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ORIGINAL PAPER

In vivo study of the anti-inflammatory effect of *Rhus toxicodendron*

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Background: **Homeopathic *Rhus toxicodendron* (*Rhus tox*) is used in various inflammatory conditions. We screened its effect compared to succussed ethanol controls and appropriate active controls.**
Method: **We initially experimented with *Rhus tox* 6, 12, 30 and 200 cH, using carrageenan-induced paw oedema in rats. The 6 cH dilution appeared most effective and was used in subsequent assays. We used pre-treatment and single treatment regimes in Wistar rats, and mice.**
Results: **We found significant reductions compared to control in carrageenan-induced paw oedema, vascular permeability, writhing induced by intraperitoneal acetic acid and stress induced gastric lesions.**
Conclusions: ***Rhus tox* in homeopathic dilution appears to interfere with inflammatory processes involving histamine, prostaglandins and other inflammatory mediators.** *Homeopathy* (2007) **96,** 95–101.

Keywords: *Rhus toxicodendron*; homeopathic medicine; inflammatory conditions; inflammatory process in animals; anti-inflammatory effect

Introduction

Rhus toxicodendron (*Rhus tox*) is the traditional homeopathic name for the plant called *Toxicodendron pubescens* in the current Botanical Code of Nomenclature. Rhus radicans (*Toxicodendron pubescens* in the current nomenclature) is a related plant.[1] Both secrete a resin called urushiol which causes a severe contact dermatitis including oedema, erythema and irritation.[2] *Rhus tox* was first used in medicine to treat a young man with an herpetic eruptions in 1798.[3] Sensitivity to *Rhus tox* is not innate, it develops through successive contact with the plant, its sensitivity tends to diminish with aging. Older people are approximately 50% less sensitive than young people and children. It is believed that 10–15% of the American population is resistant.[4] *Rhus tox* also causes muscular, articular, and ligament pain. This pain increases with rest and beginning of movements, leading to rigidity. Thus, in homeopathy it is used for articular and muscular pains.[5]

The aim of this study was to screen the homeopathic medicine *Rhus tox* in pre-clinical assays based on the inflammatory process in animals.

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Material and methods

Preparation of the medicine

The *Rhus tox* tincture and *Rhus toxicodrendon* 6, 12, 30 and 200 cH were prepared in a specialized homeopathic pharmacy, 'Farmácia de Manipulação Curanthus', in São Paulo, Brazil, following the Brazilian Homeopathic Pharmacopoeia. Thirty percent ethanol serially succussed in the same way as the active medication but without the initial addition of *Rhus tox* tincture was administered to the control group.

Evaluation of the anti-inflammatory activity

Animals

Wistar male rats (*Rattus norvegicus*) and mice (*Mus musculus*), weighing between 180–200 and 20–25 g were

used in the experiments. The animals were acquired from the Biotery of the University of Alfenas (UNIFENAS) and kept in polyethylene boxes ($n = 5$), in a controlled environment, light/dark control each 12 h (7 a.m. to 7 p.m.). They were kept without food for 12 h before the experiment, and water was *ad libitum*. The study was approved by the UNIFENAS Bioethical Committee.

Administration

Rhus tox was orally administered in different regimes, as follows:

(a) Single dose (SD, 0.5 ml/animal, p.o.) 30 min before the injection of inflammatory stimulus.
(b) Pretreatment (PT, 0.5 ml/animal/day, p.o.) during 3 days before the injection of inflammatory stimulus;
(c) Ethanol 30% (0.5 ml/animal, p.o.) was administrated using the same regimes.

Evaluation of different dynamizations of *Rhus tox*

Rat paw oedema in rats

Rat paw oedema induced by carrageenan and dextran was used to evaluate the effect of *Rhus tox*. Indomethacin (10 mg/kg, p.o.) and ciproheptadine (10 mg/kg, p.o.) were used as active comparators. Groups of rats ($n = 6$) were treated with *Rhus tox* (6, 12, 30 and 200 cH) in the oedema induced by carrageenan. The analysis was performed 4 h after the stimulus (peak of the oedema). The oedema induced by dextran was analysed after 90 min.

As inflammatory stimulus, carrageenan (1000 µg/paw–0.1 ml, Kappa carrageenan type III, Iota-Fluka-Biochemika Co.), or dextran (50 µg/paw–0.1 ml, T-70 MW, 70,000 Pharmacia) was injected in the right rear paw of the animals. The same volume (0.1 ml) of 0.9% saline solution was injected into the left rear paw. The difference of volumes between the right and left paws was the volume of oedema. A Hugo Basile (plesthymometer model 7140) was used to assess the volume of oedema volume. The recording was directly through a computerized system and the total value given in ml.[6]

Mouse ear erythema induced by croton oil

The method described by Tubaro *et al*[7] was used. Inflammation was induced by a solution of croton oil (1 mg/ear–0.1 ml, C-4755, Lot 115H0167 Sigma Co.) diluted in acetone on the right ear surface of mice. On the left ear, the same volume of acetone was applied.

The following treatment regimes were used, treatment was given 30 min after the application of the stimulus, except for the PT group:

(a) One group ($n = 10$) was treated with the SD regime.
(b) One group ($n = 10$) was treated with the PT regime.
(c) One group ($n = 10$) received dexamethasone 0.5 mg/kg.
(d) Control group ($n = 10$) was treated with control.

Vascular permeability in rats

This assay was used to estimate alterations of vascular permeability induced by intradermal injections of histamine (50 µg, histamine dihydrochloride, Fluka Ag, Buchs SG). The method used was modified from that described by Lykbe and Cummings.[8] This method consists of the spectrophotometric determination of the amount of extravasated dye in the interstitial space induced by histamine. 10 rats each were treated with:

(a) single dose of *Rhus tox* 6 cH (as above);
(b) pretreatment with *Rhus tox* 6 cH (as above);
(c) control (as above).

Evans blue (25 mg/kg) was injected intravenously. Histamine was given by intradermal injection into the animal's back 10 min after the injection of the dye. Each animal received six injections of the same histamine solution in different locations. The animals were sacrificed 20 min after the last injection. To extract the dye, areas close to the injection sites were removed (1.5 cm diameter), fragmented and placed in tube containing 3 ml of formamide, then kept at 37 °C for 24 h. This material was centrifuged at 2500 rpm for 15 min. The amount of Evans blue was measured spectrophotometrically at 620 nm. The concentrations of the dye were obtained from the optical density, multiplied by a factor calculated based on the standard deviation.

Writhing test in mice

Groups of mice ($n = 10$) were treated orally with SD, PT or control regimes (as above). Muscular contraction was induced by an intraperitoneal injection of 0.6% acetic acid solution (0.25 ml/animal). The number of muscular contractions was counted starting 5 min after injection for a period of 20 min. Data represent the average of the total writhes observed.[9]

Stress induced gastric lesions

Animals were kept with no food during 24 h. After this period, different groups ($n = 6$) were treated with *Rhus tox* 6 cH (SD and PT, p.o.), 30% ethanol (0.5 ml/animal) or Cimetidine (50 mg/kg, p.o.).[10,11] After 30 min, each animal was immobilized at an individual compartment, according to the method described by Basile *et al*,[12] and the index of lesion was determined after 17 h.[12]

Statistical analysis

The statistical analyses were done using analysis of variance (ANOVA) followed by the Tukey–Kramer multiple comparison test.[13] Results with $P<0.05$ were considered significant. Data are expressed as mean ± S.E.M.

Results

Rat paw oedema

The maximum peak after carrageenan injection in the rat paw occurred 4 h after the stimulus. In the control group, the intensity of the oedema was lower compared to the group treated with water, but this difference was not significant (Figure 1). However, the groups that received *Rhus tox* (6, 12, 30 and 200 cH, SD) showed inhibition of the maximum peak of the oedema of 53, 31, 39.63, and 39%, respectively (Figure 1). The 6 cH showed the strongest effect, the results obtained for 12, 30 and 200 cH dynamizations were not statistically different (Figure 1).

The action of *Rhus tox* medicine on rat paw oedema induced by carrageenan showed that both previous treatment and single dose decreased the oedema, formation by 38 and 33%, respectively (Figure 2). The oedema induced by dextran that the treatment with *Rhus tox* 6 cH SD was effective, decreasing the oedema in 57% when compared to the control group, treated with ETOH 30% ($P<0.05$). The inhibition of the oedema by the previous treatment for three days with RT 6 cH was not significant (Figure 3).

Dermatitis induced by croton oil

In the ear erythema test, previous treatment and single dose treatment with *Rhus tox* 6 cH was effective in inhibiting oedema caused by croton oil, decreasing oedema by 54.7 and 54%, respectively (Figure 4).

Vascular permeability in rats

The oral administration of *Rhus tox* 6 cH, either single dose or previous treatment did not inhibit significantly the action of histamine on vascular permeability. However, cyproheptadine inhibited it by 72% (Figure 5).

Writhing test in mice

In the writhing test, the previous treatment and single dose inhibited the algogenic process in 56 and 51%, respectively (Figure 6). Both treatment regimes produced similar inhibition, with no significant difference. Indomethacin also inhibited the process to a similar degree.

Stress induced gastric lesions

Treatment with *Rhus tox* 6 cH SD did not inhibit the generation of gastric ulcers. However, the pretreated group showed protection against ulcer induction, with an index of ulceration 36% lower than succussed ethanol control (Figure 7).

Discussion

In Brazil, confirmation of pharmacological efficiency of industrialized, composed, homeopathic medicines is required. Many researchers are applying pre-clinical assays to homeopathic medicines.

The effectiveness of different dynamizations (6, 12, 30 and 200 cH) of *Rhus tox* was evaluated in the experimental model of rat paw oedema caused by carrageenan compared to succussed vehicle. This is well-known model involving prostaglandins and other inflammatory mediators.[14,15] In this model, all dynamizations were effective in decreasing oedema formation, with no statistical differences between 12, 30 and 200 cH dynamizations. But the 6 cH dynamization was the most effective with an effect similar to indomethacin, used as a positive control. Both SD and PT regimes were equipotent to indomethacin (Figure 2). Hence, we used the 6 cH dynamization in the other experiments.

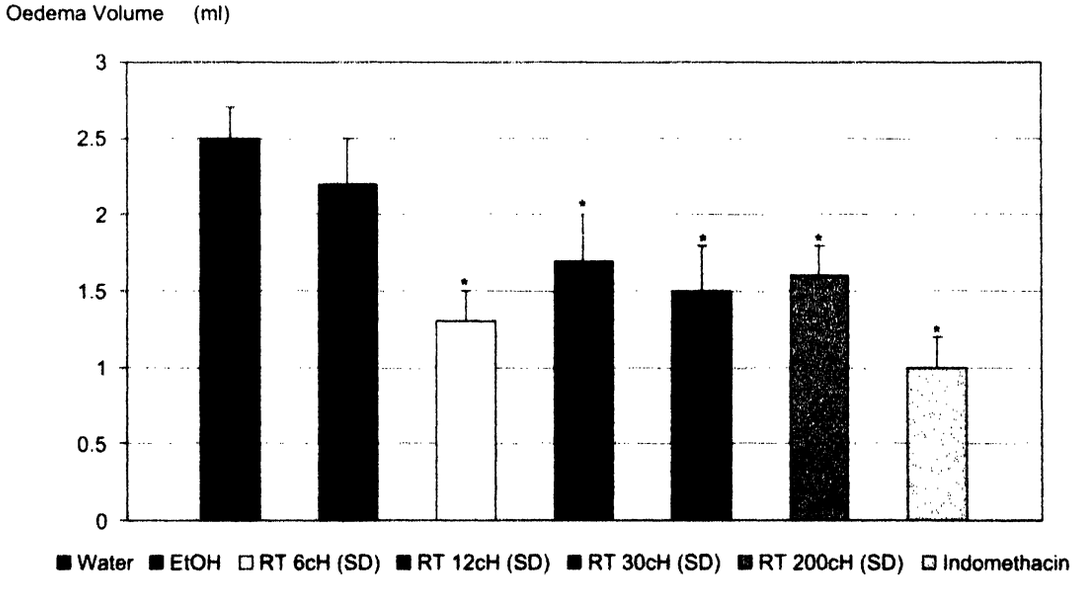

Figure 1 Effect of oral administration of distilled water (0.5 ml), succussed ethanol 30% (0.5 ml, EtOH) and *Rhus tox* (RT) 6, 12, 30, 200 cH (SD, 0.5 ml), and indomethacin (10 mg/kg), 30 min before the application of carrageenan (*$P<0.05$).

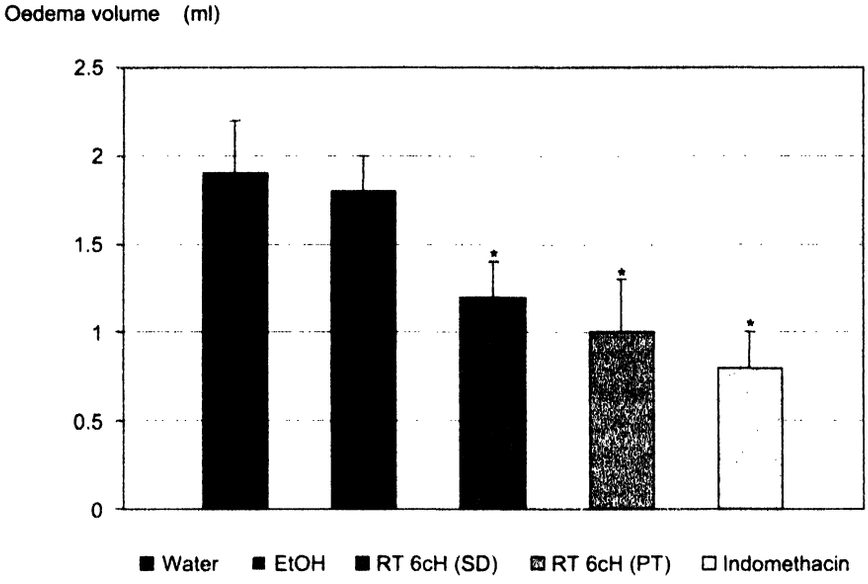

Figure 2 Effect of *Rhus tox* (RT) 6 cH (0.5 ml) single dose and pre-treatment, distilled water, succussed ethanol 30% (EtOH 0.5 ml), and indomethacin (10 mg/kg) on rat paw oedema induced by carrageenan intraplanar injection (1000 μg/paw) (*$P < 0.05$).

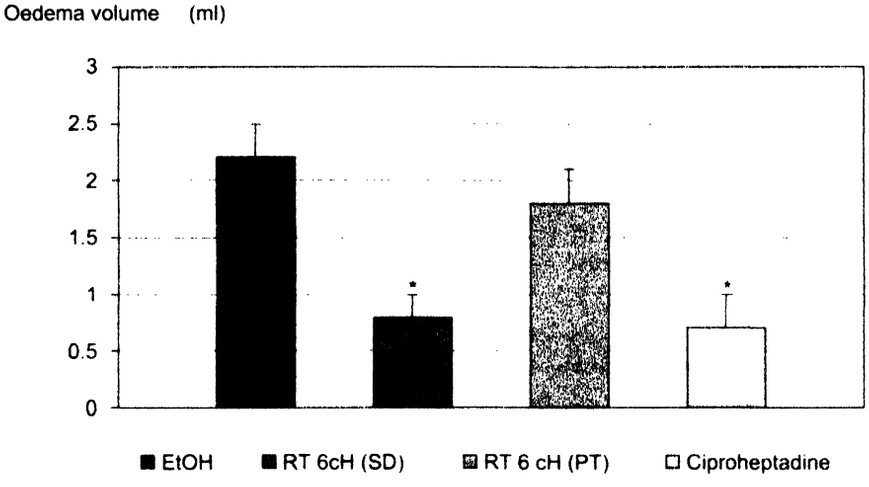

Figure 3 Effect of *Rhus tox* (RT) 6 cH (0.5 ml) single dose and pretreatment, succussed ethanol 30% (EtOH, 0.5 ml), and cyproheptadine (10 mg/kg) on rat paw oedema induced by dextran intraplantar injection (100 μg/paw) (*$P < 0.05$).

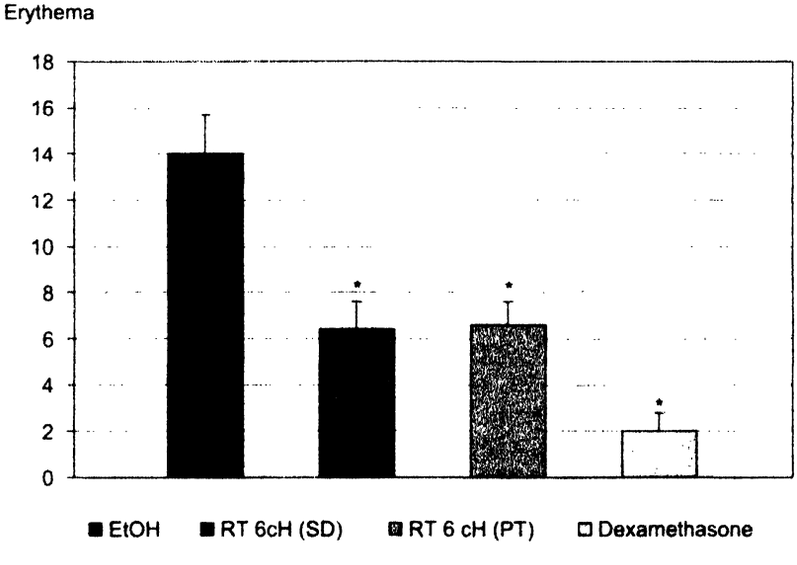

Figure 4 Effect of *Rhus tox* (RT) 6 cH (0.5 ml) single dose and pretreatment, succussed ethanol 30% (EtOH, 0.5 ml) on the mouse ear erythema induced by 0.1 ml (1 mg/ear) of croton oil (*$P < 0.05$).

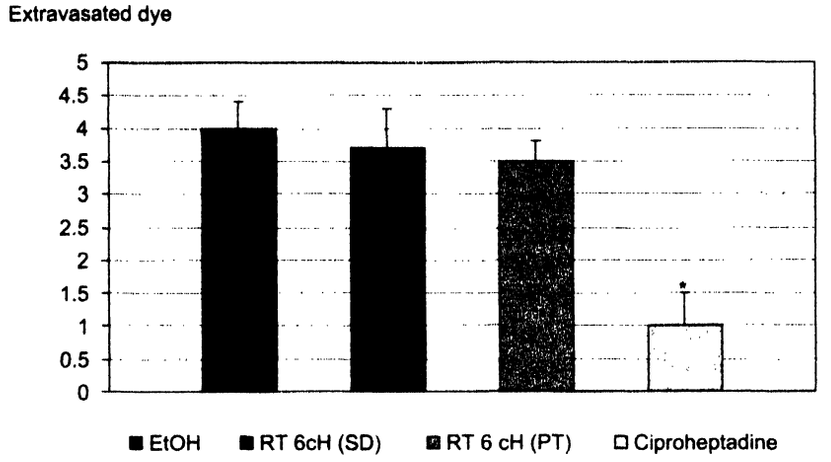

Figure 5 Effect of *Rhus tox* (RT) 6 cH (0.5 ml) single dose and pretreatment, succussed ethanol 30% (EtOH, 0.5 ml), and cyproheptadine (10 mg/kg) on the vascular permeability increase induced by histamine (50 μg/animal) (*$P < 0.05$).

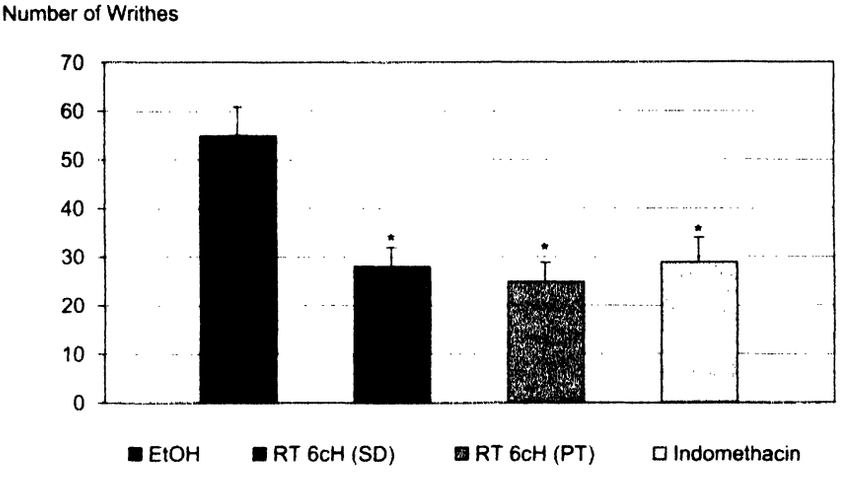

Figure 6 Effect of *Rhus tox* (RT) 6 cH (0.5 ml) single dose and pretreatment, succussed ethanol 30% (EtOH, 0.5 ml) and indomethacin (10 mg/kg) on the number of writhes induced by acetic acid in mice (0.25 ml/animal, i.p.) (*$P < 0.05$).

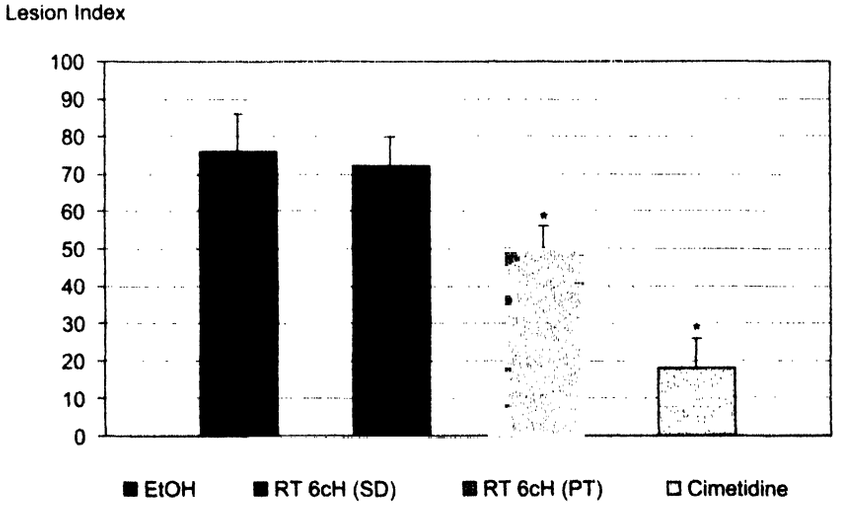

Figure 7 Effect of *Rhus tox* (RT) 6 cH (0.5 ml) single dose, and pretreatment (PT), succussed ethanol 30% (EtOH, 0.5 ml) and cimetidine (50 mg/kg, p.o.) on the gastric acute lesions induced by stress in rats (*$P < 0.05$).

Carrageenan induces protein-rich exudates, containing many neutrophils,[15] with three distinct phases. The first phase relates to the release of histamine and serotonin. The second phase involves bradikinin release, while prostaglandins are involved in the final phase.[16] Our results are in line with those of Jäggi *et al*,[17] who showed inhibition of synthesis of LTB_4 (Leukotriene B4), PGE_2, COX_1 and COX_2 isoforms by *Rhus tox* tincture in vitro. It appears that *Rhus tox* dynamizations interfere with the inflammatory process triggered by carrageenan. Dextran, the other oedematogenic agent used in this study, triggers release of histamine and 5-hydroxytriptamine mediated agents from mast cells.[18] In this assay the *Rhus tox* 6cH SD regime was effective, the PT regime had no effect.

The test of erythema in mouse ears induced by croton oil is commonly used for the evaluation of new anti-inflammatory drugs.[19,20] Dermatitis induced by croton oil represents a model of acute inflammatory response. The oedema is mediated by cycloxygenase metabolism of arachidonic acid.[21-23] *Rhus tox* 6cH might, either in pre-treatment or single dose, interfere in this process, obstructing the generation of these mediators (Figure 4).

Injection of histamine causes an increase of vascular permeability, detected by extravasation of Evans blue. Oral treatment of rats with *Rhus tox* 6cH (SD and TP) did not inhibit this response (Figure 5).[24]

Vane (1971)[25] showed that many of the effects caused by the non-steroidal anti-inflammatory drugs (NSAIDs) on the gastric mucosa are caused by inhibition of prostaglandin synthesis. The initial injury is because of the direct harm mediated by the NSAIDs, followed by a systemic effect, due to inhibition of synthesis of prostaglandins. Prostaglandin E_2 (PGE_2), PGI_2 and $PGF_{2\alpha}$, are protectors of the gastric mucosa, increasing bicarbonate transport and modifying blood-flow, avoiding ischaemia and stimulating longitudinal contraction.[26]

Rhus tox 6cH (SD) did not inhibit the formation of gastric ulcers. However, *Rhus tox* 6cH (PT) was effective in preventing the incidence of acute gastric ulcers induced by stress over 17h (Figure 7). These results corroborate with the hypotheses of Jäggi *et al*,[17] showing the *in vitro* inhibition of 5-LOX (5-Lipoxygenase) by *Rhus tox* tincture. The anti-gastric ulcer action might be due to inhibition of 5-LOX leading to lower LTB_4, an important factor in the pathogenesis of the injuries caused by NSAIDs.[27]

The methods to study pain and its relief in animals have increased in recent years.[28] We used the writhing test in mice. In this assay, both treatments (PT and SD) with *Rhus tox* were effective in inhibiting the algogenic process caused by acetic acid (Figure 6). These results reinforce those obtained in the rat's paw oedema caused by carrageenan and the erythema by croton oil, the algogenic process caused by acetic acid again involves the synthesis of prostaglandins (PGE_2 and PGF_2), and adrenergic system.[29-31]

Conclusion

Our results suggest that the homeopathic medicine *Rhus tox* (6, 12, 30 and 200cH) is effective inhibiting the oedema caused by carrageenan. The 6cH dynamization was the most effective. The same dilution demonstrated an effect on histaminergic oedema (dextran), confirming the responses to the applications described in the literature. *Rhus tox* 6cH was effective on the hyperalgesic process, demonstrating its peripheral analgesic effect. These findings suggest that *Rhus tox* may act through inflammatory mediators.

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