (Pip)			
		30min	1 h	2h	3h
Vehicle	-	18±7.3	18±7.3	24±6.0	18±7.3
Morphine	5	300±42.5**	228.4±39.1**	66.6±21.5	13.2±8.0
TPEE	200	12±7.3	85.8±22.9	148.6±36.7**	00±00
TPEE	400	26.4±16.1	144.6±22.9**	186.4±27.0**	26.6±11.2

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

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

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

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

Fig. 1: Comparison of normal and Carrageenan induced paw edema in rats.

Anti-inflammatory activity of the drug was investigated by measuring the changes in paw volume in control and experimental animals.

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 nociception (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

1. Baumgartner, W.A., Beck, F.W.J., Lorber, A., Person, C.M., Whitehouse, W.A., 1974. Adjuvant disease in rats: Biochemical criteria for distinguishing several phase of inflammation and arthritis. Proc Soc Exp Bio Med 45:625-630.
2. Wall, P.D., Melzack, R., eds., 1999, Text Book of Pain New York; Churchill, Livingstone, Edinburgh.
3. Rainsford, K.D., Whitehouse, M.W., 1980. Antiinflammatory /anti-pyretic salicylic acid esters with low gastric ulcerogenic activity, Agents Action 10,451-455.
4. Willis, W.D.,1989. The origin and destination of pathways involved in pain transmission. In: Wall PD & Melzack R (Editors), Textbook of Pain. Churchill Livingstone, Edinburg, 112-127.
5. Biren, S.N., Nayak, B.S., Seth, A.K., Jalapure, S.S., Patel, K.N., Patel, M.A., and Mishra, A.D., 2006, Search for medicinal plants as a source of anti-inflammatory and anti-arthritic agents. Pharmacognosy Magazine 2 (6), 77-86.
6. Insel, P.A., 1996. In: J.G.Hardman, Limbird, L.E. (Eds), Analgesic, Antipyretic and Antiinflammatory Agents and Drugs Employed in the Treatment of Gout. McGraw-Hill, New York, pp 617-657.
7. Shinde, V.A., Pandle, A.S., Nair, A.H., Mungantwar, A.A., Diks, V.J., Saref, M.N.,2000. Studies on the anti-inflammatory and analgesics activity cedrus denodra hill.Int. Journal of immunopharmacol 12,531-537.
8. Bisgaard, H., Kristensen, J.K., 1985. Leukotriene B4 produces hyperalgesia in humans. Prostaglandins 30, 871-877.
9. Chatterjee, G.K., Pal, S.P., 1984. Search for anti-inflammatory agents from Indian Medicinal Plants, A review. Indian Drugs. 21, 413.
10. Jachak, S.M., 2001. Natural Products: Potential source of COX inhibitors. CRIPS. 2, 12-15 (2001).

11. Chau, T., 1989. Pharmacology methods in the control of inflammation. In: Modern Methods in Pharmacology, Vol. V, Alan. R. Liss., Inc., New York, pp. 195-212.
12. Chawla, A.S., 1987. Plant anti-inflammatory agents. J. Scient. Ind. Res. 46:214-223.
13. Kaviman. S., Mounissamy V.M.,Gunasegaran,R., 2001. Analgesic and anti-inflammatory activities of Hispudulin isolated from Hlichrysum bracteatum. Indian Drugs. 37, 582-584.
14. Cheeke, PR,2006, Anti-inflammatory and anti-arthritic effects of *yucca schidigera*.
15. Kulkarni, S.K., Mehta, A.K., Kunchandy, J., 1986. Anti-inflammatory actions of clonidine, guanfacine and B-HT 920 against various inflammatory agents induced acute paw oedema in rats.
16. Duarte, J. D.G., Nakamura, M., Ferreira, S.H., 1988. Participation of the sympathetic system in acetic-induced writhing in mice. Brazilian Journal of Medicine and Biological Research 21, 341–343.
17. Mackenzie, I.R.A., 2001, Postmortem studies of the effect of anti-inflammatory drugs on Alzheimer –type pathology and associated inflammation. Neurobiology of Aging, 22, 819-822.
18. Eddy, N.B., Leimback, B., 1953. Synthetic analgesics: 11 Dithyienylbutenylamines and dithyienylbuttylamines. J. Pharmgcol Exp. Ther. 3 , 544 -547.
19. Elisabetsky, E., Amador, T.A., Albuquerque, R.R., Nunes, D.S., Carvalho, A.C.T., 1995. Analgesic activity of Psychotria colorata (wild ex R. et S.) Muell. Arg. Alkaloids. Journal of Ethnopharmacology 48, 77–83.
20. Esther N. Matu April 2003, Antibacterial and anti-inflammatory activities of some plants used for medicinal purposes in Kenya.
21. Gryglewski, R.J., 1981. Molecular mechanisms of inflammation. Eur J Rheumatol Inflamm 4, 153-9.
22. Suleyman, H., Demirezer, LO., Kuruuzum, A., Banoglu, Z.N., Gocer, F., Ozbakir, G., 1999. Anti-inflammatory effect of aqueous extract from Rumex patientia L, roots. J Ethanopharmacol 65, 141-148.
23. Gamache, D.A., Povlishock, J.T., Ellis, E.F., 1986. Carrageenan-induced brain inflammation. Characterisation of the model. Journal of Neurosurgery 65, 679-685.
24. Garcia-Pastor, P., Randazzo, A., Gomez-Paloma, l., Alcaraz, M.J., Paya, M., 1999. Effects of petrosaspongiolide M, a novel phospholipase A2 inhibitor, on acute and chronic inflammations. Journal of Pharmacology and Experimental Therapeutics 289, 166-172.
25. Morney, M.A., 1988. Selectivity of neutrophil 5-lipoxygenase and cyclo-oxygenase inhibition by an anti-inflammatory flavanoid glucoside and related aglycone flavanoids. J. Nat. Prod. 40, 787 – 792.
26. Gokhale, A.B, 2004, Preliminary evaluation of anti-inflammatory and anti-arthritic activity of *S. lappa*, *A. speciosa* and *A. aspera.*