

Activity of *Plumbago zeylanica* Linn. root and *Holoptelea integrifolia* Roxb. bark pastes in acute and chronic paw inflammation in Wistar rats

Dushyant Kumar, Kuntal Ganguly, H. V. Hegde, P. A. Patil¹, Subarna Roy, S. D. Kholkute

Regional Medical Research Centre (ICMR), ¹Universiti Sains Malaysia-Karnatak Lingayat Education Society, Belgaum, Karnataka, India

ABSTRACT

Background: The pastes prepared from roots of *Plumbago zeylanica* Linn. and barks of *Holoptelea integrifolia* Roxb. are widely used by traditional healers for the treatment of arthritis in rural northern Karnataka. **Objective:** The present study was undertaken to scientifically evaluate the safety and efficacy of traditionally used formulations in experimental animals. **Materials and Methods:** The study, approved by IAEC was carried out in male Wistar rats and dermal toxicity in rabbits. Carrageenan model was used to assess effect on acute inflammation. Paw volume were measured at 1, 2, 4, and 6th hour postchallenge. Chronic inflammation was developed by using Complete Freund's Adjuvant (CFA). Paw volume, ankle joint circumference, and body weight were assessed on 1st, 4th, 8th, 14th, 17th, and 21st day. Paste was applied once every day to the inflamed area of the paw of respective groups of animals, continuously for 14 days. **Statistics:** The data were analyzed by one way analysis of variance followed by Dunnett's *post hoc* test. $P \leq 0.05$ was considered as significant. **Results:** The formulations did not show any dermal toxicity and found to be safe. Both the pastes significantly ($P < 0.05$) suppressed, carrageenan-induced paw edema at 6th hour and *Holoptelea integrifolia* appears to be more effective than *Plumbago zeylanica*. Significant reduction was observed in paw volume, ankle joint circumference and animal body weight gained. **Conclusions:** The tested formulations (*P. zeylanica* root and *H. integrifolia* bark pastes) showed significant antiinflammatory activity. The present findings therefore support its utility in arthritic pain, inflammation and the claim of traditional practitioners.

Key words: Arthritis, *Holoptelea integrifolia*, *Plumbago zeylanica*, traditional formulation

INTRODUCTION

Rheumatoid arthritis (RA) is a symmetric, polyarticular, progressive disease that primarily affects small diarthrodial joints of the body.^[1] It is most common form of arthritis and has a substantial societal effect in term of cost, disability, lost productivity^[2] and is estimated to affect 0.5-1% of the population worldwide. It afflicts people

of all races equally and can occur at any age but most often starts at the age of 40-70 years. Patients seeking relief are subjected to treatment with nonsteroidal antiinflammatory drugs (NSAIDs), disease-modifying antirheumatic drugs (DMARDs), or immunosuppressant drugs, that provide prompt remissions at the cost of varieties of adverse drug reactions, which often force to discontinue the therapy. Most of the patients who believe that alternate systems of medicine are safer and effective usually opt for herbal medicine. Apart from qualified Ayurvedic practitioners, many traditional healers dispense a variety of herbal preparations for common ailments. In rural area of Belgaum district *Plumbago zeylanica* and *Holoptelea integrifolia* locally known as *Chilbil/Kanju/Papri* preparations are routinely dispensed for topical application to treat arthritis. *Plumbago zeylanica* Linn. belongs to the family Plumbaginaceae, which is a perennial, rambling undershrub, much cultivated and readily spread throughout India.^[3] The antiinflammatory activities of *P. zeylanica* extracts, administered orally, have been reported in animal models of acute inflammation.^[4,5] *Holoptelea Integrifolia* Roxb. belongs to the family Ulmaceae, which is large sized tree, found all over India, tropical and subtropical

Address for correspondence:

Dr. H. V. Hegde, Scientist 'B' Regional Medical Research Centre, Nehru Nagar, Belgaum - 590 010, Karnataka, India.

E-mail: harshavh@rediffmail.com

Received: 29-Mar-2013

Revised: 19-Jun-2013

Accepted: 31-May-2013

Access this article online

Quick Response Code:



Website:

www.jaim.in

DOI:

10.4103/0975-9476.128853

regions of Asia and Africa.^[6] The aqueous leaf extract of *H. integrifolia* is reported to possess antiinflammatory activity.^[7] However, there are no reports regarding their efficacy by topical application against inflammation as being used by traditional practitioners for the treatment of arthritis. Their efficacy has not been evaluated scientifically in chronic model of arthritis by topical application. Therefore, the present study was undertaken to explore their antiinflammatory activity by topical application to resembles RA in carrageen inflammation and Complete Freund's adjuvant (CFA)-induced arthritis in Wistar rats.

MATERIALS AND METHODS

Documentation of the traditional practice

The selected traditional practitioners were frequently visited and the details on disease, diagnosis, treatment, formulation and its ingredients, method of preparation, mode of administration, etc., were documented through interview and discussions.

Plant material

The plants incorporated in the formulations, that is, *Plumbago zeylanica* L. (RMRC-524) and *Holoptelea Integrifolia* Roxb. (RMRC-525) were collected from the local forest areas of Khanapur and Jamboti. Plants were authenticated and their voucher specimens were deposited at the herbaria of Regional Medical Research Centre (ICMR), Belgaum.

Preparation of paste

The formulations were prepared by following the exact procedures of traditional practitioners.

Five grams of dried root of *P. zeylanica* was weighed and 25 ml of distilled water was measured. The root piece of *P. zeylanica* was rubbed over a clean stone surface for 15 min by adding 5 ml of distilled water in five installments. The resulting paste was weighed and consistency was semisolid paste. The *P. zeylanica* root piece was weighed again to find out the weight loss due to rubbing. The process was repeated for six times, to find out statistical mean. Appearance of paste was Brownish [Figure 1], viscosity was (1333 cps) measured by Brookfield viscometer and pH was 6.3. This Paste (PZRP) was applied in sufficient quantity on the surface of the inflamed area and warmed with radiant heat (100 W electrical bulb placed at a distance of 20 cm for 15 min) to mimic the technique as practiced by traditional healers.

Bark of *H. integrifolia* was rubbed with friction on a clean stone surface with adequate quantity (25 ml distilled water) of distilled water to obtain a thick paste [Figure 1]. Appearance of paste was brownish, viscosity was 1285 cps, and pH was 6.6. This paste (HIBP) was applied in sufficient quantity on the surface of the inflamed area, wrapped

with wet cotton gauge and was covered with medical bandage (simulation of technique as used in traditional practice).

Animals

Healthy, adult Wistar rats of either sex weighing 150-200 g and New Zealand white rabbits weighing 1.5-2.0 kg were procured from Shree Venkateshwara Traders, Bangalore, India. They were housed in the laboratory for about a week for acclimatization at room temperature ($25 \pm 3^\circ\text{C}$) with natural light and dark cycle. Rats were fed with standard rat chow, tap water *ad libitum*, whereas rabbits, housed in cages, were fed with balanced diet formulated in the department (cauliflower, cabbage, Bengal gram, and wheat flour) and tap water *ad libitum*. The experiments were conducted subsequent to the approval and permission from IAEC, constituted as per CPCSEA guidelines.

Drugs and chemicals

CFA, carrageenan, and diclofenac gel were purchased from SIGMA Chemicals co (St Louis, MO). Normal saline was purchased from Claris life science Ltd and diclofenac diethylamine gel 'INAC gel'[®]: (Zydus Alidac) was purchased from local market.

Preliminary phytochemical screening

The paste was screened for the presence of various phytoconstituents, namely, alkaloid, steroids, flavonoids, glycoside, protein, carbohydrate, and phenolics.^[8,9]

Acute dermal toxicity studies

Acute dermal irritation test of traditionally used pastes of *P. zeylanica* and bark of *H. integrifolia* were carried out in individually caged healthy New Zealand rabbits, weighing 1.5-2.0 kg. The fur around both the flanks in an area measuring $6 \times 6 \text{ cm}^2$ was clipped close to the skin without injuring the skin. After 24 hours of clipping, the preparation was applied randomly on one side and the vehicle on the other and the observation were carried out as per Organization for Economic Cooperation and Development (OECD) 404 guideline 2002.^[10]

Antiinflammatory activity

Male Wistar rats with a body weight between 150 and 200 g were starved overnight, with free access to water. First group (control group) received normal saline, second group (standard group) received diclofenac diethyl amine gel, third and fourth group (test groups) received PZRP and HIBP, respectively. All were topical application to the paw of respective animal groups. After 30 min of paste application, the rats were challenged by a subcutaneous injection of 0.05 ml of 1% solution of carrageenan into the plantar region of the hind paw.^[11] The paw was marked with ink at the level of lateral malleolus to facilitate uniform

dipping in mercury to measure the paw volume. The paw volume was measured plethysmographically immediately after injection, and 1, 2, 4, and 6th hour postchallenge of carrageenan. The increase in paw volume has been expressed as percentage increase over control recorded in terms of milliliter (ml). Percent inhibition of inflammation was calculated using the formula:

$$\text{Percentage inhibition} = \frac{V_c - V_t}{V_c} \times 100$$

where V_c and V_t represents average paw volume in control and treated group, respectively.

Antiarthritic activity

Adult healthy male Wistar rats with a body weight between 150 and 200 g were used. On day 1, animals were injected intradermally into the sub plantar region of the right hind paw with 0.1 ml of CFA.^[12] Application of the paste or the standard was started on the same day and continued for 14 days as practiced in traditional practice. Paw volumes, ankle joint circumference and body weight were recorded on the day of injection. The severity of the induced adjuvant disease was assessed by observation of the injected paw. The paw volumes (ml), paw thickness (mm), ankle joint circumference (cm), and body weight (g) were assessed on day 1st, 4th, 8th, 14th, 17th, and 21st.

Statistical analysis

Data were expressed as Mean \pm SEM and were analyzed by one way analysis of variance (ANOVA) followed by Dunnett's *post hoc* test and $P \leq 0.05$ was considered as significant.

RESULTS

Preliminary phytochemical screening

PZRP was found to be positive for the presence of carbohydrate, glycoside, steroids, alkaloids, and flavonoids.



Figure 1: Pastes of *Plumbago zeylanica* roots (PZRP) and *Holoptelea integrifolia* bark (HIBP)

HIBP was found to be positive for the presence of carbohydrate, glycoside, alkaloid, phenolics, steroids, and flavonoids.

Acute dermal toxicity studies

There were no signs of toxicity and preparation on single application appears to be devoid of major dermal irritation.

Antiinflammatory activity

The severity of carrageenan-induced inflammation was significantly decreased in PZRP ($P < 0.05$) and HIBP-treated groups ($P < 0.01$) as compared with that of control group and were comparable to diclofenac gel-treated group [Figure 2].

Antiarthritic activity

The severity of CFA-induced inflammation was significantly suppressed by the treatment with PZRP ($P < 0.05$) and HIBP ($P < 0.01$) as compared with that of control group and were almost comparable to that of diclofenac gel-treated group. Paw volume was measured in milliliter (ml) [Figure 3], ankle joint circumference was measured in centimeter (cm) [Figure 4], and body weight changes in grams (g) [Figures 5].

DISCUSSIONS

Even though numbers of antiarthritic agents are available for the treatment of arthritic disorders such as Adalimumab, Infliximab, Abatacept, Rituximab and Aurothiomalate they are neither safe nor affordable for common/poor people. Most of them share common adverse effect like, upper respiratory tract infections, urinary tract infections, dermatitis, stomatitis, transient hematuria, mild proteinuria etc. In the present study PZRP showed 4%, 12%, 9% and 32% suppression in carrageenan model of inflammation at

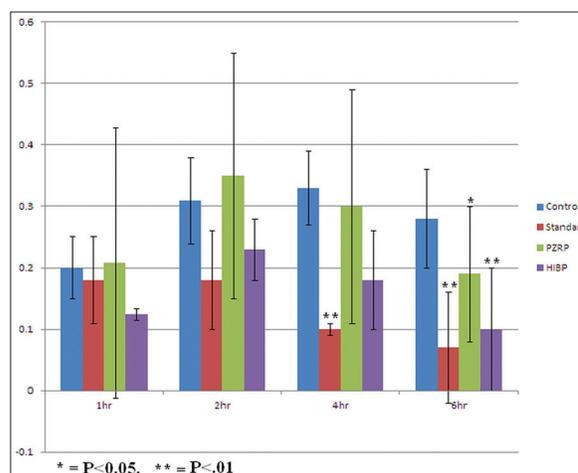


Figure 2: Antiinflammatory activity of *P. zeylanica* root and *H. integrifolia* bark pastes $N = 6$, * = $P \leq 0.05$, ** = $P \leq 0.01$

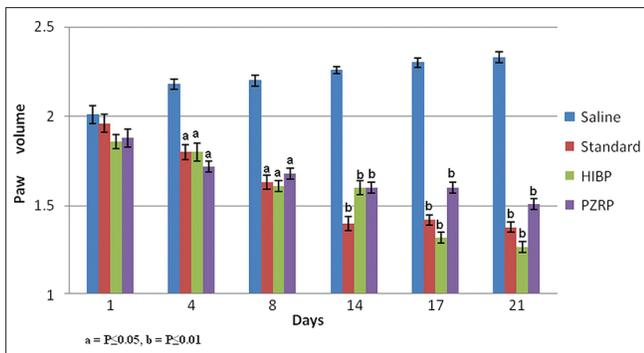


Figure 3: Paw volume changes in various treated groups of chronic inflammation $n = 6$, $a = P < 0.05$, $b = P < 0.01$

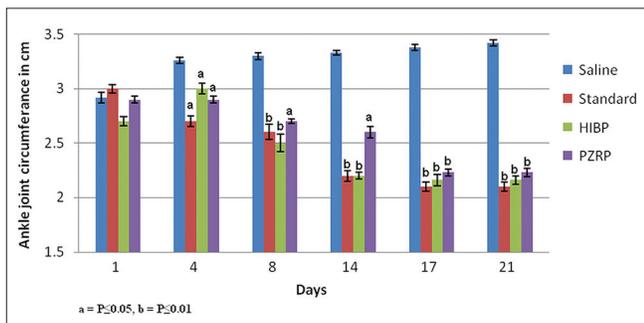


Figure 4: Ankle joint circumference changes in various treated groups of chronic inflammation $n = 6$, $a = P < 0.05$, $b = P < 0.01$

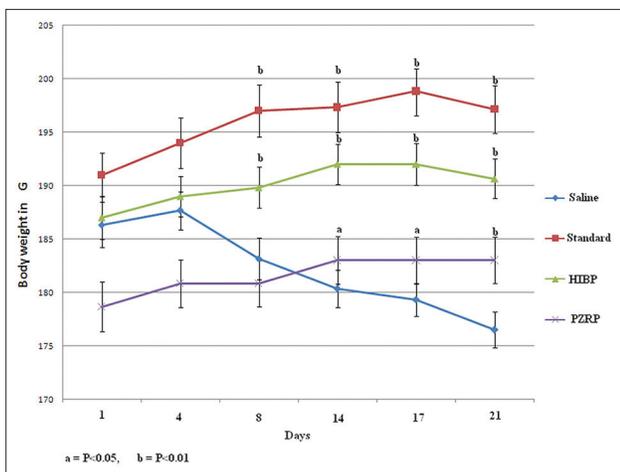


Figure 5: Body weight changes in various treated groups of chronic inflammation $n = 6$, $a = P < 0.05$, $b = P < 0.01$

1st, 2nd, 4th and 6th hours, respectively, which is insignificant compared with the control group. The standard, diclofenac diethyl amine treated group has showed 10%, 42%, 69% and 75% suppression at 1st, 2nd, 4th and 6th hour, respectively, in which suppression at 4th and 6th hour is significant with a value of $P < 0.01$. The protection in HIBP-treated group was almost comparable with that of diclofenac diethyl amine. HIBP has showed 37%, 25%, 45% and 64% suppression a 1st, 2nd, 4th and 6th hour, respectively, in comparison to control group, of which suppression at 6th hour is significant

($P < 0.01$). In CFA-induced chronic inflammation model, paw volume and ankle joint circumference are significantly suppressed in both treated groups, which are comparable to that of diclofenac gel. Paw volume was significantly suppressed on 4th and 8th day in HIBP, PZRP and diclofenac gel-treated group with a significant value ($P < 0.05$). While on 14th, 17th and 21st day, HIBP, PZRP and diclofenac gel treated group showed significant suppression with $P < 0.01$. Ankle joint circumference was progressively increased in control group, whereas in HIBP, PZRP and diclofenac gel treated group showed significant suppression with a value ($P < 0.01$) on 17th and 21st day. On 8th and 14th day, diclofenac gel, HIBP and PZRP showed significant ($P < 0.01$ and $P < 0.05$) suppression, respectively. Body weight was drastically declined in the control group, whereas in HIBP, PZRP and diclofenac gel-treated group, weight gain was shown on 8th and 14th, 17th and 21st day ($P < 0.05$ and $P < 0.01$), respectively. There is paucity of information regarding HIBP and PZRP safety and efficacy in joint pain disorder. The reported antiinflammatory activity of *P. zeylanica* and *H. integrifolia* supports its antiarthritic potential. However, both plants have been reported to have β -sitosterol as one of its active constituents, which is known to be steroid. The antiarthritic effect could be contributed due to the β -sitosterol, flavonoids, and alkaloids. It is desirable to elucidate the antiarthritic mechanism of the used plants, prior to establish its efficacy in large number of patients suffering from joint pain disorders. Therefore, we are further studying the chemical moieties responsible for antiinflammatory activities and elucidate their mechanism of action.

CONCLUSION

Based on the gathered data, we can conclude that, PZRP and HIBP are effective against CFA-induced arthritis model of Wistar rats.

ACKNOWLEDGMENT

The authors are thankful to department of AYUSH, New Delhi for financial support. Thanks are due to traditional practitioners for sharing their information through Belgaum Integrated Rural Development Society (BIRDS), Naganur, Belgaum, Karnataka, India. The facilities provided by the RMRC (ICMR) Belgaum and Department of Pharmacology, JN Medical College, Belgaum are duly acknowledged.

REFERENCES

1. Firestein GS. Evolving concept of rheumatoid arthritis. *Nature* 2003;423:356-61.
2. David ML, Michael EW. Review: Rheumatoid arthritis. *Lancet* 2001;358:903-11.

3. Saldanha CJ. vol 1. New Delhi: Flora of Karnataka, Oxford and IBH Publishing Co; 1984. p. 183.
4. Oyedapo OO. Study on the root extract of *Plumbago zeylanica*. Pharm Biol 1996;34:365-9.
5. Dang GK, Parekar RR, Kamat SK, Scindia AM, Rege NN. Anti-inflammatory activity of *Phyllanthus emblica*, *Plumbago zeylanica* and *Cyperus rotundus* in acute models of inflammation. Phytother Res 2011;25:904-8.
6. Quality standards of Indian medicinal plants. Indian Council Med Res 2004;5:246-55.
7. Sharma S, Lakshmi KS, Patidar A, Chaudhary A, Dhaker S. Studies on anti-inflammatory effect of aqueous extract of leaves of *Holoptelea integrifolia* Planch. in rats. Indian J Pharmacol 2009;41:87-8.
8. Trease GE, Evans WC. Textbook of Pharmacognosy. 12th ed. London: Balliere Tindall; 1989.
9. Harborne JB. Phytochemical methods-a guide to modern techniques of plant analysis. London: Chapman and Hall; 1998.
10. OECD (Organization of Economic Corporation Development) guidelines for the testing of chemicals/section 4 health effect. Test no 404: Acute dermal irritation/Corrosion; 2002.
11. Moody JO, Robert VA, Connolly JD, Houghton PJ. Anti-inflammatory activities of the methanol extracts and an isolated furanoditerpene constituent of *Sphenocentrum jollyanum* Pierre (Menispermaceae). J Ethnopharmacol 2006;104:87-91.
12. Newbould BB. Chemotherapy of arthritis induced in rats by mycobacterial adjuvants: Relationship of arthritogenicity and adjuvanticity in rats of vehicle composition. Immunol 1996;27:311-5.

How to cite this article: Kumar D, Ganguly K, Hegde HV, Patil PA, Kholkute SD. Activity of *Plumbago zeylanica* Linn. root and *Holoptelea integrifolia* Roxb. bark pastes in acute and chronic paw inflammation in Wistar rat. J Ayurveda Integr Med 2014;5:33-7.

Source of Support: Funded by Department of AYUSH, Government of INDIA. **Conflict of Interest:** Nil.

Staying in touch with the journal

1) Table of Contents (TOC) email alert

Receive an email alert containing the TOC when a new complete issue of the journal is made available online. To register for TOC alerts go to www.jaim.in/signup.asp.

2) RSS feeds

Really Simple Syndication (RSS) helps you to get alerts on new publication right on your desktop without going to the journal's website. You need a software (e.g. RSSReader, Feed Demon, FeedReader, My Yahoo!, NewsGator and NewzCrawler) to get advantage of this tool. RSS feeds can also be read through FireFox or Microsoft Outlook 2007. Once any of these small (and mostly free) software is installed, add www.jaim.in/rssfeed.asp as one of the feeds.