IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
August 2016 Vol.:7, Issue:1

Pharmacological Evaluation of Anti-inflammatory Activity of Mother Tincture of *Rhus Toxicodendron*

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ISSN 2349-7203
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Submission: 26 July 2016
Accepted: 1 August 2016
Published: 25 August 2016

www.ijppr.humanjournals.com

Keywords: Homeopathy, *Rhus Toxicodendron*, Urushiol, Mother Tincture

ABSTRACT

The purpose of the present study was to investigate the anti-inflammatory and anti-hyperalgesic properties of Mother tincture of *Rhus Toxicodendron* and its different sources. The Homeopathic drug, *Rhus Toxicodendron* is reported to be used in as anti-inflammatory medicine in homeopathic practice. *Rhus tox* was first used in medicine to treat a young man with an herpetic eruptions in 1798. We assessed the *Rhus tox* in the form of mother tincture and its different sources were tested through *in vivo* models including Carrageenan induced rat paw edema and decrease in hyperalgesia in Carrageenan Model. The result revealed that the anti-inflammatory activity of Mother tincture of *Rhus Tox* and its different sources like (Self Prepared, Father Muller, Heal Well, SBL, Hahnemann, Reckweg) shows significant inhibition at 3 hr, whereas decreased in Hyperalgesia in Carrageenan model using Mother tincture of *Rhus Tox* and its different sources shows significant decrease at 4^{th} hr and 6^{th} hr.

INTRODUCTION

Homeopathy is a complementary medicine system which has been controversially discussed for more than 200 years. (Ludtke, *et al*, 2008). One of the most discussed controversies about the efficacy of homeopathic medicines is the necessity to identify a perfect symptomatic analogy between patient and drug pathogenesis, that is, the necessity of individual prescription according to the similia principle. This particular feature is one of the most difficult challenges of the scientific research in this field because of the technical difficulty of its experimental demonstration.(Leoni Villano, *et al*, 2010). The most significant controversy in homeopathy involves the minute dose of the medicine (remedy). This principle states that one should use the smallest dose and lowest frequency possible. Although all good medical practitioners would agree with this principle, the degree to which homeopathy practices this defies generally known principles of molecular biology or biochemistry. Homeopathic medicines are prepared by a process of serial dilution and agitation (sucussion). Many of the homeopathic remedies are so dilute that they would not be expected to contain any of the original therapeutic substance. (Bozzuto, *et al*, 2000). The most characteristic and controversial principle of homeopathy is that the potency of a remedy can be enhanced by dilution, in a procedure known as 'dynamization' or 'potentization (Pitar, 2006).

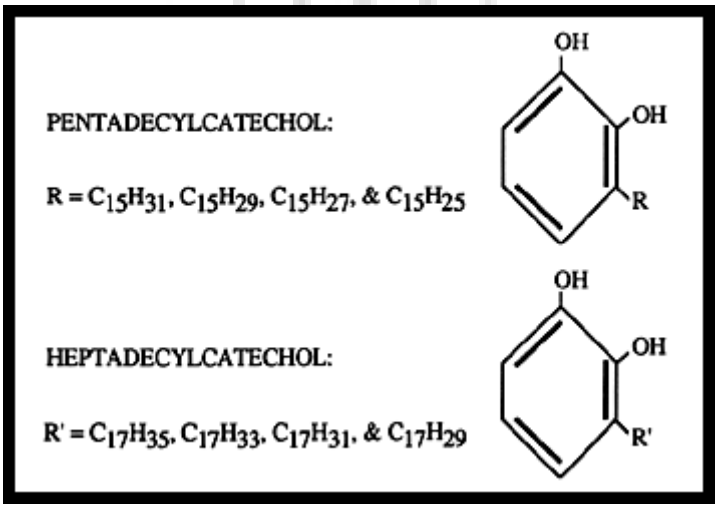
Rhus Toxicodendron

Rhus Toxicodendron- It is employed a brown-yellowish sap, of a sharp and caustic odour, distributed all through the plant (Silva Rocha, *et al*, 2008)

Fig-1

Rhus Tox secretes a resin called Urushiol. Urushiol comes from Japanese word "urushi" meaning lacquer which causes edema, erythema and irritation (Carvalho, *et al* 2007) . Urushiol is responsible for contact dermatitis caused by Rhus tox. (Patil, *et al* 2011) . The skin sensitizing property of the urushiols has been well documented. Additionally, sensitization to a single species confers cross sensitivity to the other species due to the chemical similarity of these allergens. (MURPHY, *et al* 1983). Urushiol is colorless or slightly yellow in its natural state, but oxidizes, polymerizes, and turns black when exposed to air. The oil is found in the stems, roots, leaves, and skin of the fruits of these plants. It is non-volatile and dries quickly on fomites, where it retains its antigenic potential in the dry state indefinitely (longevity is increased in dry climates and decreased in warm, moist climates). Usually, damage is required for plants to release the oil, therefore, slight contact with uninjured leaves is innocuous. More vigorous activities, such as weeding or cleaning brush, or cross-country travel and bushwhacking, can transfer the oil onto the skin. As little as 2 mg can cause a reaction in sensitive individuals. (Gladman, *et al*, 2006).

Urushiol consists of dihydric phenols with 15 or 17 carbon side chains which may or may not contain 1--3 double bonds. (Murphy, *et al*, 1983)



Chemical Constitute

- Urushiol
- Cardol,
- Phenolic acid
- Resin
- Fisetin
- Tannin

MATERIAL AND METHOD

Drug and Chemicals:

Different Homeopathic dilutions of *Rhus Tox* manufactured by Dr. Reckweg and Co. GmbH, Germany were purchased from a local vendor. Whereas different Homeopathic mother tincture of *Rhus tox* manufactured by Father Muller, Sintex, SBL, Hahnemann and Reckweg were purchased from a local vendor.

Animals:

Adult Albino Wistar rats of either sex weighing between 150-200 gm were used for study. The animals were obtained from the Department of Pharmacology, R. C. Patel College of Pharmacy, Shirpur. Animals were housed in well ventilated polypropylene cages and maintained under standard conditions at (25 ± 2^0 C, 12:12 hr L: D (Light and Dark) cycle in the departmental animal house. The animals were fed with standard pelletized feed (Amrut Rat and mice feed, Sangli) and water was provided *ad libitum*. The study was approved by Institutional Animal Ethical Committee registered with Committee for the Purpose of Control and Supervision of Experiments on Animals, India (Registration No. 651/02/C/CPCSEA).

Chemical:

Carrageenan (λ) (C3889-5G), were obtained from Sigma Aldrich. Chloroform-HPLC Grade (Case No: 67-56-1), Methanol-HPLC Grade (Case No: 67-66-3).

Equipment:

Digital Plethysmometer (Ugo Basile 7140, Italy), Von fray IITC (Life Science)

Dosage preparation and administration:

The Homeopathic dilutions of *Rhus Tox* were administered as reported earlier by **(Santos *et al.* 2007)**. 0.1 ml of each dilution was added to 1.0 ml of sterile distilled water and administered through oral gavage to respective groups ones daily. Care was taken to abstain animals from food immediately before and after drug administration for at least 2 hours.

Effect of *Rhus Tox* from different source in Carrageenan induced rat paw edema

Male Wistar rats with a body weight between 150 - 200 g were used. Rats were grouped into six groups, each groups containing six animals (Mule *et al*,2008). The rats were fasted for 12 hours prior to induction of edema, however, water was available *ad libitum* (Jain, *et al* 2010). To ensure uniform hydration, the rats receive 5 ml of water by stomach tube (controls) or the test drug dissolved or suspended in the same volume (H G Vogel *et al*. 2002). Inflammation was induced by Carrageenan (0.1ml, 1w/v% dissolved in saline) was injected into the plantar region of the right hind paw (Kim, *et al*, 2009). Negative control group received 1 ml of normal saline *(Cabrini, et al*, 2008). The remaining five groups orally received *Rhus Tox* dilution 100 µml of Self Prepared, Father Muller, Heal Well, SBL, Hahnemann, Reckweg respectively. All the drug treatments were given 1 hr before the Carrageenan injection. Subsequent readings of the volume of the same paw were carried out for 6 h at 60-min intervals and compared to the initial readings (Marzocco, *et al*, 2004).

Decreased in Hyperalgesia in Carrageenan models using different sources of *Rhus Tox* mother Tincture

Male Wistar rats with a body weight between 150 - 200 g were used. Rats were grouped into seven groups, each group containing six animals.

Table-1:

Group	Number of Animal	Group Name	Treatment
Group I	6	Control	Saline
Group II	6	Self Prepared MT	Self Prepared MT 0.1ml dil. up to 1ml with WI
Group III	6	Father Muller MT	Father Muller MT 0.1ml dil. up to 1ml water for injection
Group IV	6	Heal well MT	Heal well MT 0.1ml dil. up to 1ml water for injection
Group V	6	SBL MT	SBL MT 0.1ml dil. up to 1ml of water for injection
Group VI	6	Hahnemann MT	Hahnemann MT 0.1ml dil. up to 1ml of water for injection
Group VII	6	Reckweg MT	Reckweg MT 0.1ml dil. up to 1ml of water for injection

Animals were placed individually into Plexiglass chambers with customized platform that contains 1.5 mm diameter holes in a 5 mm grid of perpendicular rows throughout the entire area of the platform (Zhang, *et al*, 2007). 1% Carrageenan in saline, which was injected into the right hind paw plantar surface to groups of each animals (Coruzzi, *et al*, 2007). Withdrawal threshold was measured by electronic von Frey Anaesthesiometer, IITC-Life Science Instruments (Jean De Vry, *et al*, 2004). Animals were given approximately 15 min to acclimate to the testing apparatus (Fecho, *et al*, 2005). Filaments were applied to the plantar surface of a hind paw for 6–8s (Yamamoto, *et al*, 2007). Paw withdrawal latencies were recorded at 0, 2, 4, and 6 hr ((Coruzzi, *et al*, 2007). Paw withdrawal threshold was measured in grams. Negative control received 1 ml of normal saline. The remaining groups orally received Different sources of *Rhus Tox* Mother Tincture (Self Prepared, Father Muller, Heal Well, SBL, Hahnemann, and Reckweg respectively). All the drug treatments were given 1 hr before the Carrageenan injection.

RESULT

Anti-inflammatory activity

Carrageenan induced paw edema in rat

The anti-inflammatory activity of *Rhus Tox* was assessed using different sources like (Self Prepared, Father Muller, Heal Well, SBL, Hahnemann, Reckweg) administered 1hr before subplanter injection of carrageenan. The maximum in paw volume was observed at Third hour from time of inflammatory stimulus. *Rhus Tox* from different sources showed significant anti-inflammatory activity at Third hour. The Rhus Tox from different sources (Self Prepared, Father Muller, Heal Well, SBL, Hahnemann, Reckweg) showed significant inhibition at 3 hr. *Rhus Tox* showed 20.57±10%, 29.59±15%, 31.34±17%, 35.44±17%, 42.79±4.2, and 45.79±24% rise of paw volume at Heal Well, Father Muller, Self Prepared, SBL, Hahnemann and Reckweg, respectively at third hour. The percent rise of Control was found to be 46.16±14%.

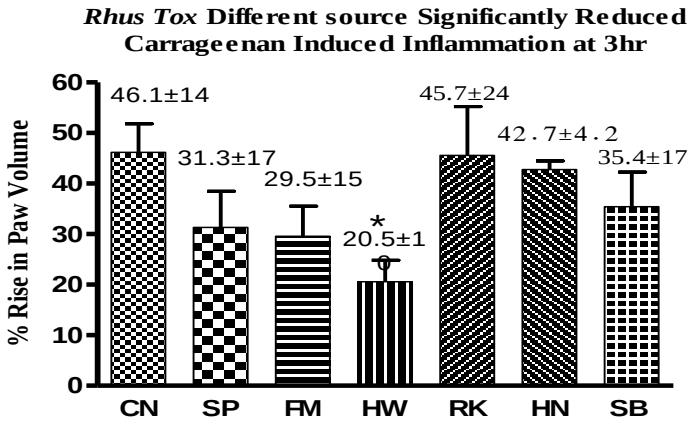


Fig-2

CN-Control, SP-Self Prepared, FM-Father Muller, RK-Reckweg, HN-Hahnemann, SB-SBL.

Data presented as Mean ± S.E.M.

ANOVO: $p<0.05$ ($F=2.3, df=6, n=42$)

Dunnett's Multiple Comparison Test

*$p < 0.05$, **$p < 0.01$, as compared to control group

Decreased in Hyperalgesia in Carrageenan models using different sources of *Rhus Tox* mother Tincture

Decreased in Hyperalgesia in Carrageenan model using Different Sources of *Rhus Tox* was assessed like (Self Prepared, Father Muller, Heal Well, SBL, Hahnemann, Reckweg) administered 1hr before subplanter injection of carrageenan. The maximum effect of was observed at Fourth hour from time of Carrageenan injection. *Rhus Tox* from different sources showed significant decreased in hyperalgesia at Fourth hour. The *Rhus Tox* from different sources (Self Prepared, Father Muller, Heal Well, SBL, Hahnemann, Reckweg) showed significant decreased hyperalgesia at 4^{th} hr . *Rhus Tox* showed 20±5.2gram, 18±9.1gram, 17±7.9gram, 17±6.2gram, 15±5.4 gram, 12±3.1gram and 9.9±3.4 gram rise at SBL, Hahnemann, Father Muller, Reckweg, Self Prepared, Heal Well and Control respectively at Fourth hour.

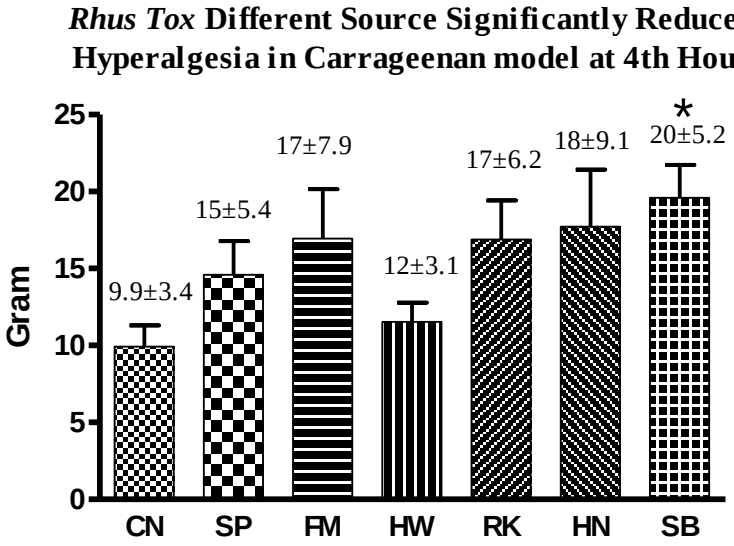


Fig-3

CN=Control, SP=Self Prepared, FM=Father Muller, HW=Heal Well, RK=Reckweg, HN=Hanhemann, SB=SBL.

Data presented as Mean ± S.E.M.

Dunnett's Multiple Comparison Test

*p < 0.05, **p < 0.01, as compared to control group

DISCUSSION

The characteristics of inflammation can be divided into acute, chronic, irritability and immunity related inflammation. Any factor that induces tissue damage could be described as the pathogenesis of an inflammation. (Hepburn, *et al*, 2005). Inflammation is a non-specific and immediate reaction of the organism against a physical, chemical or microbiological aggression to test anti-inflammatory drugs, including the inhibition of rat hind paw edema induced by oedematogenic agents, including dextran, histamine, albumin, carrageenin and others (Carvalho, *et al*, 2004) The present study uses *Rhus Tox* Mother tincture from different sources and shows a good anti-inflammatory activity. *Rhus Toxicodendron* effectively reduces the inflammation at both the phases of Carrageenan induced inflammation. This suggested that *Rhus Tox* may act by inhibiting the synthesis and/or release of mediator like histamine and serotonin.

According to recent study (Patil, *et al*, 2009) Dual effect of Toxicodendron pubescens on Carrageenan induced paw edema in rats was evaluated and found that single dose of *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 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. 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. *Rhus tox* shows pro-inflammation in chronic phase in which cytokines like mediators are involved and in acute phase, it acts as an anti-inflammatory drug through COX pathway. The inhibitory effect of *Rhus tox* on second phase of inflammation may be due to inhibition of prostaglandins, leukotriene or other late phase mediator of inflammation.

In this work we also evaluated the hyperalgesic (evaluated in von Frey electronic pressure-meter paw), study showed that treatment with Mother Tincture of *Rhus Toxicodendron* suppressed the development of the carrageenan-induced hyperalgesia and edema.

Numerous studies investigating inflammatory hyperalgesia have used Carrageenan as an inducer of acute inflammation by injecting Carrageenan into the hind paw of a rat. The responses to noxious heat and noxious pressure, which we observed, are comparable to those described after Carrageenan injection into a rat's hind paw, with peak hyperalgesia occurring within a few hours and resolving by 24 h. Similar behavioral changes to noxious heat and noxious pressure, with the same temporal pattern, were evident after a Carrageenan injection, in spite of the tail having thick keratinised skin, and the hind paw having thin glabrous tissue (Loram, *et al*, 2007). A variety of chemical mediators is released into the injury site during inflammation and injury. Many of these mediators not only maintain activity of primary afferent nociceptors but also enhance nociceptor sensitivity, such that noxious stimuli causes increased pain perception (hyperalgesia) and innocuous stimuli induce pain sensation (allodynia). Serotonin (5-hydroxytryptamine or 5-HT) is one of these mediators. *Rhus tox* in its crude form has dual effect in the Carrageenan induced rat paw edema in rat. These can be correlated that *Rhus tox* inhibits hyperalgesia. This effect may be due inhibition of serotonin and 5-HT.

CONCLUSION

In the present study, we observed that *Rhus tox* mother tincture from different sources has significant anti-inflammatory activity and anti- hyperalgesic activity.

Rhus tox was found to be effective as an anti-inflammatory agent in acute models of inflammation. *Rhus tox* has inhibitory activity on both phases of Carrageenan induced rat paw edema. This effect of *Rhus tox* may be due to inhibition of pro-inflammatory mediator like histamine, serotonin and kinins which involve in first phase of Carrageenan induced inflammation. The inhibitory effect of *Rhus tox* on second phase of inflammation may be due to inhibition of prostaglandins, leukotriene or other late phase mediator of inflammation.

According to recent study (Patil et al. 2009) *Rhus tox* in its crude form has dual effect in the Carrageenan induced rat paw edema in rat. These can be correlated that *Rhus tox* inhibit hyperalgesia. *Rhus tox* inhibits hyperalgesia, this effect may be due inhibition of serotonin and 5-HT.

Acknowledgement

I am thankful to Dr. D.R. Lohar, Director, Homeopathic Pharmacopoeia Laboratory, Ghaziabad for providing me some valuable information regarding *Rhus Toxicodendron*. I am also thankful to Dr. Shivang Swaminarayan, Head – Health Division Sintex International Limited, Kalol, Gujarat, for providing me a sample of Mother Tincture of *Rhus Toxicodendron.*

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