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ORIGINAL PAPER

Modulation of arthritis in rats by *Toxicodendron pubescens* and its homeopathic dilutions

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Background: ***Toxicodendron pubescens*** **P. Mill (Anacardiaceae) known in homeopathy as *Rhus toxicodendron* (*Rhus tox*) is used as an anti-inflammatory medicine in homeopathic practice. In this study, *Rhus tox* in its crude form and homeopathic dilutions (3cH, 6cH, 30cH, 200cH) was evaluated for effects on Complete Freund's Adjuvant (CFA) induced arthritis in rats.**

Method: **We assessed the severity of arthritis through observations including inflammatory lesions, body and organ weight and hematological parameters including C-reactive protein (CRP). Blinded radiological analysis of the affected joints and pain intensity determination was also carried out.**

Results: ***Rhus tox*** **protected rats from CFA-induced inflammatory lesions, body weight changes and hematological alterations. *Rhus tox* protected against radiological joint alterations due to arthritis. Arthritic pain scores were also favorably affected by *Rhus tox*. All the dilutions of *Rhus tox* including crude form showed anti-arthritic activity. The maximum protective effect was evident in the crude form at 10 mg/kg/day, by mouth.**

Conclusion: **This study supports claims in the homeopathic literature on the role of *Rhus tox* and its ultra dilutions in the treatment of arthritis and associated pain. Further study is needed to explain this anti-arthritic effect of *Rhus tox*.** *Homeopathy* (2011) **100**, 131–137.

Keywords: *Toxicodendron pubescens*; Homeopathy; *Rhus toxicodendron*; Adjuvant arthritis; Inflammation; Ultra dilutions

Introduction

Toxicodendron pubescens P. Mill (Anacardiaceae), known as *Rhus toxicodendron* (*Rhus tox*) is used in homeopathic practice in the treatment of inflammatory conditions.[1] It is used in the treatment of rheumatic pain, mucous membrane affections and typhoid type fever.[2] This plant contains a potent allergen, Urushiol, in its resinous sap. Urushiol is responsible for contact dermatitis caused by *Rhus tox*.[3]

Recent experimental and animal studies have demonstrated the anti-inflammatory, analgesic and immunomodulatory effects of crude and homeopathic dilutions of *Rhus tox*.[4–6] As reported by Carvalho *et al.*,[4] oral administration of *Rhus tox* as 6cH, 12cH, 30cH and 200cH dilutions exerts anti-inflammatory effects in rat paw inflammation induced by carrageenan. In the same model, the crude form of *Rhus tox* shows pro-inflammatory effect after oral administration of multiple doses.[5] *Rhus tox* exerts immunosuppressant effects in mice.[6] This report describes the evaluation of anti-arthritic activity of crude and homeopathic dilutions of *Rhus tox* in Complete Freund's Adjuvant (CFA)-induced arthritis in rats.

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Material and methods

Preparation of medicine

An authenticated sample of *Rhus tox* was generously supplied by Dr D. R. Lohar (Director), Homeopathic Pharmacopoeia Laboratory, Ghaziabad, India. The homeopathic

dilutions of *Rhus tox* were purchased from Dr Reckeweg and Company, Germany through their distributor in India. The lot numbers were as follows: crude form: authenticated sample obtained from the Homeopathic Pharmacopoeia Laboratory, Ghaziabad, Uttar Pradesh, India. Homeopathic dilutions: 3cH 56091N291273, 6cH 55751N-308083, 30cH 55801N305103, 200cH 55881N293043. The quality control certificate for the purchased product was obtained from quality control department of the company.

Diclofenac was obtained from Lord Venky Pharma Private Limited, Yanam, India as a gift sample. CFA was purchased from Sigma Aldrich, USA (Lot number 098K8729). C-reactive protein (CRP) kit was procured from Agappe Diagnostics Pvt. Ltd., Kerala, India.

Animals and experimental design

Wistar rats of either sex (150–180 g) were used in this study. They were maintained in polypropylene cages at 22 ± 2°C with free access to pellet food (Amrut Rat Food, Pune, India) and tap water. The experimental protocols were approved by the Institutional Animal Ethical Committee (IAEC) following the rules of Committee for the Purpose of Control and Supervision of Experiments on Animals, India (CPCSEA), Government of India (Regd. No. 651/02/C/CPCSEA).

Dosage preparation and administration

For oral administration dried leaves of *Rhus tox* were finely powdered and suspended in 0.5% carboxymethyl cellulose in water. The homeopathic dilutions of *Rhus tox* were administered as reported by Santos *et al.*[4] The rats were given one daily dose of 0.1 ml of respective dilutions mixed with 1.0 ml of sterile distilled water by oral gavage. The animals were starved immediately before, and after administration for at least 2 h. The treated control group rats received one dose of 0.1 ml of 70% ethanol mixed with 1.0 ml of sterile distilled water daily.

CFA-induced arthritis in rat

Each treatment group contained six Wistar rats. The rats were randomly divided into five groups: negative CFA control (vehicle), crude *Rhus tox* (10 mg/kg/day), *Rhus tox* 3cH (0.1 ml/day), *Rhus tox* 6cH (0.1 ml/day), *Rhus tox* 30cH (0.1 ml/day), *Rhus tox* 200cH (0.1 ml/day) and Diclofenac (5 mg/kg/day), all given by gavage. Arthritis was induced by injection of CFA according to the method described by Kumar *et. al.*[7] Briefly, 100 µl of CFA containing heat-killed and dried *Mycobacterium tuberculosis* was injected into the paw of left hind limb of each rat.

Evaluation of the severity of arthritis

As described by Singh *et al.*[8] the paw volumes of injected and non-injected paws, were measured using digital plethysmometer (UGO Basile 7140, Italy) before and on 7th, 14th and 21st days post adjuvant injection. The intensities of primary lesions (percentage rise in the volume of CFA injected paw) and secondary lesions (percentage rise in the volume of non-injected paw) were calculated.

The body weights of all the rats were recorded every 3rd day after adjuvant injection. The severity of arthritis was visually evaluated by a blinded observer as stated in earlier reports.[9–11] The pain associated with arthritic lesions was scored as reported by Patil *et al.*[12] The arthritis score ranged from 0 to 4; where 0 indicated the least but definite swelling and 4 represented the maximum swelling. In this scoring system the intensity of inflammation in all the four paws of each rat was scored separately.

Hematological parameters were evaluated using routine laboratory methods. The level of serum CRP was determined using commercial kit according to the manufacturers' instructions.

Radiological analysis

Radiological analysis of the affected joints of hind legs was performed as described by Patil *et al.*[12] On day 21, animals were anesthetized with ether. Radiographs of the adjuvant-injected hind paws were taken with an X-ray instrument (GE-525 DX, USA) Fuji computerized radiographic systems (Japan). The film focus distance was 60 inches (1500 mm) and machine was operated at 43 kV peak, 2 mA. X-ray of the adjuvant-injected limb of each rat was evaluated for radiographic changes by a blinded radiologist using image analysis software (Image plus version 2.4.6). As reported previously[13–16] the radiological alterations were arbitrarily graded between 0 and 4 considering severity of soft tissue swelling around the joints of hind paws, periarticular bone resorption, periarticular bone erosions and joint space narrowing.

At the end of the study the rats were sacrificed. The spleen and thymus from individual rats were isolated and weighed. The spleen weight to body weight as well as the thymus weight to body weight ratios (recorded on the 21st day post CFA injection) were determined.[17,18]

Statistical analysis

The results are expressed as the mean ± SEM for parametric data and median (minimum, maximum) for the non-parametric data sets. The significance of difference was evaluated by one-way ANOVA followed by Dunnett's multiple comparisons test for normal data and by the Kruskal–Wallis test followed by Dunn's multiple comparison test for scored data. Data were considered statistically significant if $p < 0.05$.

Results

Rhus tox reduced the primary and secondary arthritic lesions in rats

Paw volumes, body weight and arthritis scores were recorded on the 7th, 14th and 21st days after adjuvant injection. The CFA-induced arthritis control group showed signs of arthritis revealed by the increase in paw volumes in both CFA injected and CFA non-injected paws (primary and secondary arthritic lesions). Other parameters including decreased body weight and arthritis scores confirmed induction of arthritis in the CFA treated control group rats.

Compared with the control group, there was less rise in the paw volumes in the Diclofenac and *Rhus tox* treated animals (Table 1). Assessment on day 21 showed that treatment with *Rhus tox* (crude form) 10 mg/kg/day, and *Rhus tox* ultra dilutions 3cH, 6cH, 30cH and 200cH significantly ($p < 0.01$) reduced primary and secondary lesions compared with the CFA control group (Table 1).

Table 1 shows effects of various dilutions of *Rhus tox* on primary and secondary lesions in the arthritic rats. The effect of *Rhus tox* dilutions is evident from day 7. In Figure 1, it is evident that the onset of effects of *Rhus tox* is within 7 days. The 6cH dilution consistently showed less rise in the CFA injected (primary lesions) and CFA non-injected (secondary lesions) paw volumes compared to the other dilutions. At day 21, the rats receiving the 6cH dilution showed significantly lesser primary lesions ($p < 0.01$). For secondary lesions, the groups treated with crude *Rhus tox*, 3cH and 6cH had significantly ($p < 0.001$) lesser rise in paw volume than the *Rhus tox* 30 cH and 200cH dilution treated rats The rise of non-injected paw volume (secondary lesions) in the 6cH dilution treated rats was less than the crude form and 3cH dilution treated groups but this difference was not statistically significant.

The Diclofenac treated group showed significantly less primary and secondary lesions at days 7, 14 and 21. The effects of *Rhus tox* 6cH on the arthritic lesions (% rise in CFA injected and non-injected paw volumes) did not significantly differ from those of Diclofenac. Other dilutions of *Rhus tox* were less effective than Diclofenac.

Rhus tox improved weight gain and decreased relative weights of spleen and thymus in arthritic rats

It was observed that with the increased severity of arthritis, the body weight gain in rats reduced. The weight gain was significantly more ($p < 0.01$) in the groups treated with Diclofenac, *Rhus tox* crude form and 200cH dilution compared with control (Table 2). In other treatment groups, there was more weight gain compared to the arthritic group, but the differences were not statistically significant.

The Diclofenac treated group showed significant reduction in the relative weights of spleen and thymus ($p < 0.001$) compared to control. Similarly all the *Rhus tox* treated groups showed significantly lower relative weight of spleen and thymus compared to control. The reduction in the thymus weight induced by crude *Rhus tox* was comparable to Diclofenac ($p > 0.05$). The effects of crude *Rhus tox* and 3cH dilution on the relative weights of spleen were comparable to that of Diclofenac ($p > 0.05$).

Hematological alterations in CFA rats normalized after treatment with *Rhus tox*

The hematological perturbations induced by CFA arthritis such as increase WBC count, decreased RBC count and decreased hemoglobin count were also favorably influenced by *Rhus tox* treatment (Table 3).

Diclofenac (5 mg/kg/ p.o.) treatment improved hemoglobin and decreased WBC count ($p < 0.001$). The effects of *Rhus tox* on these hematological parameters were similar but less potent than Diclofenac. The RBC count significantly improved in the Diclofenac and *Rhus tox* treated groups.

The CRP levels were suppressed by treatment with *Rhus tox*

In the estimations performed on day 21, the mean serum CRP in the control group was 9.8 mg/dl. In the Diclofenac treated arthritic rats, the average CRP levels were significantly ($p < 0.001$) reduced to 3.8 mg/dl. *Rhus tox* treatment significantly reduced the serum CRP levels. The greatest decrease in CRP levels with *Rhus tox* was seen in the 200cH treated group (4.5 mg/dl) (Table 3).

Rhus tox affects pain scores in arthritic rats

The effect of *Rhus tox* on various test scores is shown in Table 4. There was reduction in the arthritis score, flexion pain test score and mobility score in rats treated with crude *Rhus tox* and homeopathic dilutions. However, statistically significant reduction was seen only in the groups treated with Diclofenac and crude *Rhus tox*. Similarly, the stance score significantly improved only in the crude *Rhus tox* treated group.

Table 1 Volume of primary and secondary paw lesions in arthritic rats

Group	7th Day		14th Day		21st Day	
	Primary lesions	Secondary lesions	Primary lesions	Secondary lesions	Primary lesions	Secondary lesions
CFA control	$79 \pm 5.0^{\phi}$	$25 \pm 1.2^{\phi}$	$111 \pm 5.5^{\phi}$	$42 + 1.0^{\phi}$	$146 \pm 3.2^{\phi}$	65 ± 1.8
Diclofenac (5 mg/kg)	30 ± 1.8^{b}	5.6 ± 0.5^{b}	57 ± 2.4^{b}	16 ± 0.9^{b}	84 ± 2.5^{a}	30 ± 1.9^{b}
Rhus tox (10 mg/kg)	40 ± 2.3^{b}	9.4 ± 0.9^{b}	$82 \pm 2.1^{b,\phi}$	$23 \pm 1.2^{b,\#}$	$105 \pm 2.7^{a,\phi}$	36 ± 1.0^{b}
Rhus tox 3cH	41 ± 1.4^{b}	$16 \pm 1.1^{b,\phi}$	$85 \pm 1.6^{b,\phi}$	$28 \pm 1.3^{b,\phi}$	$108 \pm 3.1^{a,\phi}$	$40 \pm 1.4^{b,\#}$
Rhus tox 6cH	35 ± 1.9^{b}	7.2 ± 0.4^{b}	67 ± 2.7^{b}	19 ± 0.8^{b}	87 ± 3.3^{a}	31 ± 2.2^{b}
Rhus tox 30cH	$44 \pm 1.9^{b,\#}$	$17 \pm 1.0^{b,\phi}$	$91 \pm 1.7^{b,\phi}$	$34 \pm 0.9^{b,\phi}$	$111 \pm 3.9^{a,\phi}$	$45 \pm 1.4^{b,\phi}$
Rhus tox 200cH	$56 \pm 1.7^{b,\phi}$	$20 \pm 0.5^{a,\phi}$	$98 \pm 1.4^{\phi}$	$36 \pm 1.3^{a,\phi}$	$118 \pm 1.8^{a,\phi}$	$50 \pm 1.6^{b,\phi}$
One-way ANOVA	$p < 0.0001$ $F = 41$ (df = 6, $n = 42$)	$p < 0.0001$ $F = 72$	$p < 0.0001$ $F = 42$	$p < 0.0001$ $F = 82$	$p < 0.0001$ $F = 49$	$p < 0.0001$ $F = 54$

Primary and secondary lesions indicate average % rise in the volumes of CFA injected and non-injected paws.
Data presented as mean ± S.E.M.
For the post test (Bonferroni's multiple comparison test): $^{a}p < 0.01$, $^{b}p < 0.001$ as compared to CFA control group $^{\#}p < 0.01$, $^{\phi}p < 0.001$ as compared to Diclofenac treated group.

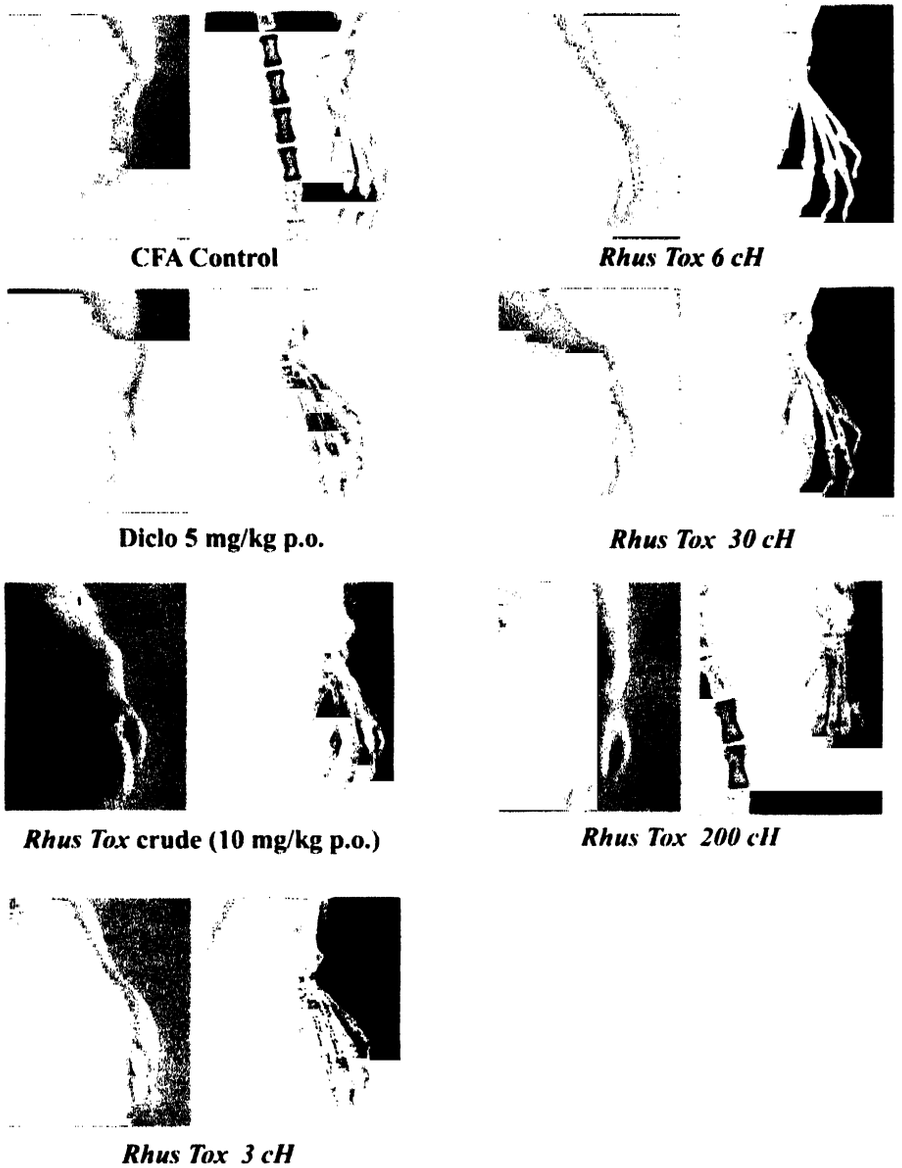

Figure 1 Photograph and radiograph of the left hind paws of rats on 21st day after CFA injection.

Rhus tox protects the rats from CFA-induced radiographic joint alterations

Figure 1 shows representative photographs of the tarsotibial joint of the left hind paws of rats from different groups on day 21 post CFA injection. The images reveal soft tissue swelling around the joints, periarticular bone resorption, periarticular bony erosions and joint space narrowing. As seen in the

Table 2 Changes in body weight and weights of thymus and spleen

Group	*Initial body weight (g)*	*Body weight gain (g)*	*Thymus/body weight (mg/g)*	*Spleen/body weight (mg/g)*
CFA control	113 ± 3.2	14 ± 0.60	1.1 ± 0.035	8.6 ± 0.23
Diclofenac (5 mg/kg)	116 ± 2.0	25 ± 1.7[b]	0.54 ± 0.031[c]	3.5 ± 0.23[c]
Rhus tox crude (10 mg/kg)	117 ± 0.8	24 ± 1.4[b]	0.62 ± 0.033[c]	4.7 ± 0.27[c]
Rhus tox 3cH	113 ± 2.4	19 ± 1.2	0.79 ± 0.029[c,♦]	4.9 ± 0.44[c]
Rhus tox 6cH	112 ± 1.6	20 ± 1.9	0.93 ± 0.05[a,♦]	6.3 ± 0.20[b,♦]
Rhus tox 30cH	118 ± 4.8	16 ± 0.64	0.74 ± 0.046[c,#]	6.1 ± 0.41[c,♦]
Rhus tox 200cH	115 ± 3.1	21 ± 1.6[a]	0.84 ± 0.032[c,♦]	6.8 ± 0.54[a,♦]
One-way ANOVA	$p = 0.701$ $F = 0.64$	$p < 0.0033$ $F = 4.1$	$p < 0.0001$ $F = 25$ (df = 6, $n = 42$)	$p < 0.0001$ $F = 22$

Data represented as mean ± S.E.M.
Bonferroni's multiple comparison test: [b]$p < 0.01$, [c]$p < 0.001$ compared with control group [#]$p < 0.01$, [♦]$p < 0.001$ as compared to Diclofenac treated group.

Table 3 Changes in hematological parameters and CRP

Group	*RBC ($\times 10^6$/mm^3)*	*WBC ($\times 10^3$/mm^3)*	*Hb (mg %)*	*CRP mg/dl*
CFA control	3.5 ± 0.15	11 ± 0.21	7.4 ± 0.48	9.8 ± 0.24
Diclofenac (5 mg/kg/day)	5.1 ± 0.18^{b}	8.2 ± 0.17^{b}	13 ± 0.24^{b}	3.8 ± 0.34^{b}
Rhus tox (10 mg/kg)	$4.3 \pm 0.076^{b,\phi}$	8.6 ± 0.41^{b}	$8.8 \pm 0.29^{a,\phi}$	$6.1 \pm 0.47^{b,\phi}$
Rhus tox 3cH	4.6 ± 0.085^{b}	9.0 ± 0.23^{b}	$8.8 \pm 0.32^{\phi}$	5.0 ± 0.20^{b}
Rhus tox 6cH	$4.6 \pm 0.072^{b,\delta}$	$9.8 \pm 0.20^{a,\#}$	$9.4 \pm 0.22^{b,\phi}$	$6.4 \pm 0.45^{b,\phi}$
Rhus tox 30cH	$4.2 \pm 0.061^{b,\phi}$	8.7 ± 0.21^{b}	$10 \pm 0.25^{b,\phi}$	$7.1 \pm 0.35^{b,\phi}$
Rhus tox 200cH	$4.6 \pm 0.064^{b,\delta}$	$9.7 \pm 0.20^{b,\#}$	$6.5 \pm 0.21^{\phi}$	4.5 ± 0.27^{b}
One-way ANOVA	$p < 0.0001$, $F = 21$	$p < 0.0001$, $F = 19$	$p < 0.0001$, $F = 55.59$	$p < 0.0001$, $F = 34$

Data represented as mean ± S.E.M.
For the post test (Bonferroni's multiple comparison test): $^{a}p < 0.01$, $^{b}p < 0.001$ as compared to CFA control group $^{\delta}p < 0.05$, $^{\#}p < 0.01$, $^{\phi}p < 0.001$ as compared to Diclofenac treated group.

Figure 1, the joints of the rats treated with *Rhus tox* appear to be protected from the CFA-induced arthritis related joint changes.

Discussion

CFA-induced arthritis in rats is used as a model to evaluate anti-arthritic activity of drugs in preclinical research.[19] CFA induces a chronic immune mediated inflammation characterized by elevation of pro-inflammatory mediators and development of swelling, pain, and deformity of joints.[18–20] Inflammation in CFA arthritis model is indicated by increase in paw volume of injected paw. Quantification of such volume helps in determination of the severity of inflammation and therapeutic efficacy of drugs.[19]

The crude form *Rhus tox* at a dose of 10 mg/kg/day and the homeopathic dilutions 3cH, 6cH, 30cH and 200cH reduced both primary and secondary inflammatory lesions induced by CFA. Rats treated with the 6cH dilution of *Rhus tox* showed minimum intensity of primary and secondary lesions at days 7, 14 and 21. This indicates that the anti-arthritic effects of *Rhus tox* begin within 7 days of the initiation of treatment. The appearances of secondary lesions (non-injected paw swelling) in the CFA treated rats is a manifestation of cell mediated immunity (T cell response) and the suppression of this response due to a test drug indicates its immunosuppressive activity.[8] *Rhus tox* effectively reduced such secondary lesions. The effects of *Rhus tox* on CFA-induced primary and secondary lesions were comparable to those of Diclofenac (5 mg/kg p.o.). The 6cH dilution of *Rhus tox* showed the most potent activity, in agreement with an earlier report.[4] In addition to this, both crude form and ultra dilutions of *Rhus tox* reduced the levels of CRP in treated rats. This indicates its efficacy in reducing immune mediated inflammation. These finding are in line with another study[6] that reported immunosuppressant effects of *Rhus tox* in experimental animals.

Table 4 Changes in pain test scores in CFA-induced arthritis in rat

Group	*Arthritis score*	*Flexion pain test score*	*Mobility score*	*Stance score*
CAF control	4 (4,3)	3 (3,3)	3 (3,3)	1 (1,1)
Diclofenac (5 mg/kg/day)	2 (4,2)^{b}	1 (2,1)^{b}	1.5 (2,1)^{b}	2 (3,1)
Rhus tox (10 mg/kg)	3 (4,3)^{a}	1.5 (2,1)^{a}	2 (2,1)^{a}	2 (3,2)^{b}
Rhus tox 3cH	3.5 (4,3)	2.5 (3,2)	2.5 (3,2)	1.5 (2,1)
Rhus tox 6cH	4 (4,3)	2.5 (3,1)	3 (3,2)	1 (2,1)
Rhus tox 30cH	3 (4,2)	2.5 (3,2)	3 (3,2)	1 (2,1)
Rhus tox 200cH	3.5 (4,3)	3 (3,2)	3 (3,2)	1 (2,1)
Kruskal–Wallis test	$p = 0.0024$	$p = 0.0015$	$p = 0.0007$	$p = 0.0054$
Kruskal–Wallis statistic	20	22	23	18

($n = 6$ per group).
Data represented as median (minimum, maximum).
Dunn's multiple comparison test: $^{a}p < 0.05$, $^{b}p < 0.01$ as compared with CFA control group.
Note: for stance score higher values represent improvement, for other scores lower scores represent improvement.

For determination of joint damage in rats, radiographic examinations were carried out and scored blind. It was observed that both *Rhus tox* and Diclofenac inhibited various radiographic changes in arthritic rats. The bone density in the group receiving Diclofenac was less than in the CFA control and *Rhus tox* treated group. Administration of Diclofenac at 5 mg/kg dose for 21 days has been reported to cause loss of bone density in rats.[21] However, this study relates to fracture healing in rats. To determine whether Diclofenac exerts similar changes in the bone density of CFA treated rats requires separate investigation.

Changes in body weight of arthritic rats are also used to assess the progression of disease and the response to anti-inflammatory therapy. Arthritis causes weight loss whereas anti-inflammatory drugs and anti-arthritic drugs impede the weight loss. During inflammatory conditions the intestinal absorption of ^{14}C-glucose and ^{14}C-leucine is reduced in rats; treatment with anti-inflammatory drugs normalizes such deranged absorption capacity.[22] The CFA treated rats in the present study showed less body weight gain than Diclofenac treated arthritic rats. The weight gain in the crude *Rhus tox* and the ultra dilutions treated groups were also higher than the CFA arthritic rats. Thus, it can be proposed that these body weight changes indicate the anti-inflammatory activity of *Rhus tox*.

Other parameters used in evaluation of the severity of arthritis included arthritis score, stance score, estimation of relative weights of lymphatic organs and hematological parameters. The secondary lesions induced by CFA in

non-injected paws indicate immunological reactions. If efficacy of a test drug in reducing primary and secondary lesions is not similar, this indicates dissociation between the anti-inflammatory and immunomodulatory activities of test drug. The immunomodulatory potential of a test drug is also revealed through its effects on lymphatic organ weights. Immunosuppressants like dexamethasone decrease relative weights of both thymus and spleen. In this investigation, *Rhus tox* caused reduction in the weights of both these organs indicating an immunosuppressant activity.

Arthritic score and secondary paw swelling indicate an autoimmune process triggered by CFA. *Rhus tox* showed the reduction in arthritis score. Of the treatment groups, the Diclofenac and crude *Rhus tox* groups showed statistically significant reduction in the pain score, mobility score and increase in stance score.

In the present study, we did not include a CFA group treated with oral 0.5 % CMC solution, comparable to the group that received crude *Rhus tox* (10 mg/kg p.o.). CMC is physiologically inert, but the lack of a vehicle-only treated control group is a shortcoming of this experimental design. However, this does not affect the results and statistical comparisons as far as homeopathic dilutions of *Rhus tox* are concerned.

Arthritis is characterized by inflammation of joints associated with hyperalgesia and functional impairment. The hyperalgesia associated with arthritis has been reported to involve prostaglandin synthesis. To assess effect of *Rhus tox* on pain in arthritic rats, the dorsal flexion pain test was carried out. *Rhus tox* and Diclofenac effectively increased the pain threshold and reduced the flexion pain test score. The frequency of leg withdrawal and squeaking was significantly reduced after treatment with *Rhus tox* in its crude form and Diclofenac. To assess the functional impairment that is a common feature of arthritis, mobility and stance score systems were used. Mobility score in the *Rhus tox* treated groups was lowered, but crude *Rhus tox* significantly increased the stance score indicating its potential to reduce pain associated with arthritis. The mechanism of such pain reduction has not been determined, but it may be that *Rhus tox* exerts such analgesic action through inhibition of prostaglandin synthesis. However, this mechanism needs to be established through further experiments.

This study and other recent reports on *Rhus tox* highlight its effectiveness in its crude form as well as homeopathic ultra dilutions. The promising results of the investigation reported here encourage further evaluation of *Rhus tox* to delineate its mechanism of action and its effects in other inflammatory ailments.

Conflict of interest

We hereby 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. We also state here that 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 of any country.

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