BVG Trust

J. Sci. Trans. Environ. Technov. 2014, 7(4) : 205-210

Scientific Transactions in Environment and Technovation

Studies on anti inflammatory, analgesic and antipyretic effect of *Tryphonium trilobatum* L.

S. Elakkiya and T.Ananthi*

P.G. Department of Biochemistry, Sengamala Thayaar Educational Trust Women's College, Sundarakkottai, Mannargudi - 614 001, Thiruvarur(Dt)., India

Abstract

Tryphonium trilobatum is a hedge plant belonged to the Araceae family, traditionally grown as ornamental plant in home gardens. In the present study, anti inflammatory, analgesic and antipyretic effect of the ethanolic extract of *T. trilobatum* was evaluated in animal models. The anti inflammatory activity of *T. trilobatum* was significant. In addition, the extract also showed significant analgesic and antipyretic activity.

Keywords: *Trphonium trilobatum*, anti inflammatory, analgesic and anti pyretic.

INTRODUCTION

Inflammation is the complex biological response of vascular tissue to harmful stimuli including pathogens, irritants, or damaged cells. It is a protective attempt by the organism to remove the injurious stimuli as well as initiate the healing process for the tissue (Barboa-filho and Piuvezam, 2005). The process of inflammation is necessary for healing of wounds, however if not controlled, lead to onset of diseases like vasomotor rhimorhoea, rheumatoid arthritis and atherosclerosis (Clark *et al.*, 1988). Inflammation is characterized by classical signs such as edema, pain, heat and loss of function. Inflammation models are two types, acute and chronic inflammatory model. Acute models are designed to test drugs that modulate erytrhema, leukocyte migration and measurement of local pain, antipyretic and local analgesic activity (Denko, 1992). Chronic models are designed to find drugs that modulate disease process and these include sponge and monoarticular arthritis which have an immune etiology (Henson and Murphy, 1989).

The genus *Tryphonium* (family: *Araceae*), includes many plant species that are being used in the treatment of various disorders including life threatening diseases (Koster *et al.*, 1959). The present study was designed to evaluate the anti-inflammatory, analgesic and antipyretic activity of ethanolic extract of *T. trilobatum* in rats.

MATERIALS AND METHODS

Collection of plant materials

T.trilobatum was collected from Vazhamarakottai, Thanjavur District,Tamil Nadu.

Extraction of plant material

Ethanolic extracts of the plants were prepared according to the methodology of Indian Pharmacopoeia (Anonymous, 1976). The plants were dried under shadow. The dried plants were subjected to pulverization to get coarse powder. The coarse powder was subjected to soxhlet extraction separately. These extracts were concentrated to dryness in flash evaporator under reduced pressure and controlled temperature (40-50^{o} C). The ethanolic extracts were put in air tight containers and stored in a refrigerator. The extract was used for anti inflammatory, analgesic and antipyretic investigation.

Animals

Healthy and male albino rats of same age group (90-100g) were used in the present investigation. Animals were breed and reared in the Departmental animal house at the temperature of 24± 2^{o} C and 65±5% relative humidity . The animals were fed with standard pellet feed and water.

Anti inflammatory activity

Carrageenan induced paw edema in rats

Pedal inflammation in albino rats were produced according to the method described by Winter *et al.* (1962). An injection was made with 0.1 ml of 1% carrageenan sodium salt into the right hind foot of each rat. The test group of rats were treated orally with 25, 50, 75 and 100 mg/Kg b.wt of chloroform and ethanolic extract of T. *trilobatum*, 1 hour before the carrageenan injection. At the same time, the control group was given 5ml/Kg of normal saline and the reference group was given 100mg/Kg of saline solution of acetyl salicylic acid. The measurements of foot volume were done by the displacement technique using a plethysmometer immediately after 30,60,120 and 240 minutes after the injection of carrageenan.

In all the above models the degree of oedema formation was determined on the basis of increase in paw thickness and per cent inhibition was calculated as follows.

*Corresponding author,
E-mail: elangani576@gmail.com

Per cent inhibition = (Vt – Vc) / Vc × 100

where Vt = Paw volume at time t

Vc = Paw volume at time 0

Analgesic activity

Mouse writhing assay

This assay was carried out according to the method described by Koster *et al.* (1959). The extract 25, 50, and 75 mg/ b. wt was orally administered to three groups of rat (and to control group) before intra peritoneal injection of acetic acid (0.6% v/v in normal saline, 10 ml/kg b. wt) acetylsalicylic acid (100 mg/kg b. wt) was used as the reference drug to the group V of rats. The number of writhes was counted for 15 minutes in all group of rats, the control group (Group I) and the three experiment group of rats (Group II, III and IV).

Formalin test

The method used was similar to that described previously (Sahni *et al.* 2001). Twenty microliters of 1% formalin was injected subcutaneously into the right hind paw of rat. The time, in seconds spend in licking and biting response of the injected paw was taken as an indicator of pain response. Responses were measured for 5 min after formalin injection (first phase) and 15-30 min after formalin injection (second phase). Plant extract (25, 50, and 75 mg/kg b. wt) was orally given and acetylsalicylic acid (100 mg/kg b. wt) was administered 30 minutes before formalin injection to the group II, III, IV and V rats . Control animals (Group I) received the same volume of distilled water orally.

Tail flick assay

The cold water tail flick assay was based on a modified method of Clark *et al.*(1988). Rats were closely restrained in a wire mesh cage and the lower half of their tail dipped in a beaker of cold water (0-1° C). Measurement of threshold was made 30 min before and after administration of extract (25, 50, 75 and 100 mg/ kg b. wt to the experiment group II, III and IV. Distilled water (5mg/kg b. wt) was administered as control. Morphine was used as a reference drug to group V.

The per cent protection against tail flicking was calculated by using the following formula.

% protection = 1-Wt/Wc × 100

Wt = Mean value of test group

Wc= Mean value of control group

Hot plate test

The modified method of Hunskaar *et al.* (1986) as previously adopted (Adzu *et al*., 2001) was used. Rat that showed antinociceptive response with 20 hours when placed on hot plate maintained at 55±0.5° C were selected and grouped into five. Group I was treated with saline. The groups II, III and IV received 25, 50 and 75 mg b.wt of the extract respectively while group V received 100mg/Kg b.wt of acetyl salicylic acid. Each rat was placed singly on the hot plate and latency to exhibit thermal stimulus was determined before and at 0, 30, 60, 120 and 240 minutes after treatment. Analgesic activity was calculated by using the following formula.

(%MPE calculated as %MPE =post- drug latency-pre-drug latency/cut-off time - pre- drug latency).

Antipyretic activity

Yeast induced pyrexia was used to screen the effect of the plant extracts (Turner, 1965; Srimal, 1984). Pyrexia was produced by injecting 2 ml of a 15% suspension of dried brewer's yeast, subcutaneously into the back of non fasted albino rats. Extracts were administered orally to those animals who showed a rise in body temperature of 1.2° C or more, 20h after the yeast injection, the rectal temperature was then recorded at 90,180 and 270 min after extract administration. Control group was administered with equivalent volumes of the vehicle used (0.9% saline). The differences between the mean temperature of the control group and that of the other three experimental groups were calculated. Paracetamol was used as the reference antipyretic agent (100mg/Kg b.wt).

Statistical analysis

The experimental data are expressed as mean± S.E.M. Statistical analysis was carried out by using one way ANOVA followed by student- t- test. The value at $p<0.05$ was considered as significant.

RESULTS AND DISCUSSIONS

Anti inflammatory activity

The extract administered 1 hour before carrageenan showed a dose dependent inhibition of the induced oedema. Carrageenan produced a swelling on the rat paw which reached a peak (41.21) in 30 min and gradually declined over the next 3 hours. The extract showed the high rate of inhibition of oedema at 100 mg/kg b wt compared to the lower doses. The standard drug produced a greater oedema inhibition (63.37%) which was not significantly different (pe" 0.05) from that of the result recorded (100mg/kg b.wt 63.35%) within the same period (Table1)

The effect of the extract was most pronounced at the later stages of the inflammatory responses, which corresponds to the phase of prostaglandin release. Carrageenan oedema consists of three distinct phases, an initial release of histamine and 5HT, a second phase mediated by kinins and finally a third phase, the mediator of which is suspected to be prostaglandin (Dharamvir Hota, 2007).

Table – 1: Anti-inflammatory activity of the ethanolic extract of *Tyhonium trilobatum* L in carrageenan induced rat hind paw oedema

S.No	Group	Dose (mg/b.wt)	Percentage of paw volume Mean +S.E (N=6) Post insult time of assay (in minutes)				Percentage of inhibition in paw volume			
			30	60	120	240	30	60	120	240
1	I	-	1.13±0.26	1.42±0.14	1.91±0.51	2.47±0.22	-	-	-	-
2	II	25	0.77±0.18	0.76±0.11	0.72±0.10	1.02±0.18	41.21	42.63	45.22	45.27
3	III	50	0.67±0.12	0.68±0.22	0.63±0.29	0.89±0.14	45.44	48.11	52.62	54.16
4	IV	75	0.79±0.22	0.84±0.17	1.03±0.15	1.68±0.17	47.82	52.39	53.01	56.27
5	V	100	0.85±0.16	0.85±0.22	1.01±0.27	1.02±0.30	48.55	55.32	57.01	61.35
6	VI	Acetyl -salicylic acid 100	0.93±0.12	0.95±0.14	1.32±0.42	1.41±0.41	57.32	61.17	65.22	63.37

Table-2: Analgesic activity of ethanolic extract of *Trphonium trilobatum L* through acetic acid induced writhing test on albino rats.

S.No	Group	Dose (mg/kg b wt)	No. of writhing (per 15 min)	Percentage of inhibition
1	I	-	48.5±3.7	-
2	II	25	34.7±4.1	46.32
3	III	50	26.5±3.8	58.22
4	IV	75	21.3±2.2	69.21
5	V	100	19.4±3.1	71.32
6	VI	Acetylsalicylic acid 100	14.3±2.2	79.48

N=6; value are expressed as mean ± S.D

(P<0.05)

Table-3: Analgesic activity of ethanolic extract of *Trphonium trilobatum L* through formalin induced pain test on albino rats

S.No	Group	Dose (mg/kg bwt)	Pain (0-5) min	Percentage of inhibition	Pain (15-30 min)	Percentage of inhibition
1	I	-	121.44.5	-	101.56.4	-
2	II	25	128.22.4	6.84	59.62.5	57.34
3	III	50	121.51.4	12.25	45.33.4	61.22
4	IV	75	119.63.2	12.82	32.42.8	59.56
5	V	100	119.63.2	12.82	32.42.8	59.56
6	VI	Acetylsalicylic acid 100	127.89.8	12.62	21.38.6	79.98

N=6; value are expressed as mean+S.D

(P<0.06)

Table-4: Analgesic activity of ethanolic extract of *Trphonium trilobatum L.* by tail flick method

S.No	Group	Dose (mg/kg b wt)	Pre treatment (in seconds)	Percentage of inhibition	Post treatment (in seconds)	Percentage of inhibition
1	I	-	5.7±3.1	-	6.3±1.4	
2	II	25	11.5±2.4	207.07	20.3±2.5	28.4±22.3
3	III	50	11.8±2.7	212.6	29.5±3.2	43.763.2
4	IV	75	12.6±2.4	217.02	33.3±3.5	51.2±23.8
5	V	100	11.6±3.3	207.14	36.2±3.5	64.1±64.2
6	VI	Morphine	12.2±4.3	234.12	49.5±3.2	79.2±54.9

N=6; value expressed as mean± S.D

(P<0.05)

Table-5: Analgesic effect of ethanolic extract of *Tryphonium trilobatum L* by hot plate method

S.No	Group	Dose (mg/kgb. wt)	Reaction time in seconds				
			0	+30	+60	+120	+180
1	I	-	3.3±0.88	3.6±0.01	3.9±0.04	3.1±0.02	3.4±0.06
2	II	25	3.3±0.48	5.9±0.04	8.1±0.03	10.1±0.02	11.8±0.04
3	III	50	3.4±0.35	6.4±0.03	8.9±0.04	10.6±0.05	12.5±0.03
4	IV	75	3.4±0.08	6.9±0.07	9.6±0.01	10.7±0.8	11.5±0.02
5	V	100	3.4±0.04	5.8±0.05	7.8±0.03	10.0±0.07	9.8±0.05
6	VI	Acetylsalicylic acid 100	3.7±0.11	6.3±0.13	9.4±0.13	12.0±0.15	12.5±0.17

Table-6: Antipyretic activity of ethanolic extract of *Trphonium trilobatum L* in brewer's yeast induced pyrexia

S.NO	Group	Dose (mg/kg b wt)	Mean total temperature + S.E.M (0^oC)				
			Before Yeast	After yeast	After treatment (minutes)		
			0h	20h	+90	+180	+270
1	I	37.01±0.00	37.01±0.00	38.62±0.08	38.67±0.25	38.72±0.04	38.69±0.11
2	II	37.01±0.00	37.01±0.00	38.71±0.05	38.64±0.04	38.55±.02	38.49±0.12
3	III	37.01±0.00	37.01±0.00	38.70±0.07	38.62±0.05	38.58±0.05	38.46±0.06
4	IV	37.01±0.00	37.01±0.00	38.76±0.16	38.59±0.09	37.49±0.05	37.42±0.15
5	V	37.01±0.00	37.01±0.00	38.74±0.12	37.76±0.21	37.41±0.06	37.43±0.07
6	VI	37.00±0.00	37.00±0.00	38.70±0.12	37.57±0.22	37.38±0.06	37.18±0.14

N=6; value expressed as mean = S.D

(P<0.05)

The anti inflammatory activity of many plants has been attributed to their sterol and flavonoids contents. Recently, flavanoids and hypolactin-8-glucoside have been shown to possess anti inflammatory activity (Vippan *et al*., 1984).

Analgesic activity

Writhing Test

In the control group of rat, the number of writhes during 15 minutes test period was 48.5±3.7. The treatment of animals with *Tryphonium trilobatum* L ethanolic extract (25,50,75 and 100mg/Kg b.wt) produced a significant dose dependent inhibition of the control writhes. The inhibition in 100mg/Kg. b.wt was similar to that produced by 100mg/Kg b.wt acetyl salicyclic acid. The extract inhibited acetic acid induced writhing in rat and hence it is suggested that the extract produced has analgesic effect (Table2).

Antinociceptive activity of opiod agonists, opoid partial agonists and nonsteroidal anti-inflammatory agents can be determined by the writhing test (Vogel,1979). It has also been shown that some plants such as *Hunteria zeylanica* (Reanmongkol *et al*., 2009) and *Rcotea suaveolens* (Beirith *et al*., 1999) decreased the stretching induced by acetic acid but did not show any effect against heat induced pain.

Formalin test

The extract demonstrated a dose dependent relationship in both phases of formalin induced pain. Significant inhibition (12.31%) was produced only with the doses of 100mg/kg b.wt extract compared to control in the first phase and in the second phase it was 59.56. However, all the doses of the extract significantly ($p<0.05$) inhibited in the second phase, similar to acetyl salicylic acid (100mg/kg b.wt) (Table 3).

Tail flick method

All the doses of the plant extract used in the test significantly ($p<0.05$) increased the reaction time compared to control. The effect of morphine (2mg/kg b.wt) was higher at highest dose of the extract.. The percentage of inhibition slowly increased from lower dosage to higher dosages. The maximum level of inhibition (64.16) was recorded for the rat treated with 100 mg of extracts. (Table -4)

Hot plate test

The response to the different concentrations of the extract varied. Higher concentrations of the extract especially 75 and 100mg/animal performed very well in more than 60 minutes. However, all the doses significantly ($p<0.05$) inhibited in the second phase, similar to acetyl salicylic acid (100 mg/kg b.wt) (Table -5)

Antipyretic activity

Subcutaneous injection of 300mg/animal of yeast produced stable pyrexia in all the animals within the 20 hours of injection. The antipyretic action of this plant extract was significant after 180 and 270 min of oral administration. The results are quite encouraging in that, the effects are appeared to be comparable to that of aspirin. At the concentration of 100mg/Kg b.wt the antipyretic activity was demonstrated within 180 minutes after administration. No mortality occurred in any of the groups (Table 6).

The ethanolic leaf extract of *P. kotschyi* demonstrated effective antipyretic activity as evident in the blocking of temperature elevation in the yeast and amphetamine models. The antipyretic action of the extract may possibly be through inhibition of prostaglandin production, leading to suppression of elevated plasma level (Akindele and Adeyemi, 2007) especially, since the extract had been shown to possess antinociceptive and anti-inflammatory activities (Musa *et al*., 2005).

Pharmacological activities in different animal models suggest that the ethanolic extract of *Tryphonium trilobatum* is a promising anti inflammatory, analgesic and antipyretic agent and could be useful for the treatment of inflammatory conditions. However, studies are required on human subjects to prove through clinical trials as an anti inflammatory, analgesic and antipyretic agent.

Acknowledgement

The authors are thankful to the Managing Trustee, S.T.E.T Women;s College, Mannargudi for providing necessary facilities to carry out this work.

References

Adzu, B., Amos, S., Wambebe, C. and Gamaniel, K. 2001. Anti nociceptive activity of the aqueous extract of *Zizyphus Spina christi* root bark. *Fitoterapia* 72: 344–350

Akindele, A.J., and Adeyemi O.O. 2007.Antipyretic activity of *Byrsocarpus coccineus* Schum and Thonn (Connecaceae). *International J' of Pharmacology*. 3(4):357-361.

Anonymous, 1976. *The wealth of India. Raw Materials,* CSIR. New Delhi, India. 12.

Barbosa-Filho, J.M., Vasconcelos, T.H.C., Alencar Batista, L.M., Oliveira, R.A.G., Guedes, D.N. and Falcao, H.S. 2005. Plants and their active constituents from South,Central and North America with hypoglycemic activity. *Rev.Bras.Farmacogn*.15: 392-413.

Beirith, T., Soft, J.D. and Stevens, A. 1999. *Theory and practice of pharmacological techniques.* Churchill Livingston, New York.

Clark, S.J, Follenfant, R.L. and Smith, T.W. 1988. Evaluation of opiod – induced antinoceptive effects in anaesthetized and conscious animals. *British J. Pharmacol.,* 95: 275-283.

Denko, C.W. 1992. A role of neuropeptides in inflammation In: *Biochemistry of Inflammation*. Whicher JT, Evans SW. Kluwer Pub.London,177-181.

Dharamvir Hota. 2007. *Bioactive medicinal plants.* Gene tech books publishing. 276.

Henson, P.M. and Murphy, R.C. 1989. *Mediators of the inflammatory process, Elsevier pub., Amsterdam*, 404-412.

Hunskaar, S, Berge, O.G. Hole, K. 1986. The formalin test in mice: disssociation between inflammatory and non – inflammatory pain.. *Pain* , 30: 103-104.

Koster, R., Anderson, M.M. and De beer, E.J.1959. Acetic acid and Dornalgesic screening, *Federation proceedings,* 18:418-420.

Musa, Y.M., Haruna, A.R., Ilyas, M., Yaro, A.H., Ahmadu, A.A. and Usman, H.2005 Analgesic and anti-inflammatory activities of the leaves of *Pseudocedrela kotschyi* Harms (Meliaceae). *Books of abstracts of the 23rd National Scientific conference of the Nigerian Society of Pharmacognosy,* 25th-28th May. 88-89.

Reanmongkol, W., Noppapan, T. and Subhadhirasakul, S.2009. Antinociceptive, antipyretic, and anti-inflammatory activities of *Putranjiva roxburghii* Wall. leaf extract in experimental animals. *J Nat Med.* 63:290–6

Sahni, R.P. and Srivastava, D.N. 2001. Analgesic activity of *Withania sominifera, Indian medicinal journal.,* 25:153-155.

Srimal, R.C. 1984. Evaluation for anti-inflammatory activity cited In: *The use of pharmacological techniques for the evaluation of natural products* UNESCO. 39.

Turner, R.A. 1965. *Screening methods in pharmacology.* Vol.1. Academic press. New York. 298-299.

Vippan,A., Casco, M.A. and Al-Caraz, M.J. 1984. Anti-inflammatory and anti-ulcer properties of hypolactis 8 glycoside, a novel plant flavonoid. *J.pharm.,* 22: 820-823.

Vogel, H.G. 1979. Inûuence of maturation and aging on mechanical and biochemical parameters of rat bone. *Gerontology*, 25: 16–23.

Winter, C.A., Risely, E.A. and Nuss, G.W. 1962. Carrageenan induced oedema in hind paw of the rat as an assay for anti-inflammatory drugs. *Proceedings of the society for experimental biology and medicine* v.III: 544-547.