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Research Article

Anti-inflammatory activity of *Rumex vesicarius* L. leaves

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ABSTRACT

The *Rumex vesicarius* ethanolic extract (RVEE) of leaves was studied for anti-inflammatory activity in carrageenan induced paw edema and cotton pellet induced granuloma in rats. Wistar rats were orally administered RVEE (200 mg/kg and 400 mg/kg) and the standard drug diclofenac sodium (40 mg/kg) 60 min prior to a subcutaneous injection of carrageenan (0.1 ml of 1% w/v) into their right hind paws to produce edema. The paw volumes were measured at various time intervals to assess the effect of drug treatment. In the granuloma model, 1 sterile cotton pellet were implanted in the axilla region of each rat. RVEE (200 mg/kg and 400 mg/kg) and the standard drug diclofenac sodium (40 mg/kg) were administered orally for 8 days to the pellet implanted rats. The granuloma tissue formation was calculated from the dissected pellets. A significant reduction in paw edema and cotton pellet granuloma was observed with RVEE treatment when compared with the carrageenan treated and cotton pellet implanted animals respectively. It may be concluded that RVEE possesses anti-inflammatory activity in a dose dependent manner which may be due to an underlying antioxidant activity and/ or lysosomal membrane stabilization by virtue of its poly phenolic constituents.

Keywords: Anti-inflammatory Activity; *Rumex vesicarius*; Carrageenan induced paw edema; Cotton pellet induced granuloma in rat.

INTRODUCTION

Inflammation is a local response (reaction) of living vascularized tissues to endogenous and exogenous stimuli. The term is derived from the Latin "inflammare" meaning to burn. Inflammation is fundamentally destined to localize and eliminate the causative agent and to limit tissue injury. Inflammation usually involves complex biological response of vascular tissues to invasive stimuli, such as pathogens, damaged cells, or irritants. Inflammation is basically a protective immune response that involves immune cells, blood vessels, and some mediators. The basic purpose of inflammation is elimination of the initial cause of cell injury, eradicate out necrotic cells and tissues damaged from the original inflammation. (bourne et al., 1974)

The signs of acute inflammation counts to be pain, heat, redness, swelling, and loss of function. Inflammation is a generic response, and therefore it is considered as a mechanism of innate immunity, as compared to adaptive immunity, which is specific for each pathogen. Inflammation can be categorised as either acute or chronic. Acute inflammation defined as initial response while prolonged inflammation, is

known as chronic inflammation which leads to a progressive damage in the type of cells present at the particular site of inflammation. (Brownlee et al., 1950)

Rumex vesicarius (L.) is a well known medicinal herb, belonging to family of Polygonaceae, commonly known as "Bladder dock or Chukkakura or Khatta palak". It grows in several parts of India. The whole plant is known to be medicinally important and cures several diseases. It is an annual monoecious, glabrous, branched from the root, rather fleshy, pale green, 15 to 30 cm high dichotomously branched, succulent herb. It is found in the areas of desert and semi desert such as west Punjab, trans indus hills, Afghanistan and Pakistan. It is a well branched annual herb of 0.1 and 0.8 m tall. (Rao et al & Andrea et al)

MATERIAL AND METHODS

Drug and chemicals

All the chemicals were used under analytical grade

Animals

Male Wister rats weighing in the range of 160-200gm were used for the study of anti-inflammatory activity. They were maintained in a well-ventilated room with 12:12 hour day/light circle in polypropylene cages and fed with standard pellet diet with water ad libitum. Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) CPCSEA.

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Plant material

The leaves of *Rumex vesicarius* plant were used for investigation which was collected in the month of September, 2014 from local vegetable market of Nellore district of Andhra Pradesh, India. The leaves were identified and authenticated by Dr. C.V.S. Bhaskar, Principal, Department of Botany, S.P.S.R, Nellore.

Preparation of Extract

The leaves of the plant were collected and dried under shade. It was then processed through 40 mesh sieve. A weighed quantity of the powder was then subjected to cold maceration. (Mona *et al.*, 2013) The ethanolic extract of *Rumex vesicarius* yielded thick dark green residue. Percentage yield was found to be 8.75 % w/w.

Preliminary phytochemical screening

The *Rumex vesicarius* ethanolic extract was tested for the presence of alkaloids, glycosides (cardiac & anthraquinone), sterols, triterpenoids, flavonoids, tannins, carbohydrates, saponins, phenols, proteins, quinones and amino acids by commonly used precipitation & coloration reaction. (Remington JP) General test in the analysis revealed the presence or absence of the compounds in the extract.

Preparation of the drug for the experimental study:

The drug extracts were administered in form of aqueous suspension.

Acute Toxicity Studies

As per OECD-423 guidelines acute toxicity studies were performed. Male Wistar rats were used in this study. The animals were divided into six groups each containing six. The extract & drug were given orally ranging in the doses from 200- 2000mg/kg. Toxicity & mortality signs were nil as observed up to dose of 2000mg/kg. (OECD., 2002)

ANTI- INFLAMMATORY METHODS

Carrageenan induced rat paw edema

All the animals were divided into 4 groups, in which each group contained 6 animals. Animals were weighed and marked for identification. All four groups were treated with extract and standard drugs used for the study respectively. After 30 minutes of administering doses, 0.1ml of 1% carrageenan in saline was injected in the sub plantar region of the left hind paw of each rat in order to induce edema.

Group-1: Served as toxicant control, which received orally 1ml/kg of 1% sodium CMC solution + 0.1 ml of 1% (w/v) of carrageenan by subcutaneous injection.

Group-2: Served as positive control and received diclofenac sodium 40 mg/kg (Mariyammal *et al.*, 2013) orally (30 min prior to carrageenan injection) + 0.1 ml of 1% (w/v) of carrageenan by subcutaneous injection.

Group-3: Received RVEE 200mg/kg orally (30 min prior to carrageenan injection) + 0.1 ml of 1% (w/v) of carrageenan by subcutaneous injection.

Group-4: Received RVEE 400mg/kg orally (30 min prior to carrageenan injection) + 0.1 ml of 1% (w/v) of carrageenan by subcutaneous injection.

The paw volume was measured initially and at intervals of 0, 60, 120, 180, 240, 300, 360 min. Paw volume was measured by volume displacement method using Plethysmometer by immersing the paw in mercury cell. The percentage inhibition of paw volume in drug treated group was compared with control group. Diclofenac 40mg/kg was used as standard drug. The percentage inhibition of paw edema was calculated by using the following formula

$$\text{Percentage of edema inhibition} = \left[\frac{(V_c - V_t)}{V_c} \right] \times 100$$

V_c = Volume of edema in control group.

V_t = volume of edema in treated group.

Cotton pellet granuloma: The animals were divided into 4 groups, containing 6 in each group. The animals of each group were treated with both standard and test drugs orally. After 30 minutes of administration of doses, the animals were anaesthetized with diethyl ether. The sterile cotton pellets weighing 20mg were inserted surgically in axilla region of rats by making incision in the subcutaneous region. The incisions were later sutured using sterile catgut. The extract and drugs were administered for seven days. After 8th day all the animals were sacrificed under anaesthesia and the cotton pellets were removed surgically. Later they were separated from extraneous tissues. The pellets were weighted separately and dried at 70°C for 6 hour and weighted again to measure dry weight of the pellets.

Group I served as Control, Group II as positive control receiving only standard- diclofenac- 40mg/kg, Group III received RVEE at a dose of 200mg/kg, Group IV received RVEE at a dose of 400mg/kg. The percentage inhibition of granuloma formed was calculated by using the following formula;

$$\text{Percentage of edema inhibition} = \left[\frac{(W_c - W_d)}{W_c} \right] \times 100$$

W_c = Pellet weight in control group.

W_d = Pellet weight in drug treated group.

STATISTICAL ANALYSIS

All experiments were performed and the calculations were processed by Tukeys multiple comparison test followed by one way ANOVA. *P* value < 0.05 was considered as significance. All the calculations were done using Graphpad Prism software (version 2.5).

Table 1: Effect of Ethanolic extract of *Rumex vesicarius* (L.) leaves on inhibition of carrageenan induced paw edema in rats

Groups	0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
Control	0.7167 ± 0.0408	1.3700 ± 0.0752	1.6500 ± 0.0547	1.85000 ± 0.05477	2.15000 ± 0.08367	2.31700 ± 0.02582	2.37500 ± 0.02739
Standard	0.7167 ± 0.0706	1.0170 ± 0.0752 ***	0.9333 ± 0.0516***	0.9333 ± 0.0516***	0.7500 ± 0.0547***	0.6333 ± 0.0516***	0.5833 ± 0.0428***
RVEE low dose	0.7000 ± 0.0894	1.2000 ± 0.0632 *	1.2170 ± 0.0408***	1.3170 ± 0.0408***	1.1170 ± 0.0983***	1.0170 ± 0.0408***	0.9833 ± 0.0408***
RVEE high dose	0.7083 ± 0.0147	1.1830 ± 0.0408 *	1.0170 ± 0.0408***	1.1170 ± 0.0408***	0.9000 ± 0.0632***	0.8667 ± 0.0816***	0.7167 ± 0.0408***

Values are expressed as Mean ± S.D; n = 6 animals in a group; one way ANOVA followed by Tukey's test

* = P<0.05

***=P<0.001

As compared to control group.

Table 2: Percentage inhibition of oral dose of Ethanolic extract of *Rumex vesicarius* (L.) leaves on carrageenan induced paw edema in rats

Groups	Treatment & Dose	Percentage Inhibition
I	Carrageenan control (0.1 ml of 1%)	--
II	Diclofenac sodium (40mg/kg b.w)	75
III	RVEE low dose(200mg/kg b.w)	58
IV	RVEE high dose(400mg/kg b.w)	69

Table 3: Percentage inhibition of oral dose of Ethanolic extract of *Rumex vesicarius* (L.) leaves on cotton pellet induced granuloma in rats

Groups	Treatment & Dose	Percentage Inhibition
I	Carrageenan control (0.1 ml of 1%)	--
II	Diclofenac sodium (40mg/kg b.w)	68
III	RVEE low dose(200mg/kg b.w)	53.4
IV	RVEE high dose(400mg/kg b.w)	61

RESULTS

Carrageenan induced rat paw edema method

Table no. 2 shows the percentage inhibition of edema in rats treated with Diclofenac sodium, RVEE is calculated with reference to the control group by applying Tukey's test. The percentage inhibition of edema at the end of 6 hours with Diclofenac sodium was 69 %, whereas Anti-inflammatory activity of RVEE in low dose was 48 %. At the same time high dose executed 61% inhibition which was almost equivalent to standard.

Cotton pellet granuloma method

Percentage inhibition of granuloma formation (table no.3) exhibited by higher dose of RVEE is around 61% which is almost near to the standard inhibition of 68% where as lower dose of RVEE exhibits only 53.4%

DISCUSSION

The ethanolic extract of the leaves of *Rumex vesicarius* did not show any toxic or deleterious effects by oral

route up to 2000 mg/kg indicating low toxicity of the leaves at high doses.

The results of the present investigation revealed that the leaves of *Rumex vesicarius* possesses moderate anti-inflammatory effect that was evidenced by the significant reduction in carrageenan induced paw edema and cotton pellet granuloma.

In this model generation of inflammation is performed by injection of materials such as formalin, 5 - hydroxytryptamine and dextran into the hind paw of rats. The material used to produce acute inflammation is carrageenan, which is a sulphated polysaccharide available from red green algae belonging to family (Rhodophyceae). (Winter et al., 1962)

Carrageenan- is a chemical used for the release of inflammatory and proinflammatory mediators such as prostaglandins, histamine, leukotrienes, bradykinin, TNF- α , etc. The nature of the induced inflammation is usually biphasic. First phase involves the release of

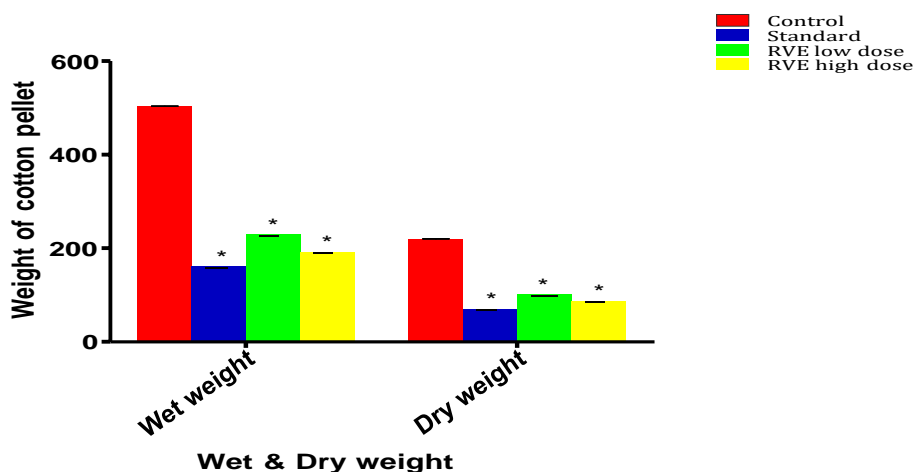


Figure 1: Effect of Ethanolic extract of *Rumex vesicarius* (L.) leaves on inhibition of cotton pellet granuloma in rats.

serotonin, kinins and histamine from the mast cells in the first 1-2 hours, after injection of the phlogistic agent. While the second phase is mediated by release of prostaglandins, cyclo oxygenase (COX) and lipoxy

genase (LOX) products in 2-3 hours. Phase 2 is sensitive to both the steroidal and non-steroidal anti-inflammatory drugs. The basic two important types of inflammatory mediators are: prostaglandin E₂ is main mediator to cause the acute inflammation and leukotriene B₄ is the mediator of leukocyte being responsible for activation of inflammatory event. (Ravichandran et al., 2014)

The percent inhibition of edema in rats treated with Diclofenac sodium, RVEE is calculated with reference to the control group by applying Tukey's test. The percent inhibition of edema at the end of 6 hours with Diclofenac sodium was 75 %, whereas Anti-inflammatory activity of RVEE in low dose was 58 %. At the same time high dose executed 69% inhibition which was almost equivalent to standard. RVEE most probably inhibits the COX, which leads to blocking of prostaglandin synthesis. Oral dose of RVEE in both low & high doses significantly regulates the pro inflammatory cytokines.

The cotton pellet granuloma model has been usually employed to assess the transudative, exudative and proliferative components of chronic inflammation. This model involves 3 phases in the inflammatory response. In the first phase inhibition of fluid containing low protein takes place at the site of cotton pellet implantation followed by second phase, exudation of fluid containing the protein takes place after 2-3 days of pellet implantation. In the third phase, also known as proliferative phase, consist of appearance of collagen followed by muco polysaccharide synthesis, and increase in the number of fibroblasts around the implanted cotton pellets. (Vandana et al., 2013)

The amount of newly formed connective tissue is measured after removing and weighing the dried pellets. RVEE extract significantly decreased the final dry weight of the cotton pellets, i.e. it decreased the amount of granulomatous tissue, suggesting that it has the capability of reducing the synthesis of muco polysaccharides and collagen with the number of fibroblasts, which are considered as natural proliferative events of granulation during tissue formation. RVEE extract decreased the weight of granuloma tissue in a dose-dependent manner, confirming its activity and effectiveness in the chronic phase of inflammation.

The wet weight of the cotton pellets always correlates with the exudates and at the same time the dry weight of the pellets correlates with the amount of the granulomatous tissue (Snehalata et al., 2013) which shows dose dependent anti inflammatory activity.

The *Rumex vesicarius* exhibited significant anti-inflammatory activity at the doses of 200, 400 mg/kg body weight

CONCLUSION

Thus to summarise, the results of the present study supports the traditional use of *Rumex vesicarius* in inflammation. *Rumex vesicarius* exhibited significant anti-inflammatory activity at the doses of 200 and 400mg/kg. The results produced by *Rumex vesicarius* were comparable with that of standard diclofenac.

Hence, the ethanolic extract of *Rumex vesicarius* (L.) leaves can be advocated as anti-inflammatory agent. Further studies are required which compounds its acceptance as an anti inflammatory agent.

The efficacy of *Rumex vesicarius* (L.) for curing or alleviating inflammation may be a light for developing a potential herbal medicine for the future.

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