ISSN: 2322 - 0902 (P)
ISSN: 2322 - 0910 (O)

International Journal of Ayurveda and Pharma Research

Research Article

ANTI-INFLAMMATORY ACTIVITY OF AQUEOUS BARK EXTRACT OF *THESPESIA POPULNEA* IN RATS

U.D.S. Sewwandi[1], E.R.H.S.S.Ediriweera[2]*, W.D.Ratnasooriya[3]

[1]Specialist, Bandaranaike Memorial Ayurvedic Research Institute, Nawinna, Maharagama, Sri Lanka.

*[2]Senior Professor, Department of Nidana Chikithsa, Institute of Indigenous Medicine, University of Colombo, Rajagiriya, Sri Lanka.

[3]Professor Emeritus, Department of Zoology, University of Colombo, Sri Lanka.

ABSTRACT

This study examined the oral anti-inflammatory potential of aqueous extract of *Thespesia populnea* Linn bark. Soland. Ex. Corr. (Family: Malvaceae) which is used by Sri Lankan native practitioners to treat skin ailments and arthritis. This was tested in conscious male Wistar rats using carrageenan induced paw edema model and three oral doses; 1250, 2500 and 5000mg/kg. Indomethacin was used as the reference drug. The result showed that the aqueous extract of bark significantly $P<0.05$ and dose dependently inhibited both early (1-2 h) and late phase (4-5 h) of inflammation in the carrageenan model. In addition, it inhibited the intermediated phase (3 h). The anti-inflammatory activity of the highest doses of extract was comparable to that of indomethacin. The extract did not display overt signs of toxicity and was neither hepatotoxic, renotoxic nor hematotoxic even with chronic administration. It is concluded that aqueous bark extract of *Thespesia populnea* can function as an orally active, safe and potent anti-inflammatory agent.

KEYWORDS: *Thespesia populnea*, Gan Sooriya, Anti-inflammatory, Aqueous bark extract.

INTRODUCTION

Thespesia populnea Linn. Soland. Ex. Corr. (Family: Malvaceae) Gan Sooriya in Sinhala and Kavarachu in Tamil, is a fairly large tree or shrub growing up to 10-15m with branches spreading profusely. Its bark is gray to brown in color, fibrous, knobby fissured. Leaves are simple alternate and ovate to cordate. Flowers are large and with axillary's on the stem. It is found in many tropical countries including Sri Lanka. In Sri Lanka, the plant is very common near sea side and in dry zone up to an altitude of 665 m [1,2].

Phytochemical studies indicated that the ethanolic extract of bark of *T. populnea* contains alkaloids, carbohydrates, protein, tannins, phenols, flavonoids, gums, mucilage, saponins and terpenes [3]. Pharmacologically, various parts of *T. populnea* have shown to possess anti-bacterial, anti-oxidant, anti-fungal, anti-yeast, anti-implantation, anti-spasmodic, haemostatic, hepatoprotective, purgative, memory enhancing and anti-inflammatory activities[4]. However, in most of these studies organic solvents have been used in the extraction procedure, although water extracts are normally used in Ayurvedic, traditional and folk medicines. Native physicians of Sri Lanka recommend external application and internal administration of decoction made from bark of *T. populnea* plant in the treatment of several skin diseases such as eczema and boils [5]. Further, some traditional practitioners' claimed that decoction made from the bark of the plant is used in the treatment of arthritis. The use of the bark of *T.populnea* decoction in the treatment of these conditions suggests that the aqueous extract may possess anti-inflammatory activity. However, to our knowledge, this has not been tested by scientific experimentation. If aqueous bark extract is shown to have safe anti-inflammatory activity, it could also be used as an anti-inflammatory drug in native medicine. This study was undertaken to investigate the anti-inflammatory potential of aqueous bark extract of *T. populnea* using male Wistar rats.

MATERIAL AND METHODS

Animals

Healthy adult male Wistar rats (Weight: 200 ± 15g) (Mean±SEM) were used. The rats were purchased from Medical Research Institute, Colombo. The animals were kept at the animal house of the Department of Zoology, University of Colombo. They were kept under standardized animal house conditions (temperature: 28-30 º C; photoperiod: 12 h natural light and 12 hours dark; relative humidity 50-55%) with free access to pelleted food (Vet house Ltd. Colombo, Sri Lanka) and tap water. All animal

experiment were conducted in accordance with the internationally accepted laboratory animal use and care, and guidelines (guiding principles in the use of animals in toxicology adopted by the society of Toxicology in 1999) and rules of the Department of Zoology, Faculty of Science, University of Colombo, for animal experimentation.

Collection of plants and preparation of *T. populnea* decoction

Fresh barks of *T. populnea* were collected from the sea-shore at Moratuwa on April 2005 and was identified and authenticated by the scientist in Botany division, at Bandaranaike Memorial Ayurveda Research Institute (BMARI), Maharagama, Sri Lanka. A voucher specimen is deposited at BMARI (UDS/ KSSS 1). The fresh bark was cut into small pieces (2-2.5cm) and washed in tap water. 120 mg of the cut bark was boiled with 1920 ml of water in an earthen pot under low flame until the volume of the extract was reduced to 240ml. The aqueous extract was filtered using a sieve and heated in a water bath until a dark brown semisolid residue (*Ghanasara*) was formed [6,7] (yield 4.6%w/w). This was reconstituted in 1 ml distilled water (DW) to prepare the desired concentrations (1250,2500,5000mg/kg) immediately before oral administration. The mid dose is equivalent to ten times of the human recommended dose. [8]

Evaluation of anti-inflammatory activity

Forty five rats were selected and randomly divided into 5 groups (n =9/group) and was orally treated in the following manner; group 1: 1 ml of DW; group 2: 1250mg/kg of aqueous extract; group 3: 2500 mg/kg of aqueous extract; group 4: 5000 mg/kg of aqueous extract; group 5: 5mg/kg of indomethacin (State Pharmaceutical Corporation, Colombo, Sri Lanka). After 1 h, these rats were injected sub-cutaneously with 0.05ml of 1% (W/V) carrageenan (Sigma chemical Company, St. Louis, USA) suspension into the subplantar surface of foot pad of the left hind paw under mild ether anesthesia using a 27G needle and 1ml syringe. The paw volume of these rats were measured using a plethysmometer (Letica Scientific Instruments, Barcelona, Spain) 1 h before, and 1, 2, 3, 4 and 5 h after the carrageenan injection.

Evaluation of chronic toxicity

Eighteen rats were randomly assigned into two equal groups (n = 9). The first group was orally treated daily, for 30 consecutive days, with the highest dose (5000mg/kg) of *T. populnea* aqueous bark extract and the other with 1ml of DW. During this period, each rat was observed daily for overt signs of toxicity (salivation, lachrymation, ptosis, squinted eyes, writhing, convulsions, tremors, yellowing of fur, loss of hair), stress (erection of fur and exophthalmia), behavioral abnormalities (such as impairment of spontaneous movement, climbing, cleaning of face and ataxia, and other postural changers) and aversive behaviors (biting and scratching behavior, licking of tail, paw and penis, intense grooming behavior and vocalization) and diarrhoea. On day 1 post treatment, these rats were anaesthetized with ether. Blood was collected from tails using aseptic precautions, serum separated and, urea and creatinine (to examine renal toxicity), and GOT and GPT (to judge liver toxicity) levels determined using respective assay kits[9] (Randox Laboratories Ltd. Antrim, UK).

Red blood cells (RBC) count, white blood cell (WBC) count and haemoglobin concentration were determined using standard techniques.

In addition, food and water intake and weight of these rats were determined on day 1 of treatment and day 1 of post treatment. On these two days, urine was also collected (for 5 hours) using metabolic cages and presence of glucose, bilirubin, red blood cells, leukocytes and proteins were checked and pH, and specific gravity were determined using urinalysis reagent strips. (ACON Biotech Co. Ltd., Hangzhou, China).

Statistical analysis

Data are represented as mean ± SEM. Data was analyzed with Mann-Whitney U test. Significance was set at $P< 0.05$. Dose dependency was determined using regression analysis.

RESULTS

As shown in Table 1, oral treatment of low dose of *T. populnea* aqueous bark extract significantly reduced the paw edema at 1st h (by 30%), 4th h (by 26%), and 5 th h (by 26%). The mid dose significantly reduced the paw edema at 1st h (by 33%), 2nd h (by 29%) 4th h (by 24%), and 5 th h (by 21%). The highest dose of aqueous bark extract of *T.populnea* significantly ($P<0.05$) reduced the paw edema at each hour up to 5th h.1st h (by 60%), 2nd h (by 38%), 3rd h (by 24%) 4th h (by 32%), and 5th h (by 32%). A similar reduction of paw edema was evident with the reference drug indomethacin: 1st h (by 63%), 2nd h (by 50%), 3rd h (by 42%) 4th h (by 43%), and 5th h (by 42%). Impairment of paw edema induced by *T. populnea* aqueous extract was dose-dependent ($r^2 = -0.7874$; $P<0.05$).

Table 1: Effect of the oral treatment of aqueous bark extract of *Thespesia populnea* on the carrageenan induced paw edema in rats. (Means ± SEM, n = 09)

Paw volume (ml)					
Treatment	**1h**	**2h**	**3h**	**4h**	**5h**
Control	0.30 ± 0.04	0.64 ± 0.06	0.78 ± 0.09	0.80 ± 0.07	0.71 ± 0.07
1250 mg/kg E	0.21 ± 0.01*	0.64 ± 0.01	0.71 ± 0.04	0.59 ± 0.04**	0.52 ± 0.04*
2500 mg/kg E	0.20 ± 0.02*	0.45 ± 0.03*	0.65 ± 0.05	0.61 ± 0.05*	0.56 ± 0.06*
5000 mg/kg E	0.12 ± 0.04*	0.40 ± 0.05*	0.59 ± 0.07*	0.54 ± 0.06*	0.48 ± 0.06**
5mg/kg indomethacin	0.17 ± 0.05*	0.32 ± 0.05**	0.45 ±0.08**	0.46 ± 0.06**	0.41 ± 0.07**

As compared with controls: * P < 0.05, ** P < 0.01 (Mann-Whitney U - test).

E: aqueous bark extract of *T. populnea*

Table 2: Effect of oral administration of aqueous bark extract of *Thespesia populnea* on body weight, water and food intake, some serum and urine parameters (Means ± SEM)

Parameters	**Control Means ± SEM**	**Extract Means ± SEM**
Body weight (g)	247.2 ± 3.7	242.5 ± 2.2
Water intake (ml)	18.1 ± 0.4	17.7 ± 0.6
Food intake (g)	15.8 ± 0.5	15.9 ± 0.2
Serum urea (mg/dl)	30.4 ± 0.7	31.5 ± 0.6
Serum creatinine (mg/dl)	0.8 ± 0.1	0.7 ± 0.5
SGOT (mg/dl)	20.3 ± 1.0	19.9 ± 0.9
SGPT (mg/dl)	11. 6 ± 1.0	11.8 ± 1.0
RBC (10^6 mm^3)	3.6 ± 0.0	3.6 ± 0.1
Hb (g/dl)	18.4 ± 0.3	18.7 ± 0.3
WBC (mm^3)	10876 ± 67	9986 ± 44
Urine pH	5.3 ± 0.2	5. 6 ± 0.2
Urine specific gravity	1.0 ± 0.0	1.0 ± 0.0

As compared with controls: (Mann-Whitney U - test)

In the toxicity study, overt signs of toxicity, stress, aversive behaviors or behavioral abnormalities were evident with aqueous bark extract of *T. populnea* treatment. As shown in Table 2, none of parameters monitored were significantly (P> 0.05) altered by the aqueous bark extract of *T. populnea*. In addition, in the urine of both, control and treated rats glucose, protein, bilirubin, erythrocyte and leukocytes were not detected.

DISCUSSION

This study examined the anti-inflammatory potential of aqueous bark extract of *T. populnea* in rats using carrageenan-induced paw edema model. This model is a widely used sensitive and reliable test for the investigation of potential acute inflammatory agent. The result showed, that the aqueous bark extract of *T. populnea* possesses safe acute anti-inflammatory activity. This is a therapeutically important finding since aqueous bark extract of *T. populnea* of plants are usually used in Ayurveda, traditional and folk medicines [10]. The anti-inflammatory activity was dose-dependent indicating that the effect was intrinsic and causal and may not have been resulted from non specific action of the aqueous extract. Further, the effectiveness of anti-inflammatory action of the aqueous bark extract of *T. populnea* is comparable to that of reference drug, indomethacin, which is widely used non-steroidal anti-inflammatory agent [11].

In the carrageenan edema model, the early phase (1-2 h) is attributed to the release of histamine and serotonin and increase synthesis of prostaglandins in the surrounding tissue [12]. While the late phase (4-5 h) is mediated by leukotrienes, mobile phagocytic cells, polymorphoneuclear cells, monocytes, macrophages and prostaglandins are produced by the tissues macrophages. The edema maintained between the early and late phase (3 h) is due to kinin like substances, especially bradykinin [13].

The aqueous extract of *T. populnea* impaired all these three phases in the paw edema test. This indicates the aqueous extract of *T. populnea* induced anti-inflammatory action may be mediated by impairing the synthesis and/or release of chemicals and infiltration cells mentioned earlier.

The aqueous bark extract of *T. populnea* did not induce overt signs of toxicity even following chronic administration. Further, it was not renotoxic (in terms of blood urea and serum creatinine), hepatotoxic (in terms of SGOT, SGPT) and, haemotoxic (in terms of RBC, WBC count and, Hb levels) displaying an encouraging safety profile.

CONCLUSION

In conclusion, this study shows for the first time, safe and potent oral anti-inflammatory activity of aqueous bark extract of *T. populnea.* Further, the results provide a scientific basis for its use in traditional ethnomedical use in Sri Lanka.

ACKNOWLEDGEMENT

We wish to thank Mr. J.R.A.C. Jayakody and Mr. R.R. Kumara for technical assistance.

REFERENCES

1. Jayaweera DMA. Medicinal plants Part -4. Sri Lanka; The national council of Sri Lanka; 1982. p.37.
2. Dassanayake MD, Fosberge FR. A revised handbook to the flora of Ceylon. Vol. -II. New Delhi; Oxford and IBH publishing co.PVT. LTD; 1997.p.312.
3. Vasudevan M, Gunnam KK, Parle M. Antinociceptive and anti-inflammatory effects of Thespesia populnea bark extract. J Ethnopharmacol. 2006.109: p. 264-270.
4. Vasudevan M, Parle M. Memory- Enhancing activity of Thespesia populnea in rats. Pharma Biol. 2007; 45 (4): p. 267-273.
5. Anonymous, The Wealth of India. Raw Materials. Vol.-10. New Delhi; Council of Scientific and Industrial Research. 1962; p.223-225.
6. Javaj T. Shaarangadhara Samhita, Madhyma Khanda 8/1. India; N. S. Press 23, kolbhat Lane, Bombe, 1914; p.98.
7. Savrikr SS, Ravishankar B. Bhaishajya Kalpana-The Ayurveda Pharmaceutics. Afr.J. Trad.CAM. 2010; 7 (3): p.180.
8. Rathnasooriya WD, Daraniyagala SA & Priyadarshani S. Antinociceptive activity of the aqueous leave of Pongamia pinnata in rats. IOSR journal of pharmacy. 2016; 6: p.15-22.
9. Daraniyagala SA, Rathnasooriya WD & Gunasekara CL. Antinociceptive effect and toxicology study of the aqueous extract of Barringtonia racemosa on rats. Journal of Ethnopharmacology. 2003; 86: p.21-26.
10. Anonymous, Ayurveda Pharmacopia. Part 2, Vol.-I. Sri Lanka; Department of Ayurveda. 1979; p.73.
11. Rang HP, Dale MM. Pharmacology. 2nd edition,UK; Churchill Livingston, 1991.
12. Vinegar R, Scheriber W, Hugo R. Biphasic development of carrageenan oedema in rats. J Pharmacol. Exp. Ther. 1969; 166: p.96-103.
13. Vinegar R, Trvax JF, Selph JL, Johnston PR, Venable AL, Makenzie KK. Pathway to carrageenan- induced inflammation in the hind limb of the rat, Fed proc. 1987; 46: p.118-126.

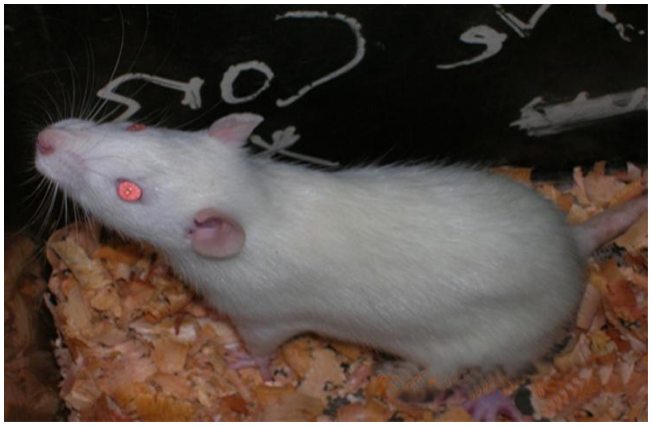
Cite this article as:
U.D.S.Sewwandi, E.R.H.S.S.Ediriweera, W.D.Ratnasooriya. Anti-Inflammatory Activity of Aqueous Bark Extract of Thespesia Populnea in Rats. International Journal of Ayurveda and Pharma Research. 2019;7(12):14-19.
Source of support: Nil, Conflict of interest: None Declared

***Address for correspondence**
Dr E.R.H.S.S. Ediriweera
Senior Professor, Department of Nidana Chikithsa, Institute of Indigenous Medicine, University of Colombo, Rajagiriya, Sri Lanka.
Email: ayurvedadocsujatha@yahoo.com

Branch of *Thespesia populnea* Linn.

Flower of *Thespesia populnea* Linn.

Bark of *Thespesia populnea* Linn.



Wistar Rat

Measuring paw volume

Digital Plethysmometer