Local Antiinflammatory Activity of *Jatropha curcas* L. Root in Mice

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The roots of *Jatropha curcas* were extracted successively with petroleum ether and methanol to investigate local antiinflammatory activity based on ethnobotanical lead. Methanol extract was studied against 12-O-Tetradecanoylphorbol-13-acetate, Ethyl phenylpropiolate and Arachidonic acid induced local inflammatory response in albino mice. The methanol extract showed dose dependant local antiinflammatory activity against all these phlogestic agents. Net local antiinflammatory activity is complex mainly evident due to inhibition of biosynthesis of prostaglandin, leukotrienes and protein kinase C and action on peripheral vascular permeability.

Jatropha curcas L. (Euphorbiaceae) is a soft wooded shrub, commonly grown in rural areas as a fence in India. The oil of this plant is used for manufacture of various household commodities and industrially useful products. It is also used traditionally for the treatment of sciatica, dropsy, paralysis, rheumatism, dysentery, diarrhoea and certain skin diseases[1-6]. Recently, based on ethnobotanical practice the methanol extract and JC fraction of this extract was investigated for antidiarrhoeal activity in albino mice[7,8]. The roots of this plant are applied locally in paste form after crushing for the treatment of inflammation by *Bhil* tribes from Rajasthan area in India on empirical basis[9]. Considering above information, these roots were collected in bulk quantity and its extracts were evaluated for local antiinflammatory activity using various animal models in the present investigation.

Roots of *Jatropha curcas* were collected , from Awasari ghat near Pune, in winter season of 1999 in bulk quantities from the field. After cleaning they were shade dried. Routine pharmacognostic studies were carried out to confirm identity of the material[10]. The specimen of the collected material was matching with voucher specimen number, AHMA: 17567 at Agharkar Herbarium of Maharashtra Association at Agharkar Research Institute (ARI), Pune.

These roots were coarsely powdered and subjected to successive solvent extraction in Soxhlet apparatus using petroleum ether (60-80°) and methanol. These extracts were concentrated for further studies at reduced temperature and pressure in a rotary evaporator. (yields 1.62% and 5.51%, respectively). In pilot studies only methanol extract (extract) showed activity against 12-O-tetradecanoylphorbol-13-acetate (TPA) induced ear inflammation model in mice. The High Performance Thin Layer Chromatography (HPTLC) studies of this extract were carried out for authentication and consistency by earlier protocol reported by us[7].

Swiss albino mice of either sex were used from animal house facilities at ARI. They were housed in polypropylene cages in an air-conditioned area at 25±2° with 10:14 h light and dark cycle. They were given Amrut brand balanced animal feed and water *ad libitum*. The optimum conditions for experiments were decided on the basis of pilot experiments carried out using three animals per group. For further experiments a group of at least six animals was used for individual treatment. In all experiments the thickness of mouse ear was measured by using a micrometer (Digitrix mark II, Japan). These experiments were carried out after approval of Institutional Animal Ethics Committee of ARI.

The following chemicals were used for pharmacological studies. TPA and arachidonic acid (AA) were purchased from Sigma Chemical CO., St. Louis. Indomethacin, Ethyl phenyl propiolate (EPP) were obtained from Fluka, Switzerland. Solvents-SQ grade solvents of Qualigens fine chemicals were used.

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An edema was induced on the right ear by topical application of 2.5 μg of TPA or 1mg of EPP or 2 mg of AA in 20 μl of acetone. The left ear acts as a control received the vehicle acetone. Extract 0.5 and 1 mg/ear was dissolved in acetone (20 μl) and indomethacin 0.5 mg/ear or dexamethasone 0.05 mg/ear or phenidone 1 mg/ear respectively were used as a positive control applied to right ear simultaneously with TPA or EPP or AA. The edema was measured initially and after 4 h, 1h and 1h respectively after challenge of phlogestic agent to assess an increase in the ear thickness due to treatment[11-14]. The results of these studies are presented in Table 1, 2 and 3, respectively. The results of all experiments were reported as mean±SEM. These results were further analyzed by using Student's *t*-test to calculate significance of the results. P values less than 0.05 were considered as statistically significant.

Based on ethnobotanical practice followed by Bhil tribes from Rajasthan area for the treatment of local inflammation. In the present study the methanol extract of these roots was examined by employing local application of TPA or EPP or AA on mouse ear to understand the mechanism of action. The extract showed significant and dose dependent reduction in TPA, EPP and AA - induced local inflammatory changes, however it was less as compare to respective positive controls viz. indomethacin, dexamethasone and phenidone application groups.

TPA a phorbol ester provides a skin inflammation model suitable for evaluation of topical and systemic antiinflammatory agents. The majority of its activities appear to involve or depend on arachidonic acid release and metabolism and interaction with protein kinase C[11]. Topical application of TPA induces long lasting inflammatory response, associated with increase in prostanoid production. Moreover, topical administration of cyclo-oxygenase appear more effective at inhibiting the edema response than lipo-oxygenase response[15]. While, AA-induce ear inflammatory response is paralleled to the generation of prostaglandin and leukontrienes[11,15]. The local inflammatory response by EPP is mainly vascular in nature, involving increase in permeability and blood flow, infiltration of leukocytes[16]. Thus, local antiinflammatory activity of extract appears to be complex in nature involving mainly an action on pathways associated with biosynthesis of prostaglandin and leukotrienes or involving arachidonic acid release and metabolism or interaction with protein kinase C. Possible effects on vascular permeability leading to increases in blood flow and migration of leukocytes to periphery can not be ruled out also. It is necessary to fractionate this extract to isolate specific

TABLE 1: EFFECT OF EXTRACT ON TPA-INDUCED LOCAL INFLAMMATION.

Treatment	Difference in ear thickness (mm^{-3})±SEM	% inhibition of inflammation
Control	69.2±2.0	-
Extract (0.5 mg/ear)	23.0±3.7*	65.5
Extract (1 mg/ear)	14.2±1.8*	80.2
Indomethacin (0.5 mg/ear)	15.0±1.7*	79.2

*Significant as compared to control P< 0.05

TABLE 2: EFFECT OF EXTRACT ON EPP-INDUCED LOCAL INFLAMMATION.

Treatment	Difference in ear thickness (mm^{-3})±SEM	% inhibition of inflammation
Control	56.3±1.1	-
Extract (0.5 mg/ear)	21.7±1.4*	71.0
Extract (1 mg/ear)	14.3±2.5*	80.2
Dexamethason (0.05 mg/ear)	8.5±1.1*	88.4

*Significant as compared to control P< 0.05

TABLE 3: EFFECT OF EXTRACT ON AA-INDUCED LOCAL INFLAMMATION.

Treatment	Difference in ear thickness (mm^{-3})±SEM	% inhibition of inflammation
Control	65.2±3.0	-
Extract (0.5 mg/ear)	54.3±1.9*	30.2
Extract (1mg/ear)	27.5±3.6*	64.4
Phenidone (1 mg/ear)	10.7±2.1*	84.9

*Significant as compared to control P< 0.05

components responsible for the pharmacological action before further delineating the mechanism of action.

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