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Protective Effects of *Thespesia populnea* (L.) Sol ex. Correa in Inflammatory, Nociceptive and Arthritic Conditions on Experimental Animals

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Authors' contributions

This work was carried out in collaboration between all authors. PSP designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. PDA wrote the protocol, and helps to write the first draft of the manuscript. RSG and RV managed the analyses of the study. PRS managed the literature searches. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: To evaluate the anti-inflammatory, antinociceptive and antiarthritis activities of methanolic extract of *T. populnea* flower (TPF) and root (TPR) extract; yet unreported.
Study Design: Extraction and administration of bioactive extract.
Place and Duration of Study: Department of Pharmacology and Department of Pharmacognosy, R.V.S. College of Pharmaceutical Science, Sulur, Coimbatore, Tamilnadu, India, between June 2010 and July 2011.
Methodology: *Thespesia populnea* flowers and roots were extracted by soxhlet extraction using methanol. Anti-inflammatory activity of TPF and TPR was studied by using acetic acid induced vascular permeability and cotton-pellet granuloma. The antinociceptive activity of TPF and TPR was evaluated using formalin-induced paw licking response and the hot-plate test. The antiarthritic activity was studied by using adjuvant-induced arthritis model in rat. In addition total flavonoid content was determined with spectrophotometric method.
Results: Administration of TPF and TPR (400 mg/kg) significantly ($P < 0.01$) decreased

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the formation of granuloma tissue induced by cotton pellet at a rate of 37.06% and 25.76% respectively. TPF and TPR inhibited acetic acid-induced vascular permeability in mice. In the adjuvant-induced arthritis test TPF and TPR inhibited 50.68% and 30.13% of paw thickness respectively. TPF and TPR also produced significant ($P < 0.01$) analgesic activity in formalin-induced paw licking response. In the hot-plate test, TPF and TPR have shown significantly ($P < 0.01$) increased in latency time when compared with control.
Conclusion: Altogether, the present data demonstrate the anti-inflammatory antinociceptive and antiarthritis properties of flower and root of *Thespesia populnea* suggesting its potential role as adjuvant therapeutic tool for the management of inflammatory-related diseases.

Keywords: Antinociceptive; anti-inflammatory; antiarthritis; flavonoid content; cotton pellet; Thespesia populnea.

1. INTRODUCTION

In analysis the research during the last decades, it is estimated that the analgesics are one of the highest therapeutic categories on which research efforts are concentrated [1]. Analgesic compounds available in the market, still present a wide range of undesired effects [2] leaving an open door for new and better compounds. Natural products are believed to be an important source of new chemical substance with potential therapeutic applicability [3].

Thespesia populnea (L.) Soland ex Correa (family: Malvaceae) is a large avenue tree found in the tropical regions and coastal forests in India. The bark, leaves, flowers and fruits are useful in cutaneous infections, such as scabies, psoriasis, eczema, ringworm and guinea worm. The leaves are applied locally for their anti-inflammatory effects in swollen joints [4,5]. A decoction of the bark is commonly used for the treatment of skin and liver diseases. Oil of bark mixed with vegetable oil is useful in urethritis and gonorrhea [6]. The alcoholic bark extract was also evaluated for hypoglycemic and anti-hyperglycemic studies [7]. The astringent bark, roots and fruits were used in dysentery, cholera and hemorrhoids; bark is employed as a poultice for wounds. In the indigenous system of medicine, the paste of fruits, leaves and roots are applied externally for various skin diseases. The barks possess astringent, hepatoprotective and antioxidant activity in rats [8,9]. A polyherbal formulation containing *T. populnea* as one of the ingredient was shown useful remedy for Alzheimer's disease [10]. Four naturally occurring quinines, viz. thespone, mansonone-D, mansonone-H, and thespesone, have also been extracted from heartwood of *T. populnea* [11]. Much work has been done and reported on various plant parts especially on bark and leaves. But mechanisms are not clearly reported, whether activity is due to suppression of central and peripheral pathways of inflammation or both.

In view of this and on account of the reported usefulness of this plant in traditional system of medicine and inflammatory conditions, the present study was aimed at investigating possible antinociceptive, anti-inflammatory and anti-arthritis activity of methanolic extract of flower and root to ascertain ethnopharmacological claims; which is not yet evaluated.

2. MATERIALS AND METHODS

2.1 Plant Materials and Extract

The flower and root of *T. populnea* (International Plant Names Index number 52854-3:1.1) was obtained from Nasik district (M.S.) and authenticated by Dr. D.A. Patil, reader and the authorized plant identifier of Department of Botany, S.S.V.P.S. College, North Maharashtra University, Dhule (M.S); a specimen is preserved in the college herbarium (KBHSS/PCG/2009/12).

2.2 Preparation of Extracts

The collected flowers and roots were sun dried for 5 days and pulverized into a dry powder. The powder (1.5 kg) was subjected to extraction in soxhlet extractor using methanol (5 Lit) at boiling point for 72h. The extracts were filter and each filtrate was evaporated by distillation under reduced pressure using rotary vacuum evaporator at 30ºC and stored in the dark at 4ºC. The extraction yielded 8.7% (w/w) of methanolic extract of flower (TPF) and 12.4% w/w methanolic extract of root (TPR).

2.3 Phytochemical Investigation

Phytochemical screening of TPF and TPR were carried out employing standard procedures and tests [12,13] for the presence of chemical constituents such as tannins, saponins, unsaturated sterols, triterpenes, alkaloids, anthraquinones, flavonoids, lactones/ esters, protein/amino acids and carbohydrates and/or glycosides.

Total flavonoids content was determined by employing standard procedure [14]. TPF and TPR was mixed with 1.5 mL of methanol, 0.1 mL of 10% Aluminium chloride, 0.1 mL of 1M potassium acetate and 2.8 mL of purified water. After 30 min, the absorbance was measured at 415 nm. Calibration curve was obtained by preparing quercetin solutions (12.5 to 100 µg/ mL in methanol).

2.4 Animals

The study was performed with wistar rats (180–200 g) and swiss albino mice (25–30 g) of either sex. Animals were obtained from Department of Pharmacology, RVS College of Pharmaceutical Sciences, Sulur, Tamilnadu, India. Animal quarters were maintained at a temperature of 24± 2ºC and with 12-h light/12-h dark cycle. Rats were acclimatised, fed commercial pelleted feed and water *ad libitum*.

Experimental protocols reported in this study were approved by the Institutional Animal Ethical Committee of CPCSEA, Govt. of India (1012/C/06/CPCSEA) and carried out accordance with local IAEC guidelines.

2.5 Acute Toxicity Testing

The procedure was followed by using OECD guidelines (Organization of Economic Cooperation and Development) 423 (Acute Toxic Method) [15]. Rats of either sex were divided into three groups of three each. Vehicle control group received 0.2% Carboxy Methyl Cellulose (CMC) while, second and third group 2000 mg/kg b.w. of TPF and TPR suspended

in 0.2% CMC respectively. Immediately after the dose, animals were observed continuously for first 4 h and next 14 days for signs of toxicity or mortality.

2.6 Anti-Inflammatory Activity

2.6.1 Acetic acid induced vascular permeability in mice (acute test)

One hour after oral administration of the extracts TPF and TPR at doses of 100, 200 and 400 mg/kg, mice were injected with 0.25 ml of 0.6% solution acetic acid intraperitoneally. Indomethacin (5 mg/kg, p.o.) served as the reference drug, while animals in the control group received 0.2 ml CMC suspension at a dose of 10 ml/kg. Immediately after administration, 10 ml/kg of 10% (w/v) Evan's blue was injected intravenously through the tail vein. Thirty minutes after Evan's blue injection, the mice were killed and the viscera exposed. The animals were held by a flap of abdominal wall and the viscera irrigated with distilled water over a petridish. The exudate was then filtered and made up to 10 ml. The vascular permeability was represented in terms of the absorbance (A_{610}) and % inhibition [16,17].

2.6.2 Cotton pellet granuloma in rats (chronic test)

The effect of TPF and TPR on chronic or proliferative phase of inflammation was assessed in cotton pellet granuloma rat model. [18] Autoclaved cotton pellets weighed 20 ± 1 mg each was implanted subcutaneously through small incision made along the axilla or flank region of the rats anesthetized with ether. The different groups of rats were administered the TPF and TPR (100, 200 and 400 mg/kg, p.o.) and Indomethacin (5 mg/kg, p.o.) once daily for seven consecutive days from the day of cotton pellet insertion. The control group received 0.2 mL CMC suspension. On the eighth day, all the rats were anesthetized and the cotton pellets covered by the granulomatous tissue were carefully removed. The moist pellets were weighed and then dried at 60ºC for 48 h and again weighed. The reduced weights of the cotton pellets were observed for the test compounds and compared with that the respective controls. This provides a measure to assess the anti-inflammatory effect of the test compounds [19].

2.7 Antinociceptive Activity

2.7.1 Hot plate method in mice

Mice that showed nociceptive responses within 20 s when placed on hot plate maintained at 55 ± 0.5ºC were selected and grouped into eight (n=6). Group I was control and treated with CMC suspension; groups III- V and VI-VIII received 100, 200 and 400 mg/kg p.o. of the extract TPF and TPR respectively; while group II received 2 mg/kg i.p. of Pentazocine. Each mouse was placed singly on the hot plate and the latency to exhibit thermal stimulus were determined before and at 30, 60, 90 and 120 min after treatment. Licking of paws and jumping were the parameters evaluated as the thermal stimulus. 30 sec. was taken as the cut-off time to avoid animal tissue damage [20,21]. Each mouse served as its own control. Before treatment, its reaction time was taken as initial reaction time. The mean reaction time for the all groups was pooled to obtain the final control mean reaction time (Tb). This was pooled for the mice in each treatment group and the final test mean value for each treatment group at each measurement was calculated [22]. This final test mean value represented the

after treatment reaction time (Ta) and was subsequently used to determine the percentage thermal pain stimulus or protection by applying the following formula:

% protection against thermal induction = Test mean (Ta) – Control mean (Tb) / Control mean (Tb)

2.7.2 Formalin induced nociception in mice

The mice were observed in a chamber with mirrors were mounted on three sides to allow clear observation of the paws of the animals. The animals (8 groups, n=6) were treated orally with CMC suspension or with the methanolic extract of TPF and TPR (100, 200 and 400 mg/kg, p.o.) 1-h before formalin injection. Each mouse was placed in the chamber more than 5 min before treatment in order to allow acclimatization to the new environment. 20 µL of a 2.5% formalin solution was injected in the dorsal surface of the left hind paw. Each animal was then returned to the chamber and the amount of time that the animal spent licking the injected paw was considered as indicative of pain. Two distinct phases of intensive licking activity were identified; an early acute phase (0–5 min) and a late or tonic phase (15–30 min after formalin injection, respectively) [23,24].

2.8 Antiarthritis Activity

2.8.1 Adjuvant induced arthritis in rats

Rats of either sex were divided into four groups of six each. Group I served as control. Group II served as standard and received Diclofenac sodium (DF) (5 mg/kg, p.o.) one hour prior to the induction of arthritis. Group III was treated with TPF, and groups IV with TPR at the doses of 400 mg/kg, p.o. respectively. Arthritis was induced in rats by injection of 0.1 ml of Freund's complete adjuvant (FCA) into the subplantar region in the left hind paw one hour after the administration of doses (day 0). Subsequently, treatment was continued daily until day 14, and paw volume was measured by using digital plethysmometer (UGO Basile 7140, Italy). The body weights and hematological parameters like White blood cell (WBC) count, Erythrocyte sedimentation rate (ESR) of the rats were also measured [25,26].

2.9 Statistical Analysis

Statistical analysis was performed by comparing the treatment groups with the vehicle control group using GraphPad Prism4 (GraphPad Software, Inc., CA, USA). All the variables were subjected to a one-way analysis of variance, followed by Dunnett's multiple comparison test. Results with $P<0.05$ were considered statistically significant.

3. RESULTS

3.1 Phytochemical Investigation

The phytochemical screening of methanolic extract of *Thespesia populnea* flower and root shows the presence of flavonoids, terpenoids, tannins, saponins and glycoside. Test for alkaloids, anthraquinones, lactones/ester, and protein/amino acids showed negative responses. Total flavonoid content in TPF (1.20%) is greater than TPR (0.344%).

3.2 Acute Toxicity Testing

Preliminary acute toxicity studies showed no toxic side effects and mortality in animals of both sexes. Hence, the extracts TPF and TPR were safe up to the dose of 2000 mg/kg b.w., from that three doses (100, 200 and 400 mg/kg b.w.) were selected for pharmacological studies.

3.3 Anti-Inflammatory Activity

3.3.1 Acetic acid induced vascular permeability in mice

T. populnea reduced the extent of peritoneal inflammation induced by injection of acetic acid. The absorbance was considerably (P<0.01) reduced in all doses of TPF and TPR. Standard drug treatment indomethacin exhibited strongest activity with 65.31% inhibition in dye leakage as compared to control (Table 1).

Table 1. Effect of TPF and TPR on acetic acid induced vascular permeability in mice

Groups	Dose (mg/kg)	Absorbance (% inhibition)
Control	CMC suspension	1.848 ± 0.12
Indo	5	0.641 ± 0.10** (65.31)
TPF	100	1.560 ± 0.15 (15.58)
	200	1.141 ± 0.04* (38.26)
	400	0.710 ± 0.11** (61.58)
TPR	100	1.564 ± 0.09 (15.37)
	200	1.397 ± 0.10 (24.40)
	400	0.878 ± 0.80** (52.49)

*Indo: Indomethacin; CMC: Carboxy Methyl Cellulose; TPF: methanolic extract of flower; TPR: Methanolic extract of root; Values are expressed as mean ± SEM. n = 6. * P < 0.05 compared with control (ANOVA followed by Dunnett's t-test). ** P < 0.01 compared with control (ANOVA followed by Dunnett's t-test).*

3.3.2 Cotton pellet induced granuloma in rats

Table 2 shows chronic anti-inflammatory effects of the TPF and TPR at the doses of 100, 200 and 400 mg/kg. Changes in the cotton pellets weights (wet weight–dry weight) of the test substances were compared with the controls. TPF and TPR showed significant (P< 0.01), dose dependant chronic anti-inflammatory effect. TPF and TPR (400 mg/kg) showed 37.06% and 25.76% inhibition respectively; whereas Indo treated group exhibited 46.52%. Their effects are in comparable magnitude with the standard anti-inflammatory drug.

3.4 Anti-Nociceptive Activity

3.4.1 Hot plate method in mice

TPF at the dose 400 mg/kg showed a significant analgesic effect (P<0.01) against thermally induced pain at 30, 60, 90 and 120 min of the study period (Table 3), with more than 50% protection was achieved at 60 min. TPR at the dose 400 mg/kg significantly increased the hot-plate latency time in mice starting from 30 min (P< 0.05), at 120 min it showed the maximum protection (~112%) which is fairly similar to TPF (400 mg/kg). By contrast,

Pentazocine was shown to provide protection against thermally induced stimuli throughout the observation period (P<0.01 at 30, 60, 90 and 120 min), with maximum protection (~157%) conferred after 120 min. (Fig. 1).

Table 2. Effect of TPF and TPR on % inhibition on cotton pellet induced chronic inflammation in rats

Groups	Dose (mg/kg)	Wet weight (g)	Dry weight (g)	Difference (g)	% Inhibition
Control	CMC suspension	154.62± 2.04	56.87± 3.70	97.75	0.00
Indo	10	73.98±2.03**	21.7±1.03**	52.28	46.52
TPF	100	138.57±2.40*	41.05±1.61**	97.52	0.24
	200	108.87±4.37*	33.47±1.18**	75.4	22.86
	400	84.12±2.25**	22.6± 1.24**	61.52	37.06
TPR	100	142.18±1.87*	44.62±1.87**	97.56	0.19
	200	120.33±1.50*	38.83±1.82**	81.5	16.62
	400	101.6±2.07**	29.03±2.96**	72.57	25.76

*Indo: Indomethacin; CMC: Carboxy Methyl Cellulose; TPF: methanolic extract of flower; TPR: Methanolic extract of root; Values are in milligrams. Mean ± SEM, (n=6) was computed over six animals/ group. * P < 0.05 compared with control (ANOVA followed by Dunnett's t-test). ** P < 0.01 compared with control (ANOVA followed by Dunnett's t-test). Percentage inhibition was computed over respective negative control groups.*

Table 3. Effect of TPF and TPR on latency time and % protection against thermal induction on hot plate method in mice

Groups	Dose (mg/kg)	Latency time/ reaction time (sec)				
		Pre drug latency	30 min	60 min	90 min	120 min
Control	CMC Suspension	4.34± 0.17	4.41± 0.18	4.8± 0.46	4.93± 0.44	6.14± 0.47
Penta	2	5.07± 0.21	6.60± 0.51** (30.18)	10.25± 0.64** (102.17)	13.18± 0.51** (159.96)	13.06± 0.72** (157.59)
TPF	100	4.78± 0.24	5.03± 0.15 (5.23)	7.55± 0.41** (57.95)	7.12± 0.42* (48.95)	8.39± 0.44* (62.30)
	200	5.12± 0.39	5.29± 0.35 (3.32)	6.73± 0.34* (31.45)	8.31± 0.57** (62.30)	9.92± 0.40** (93.75)
	400	5.37± 0.26	7.33± 0.49** (36.50)	8.63± 0.52** (60.71)	11.03± 0.75** (105.40)	11.41± 0.41** (112.48)
TPR	100	4.59± 0.31	4.78± 0.22 (4.14)	6.82± 0.41* (48.58)	7.82± 0.54** (70.37)	8.33± 0.35* (81.48)
	200	5.37± 0.26	4.77± 0.23 (-11.17)	7.04± 0.29* (31.10)	8.06± 0.53** (68.72)	8.8± 0.38** (63.87)
	400	4.49± 0.25	5.9± 0.51* (31.40)	7.81± 0.73** (73.94)	9.1± 0.47** (102.67)	9.54± 0.53** (112.47)

*Penta: Pentazocine; CMC: Carboxy Methyl Cellulose; TPF: methanolic extract of flower; TPR: Methanolic extract of root; Values are expressed as mean ± SEM. n = 6. * P < 0.05 compared with control (ANOVA followed by Dunnett's t-test). ** P < 0.01 compared with control (ANOVA followed by Dunnett's t-test). Values in parentheses indicate % protection against thermal induction.*

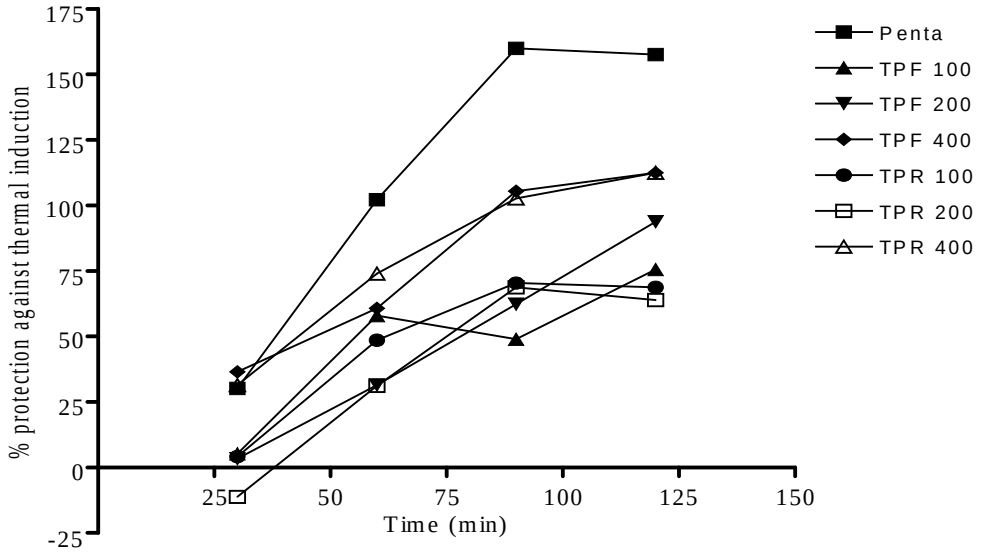

Fig. 1. Effect of TPF and TPR on % protection against thermal induction on hot plate method in mice.

Penta: Pentazocine; TPF: methanolic extract of flower; TPR: Methanolic extract of root.

3.4.2 Formalin induced nociception in mice

Table 4 depicts the effect of TPF and TPR on formalin induce nociceptive response in mice. As shown, all the three doses of the TPF and TPR were significantly (P<0.01) impaired the time spent on licking of injected paw, both in the early (0- 5 min) and late phases (15- 30 min) of nociception. However, the effect in the early phase appears to be rather higher than in the late phase.

Table 4. Effect of TPF and TPR on nociceptive response on formalin induced nociception in mice

Groups	Dose (mg/kg)	Nociceptive response (sec)		% inhibition	
		Early Phase	Late phase	Early phase	Late phase
Control	CMC Suspension	58.1± 1.09	39.28± 1.04	--	--
Penta	2	13.52 ± 1.36**	7.98± 1.06**	76.73	79.68
TPF	100	44.77± 1.70*	33.38± 1.70	22.94	15.02
	200	35.1± 1.48*	20.57± 1.19*	39.59	47.63
	400	18.3± 1.47**	13.7± 1.41**	68.50	65.12
TPR	100	44.63± 1.13*	36.37± 2.27	23.18	7.41
	200	36.43± 1.51*	34.7± 1.16*	37.29	11.66
	400	24.9± 1.72**	16.34± 1.08**	57.14	58.40

*Penta: Pentazocine; CMC: Carboxy Methyl Cellulose; TPF: methanolic extract of flower; TPR: Methanolic extract of root; Values are expressed as mean ± SEM. n = 6. * P < 0.05 compared with control (ANOVA followed by Dunnett's t-test). ** P < 0.01 compared with control (ANOVA followed by Dunnett's t-test).*

3.5 Adjuvant Induced Arthritis in Rats

In Table 5, on day 14, a dose-dependent reduction in % rise in paw volume was exhibited by the extract of *T. populnea*. At a dose of 400 mg/kg per day of the extract, arthritic swelling was inhibited by 50.68%, compared with DF, which produced an inhibition of 63.01%. The body weights of extract treated rats were also found to have increased significantly ($P<$ 0.01), compared with CMC suspension treated animals. White blood cells (WBC) count and Erythrocyte sedimentation rate (ESR) were significantly ($P<0.01$) reduced in treated groups as compared to control.

Table 5. Effect of TPF and TPR on % paw volume, body weight, WBC count, and ESR on adjuvant induced arthritis in rats

Group	Dose (mg/kg)	Difference in paw volume (mL)	Body weight on day 14 (g)	WBC count (X $10^3/mm^3$)	ESR (mm/hr)
Control	CMC Suspension	1.46 ± 2.32	164.7 ± 1.25	13.19 ± 0.32	6.19 ± 0.20
DF	5	0.54 ± 3.61** (63.01)[a]	184.7 ±1.78** (3.8)[b]	9.53 ± 0.24**	3.42 ± 0.15**
TPF	400	0.72 ± 3.42** (50.68)[a]	195.5 ±5.58** (5.5)[b]	9.82 ± 0.37**	3.94 ± 0.25**
TPR	400	1.02 ± 4.87** (30.13)[a]	191.5 ±5.50** (5)[b]	11.28 ± 0.30**	5.22 ± 0.21**

*DF: diclofenac; CMC: Carboxy Methyl Cellulose; TPF: methanolic extract of flower; TPR: Methanolic extract of root; Values are expressed as mean ± SEM. n = 6. * P < 0.05 compared with control (ANOVA followed by Dunnett's t-test). ** P < 0.01 compared with control (ANOVA followed by Dunnett's t-test). Values in parentheses indicate, a: % reduction in paw volume; b: % gain in body weight.*

4. DISCUSSION

T. populnea is used in the indigenous system of medicine for the treatment of inflammatory conditions. However, there is no reported literature on detailed investigation on flower and root of the plant for rationality behind their use in inflammation. With the view of screening natural products to find novel anti-inflammatory drugs, we have evaluated the methanolic extracts of this plant in models of acute (acetic acid induce vascular permeability) and chronic (cotton pellet induced granuloma) inflammation, nociception (hot plate method and formalin test) and arthritis (adjuvant induced arthritis). The anti-inflammatory activity of methanol extract of *T. populnea* flower was found to be comparatively effective in chronic inflammation as well as in acute inflammation.

In order to understand the mechanism(s) of the anti-inflammatory activity of *T. populnea,* the effects of the extracts on vascular permeability was evaluated. This phenomenon is one of the hallmarks of the inflammatory process. Because of increased permeability; platelet activating factor aggregates platelets, releases from their granules, histamine and serotonin, activates and degranulates leukocytes, and induces chemotaxis, edema, and bronchospasm [27]. PGD2 from mast cell along with PGE2 causes vasodilation and increased vascular permeability of post capillary venules, thus potentiating the edema formation [28]. The ability of TPF and TPR to reduce the vascular permeability by reduction of dye leakage into the peritoneum maybe indicates its ability to inhibit permeability of small blood vessels and an

effect on leukocyte migration and antagonism of the phlogistic actions of mediators of inflammation.

Chronic inflammation is generated when the body fails to respond against inflammatory agents. This leads to fibroblast proliferation and formation of granulomatous tissues [29]. The cotton pellet-induced granuloma formation may be considered an example of chronic inflammation responses which include a transudative phase and a proliferative phase [30]. The fluid absorbed by the pellet greatly influences the wet weight of the granuloma whereas the dry weight correlates well with the amount of granulomatous tissue formed [31]. The extracts of *T. populnea* exhibited significant anti-inflammatory activity in the cotton pellet test. This effect possibly shows the ability of the extracts in reducing the number of fibroblasts, and synthesis of collagen and mucopolysaccharide, which are natural proliferative events of granulation tissue formation.

Hot-plate test is one of the most common tests of nociception that are based on a phasic stimulus of high intensity [32]. Pain induced by thermal stimulus of the hot-plate is specific for centrally mediated nociception [33]. All doses of the extracts were prolonged the hot-plate latency with time, which was nearly similar to standard Pentazocine, may indicating that the extracts' analgesic effect is comparable to that of Pentazocine though it is less potent and slow acting. The ability of the extract to prolong the reaction latency further suggests that the extract is endowed with a central analgesic activity.

The formalin test, however, is sensitive to non-steroidal anti-inflammatory drugs and other mild analgesics. The test possesses two distinct phases, possibly reflecting different types of pain. The earlier phase reflects a direct effect of formalin on nociceptors (non-inflammatory pain), whereas the late phase reflects inflammatory pain [23,34]. The extracts of *T. populnea* were produced significant analgesic effect in both phases. This probably indicates that the analgesic effect of the extracts was mediated by both neurogenic (central) and inflammatory (peripheral) mechanisms.

An anti-arthritic effect was also exhibited by the extract of *T. populnea* flower and root at the dose of 400 mg/kg, with a resulting increase in body weight, compared with control animals. The determinations of paw volume and changes in body weight of the animals have been used in evaluating anti-inflammatory activity and therapeutic effects of treatment. Earlier findings suggest that, absorption of ^{14}C-glucose and ^{14}C-leucine in rat's intestine was reduced in the case of inflammed rats. But on treatment with anti-inflammatory drugs, the decrease in absorption was nullified and this showed that the anti-inflammatory drugs correct the decrease or deranged absorption capacity of intestine during inflammation [35,36]. In arthritic condition release of IL-1β increases the production of granulocytes and macrophages colony stimulating factor, these increased values suggest inflammation or infection. In the present study TPF and TPR showed decrease WBC count as compared to arthritic control rats. This may indicates that migration of leucocytes into the inflamed area was suppressed by extracts and standard drug DF. Increase in ESR is a common feature in arthritis [37]. TPF and TPR significantly lowers the ESR as compared with control group.

Thus, the strong anti-inflammatory and antinociceptive activity of *Thespesia populnea* may be due to cumulative effects of different active constituents in reducing the synthesis, release and action of histamine, serotonin, and prostaglandins. The evidence that the extract has significant anti-inflammatory activity as well as antinociceptive efficacy might be the pharmacological basis for the folkloric use of the plant as a remedy against acute

rheumatism. Such association is well known for a lot of analgesics especially the NSAIDs [38].

5. CONCLUSION

In conclusion, the present study demonstrated the anti-inflammatory and antinociceptive activities associated with the flower and root of *Thespesia populnea*, presenting evidence for the ethnobotonical use of the plant for painful conditions such as arthritis. The findings also suggested that the analgesic activity of methanolic extract could involve both peripheral and central mechanisms. Moreover, the extracts appeared to be very safe when taken orally as they were devoid of any overt toxicity in the experimental animals used in this study.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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