DOI: 10.7860/JCDR/2017/25081.10257

Experimental Research

Pharmacology Section

Anti-inflammatory and Analgesic Activities of Topical Formulations of *Pterocarpus Santalinus* Powder in Rat Model of Chronic Inflammation

PRITI PRAVIN DHANDE[1], AMIT O GUPTA[2], SOURAV JAIN[3], JAYSHREE SHRIRAM DAWANE[4]

ABSTRACT

Introduction: The incidence of arthritis is quite high and there is a need for the search of natural products to halt the progression of disease or provide symptomatic relief without significant adverse effects.

Aim: This study aimed at evaluating the anti-inflammatory and analgesic activities of topical *Pterocarpus santalinus* in an animal model of chronic inflammation.

Materials and Methods: Albino rats of either sex were divided into five groups of six rats each (Group I – Control, Group II –Gel base, Group III –*P. santalinus* paste, Group IV –*P. santalinus* gel, Group V– Diclofenac gel). Chronic inflammation was induced on day 0 by injecting 0.1 ml Complete Freund's Adjuvant (CFA) in sub-plantar tissue of left hind paw of the rats. Topical treatment was started from day 12 till day 28. Body weight and paw volume (Plethysmometer) were assessed on day 0, 12 and 28. Pain assessment was done using Randall and Selitto paw withdrawal method. Data was analysed using GraphPad Prism version 5. Unpaired students t-test and ANOVA followed by Tukey's test was used for comparison among groups.

Results: Only topical *P.santalinus* gel significantly reduced the body weight ($p=0.02$) due to reduction in inflammatory oedema of the left limb. *P. santalinus* gel also showed significant reduction ($p=0.03$) in paw volume of rats compared to the other groups. There was significant reduction in pain threshold (gm/sec) due to chronic inflammation, with all the study drugs ($p<0.05$) but with *P. santalinus* gel, this reduction was less ($p<0.001$).

Conclusion: Gel showed significant anti-inflammatory and mild analgesic activity on topical application in rat model of chronic inflammation.

Keywords: Bark wood, Gel, Oedema, Raktachandan

INTRODUCTION

India has a high prevalence of arthritis, which is a chronic inflammatory condition with about 15% people i.e. over 180 million people affected by it [1]. It is associated with joint inflammation with immune cell infiltration, pain, swelling and synovial hyperplasia. Chronic inflammation leads to joint deformity and compromises the activities of daily living. The treatment agents used for chronic inflammatory conditions like non-steroidal inflammatory agents and glucocorticosteroids are associated with serious adverse effects on prolonged use with unsatisfactory response [2,3].

Pterocarpus santalinus is commonly known as Red sandalwood (English) and Raktachandan (Sanskrit). The paste of its wood has been considered as a cooling agent for external application which helps in treating inflammations and headache, mental aberrations, and ulcers [4]. From ancient times, it has shown to reduce burning sensation, arrest bleeding and alleviate oedema, because of its anti-inflammatory activity. Wood paste is applied on boils and other skin eruptions, infections, inflammation, and on forehead to relieve headache. The antibacterial, anticancer, hepatoprotective, and wound healing properties of this drug have been established recently [5].

Reactive Oxygen Species (ROS) participate in the process of inflammation in various tissues [6]. Thus, substances having radical scavenging activities, may be expected to have therapeutic potential in several inflammatory diseases. Authors have revealed the antioxidant potential of *P. santalinus* leaf extract and demonstrated the free radical scavenging activities of the same [7].

Literature search has revealed anti-inflammatory activities of *P. santalinus*, studied using extract preparation of bark wood powder [8]. This concept of using an extract preparation of *P. santalinus* bark wood does not match with the clinical practices of Ayurveda, where its bark powder is utilized in diseases. Also, it has not been studied topically in chronic inflammatory models. Hence, this study was planned to evaluate the effect of topical *P. santalinus* powder in CFA induced chronic inflammation in albino rats.

MATERIALS AND METHODS

The experimental study was started after getting approval from Institutional Animal Ethics Committee from Bharati Vidyapeeth Deemed University Medical College, Pune, Maharashtra, India, (BVDUMC/30-78/2015/001/005) and conducted according to CPCSEA guidelines. The study duration was 40 days (March and April 2015). Albino rats used for the study were housed in separate cages with free access of rodent food and aquaguard water.

Chemicals: CFA (Sigma-Aldrich), *Pterocarpus santalinus* bark wood (made into powder after authentication), injection pentobarbitone (LOBA-CHEMIE INDOAUSTRANAL CO., BOMBAY), diclofenac gel (Cipla Pharmaceuticals Ltd.,), carbopol 934P as gelling agent (Thomas Baker Pvt. Ltd.,), triethanolamine (Thomas Baker Pvt. Ltd.,).

Authentication and preparation of study material: *Pterocarpus santalinus* bark wood was obtained from ayurvedic medical stores (Vaidya Khadiwale) and sent for authentication to Agharkar Research Institute, Maharashtra, India. The sample was critically studied with macroscopic, organoleptic, microscopic and TLC profile and authenticated by AS Upadhye, Scientist, Plant Drug Authentication Service, Botany group, Plant Sciences Division as mentioned in the certificate number Auth.15-072. It was confirmed to be the heartwood of *Pterocarpus santalinus* Lf (Family: Fabaceae). The bark wood was then dried in shade and powdered finely to be used for preparation of the study formulations.

Preparation of *P. santalinus* paste formulation: A 10 gm of powder of *P. santalinus* was added to 10 ml of distilled water and mixed to form a uniform paste. Equal amount of paste (1.5 gm) was applied topically on the arthritic joint of the animal.

Preparation of *P. santalinus* gel formulation: Weighed (0.1 gm) quantity of carbopol 934P was taken and added to distilled water (5 ml). About 10 gm powder of *P. santalinus* was mixed with 20 ml distilled water and transferred to appropriate quantity of aqueous dispersion of carbopol 934P. The mixture was then stirred gradually by means of a stirrer until a cloudy, lump free dispersion was obtained. Then it was allowed to stand for air bubbles to separate. Nearly, 0.1 ml triethanolamine then added to neutralize the carbopol 934P solution and to form the gel [9]. This preparation was divided into six equal parts on the tile and one part was applied topically to each rat every day.

Induction of inflammation: Induction of inflammation was done with CFA as described below and evaluation of the inflammatory activity was done by measurement of oedema size resulting from sub-plantar CFA injection unilaterally in the left hind paw region of the body. Animals were anesthetized by injecting pentobarbitone intraperitoneally in the dose of 6 mg/100 gm body weight. Then 0.1 ml of CFA was injected in the sub-plantar tissue of the left hind paw. According to Bendele A, acute inflammatory reaction develops rapidly but chronic inflammation appears in the later stages (day 12+) as severe paw swelling [10].

Groups: Albino rats of either sex weighing 150-200 gm were divided into five groups as follows with six rats in each group-

Group I – Negative control (distilled water)

Group II – Vehicle control (Gel base)

Group III –*P. santalinus* paste

Group IV –*P. santalinus* gel

Group V– Diclofenac gel (standard treatment)

Topical treatment was started, as per the respective groups from day 12 till day 28. After applying the drug on the diseased part, that area was covered by sterile gauze to prevent the inadvertent consumption of the medication by the animals.

Parameters Assessed

1. Body weight of the study animals was observed on day 0, 12 and 28.
2. Measurement of paw volume: Paw volume was measured with plethysmometer on day 0, 12 and 28. The right hind paw volume served as the control reading against diseased left hind paw volume for each animal. Paw volume in the test group was compared with that of control and standard treatment groups.
3. Pain assessment: Pain threshold was assessed by using the Randall and Selitto paw withdrawal method [11]. A paw pressure test was carried out in which pressure was measured in grams/second using an Ugo Basile Analgesy Meter by applying an increasing amount of force to the left hind paw of rats. Pressure was applied until they reacted either by paw withdrawal or squealing. Pain threshold was determined for each animal at the start of the experiment (day 0) to obtain a baseline reading [12]. After 28 days, pain threshold (in grams) was again estimated. Each animal served as its own control.

Keeping the ethical issues of 3R's (Replacement, Reduction and Refinement) in review, the animals were handled by competent, empathetic capture using the cupping method and not lifted by their tail. While injecting the inducing agent, anaesthesia was provided to reduce the suffering in the animals. We ensured that they could easily reach water and food provided to them in the cage. We also implemented husbandry refinements in the form of long nozzles on drinking bottles and soft sawdust bedding material. They were provided appropriate environmental enrichment and housed them in groups of three animals per cage [13].

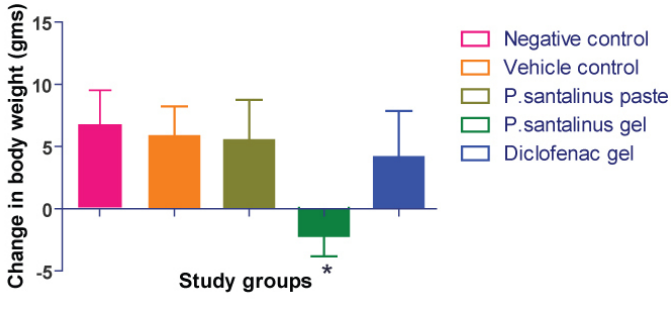
STATISTICAL ANALYSIS

Data was analysed using Graph Pad Prism version 5. Paired and Unpaired students t-test was used for comparison within and among groups respectively. The $p<0.05$ was considered statistically significant.

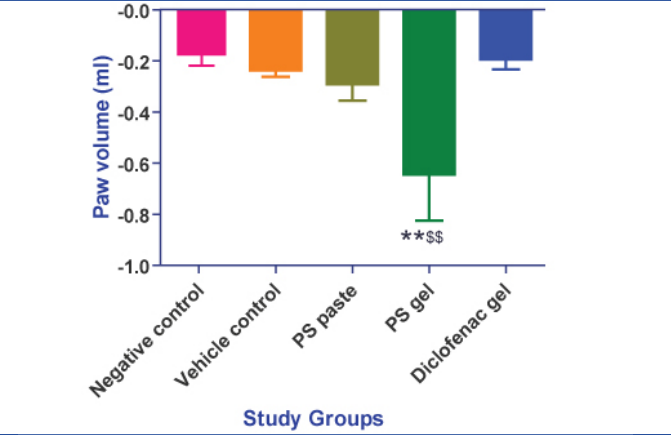
RESULTS

Body weight of the study animals was observed on day 12 when the chronic inflammation model developed and also on day 28 to see the response of the drug treatment on this parameter. There was significant reduction in body weight in rats treated with *P.santalinus* gel (p=0.02) when compared to other groups on day 28 of the experiment [Table/Fig-1].

Paw volume calculated using plethysmometer also showed reduction in paw volume in all the groups on day 28 as compared to day 12; but this parameter was statistically significant (p=0.03) only in rats treated with *P.santalinus* gel as compared to control group and standard treatment group [Table/Fig-2,3].



[Table/Fig-1]: Effect of topical *Pterocarpus santalinus* formulations and diclofenac on body weight in rat chronic inflammation model (Day 12 - Day 28).
*p = 0.02 using unpaired t-test when compared to negative control group (I)



[Table/Fig-2]: Reduction in paw volume with topical *Pterocarpus santalinus* formulations and diclofenac in rat chronic inflammation model (Day 12 - Day 28).
** p = 0.03 using unpaired t-test when compared to negative control group (I).
$$ p= 0.03 using unpaired t-test when compared to diclofenac gel group (V).

Sr.No.	Group	Day 0	Day 12	Day 28	Change in paw volume on Day 28 from Day12
I	Negative control	1.3 ± 0.04	2.1 ± 0.04	1.9 ± 0.05	-0.2 ± 0.04
II	Vehicle control	1.5 ± 0.14	2.6 ± 0.16	2.3 ± 0.14	-0.3 ± 0.02
III	Pterocarpus paste	1.3 ± 0.08	2.2 ± 0.08	1.9 ± 0.08	-0.3 ± 0.06
IV	Pterocarpus gel	1.2 ± 0.05	2.6 ± 0.19	1.9 ± 0.03	-0.7 ± 0.18**$$
V	Diclofenac gel	1.3 ± 0.03	2.2 ± 0.05	2.1 ± 0.06	-0.1 ± 0.03

[Table/Fig-3]: Paw volume of study rats on day 0, 12 and 28.
Values are expressed as Mean+SEM
** p = 0.03 using unpaired t-test when compared to negative control group (I)
$$ p= 0.03 using unpaired t-test when compared to diclofenac gel group (V)

Pain assessment was done using Randall LO and Selitto JA paw withdrawal method which showed decrease in pain threshold in all the groups on Day 28; but this change in pain threshold was less in *P.santalinus* gel group (158±8.1) on day 0 , (117±9.8) on day 28 [Table/Fig-4].

Group No.	Treatment	Pain threshold (gm/sec) Day 0	Pain threshold (gm/sec) Day 28	p-value
I	Distilled water	168 ± 16	107 ± 4.1**	0.005
II	Gel base	135 ± 2.3	98 ± 5.9**	0.002
III	P.santalinus paste	144 ± 5	96 ± 5.6**	0.003
IV	P.santalinus gel	158 ± 8.1	117 ± 9.8 *	0.01
V	Diclofenac gel	139 ± 4.2	97 ± 6.6**	0.003

[Table/Fig-4]: Effect of topical *P.santalinus* formulations and diclofenac on pain threshold (from day 0 to day 28) in rat chronic inflammation model.
* $p < 0.05$, ** $p < 0.001$ using paired t-test when compared between day 0 and day 28 readings of each group

DISCUSSION

CFA induced inflammation of the rat hindpaw has been extensively used as model of acute and chronic inflammation and arthritis [10]. Studies have shown that when CFA is injected subcutaneously at a relatively high dose, initially it leads to unilateral paw inflammation and acute arthritis, followed by contralateral paw swelling and a rheumatoid-arthritis like disease in later stages. But when a low dose of CFA is injected, both the hind paw oedema and arthritis remain strictly unilateral [14,15]. This was the model chosen for developing chronic inflammation in our study rats.

The standard drug used in this study, diclofenac was applied in the form of a gel preparation. Diclofenac, a non-steroidal anti-inflammatory agent is known for its prominent anti-inflammatory and analgesic properties even in arthritic conditions.

Two formulations of the study drug, *Pterocarpus santalinus* were tested for their anti-inflammatory and analgesic activities in this model. *P. santalinus* in the form of paste and gel were used for this purpose. Gels generally have a high rate of acceptance amongst consumers due to cosmetic elegance. Gels are generally thixotropic materials, exhibiting a stable form at rest but becoming fluid when agitated hence it shows better performance effect in terms of stability and therapeutic applicability [7,16]. Hence, for topical application of *P. santalinus*, gel was selected compared to other semisolid dosage form.

Our study revealed that topical treatment with *P.santalinus* gel in chronic inflammation model of rats, significantly reduced the paw volume in the rats [Table/Fig-2,3]. This significant reduction in paw volume might also have contributed to the decrease in body weight in these animals as seen in [Table/Fig-1]. Rats treated with topical *P.santalinus* gel also have demonstrated mild analgesic effect of this drug as can be seen from the minimal reduction in pain threshold in this group [Table/Fig-4] when compared to its baseline readings. The mild analgesic property of *P.santalinus* gel might correlate with the reduction in inflammation due to this drug which in turn reduced the inflammation associated pain.

It is well known that cell injury leads to activation of the arachidonic acid pathway and thereby production of prostanoids and leukotrienes and recruitment of immune cells at this site further releases mediators including cytokines and growth factors. Some of these inflammatory mediators activate peripheral nociceptors directly [17]. In the groups I, II, III and V, as there was no significant reduction in paw volume (representing inflammation), pain threshold was significantly lower than baseline values. This indicates that inflammation associated pain was still persisting in the other groups due to the release of inflammatory mediators from damaged cells like bradykinin, histamine, 5-hydroxytryptamine (5-HT), ATP and nitric oxide.

The qualitative phytochemical analysis of *P. santalinus* has confirmed the presence of various components, such as carbohydrates, steroids, anthocyanins, saponins, tannins, phenols, triterpenoids, flavonoids, glycosides, and glycerides [18]. Savinin, a lignan from *Pterocarpus santalinus* has been shown to inhibit TNF-α production and T cell proliferation which may act as an active principle in the anti-inflammatory effect [19]. A previous study has shown that anti-inflammatory effect of methanolic extract of *P. santalinus* as gel formulation was better than that of diclofenac gel in Carrageenan induced rat paw oedema method (acute inflammation) [7]. Our study has revealed its efficacy (gel formulation) even in chronic inflammatory model in which *P. santalinus* gel significantly reduced oedema and there was only slight reduction in pain threshold.

It is known that ROS participate in the process of inflammation in various tissues and authors have demonstrated in vitro free radical scavenging activities of *P. santalinus* methanolic wood extract [5]. This property might provide the basis of anti-inflammatory activity of *P. santalinus* in this model of chronic inflammation.

Our study has shown that the anti-inflammatory and analgesic activities of *P.santalinus* gel were better than the standard diclofenac gel applied topically in chronic inflammation model of rats. Previous studies have used extract preparations of *P.santalinus* for evaluating its anti-inflammatory and analgesic activities and the authors have observed that methanolic extract of this drug given orally or applied topically had comparable efficacy to standard drug diclofenac intraperitoneally or topically respectively [6,7]. In a clinical study which compared diclofenac concentrations in the soft tissues and blood on topical and oral use, topical application of diclofenac has shown higher concentrations in adjacent adipose tissue and skeletal muscle than oral preparations, but synovial concentrations were lower than with oral use [20]. It has been shown that diclofenac achieves higher concentration in synovial fluid on oral and intramuscular administration which peaks within 2-4 hours. But gastric irritation is known to be the most common adverse effects of NSAIDs including diclofenac. An added advantage of *P.santalinus* is its gastroprotective action proven in various studies [21,22]. Further studies should be done to test the anti-inflammatory activity of oral *P.santalinus* bark wood powder as mentioned in ayurvedic literature.

This study demonstrates a significant anti-inflammatory and mild analgesic activity of topical *P.santalinus* gel in CFA induced chronic inflammation in rats. *P.santalinus*/Raktachandan in the form of a gel formulation can be a promising and safe alternative to topical NSAIDs in chronic inflammatory conditions.

LIMITATION

Limitations of our study were that we were not able to perform the histopathological examination of the inflammed area which could have revealed the effects of the drugs at the tissue level.

CONCLUSION

Pterocarpus santalinus bark wood powder gel showed significant anti-inflammatory and mild analgesic activity on topical application in rat model of chronic inflammation. Future studies can be planned to study the mechanism of action of our study drug as anti-inflammatory agent in acute and chronic inflammation models.

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PARTICULARS OF CONTRIBUTORS:
1. Associate Professor, Department of Pharmacology, Bharati Vidyapeeth Deemed University Medical College, Pune, Maharashtra, India.
2. Postgraduate Student, Department of Pharmacology, Bharati Vidyapeeth Deemed University Medical College, Pune, Maharashtra, India.
3. Postgraduate Student, Department of Pharmacology, Bharati Vidyapeeth Deemed University Medical College, Pune, Maharashtra, India.
4. Assistant Professor, Department of Pharmacology, Bharati Vidyapeeth Deemed University Medical College, Pune, Maharashtra, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Priti Pravin Dhande,
9. Natasha Society, D.P. Road, Aundh, Pune-411007, Maharashtra, India.
E-mail: ppdhande@yahoo.com

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Oct 27, 2016**
Date of Peer Review: **Nov 28, 2016**
Date of Acceptance: **Apr 25, 2017**
Date of Publishing: **Jul 01, 2017**