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Anti-inflammatory, analgesic and antipyretic properties of *Thespesia populnea* Soland ex. Correa seed extracts and its fractions in animal models

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ABSTRACT

Ethnopharmacological relevance: Thespesia populnea Sol. ex Correa (Malvaceae) is commonly known as "Indian tulip tree". The plant has been used as an astringent, antibacterial, anti-inflammatory, antinociceptive and hepatoprotective in Indian system of traditional medicine.

Materials and methods: Thespesia populnea seeds were successively extracted by soxhlet extraction using petroleum ether (40–60 °C) (TPO) and ethanol (TPE). Unsaponifiable matter (TPOUM) and fatty acids were separated from seed oil. A GC–MS analysis of fatty acid methyl esters was carried out. Ethanolic extract was fractionated using $CHCl_3$, EtOAc, n-BuOH and H_2O. Acute arthritis was induced by sub-plantar injection of carrageenan into the left hind paw of rats. The paw volume was measured using plethysmometer. Analgesic activity was assessed by heat induced pains (tail immersion model) and antipyretic activity assessed using brewers yeast-induced pyrexia model.

Results: Oral administration of TPO and TPE at 200 and 400 mg/kg b.w. and tested fractions at 200 mg/kg significantly reduced carrageenan induced paw edema and brewers yeast-induced pyrexia. In tail immersion method also extracts and fractions showed significant analgesic activity. Amongst all fractions EtOAc showed most significant results. TPOUM showed significant anti-inflammatory and analgesic activity. GC–MS analyses showed the presence of fourteen fatty acids, predominant fatty acids were palmitic and stearic acid.

Conclusion: These results highlighted anti-inflammatory, analgesic and antipyretic effects of *Thespesia populnea* seeds.

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1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are used worldwide for the treatment of inflammation, pain and fever as well as for cardiovascular protection. However, the side effects of currently available anti-inflammatory drugs include gastric ulcer, renal damage, bronchospasm and cardiac abnormalities have limited their use (Burke et al., 2006). Many researchers have focused in recent years on medicinal plants derived natural products such as flavonoids, steroids, polyphenols, coumarins, terpenes and alkaloids due to their wide range of pharmacological significance including anti-inflammatory, analgesic and antipyretic activities with lesser side effects (Shukla et al., 2010).

Thespesia populnea (L.) Sol. ex. Correa (Malvaceae) is evergreen shruby tree, commonly known as "Indian tulip tree" and found in tropical regions and coastal forests of India (Anonymous, 1995; Belhekar et al., 2009). The bark, leaves, flowers and seeds are useful in cutaneous infections such as scabies, psoriasis, eczema, ringworm and guinea worm (Chopra et al., 1956). In the indigenous system of medicine, the paste of fruits, leaves and roots are applied externally for various skin diseases. The leaves are applied locally for their anti-inflammatory effects in swollen joints (Anonymous, 1995; Vasudevan et al., 2007).

The plant have been thoroughly studied scientifically in the last four decades and have been reported to possess a number of medicinal properties such as antihyperglycemic (Belhekar et al., 2009), hepatoprotective (Yuvaraj and Subramoniam, 2009), antinociceptive and anti-inflammatory (Vasudevan et al., 2007), antidiarrheal (Viswanatha et al., 2007) and antifertility (Waller et al., 1983).

Four naturally occurring quinines viz. thespone, thespesone, mansonone-D and mansonone-H have been extracted from plant heart wood. The phytochemical study of bark reveals the presence of gossypol, tannin, acacetin, quercetin, coloring matter and leaf extract indicates the presence of lupeol, lupenone, β-sitosterol (Parthasarathy et al., 2009). The flowers contained kaempferol, kaemperol-7-glucoside and gossypetin. The fruit kernels were reported to contain β-sitosterol, ceryl alcohol and a yellow pigment, thespesin (Ghosh and Bhattacharya, 2004).

Although much work has been done on various parts of this plant especially on bark, information on its anti-inflammatory,

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analgesic and antipyretic properties of seeds are lacking. Therefore the present study was designed to evaluate anti-inflammatory, analgesic and antipyretic activities of *Thespesia populnea* seed oil, unsaponifiable matter, ethanolic extract and fractions to ascertain ethnopharmacological claims.

2. Materials and methods

2.1. Collection and authentication of plant material

The *Thespesia populnea* seeds were collected from the campus of Sitabai Thite College of Pharmacy, Shirur, Dist. Pune in February. The plant was identified taxonomically by Prof. T. Chakraborty, Scientist D, Botanical Survey of India, Pune (M.S.) with a voucher specimen No. AMSTP-1 and herbarium was deposited for future reference.

2.2. Preparation of extracts and fractionation

The collected seeds were sun dried in open air for 5 days and reduced to coarse powder (200 g). The powder was subjected to successive extraction in soxhlet extractor as per standard American Oil Chemical Society procedure (AOAC, 1995) using petroleum ether (40–60 °C) and ethanol at their boiling points for 24 h and 48 h respectively. The extracts were filtered and filtrate was evaporated by distillation under reduced pressure using rotary vacuum evaporator at 30 °C and stored in the dark at 4 °C. The extraction yielded 29.5% (w/w) of petroleum ether extract (TPO) and 11.04% (w/w) of dried ethanolic extract (TPE).

The ethanolic extract (10 g) was suspended in water and fractionated with $CHCl_3$, EtOAc, n-BuOH, and water soluble layer. Each fraction was evaporated to obtain the chloroform (1.5 g), EtOAc (3.5 g), n-BuOH (3.1 g) and H_2O (1.3 g).

2.3. Separation of unsaponifiable matter and fatty acids from TPO

10 g of oil was saponified with 5% methanolic KOH (200 ml) by heating under reflux for 6 h, methanol evaporated and 200 ml water was added to the residue. The mixture was extracted three times with diethyl ether (3 × 100 ml). The combined ether extracts were washed with water till free from alkalinity, dried using anhydrous sodium sulphate and filtered, filtrate was evaporated. The obtained unsaponifiable matter was subjected to sterol tests and pharmacological screening. The aqueous layer remaining after extraction was acidified with 6 N HCl to liberate the free fatty acids, which was extracted three times with diethyl ether (3 × 100 ml). The combined ether extracts were washed with water till acid free, dried using anhydrous sodium sulphate and filtered, filtrate was evaporated to obtain the fatty acids (Al Ashaal et al., 2010).

2.4. Preparation of fatty acid methyl esters (FAMEs) and GC–MS analyses of FAMEs

Fatty acid residue (200 mg) was dissolved in 100 ml methanol, 10 ml benzene and 1 ml concentrated sulphuric acid. The mixture was heated under reflux in boiling water bath for 6 h and then evaporated. 100 ml water was added to residue. The mixture was extracted three times with diethyl ether (3 × 75 ml). The combined ether extracts were washed with water, dried using anhydrous sodium sulphate and filtered, filtrate was evaporated. The produced fatty acid methyl esters were analyzed by GC–MS.

GC–MS analyses was performed using Shimadzu GC2010A (Kyoto, Japan) gas chromatography instrument coupled to a GCMS-QP2010 mass spectrometer (Compaq-Pro Linear data system, class 5K software). In the gas chromatographic system, a Rtx-WAX capillary column (30 m × 0.32 mm I.D., film thickness 0.25 μm) was used. The GC oven temperature was programmed to increase after 1 min from 80 °C to 220 °C at the rate of 5 °C/min, the latter temperature being held for 10 min. The total GC run time was 38 min and equilibrium time 3 min. Injection temperature was kept at 220 °C. Helium carrier gas was used at a constant flow rate of 1.5 ml/min with linear velocity of 45.1 cm/s. Sample of 0.2 μl was injected, the split ratio of the injector being 1:100. Mass conditions were: ionization voltage 80 eV; ion source temperature 200 °C; interface temperature 220 °C; ACQ scan mode in the 40–550 *m*/*z* mass range with 0.5 s/scan. Fatty acids were identified by comparison with standard MS-library NIST05 and WILEY7.

2.5. Phytochemical screening

Phytochemical screening of TPO, unsaponifiable matter (TPOUM), TPE and fractions were carried out employing standard procedures and tests (Kokate, 1991; Trease and Evans, 1996) to reveal the presence of chemical constituents such as alkaloids, flavonoids, tannins, saponins, carbohydrates, steroids, triterpenoids, fats and oils, glycosides and proteins.

2.6. Animals

Female Wistar albino rats (Sri Venkateshwara Traders, Bangalore) weighing 150–200 g were selected for this study. They were housed in acryl fiber cages at 24 ± 2 °C, humidity 50 ± 1.0% and were kept on a 12 h light/dark cycle. They were fed with standard pellet diet (Amrut laboratories, Sangli) and water *ad libitum* and acclimated for 7 days before experimentation. Rats were fasted for 12 h before each test. Experimental protocols reported in this study were approved by the Institutional Animal Ethical Committee of CPCSEA, Govt. of India (IAEC-Resolution No. 13, 31-7-2010) and carried out accordance with local IAEC guidelines.

2.7. Chemicals and drugs

Pure chemicals carrageenan, brewers yeast and tween 80 were purchased from Himedia Lab. (Mumbai), Sigma–Aldrich (USA) and Loba Chemie (Mumbai), respectively. Reference drug Aspirin (Aspin-100, Cipla Pharmaceuticals), Paracetamol (Crocin, GSK Lab.), Diclofenac sodium (Voveran, Novartis), Pentazocine (Fortwin, Ranbaxy Lab.), sterile water for injection (Core Health Care Ltd.) and analytical grade chemicals/solvents were obtained from local market.

2.8. Drug administration

The drugs and extracts used were orally administered except pentazocine (i.m.) with the aid of stainless metallic feeding canula in equivalent volume of 0.5 ml/100 g body weight of animal. A 1% tween 80 was used for preparation of dose of extracts.

2.9. Acute toxicity assay

Acute toxicity assay was performed as per OECD guidelines 423 (limit test). Six female Wistar albino rats (three animals in each step) were randomly selected. The animals were kept fasting for overnight providing only water. The test drug was administered orally at one dose level of 2000 mg/kg b.w. In further, rats were observed continuously for the first 4 h and then periodically up to 24 h for toxic symptoms and mortality.

2.10. Evaluation of carrageenan induced inflammation

Acute inflammation was produced by injecting 0.1 ml of 1% carrageenan in sterile WFI into sub plantar region of rat left hind paw

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