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HPTLC analysis and Anti-inflammatory activity of *Jatropha gossypifolia* L. root in mice and Wistar rats

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Abstract

Jatropha gossypifolia has been used in Indian traditional system but there is paucity of scientific data on anti-inflammatory activity of root. Anti-inflammatory activity was evaluated by TPA (12-*O*-tetradecanoylphorbol-13-acetate) induced ear inflammation, carrageenan-induced paw edema and cotton pellet granuloma. Topical application of 0.5 and 1 mg extract significantly reduced the TPA induced ear inflammation. The extract (125 mg/kg p.o) significantly reduced the carrageenan induced edema. Seven days administration of 50 and 100 mg/kg of extract significantly reduced the cotton pellet granuloma. The activity might be due to effects on several mediators involving cyclooxygenase pathway resulting in prostaglandin formation and leukocyte migration.

Key words: *J. gossypifolia* root, HPTLC, TPA, carrageenan, cotton pellet granuloma

1. Introduction:

Inflammation is a patho-physiological response to the injury. Its complex mechanism involves many mediators leading to accumulation of fluid. Currently available treatments for inflammation lead to many side effects; therefore development of new anti-inflammatory drugs which are safe with fewer side effects is needed. Plants provide drugs in the past and they remain a rich source of novel compounds based on nature's combinatorial natural products chemistry during million of years of evolution, they are rich source of novel therapeutic agents[1]. *Jatropha gossypifolia* L. (Euphorbiaceae) is medium sized shrub found throughout India. It is well naturalized shrub found commonly on waste land. It is used in the traditional system of medicine for the treatment of various ailments, viz. arthritis, asthma, washing wounds, blood purifier, bronchitis, carbuncles, diarrhoea, dysentery, as an antidote for snake bite, in piles, eczema, fever, gum infections, inflammation, itching, leprosy, stomach ache and ulcer[2, 3, 4, 5, 6, 7]. Three new antitumour derivatives of jatrophone, 2α-hydroxyjatrophone, 2β-hydroxyjatrophone and 2β-hydroxy-5, 6-isojatrophone were reported from roots. Jatrophone, a novel macrocyclic diterpenoid, are shown cytotoxic and tumour inhibitory metabolites that exhibits anticancerous activity[8, 9]. Whole plant was reported as hypothermic, Central Nervous System (CNS) depressant and antileukaemic [10]. The other chemicals reported from roots includes-2, 3-*bis* (hydroxymethyl)-6, 7-methylenedioxy-1-(3′, 4'-dimethoxyphenyl)-napthalene i.e. arylnapthalene lignan, triterpenoides[11,12], 2-piperonylidene 3-verytryl-3-R-γ-butyrolactone[13, 14]. Only brief report is available for anti-inflammatory activity of *J. gossypifolia* leaf by carrageenan induced rat paw edema model[15]. Ethnobotanical and ethnopharmacological survey revealed that roots of this plant is used for treatment of inflammatory conditions therefore on the basis of ethno-botanical claim the anti-inflammatory activity of *J. gossypifolia* root was carried out in detail as there is s paucity of scientific data. Therefore, in the present investigation anti-inflammatory activity evaluation of methanol extract of *J. gossypifolia* roots using battery of pharmacological tests has been carried out on suitable animal models and also an effort was made to find out the probable mechanism of action.

2. Material and Methods

2.1. Plant Material: Roots of *J. gossypifolia* were collected from Pune district, Maharashtra, India in November-January 2009. Plant material was identified by regional floras and microscopic study[16] and specimens were deposited in Agharkar Herbarium of Maharashtra Association (AHMA) at Agharkar Research Institute (ARI), Pune, India (Voucher No. 024929). The shade dried roots were coarsely powdered and macerated successively with petroleum ether (60-80 ºC) and methanol at room temperature. These extracts were dried under reduced temperature and pressure in a rotary evaporator. Yield of petroleum ether was 0.72 % and that of methanol extract 4.34 %, respectively. Methanol extract was prepared in acetone

for topical application. The methanolic extract was suspended in 1% carboxy methyl cellulose and administered by oral route.

2.2. Drugs and Chemicals: Carboxy Methyl Cellulose (CMC), TPA (12-*O*-tetradecanoylphorbol-13-acetate) was purchased from Sigma Chemical CO., St. Louis, MO. Carrageenan sodium was procured from S.D. Fine Chemical Ltd., Mumbai, India. Indomethacin was obtained from Fluka, Switzerland. Petroleum ether (60-80ºC), methanol, acetone from Qualigens were used.

2.3. High Performance Thin Layer Chromatography (HPTLC) Analysis: The standardization of methanol extract was carried out by HPTLC fingerprints. The methods described [17] were followed for the development of fingerprints. The samples of petroleum ether and methanol extract of root were spotted in the form of streaks with Linomat-IV on the precoated plate, silica gel Merk-60F 254 aluminium (Merk) of 100 x 100 mm dimensions. The known volume, 10 µl of each sample was spotted on the plates. These were developed in the Camag Twin Trough Chamber at 25º C. Densitometric analysis was carried out at 254 nm using CAT's software on Camag III Scanner. The extracts were also standardized with marker compound β-sitosterol in Toluene: Methanol (90:10) system[18].

2.4. Experimental animals and research protocol approval: Wistar albino rats of either sex (100-150g) and Swiss albino mice (18-22g) were obtained from in house experimental Animal facility of ARI. Animals were housed in polypropylene cages at temperature 25 ± 5 ºC, relative humidity of 45-55 % and 10:14 h Light : Dark cycle. Animals had free access to food (Standard chow pellet, Amrut brand Chakan oil mills, Sangli) and water was made available *ad libitum*. The animals were quarantined for 7 days before starting the experiments. Food but not water was withdrawn from rats 12 h before and from mice 3 h before commencement of experiment. Animal experiments were carried out by following the CPCSEA (Committee for Purpose of Control and Supervision of Experimental Animals", India) rules, regulation and guidelines. The animal experiment was started after obtaining the approval from Institutional Animal Ethical Committee (IAEC) of ARI, Pune.

2.4.1. Acute oral toxicity (OECD Guideline No. 423): The acute toxicity study was performed as per the OECD guidelines 423 at a limit dose of 2000 mg/kg. The doses administered were 175, 550 and 2000 mg/kg by oral route in mice. Animals were observed after dosing individually at least once during the 30 minutes for 4 h, periodically during the first 48 h and daily thereafter, for 14 days for sign of toxicity and mortality if any.

2.4.2. TPA-induced mouse ear edema: Swiss albino mice of either sex weighing between 18-22 g were divided into four groups (n=6). Left ear of each animal in all groups was served as control and received the topical application of vehicle (acetone). TPA (2.5 µg of TPA in 20 µl of acetone) was applied on the right ear of animals in group 1. Methanol extract (ME) of roots in acetone at concentration 0.5 and 1 mg was applied simultaneously with TPA (2.5 µg of TPA in 20 µl of acetone) on right ear of animals in group 2 and 3 respectively. The Standard drug indomethacin 0.5 mg per ear simultaneously with TPA (2.5 µg of TPA in 20 µl of acetone) was applied on right ear of animals in group 4. The thickness of both the ears was measured using a micrometer before and at four hour after TPA application[19] (Figure 1).

2.4.3. Carrageenan-induced rat paw edema: The Wistar rats weighing between (100-150 g) were divided into 5 groups (n=6 rats/group). Group 1 received vehicle (1% CMC, p.o) group 2, 3 and 4 received ME 250, 125 and 75 mg/kg p.o. respectively and group 5 received indomethacin (10 mg/kg, p.o.). 0.1 ml of 1 % carrageenan was injected in subplanter region of right hind paw of all animals after half hour of vehicle, extract or indomethacin pretreatment [20]. The paw volume was measured before and 1, 2, 3 and 4 h after the carrageenan injection by using plethysmographic method [21] (Figure 2).

2.4.4. Cotton pellet-induced granuloma: Wistar rats were divided into 4 groups (n=6 rats/group). The animals were anesthetized with anesthetic ether. The back skin was shaved and disinfected with 70 % ethanol. A small incision was made at scapular region on both sides. By a blunted forceps subcutaneous tunnels were formed and a sterilized cotton pellet (10 mg each) was placed on both sides in the scapular region. The animals were treated for 7 days orally, treatment started after 24 h of the surgical procedure. 1 % CMC and indomethacin were made in distilled water. Group 1 received vehicle (1% CMC), group 2 and 3 received ME 50 and 100 mg/kg respectively and group 4 received indomethacin 5 mg/kg. On 7th day animals were sacrificed and the cotton pellets were removed and dried at 60° C for 24 h. The net dry weight of granuloma, i.e. after subtracting the weight of the cotton pellet was determined. The percent change of granuloma weight relative to vehicle control group was determined [22] (Figure 3).

3. Results

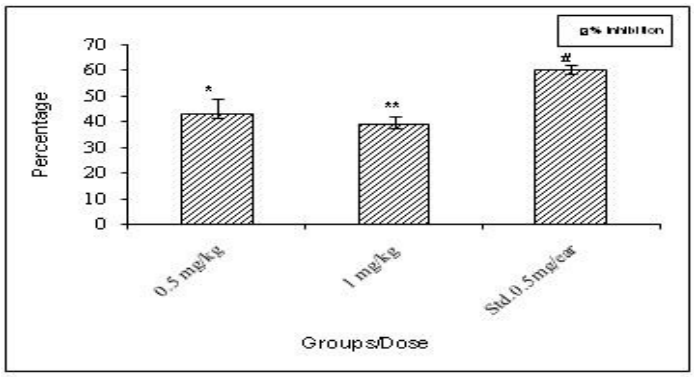
3.1. HPTLC analysis: The number of spots at 254 and 366 nm in methanol extract was 8 and 6 in Toluene: Ethyl acetate (80:20) solvent system. The R*f* of spots at 254 nm were 0.05, 0.11, 0.21, 0.33, 0.54, 0.76, 0.88, 0.97 and at 366 nm were 0.03, 0.09, 0.17, 0.69, 0.85, 0.99.

3.2. Acute oral toxicity: The ME of *J. gossypifolia* root was found to be safe in the doses used and there was no sign of toxicity and mortality up to a dose of 2000 mg/kg p. o.

3.3. TPA-induced mouse ear edema: In control group application of TPA (2.5µg/ear) produced ear edema, which was

measured as increase thickness of ear. ME 0.5 mg/ear and 1 mg/ear significantly (P<0.05, P<0.01, respectively) inhibited the induction of ear edema by TPA. Similarly the indomethacin significantly (P<0.01) inhibited the induction of ear edema (Figure 1).

Figure 1. Effect of JGO-R ME on TPA induced mouse ear edema

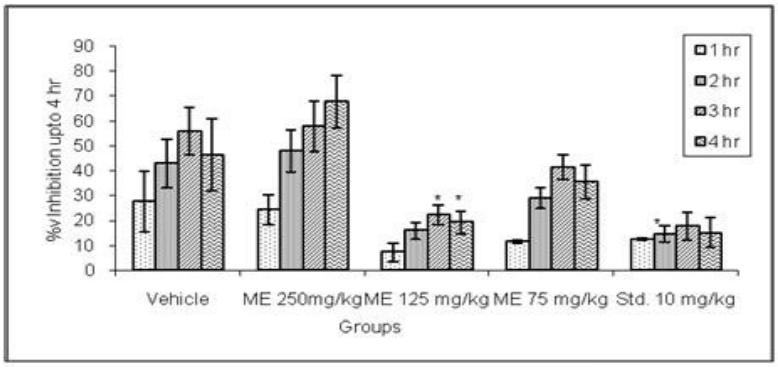


Data represent mean ± S.E.M. % inflammation (n=6)

*p < 0.05, ** p < 0.01, #*P*< 0.001 Significant as compared to control

3.3. Carrageenan-induced rat paw edema: Injection of carrageenan (1%) induced inflammation in rat paw edema. In ME treated group (250 mg/kg) extract was administered to the animals by oral route 30 min prior to the carrageenan injection, the inflammation was reduced significantly at 1st, 2nd, 3rd and 4th hours (P<0.05). Similarly pretreatment of standard drug indomethacin (10 mg/kg p. o) also reduced inflammation up to 1-4 hours significantly (P<0.05 and P<0.001). The anti-inflammatoty activity of ME is comparable with the standard drug indomethacin used in the study (Figure 2).

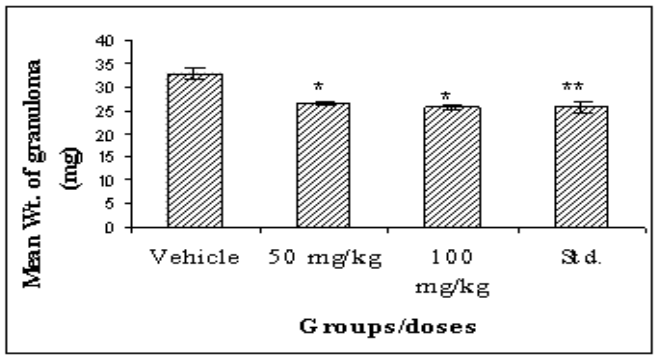
Figure 2. Effect of JGO-R ME in carragennan-induced rat paw edema



Data represented as mean ± S.E.M. (n=6), analyzed by ANOVA followed by Dunnet's test, * *P*<0.05 Significant as compared to control

3.4.Cotton pellet implantation: Seven days pretreatment with ME 50 and 100 mg/kg (p<0.05) showed significant reduction in cotton pellet granuloma in albino rats. Indomethacin (5 mg/kg p. o) pretreatment for seven days significantly (P<0.01) reduced cotton pallet granuloma (Figure 3).

Figure 3. Effect of JGO-R ME on Cotton pellet induced granuloma in Wistar rat



Data represent the mean ± S.E.M. (n=6)

*p< 0.001 significant as compared to the control

** p< 0.005 significant as compared to the control

4. Discussion

J. gossypifolia root in the form of paste were traditionally used for the treatment of inflammation. However there is paucity of scientific data about anti-inflammatory activity of *J. gossypifolia.* Topical application of TPA produces a long-lasting edema. However, exact mechanism of TPA-induced inflammation is not completely revealed, it is suggested to be dependent mainly on leukotrienes (LT), which are synthesized by the lipoxygenases pathway [23]. TPA strongly increases the epidermal content of the cysteinyl LTs, LTC4, LTD4, and LTE4 in mouse skin [24]. The inhibition of TPA induced ear edema by the application of extract suggests that the topical anti-inflammatory activity of the extract is mainly due to the inhibition of leukotriene activity or their synthesis. The observed anti-inflammatory activity was less than indomethacin. Carrageenan-induced hind paw edema occurs as a biphasic event. The initial phase (90– 180 min.) of the inflammation is due to the release of histamine, serotonin and similar substances. The later phase (270–360 min) is associated with the activation of prostaglandins, proteases, lysosomes and other kinin-like substances[25]. The generation of prostaglandins, TNFα, IFNγ, IL-1 and IL-2 like mediator are able to stimulate nociceptors inducing inflammation and nociception[26]; they also activate nitric oxide synthase and cyclo-oxygenase 2[27]. The second phase of the inflammation provoked by carrageenan is mostly relevant to the mechanism of clinically effective anti-inflammatory drugs; this assay is useful to study the anti-inflammatory effect of natural products[28]. The methanolic extract at 125 mg/kg significantly inhibited hind paw edema induced by carrageenan in both phases i.e. at 2 to 3 h. The observed anti-inflammatory activity is less than the indomethacin. Chronic inflammation is the reaction arising when the acute response is insufficient to eliminate the pro-inflammatory agents. Chronic inflammation includes a proliferation of fibroblasts and infiltration of neutrophils and exudation. It occurs by means of development of proliferative cells which can either spread or form granuloma. Efficacy of anti-inflammatory agents in chronic inflammatory states is indicated by their ability to inhibit the increase in the number of fibroblasts during granular tissue formation[29].

The cotton pellet granuloma method has been widely employed to assess the proliferative components of chronic inflammation. NSAIDs produce the inhibitory effect in cotton pellet granuloma test by inhibiting granulocyte infiltration to foreign body (cotton pellet), preventing the generation of collagen fibers and mucopolysaccharides [30]. Seven day pre-treatment with ME reduced cotton pellet granuloma formation indicating inhibition of proliferative phase of chronic inflammation similar to indomethacin potency compared with indomethacin.

5. Conclusion

The results of present study indicate that the ME of *J. gossypifolia* root possess topical and systemic anti-inflammatory activity respectively. Indicating anti inflammatory activity of ME through inhibition of phase 1 and phase 2 of acute inflammation and also inhibited the proliferation phase of chronic inflammation.

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