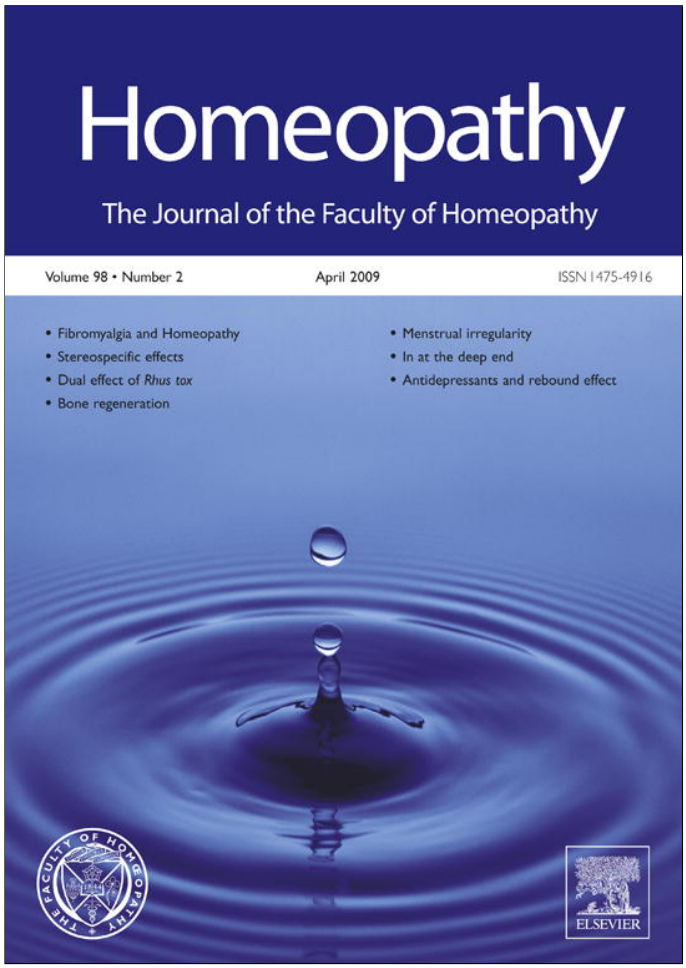

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ORIGINAL PAPER

Dual effect of *Toxicodendron pubescens* on Carrageenan induced paw edema in rats

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Background: ***Toxicodendron pubescens*** **is the current botanical name of homeopathic** ***Rhus toxicodendron*** **(*Rhus tox*). *Rhus tox* drug is widely used in homeopathically diluted form in the treatment of inflammatory and edematous conditions. We studied the effect of crude form of this plant, after single and multiple doses in Carrageenan induced paw inflammation in rats.**
Method: **We evaluated effects of single dose and multiple doses of orally administered *Rhus tox* on Carrageenan induced paw inflammation in rats. We tested 10 mg/kg, 20 mg/kg and 40 mg/kg doses of *Rhus tox*.**
In the single dose study, *Rhus tox* was administered 1 h prior to the subplantar injection of Carrageenan. In the multiple dose study, *Rhus tox* was administered twice daily for three days and Carrageenan was injected 1 h after the last dose.
Paw volume was measured using a digital plethysmometer.
Results: **Administration of a single dose of *Rhus tox* 1 h prior to injection of Carrageenan significantly reduced the paw inflammation in a dose dependent manner. Administration of multiple doses of *Rhus tox* increased the intensity of inflammation induced by Carrageenan, but this was not statistically significant.**
Conclusion: ***Rhus tox*****, in crude form, exerts anti-inflammatory effects after a single dose and proinflammatory effect after multiple doses in Carrageenan induced paw inflammation in rats. Further study is needed to explain this dual effect.** *Homeopathy* (2009) **98,** 88–91.

Keywords: *Toxicodendron pubescens*; anti-inflammatory; proinflammatory; Carrageenan; dual effect; *Rhus tox*

Introduction

Toxicodendron pubescens (Family: *Anacardiacea*) is botanical name of the *Rhus toxicodendron* (*Rhus tox*) according to the current botanical nomenclature system.[1,2] The resinous content of this plant, called Urushiol induces contact dermatitis.[1] This hypersensitivity reaction has been attributed to some constituents of Urushiol; [1, 2-dihydroxy-3-(pentadec-8-ynyl) benzene]. Urushiol has been shown to act as a hapten in the induction of such hypersensitivity reaction.[3]

In homeopathy, dilutions of *Rhus tox* are indicated in treatment of joint pain with stiffness. A recent study on homeopathic dilutions of this plant revealed that, in its dynamized form, it possesses anti-inflammatory activity in Carrageenan induced paw inflammation in rats.[2] In this study the effects of *Rhus tox* 6cH, 12cH, 30cH and 200cH were evaluated in the inflammation induced by Carrageenan, Histamine and Serotonin in Wistar rats. *Rhus tox* possesses anti-inflammatory and peripheral analgesic activity in these experimental models. The 6cH dilution was shown to possess more potent anti-inflammatory effects than other dilutions studied. The study further proposed that *Rhus tox* acts through modulations in the inflammatory

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mediators. The same report also states that it has no effect on histamine induced rise in vascular permeability in rats.

Even though thus documented in literature, the reports on systematic evaluation of the effect of substantial doses of *Rhus tox* on the process of induced inflammation are rare. Hence we evaluated the effects of single and multiple doses of *Rhus tox* in crude form in Carrageenan induced inflammation in rats. The duration of three days for multiple dose study was used on the basis of an earlier report on this drug.[2]

Material and methods

Authenticated sample of leaves of *Rhus tox* was procured from Homeopathic Pharmacopoeia Laboratory, Ghaziabad, New-Delhi, India. The leaves were dried and crushed to powder form. For oral administration, the powder of leaves was suspended in 0.5% Carboxymethyl cellulose (CMC) solution, prepared fresh not more than 1 h prior to administration.

Animals

Male Adult Albino rats (Wistar strain) weighing 150–200 g and Swiss albino mice weighing 20–25 g were used for this study. The animals were procured from our Institutional animal house facility. They were housed in polyethylene cages under standard conditions of 12 h light and dark cycle at $22 \pm 2^{\circ}$C. Animals were provided with standard pelletized feed (Amrut Rat Feed, Pune, India) and water was made available ad libitum.

Approval for this study was obtained from the Institutional Animal Ethical Committee constituted under the 'Committee for the Purpose of Control and Supervision on Experiments on Animals' (CPCSEA) regulations, Government of India.

In the single dose study, animals were deprived of feed for 18 h prior to drug administration, but water was available. In the multiple dose experiments, the rats were administered *Rhus tox* suspensions twice a day; in the morning between 09.00 and 10.00 and between 17.00 and 18.00. Dosing was scheduled in such a way that the rats did not have access to feed for at least 2 h before and 2 h after dosing. On the third day of experiment, the rats were deprived of feed and only drug was administered twice, water was available.

Toxicity studies

Toxicity study was performed as per 'Organization for Economic Co-operation and Development' (OECD) guideline number 425.[4] The limit test was performed initially. Swiss albino mice weighing 20–25 g were used. Six mice were serially administered 2000 mg/kg dose of *Rhus tox* suspended in 0.5% CMC as recommended in the guideline. After dose administration, each animal was observed every hour for signs of toxicity and abnormality in the behavior up to the 48th hour, followed by daily observations for toxicity and mortality up to 14 days. Body weights of the animal were recorded every third day. On 14th day post dosing, all the mice were sacrificed and processed for gross necropsy.

Administration of doses

Rhus tox was orally administrated as per their treatment groups, as follows:

Single dose study

(a) *Rhus tox* (10 mg/kg p.o.) 1 h before the injection of inflammatory stimulus
(b) *Rhus tox* (20 mg/kg p.o.) 1 h before the injection of inflammatory stimulus
(c) *Rhus tox* (40 mg/kg p.o.) 1 h before the injection of inflammatory stimulus

Multiple dose study

(a) *Rhus tox* (10 mg/kg p.o.) two times daily for 3 days
(b) *Rhus tox* (20 mg/kg p.o.) two times daily for 3 days
(c) *Rhus tox* (40 mg/kg p.o.) two times daily for 3 days

The last dose of *Rhus tox* was administered 1 h prior to Carrageenan administration.

Diclofenac at dose of 10 mg/kg was orally administered 1 h prior to Carrageenan injection to the rats in standard drug treated groups.[5,6] For administration, Diclofenac was suspended in 0.5% CMC solution in water (positive control).

In control group animals, Carrageenan was injected as above and an equal volume of 0.5% CMC was administered.

Induction and measurement of paw edema

As an inflammatory stimulus, Carrageenan (λ4, Iota-Fluka-Biochemica Co.) was injected in the right rear paw of the animals at a dose of 0.1 ml of 10 mg/ml solutions.[7,8] An Ugo Basile plethysmometer (Model 7140, Italy) was used to measure the paw volumes. The recording of paw volumes (in ml) was performed at an interval of 1–6 h.

The volume of paw recorded just before Carrageenan injection was recorded as initial volume (V_0) in each case. The mean percent rise in the paw volume was determined by following formula, where Vt indicates volume at different intervals after Carrageenan injection.

$$\% \text{ Rise} = \frac{(Vt - V_0)}{V_0} \times 100$$

Statistical analysis

For statistical comparison, 4th hour readings of percentage rise in the paw volume were considered. The statistical analysis was carried out by One-way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison test; $p < 0.05$ was taken to be statistically significant.

Line charts indicating percentage rise in paw volume in different treatment groups at different time intervals have also been included in the results.

Results

In the acute toxicity study of *Rhus tox* in mice none of the six animals administered with 2000 mg/kg oral dose of

Rhus tox died or revealed any observable neurobehavioral effects during the observation period of 14 days. In the necropsy following euthanasia, no significant alterations in the histology of vital organs were observed. Hence, as in line with OECD Guideline number 425, *Rhus tox* cannot be categorized under Global Harmonization System classes (GHS classes). Due to lack of observable toxicity at 2000 mg/kg dose, no determination of actual LD50 was carried out.

The effects of *Rhus tox* single and multiple dose treatments on Carrageenan induced inflammation in rats are shown in Figure 1a and b as percentage rise in the paw volume over 6 h following Carrageenan injection. Figure 1a shows that the single dose treatment with *Rhus tox* exerted dose dependent inhibition of paw inflammation induced by Carrageenan. However, this effect was less potent than Diclofenac (10 mg/kg p.o.) active control.

Figure 1b shows the effect of multiple doses of *Rhus tox* treatment on Carrageenan induced paw inflammation. In this treatment group, the percentage rise in the paw volume in the *Rhus tox* treated animals was greater than the control group rats. This proinflammatory effect was not found to be dose dependent; the increase in paw volume was maximum in 10 mg/kg *Rhus tox* treated group. The rise in paw inflammation compared to control group rats was consistent in all the three groups of rats treated with multiple doses of *Rhus tox*.

Table 1 shows the average 4th hour readings of percentage rise in the paw inflammation in all the treatment groups. Statistical tests were applied to readings of single dose treatment and multiple dose treatment separately. It was observed that the difference in the means of groups in both the cases was statistically significant as revealed by the *p* values obtained in the ANOVA.

With single dose treatment, there was a dose dependent anti-inflammatory effect and this was statistically significant (Dunnett's test). In the multiple dose treatment, the percentage rise in the paw inflammation in *Rhus tox* treated groups was higher than that of the corresponding control group. This proinflammatory effect was not dose dependent and the increase in inflammation was not statistically significant as compared to that of control group.

Discussion

The results of our study show that *Rhus tox*, in crude form, when administered as a single oral dose prior to inflammatory stimulus shows dose dependent anti-inflammatory activity. Whereas, the same drug when repeatedly administered for three days, exerts proinflammatory effects in the carrageenan induced inflammation model. An earlier report by Dos Santos *et al*.[2] states that the homeopathic dilutions of *Rhus tox* of 2cH, 12cH, 30cH and 200cH show anti-inflammatory activity in Carrageenan induced paw inflammation model in rats. This study tested the effect of a single dose and three days dose schedule. It has been reported that *Rhus tox* exerts anti-inflammatory effects in both single dose and multiple dose treatments. However in this study, crude form or mother tincture of *Rhus tox* was not evaluated for its anti-inflammatory effects.

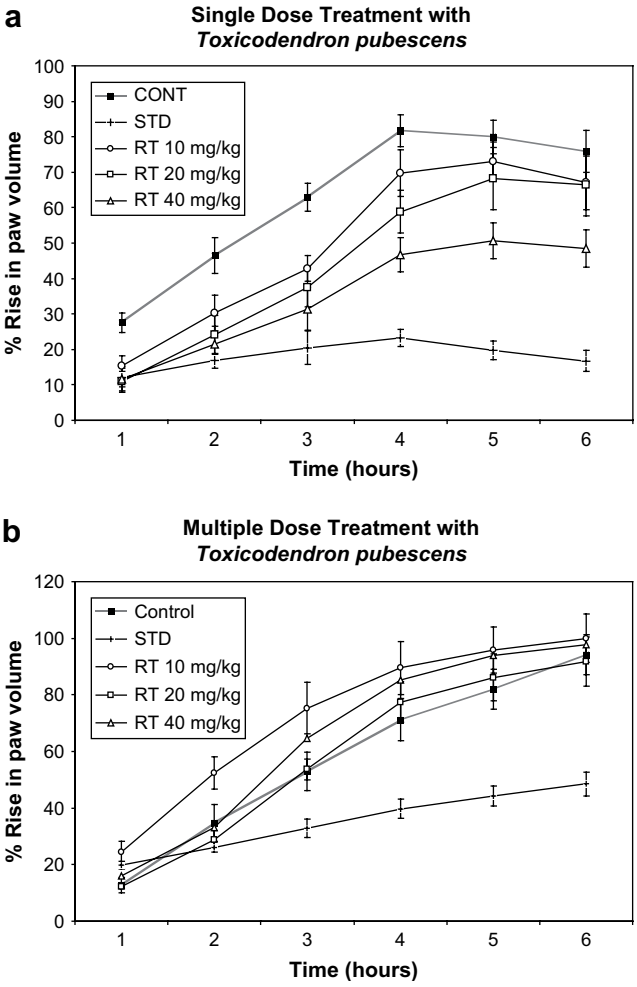

Figure 1 (a) Effect of single dose of *Toxicodendron pubescens* administration on paw inflammation induced by Carrageenan. (b) Effect of multiple doses of *Toxicodendron pubescens* administration on paw inflammation induced by: Carrageenan. —■— Control (saline), — + — Standard (Diclofenac 10 mg/kg/p.o.) —○— *Rhus tox* 10 mg/kg/p.o. —□— *Rhus tox* 20 mg/kg/p.o. —△— *Rhus tox* 40 mg/kg/p.o.

In our study, we evaluated the effects of crude form of *Rhus tox*. In the single dose study, *Rhus tox* in its crude form inhibited both phases of inflammation induced by Carrageenan. This suggests an inhibitory effect of *Rhus tox* on the release of

Table 1 Effect of *Toxicodendron pubescens* on Carrageenan induced rise in the paw volume in rats (at 4th hour)

Treatment groups	*% Rise in paw volume*	
	Single dose Rhus tox	*Multiple dose* Rhus tox
Control	81.7 ± 3.8	71.2 ± 7.4
Diclofenac 10 mg/kg/p.o.	23.3 ± 2.0*	39.7 ± 3.4*
Rhus tox 10 mg/kg/p.o.	69.8 ± 5.6	89.5 ± 9.4
Rhus tox 20 mg/kg/p.o.	58.9 ± 5.1†	77.5 ± 7.1
Rhus tox 40 mg/kg/p.o.	46.7 ± 4.1*	85.4 ± 4.3
Statistics (ANOVA followed by Dunnett's multiple comparison test)	ANOVA: $p < 0.0001$	ANOVA: $p < 0.0002$

$n = 6$ in each group.
* Post test: $p < 0.01$ as compared to control group.
† Post test: $p < 0.05$ as compared to control group.

mediators like Histamine and Serotonin which are involved in the initial phase of Carrageenan induced inflammation. Inhibition of subsequent phase of inflammation suggests suppression of prostaglandin mediated inflammation.[9]

After multiple doses there was a rise in inflammation in both these phases. The effects of single doses were dose dependent however; such dose dependency was not observed in the proinflammatory effect of multiple doses.

The present study does not involve elucidation of the mechanism of the dual effects of *Rhus tox*. But two similar reports, one on a homeopathic drug and another on a synthetic drug give hints about the type of mechanism that may be involved in such dual effects. A study on homeopathic formulation containing minerals reported its dual effects on Carrageenan induced inflammation. When this drug was administered 60 min prior and 30 min after Carrageenan injection, it exerted proinflammatory and anti-inflammatory effects, respectively.[10] The authors concluded that the mineral complex they investigated acts through an unconventional mechanism, activating endogenous regulatory mechanisms of the body that lead to its anti-inflammatory activity. Celecoxib, a potent conventional anti-inflammatory drug has been reported to lose its anti-inflammatory activity at higher plasma concentrations.[11] It has been shown that at higher concentrations Celecoxib increases COX-2 expression and IL-1β-induced NF-kB activation which leads to negation of its anti-inflammatory activity.

According to homeopathic principles, a drug able to induce symptoms of a disease at higher doses is considered to be effective in the treatment of such diseases at ultrahigh dilutions (in potentized form).[12] Over the past few years, there has been an increase in the number of preclinical (*in vitro* and *in vivo*) studies aimed at evaluating the pharmacological activity and efficacy of homeopathic remedies; however, in addition to major differences of experimental models, these studies have also highlighted a series of methodological difficulties and lack of independent replication.[13,14] Hence, simple and reproducible experiments are needed to investigate the efficacy and mechanism of actions. Such experiments are also necessary to establish whether these drugs really abide by the principle of 'Similia' by inducing symptoms of the diseases in their crude form (or at higher doses). In case of *Rhus tox* we found that, in crude form, it possesses dose dependent anti-inflammatory activity when administered as a single dose, but after multiple doses proinflammatory effect becomes evident.

Further evaluation of effect of *Rhus tox* on the mediators of inflammation may reveal the basis of its anti-inflammatory and proinflammatory effects. Our results suggest *Rhus tox* as a prototype that can be used to prove validity of principle of 'Similia'.

Conflict of interest

We declare that the research involved in the above manuscript has been carried out at an educational Institute as a part of dissertation work. We did not receive any funds that could influence our work. The Institutes where we are working have not paid us any honoraria, consultancy fees and the findings of this study have not been submitted as a part or as a whole to the patenting authorities on any country.

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