Original Article

Anti-Arthritic Activity of *Premna serratifolia* Linn., Wood against Adjuvant Induced Arthritis

Rekha Rajendran [1*] and Ekambaram Krishnakumar [2]

1. Department of Pharmacognosy and Phytochemistry, Mohamed Sathak A. J. College of Pharmacy, Tamil Nadu, India
2. Department of Pharmaceutical Biotechnology, Balaji Institute of Pharmaceutical Sciences, Warangal, AP, India

Abstract

Adjuvant induced arthritis is a chronic crippling, skeleton-muscular disorder having nearest approximation to human rheumatoid arthritis for which there is currently no medicine available effecting a permanent cure. Even modern drugs used for the amelioration of the symptoms, offer only temporary relief and also produce severe side effects. In the indigenous system of medicine, wood of *Premna serratifolia* Linn., is reported to be useful in the treatment of arthritis. It is a large shrub, distributed throughout Asia, used against a wide variety of diseases. However, no systematic study has been reported regarding its anti-arthritic activity. This work was aimed at the scientific validation of the ethno-pharmacological claim about its anti-arthritic property. In the present study, anti-arthritic activity of ethanol extract of *Premna serratifolia* Linn., wood is done by Freund's adjuvant induced arthritis model. Loss in body weight during arthritis condition was corrected on treatment with ethanol extract and standard drug, indomethacin. Biochemical parameters such as hemoglobin content, total WBC, RBC, erythrocyte and sedimentation rate were also estimated. The ethanol extract at the dose of 300 *mg/kg* body weight inhibited the rat paw edema by 68.32% which is comparable with standard drug indomethacin 74.87% inhibition of rat paw edema after 21 days. The results of the current investigation concluded, ethanol extract of *Premna serratifolia* Linn., wood possess a significant anti-arthritic activity against adjuvant induced arthritis and justifying its therapeutic role in arthritic condition. The observed anti-arthritic activity may be due to the presence of phytoconstituents such as irridiod glycosides, alkaloids, phenolic compounds and flavonoids.

*** Corresponding author:**
Rekha Rajendran,
M. Pharm., DCA.,
Department of Pharmacognosy and Phytochemistry, Mohamed Sathak A. J. College of Pharmacy, Tamil Nadu, India
Tel: +91 98 84292139
E-mail: rekhacognosy@rediffmail.com
Received: 15 Apr 2010
Accepted: 12 Jun 2010

Avicenna J Med Biotech 2010; 2(2): 101-106

Keywords: Arthritis, Freund's adjuvant, Indomethacin

Downloaded from http://www.ajmb.org

Introduction

Premna serratifolia Linn., (Verbenaceae) is an important plant belonging to the family Verbenaceae, and is one of the most wide-spread large shrubs in the forests of India, usually occurring in deciduous forests. The whole plant possesses medicinal properties, useful in the treatment of cardiovascular diseases, skin diseases, inflammatory diseases, arthritis, gonorrhea, rheumatism, anorexia and jaundice. It is an important Ayurvedic medicinal herb and its synonym is *Premna integrifolia*. It is popularly known as

Copyright © 2010, Avicenna Journal of Medical Biotechnology. All rights reserved.

"Munney" in Tamil, and "Agnimantha" in Ayurvedic system of medicine. Root forms an ingredient in well known Ayurvedic formulation "Dasamula" which is used for variety of affections [1]. It is widespread throughout Micronesia and much of the tropical Pacific and tropical Asia. It is common along the Indian and Andaman coasts. Infusion of the leaves is administered with pepper in cold and fever. Leaves are used to cure "weakness of limbs" and the leaves and leaf sap were used to alleviate headache [2].

Premna serratifolia Linn., has cardiotonic [3], anti-coagulant [4], anti-inflammatory [5], anti hyperglycaemic [6], anti-parasitic [7], antioxidant [8] and antimicrobial [9] properties. Most of the plant parts of *Premna serratifolia* Linn., have been used in the traditional system of medicine in India to treat various infectious diseases. Adjuvant induced arthritis is a chronic crippling, skeleton-muscular disorder having nearest approximation to human rheumatoid arthritis for which there is presently no medicine available effecting a permanent cure. The modern drugs both steroidal and non-steroidal anti-inflammatory drugs are used for the amelioration of the symptoms of the disease, however they offer only temporary relief and also produce severe side effects [10]. In the Ayurvedic system of medicine, this plant has been used in the treatment of rheumatoid arthritis [1].

Literature survey revealed that there is no systematic study regarding the anti-arthritic activity of the wood of *Premna serratifolia*. Hence in the present study, an attempt has been made to evaluate the anti-arthritic activity of the ethanol extract of wood of *Premna serratifolia* Linn., using adjuvant induced arthritis in rats.

Materials and Methods

Plant material

Fresh wood (without bark) of *Premna serratifolia* Linn., was procured from The Indian Medical Practitioners Co-operative Pharmacy and Stores (IMPCOPS) garden, Thiruvanmiyur, Chennai, Tamil Nadu. The plant was identified [11], authenticated by Botanist, Dr. P. Jayaraman, Plant Anatomical Research Centre (PARC), Tambaram, Chennai and the voucher specimen (PARC/ 2007/ 71) have been kept in the Department of Pharmacognosy, Madras Medical College, Chennai, for future reference. Care was taken to select the healthy plants and for normal organs.

Extraction

The freshly collected wood was chopped, shade dried and coarsely powdered (40 mesh size). The powder was defatted with petroleum ether (60 - 80 ℃) and then extracted with 90% ethanol in a Soxhlet extractor. The extract was dried under reduced pressure using a rotary vacuum evaporator and the percentage yield was 7.90% *w/w*. The obtained ethanol extract was suspended in 5% gum acacia for the pharmacological screening.

Phytochemical screening

Phytochemical screening [12] of the extract was performed using the following reagents and chemicals: Alkaloids with Dragendorff's reagent, flavonoids with magnesium and hydrochloric acid, tannins with ferric chloride and potassium dichromate, Trim-hill test for iridoid glycosides, Libermann-Burchard test for steroids and for phenolic compounds with ferric chloride.

Animals

For acute toxicity and anti-arthritic activities, Wistar albino rats weighing between 150 - 200 *gm* were selected. The animals were acclimatized to the standard laboratory conditions (temperature 25±2 ℃) and maintained on 12 *hr* light, 12 *hr* dark cycle. The animals were fed with standard diet and water *ad libitum*. The animals were maintained as per the norms of CPCSEA and the experiments were cleared by CPCSEA and the institutional ethics committee.

Acute toxicity studies

Acute toxicity study was performed for methanol extract according to the acute toxic classic method as per OECD guidelines [13]. Female albino rats were used for acute toxicity study. The animals were kept fasting for

Downloaded from http://www.ajmb.org

overnight providing only water, after which the extract was administered orally at the dose of 300 *mg/kg* and observed for 14 days. If mortality was observed in two animals out of three animals, then the dose administered was assigned as toxic dose. If the mortality was observed in one animal, then the same dose was repeated to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher doses such 50, 200 and 2000 *mg/kg* body weight. The animals were observed for toxic symptoms such as behavioral changes, locomotion, convulsions and mortality for 72 *hr*.

Anti-arthritic activity

Freud's adjuvant induced arthritis [14] model was used to assess the anti-arthritic activity in albino rats. Animals were divided into three groups of six animals each. Group I served as control, which received 5% gum acacia suspension, Group II served as reference standard, which received 10 *mg/kg* body weight *IP* of indomethacin, and Group III served as test, which received the ethanol extract of *Premna serratifolia* Linn., wood at the dose of 300*mg/kg* body weight *PO*, respectively.

Arthritis was induced by injecting 0.05 *ml* of suspension of killed *Mycobacterium tuberculosis* bacteria (0.5% *w/w*) homogenized in liquid paraffin into the left hind paw. Drug treatment was started from the initial day i.e. from the day of adjuvant injection (0 day), 30 *min* before adjuvant injection and continued till 21st day. Paw volume was measured on 4th, 8th, 14th and 21st day with the help of Plethysmometer.

The mean changes in injected paw edema with respect to initial paw volume, were calculated on respective days and percentage inhibition of paw edema with respect to untreated group (control) was calculated using the formula: Percentage inhibition of paw edema = [1- (mean change in paw volume of treated rat/ mean change in paw volume of untreated rat)] x 100.

The changes in body weight were recorded daily. On the 22nd day, blood was withdrawn through retro-orbital vein puncture of all groups by anaesthetizing the animals with diethyl ether and the biochemical parameters such as hemoglobin content, total WBC count, ESR and RBC were analyzed.

Statistical analysis

Results were expressed as mean ± SD. The significance of difference among the groups was assessed using One way analysis of variance (ANOVA) followed by Dunnet's test. $P<0.05$ was considered significant.

Results

The results of the preliminary phytochemical screening of the ethanol extract of *Premna serratifolia* Linn., revealed the presence of phytoconstituents such as alkaloids, steroids, flavonoids, phenolic compounds, tannins and glycosides specifically iridoid glycosides.

In acute toxicity studies, the ethanol extract of *Premna serratifolia* Linn., did not produce any toxic symptoms or mortality up to the dose level of 2000 *mg/kg* body weight in rats, and hence the extract was considered to be safe and non-toxic for further pharmacological screening. In adjuvant induced arthritis model, rats developed a chronic swelling in multiple joints with the influence of inflammatory cells, erosion of joint cartilage and bone destruction and remodeling.

Table 1. Mean changes in paw volume using Plethysmometer in adjuvant-induced arthritis in rats

Treatment	4th day	8th day	14th day	21st day
Group I (Arthritic control)	5.26±0.26	5.34±0.20	5.24±0.21	5.13±0.22
Group II (Standard drug)	4.63±0.15^{a*}	3.95±0.25^{a*}	3.13±0.29^{a*}	1.31±0.18^{a*}
Group III (Ethanol extract)	4.69±0.22^{a*}	4.1±0.24^{a*}	3.19±0.29^{a*}	1.61±0.11^{a*}

n=6, values are expressed as mean±SEM, $p<0.05$ - significant, $^{a*}p<0.01$ - more significant when compared to the control

Downloaded from http://www.ajmb.org

Table 2. Percentage inhibition of paw volume in adjuvant-induced-arthritis in rats

Treatment	4th day	8th day	14th day	21st day
Group I (Arthritic control)	0	0	0	0
Group II (Standard drug)	11.41	25.84	40.59	74.87
Group III (Ethanol extract)	10.91	23.30	38.74	68.32

These inflammatory changes ultimately result in the complete destruction of joint integrity and functions in the affected animal [15]. The ethanol extract inhibited the rat paw edema by 68.32%, which is comparable with standard drug, indomethacin 74.87% inhibition of rat paw edema after 21 days (Tables 1 and 2). As shown in table 3 standard drug, indomethacin and the ethanol extract have shown to increase the hemoglobin content when compared to control group. The total WBC counts were remarkably increased in adjuvant-induced rats (Table 3-control group).

However, *Premna serratifolia* Linn., wood ethanol extract and the standard drug treated groups significantly decreased ($p<0.01$) the total WBC count. The ESR count, which drastically increased in arthritic control group, has been remarkably counteracted by the standard drug; indomethacin and ethanol extract, restoring it back to normal, thus justifying its significant roles in the severe arthritic conditions. The loss of body weight observed during the arthritis condition, which is in the arthritic control group, the standard drug and the ethanol extract treatment has significantly increased the body weight further confirming the significant anti-arthritic activity of the ethanol extract of *Premna serratifolia* Linn., wood and it is shown in the table 4.

Discussion

In the present study, the rats were selected to induce arthritis because rats develop a chronic swelling in multiple joints with the influence of inflammatory cells, erosion of joint cartilage and bone destruction. It has close similarities to human rheumatoid disease [16]. The determination of rat paw swelling is apparently simple, sensitive and one of the quick procedures for evaluating the degree of inflammation and the therapeutic effects of drugs. The chronic inflammation involves the release of number of mediators like cytokines, GM-CSF, interferons and PGDF. These mediators are responsible for the pain, destruction of bone and cartilage that can leads to severe disability [17]. However, the standard drug, indomethacin and the ethanol extract significantly suppressed the swelling of the rat paws.

In arthritic condition, there is a mild to moderate rise in WBC count due to the release of IL-IB inflammatory response, IL-IB increases the production of both granulocyte

Table 3. Effect on hematological parameters in adjuvant-induced arthritis in rats

Treatment	Total WBC count (*cells/cu.mm*)	RBC count (*million/cu.mm*)	Hb (*gm*%)	ESR (*mm/hr*)
Group I (Arthritic control)	7.85±0.08	5.2±0.1	13.7±0.2	4.05±0.1
Group II (Standard drug)	7.15±0.1[a*]	5.22±0.1[a*]	14.08±0.1[a*]	3.25±0.1[a*]
Group III (Ethanol extract)	7.03±0.08[a*]	5.58±0.1[a*]	14.5±0.1[a*]	3.22±0.1[a*]

n=6, values are expressed as mean±SEM, $p<0.05$ - significant, [a*]$p<0.01$ - more significant when compared to the control

Table 4. Changes in the body weight in adjuvant-induced arthritis in rat

Groups	Before induction (*gm*)	On 21st day (*gm*)	Mean changes in the body weight
Group I (Arthritic control)	158	166	8.33±1.66
Group II (Standard drug)	155	195	40±2.6[a*]
Group III (Ethanol extract)	150	174	23.3±1.5[a*]

n=6, values are expressed as mean±SEM, $p<0.05$ - significant, [a*]$p<0.01$ - more significant when compared to the control

Downloaded from http://www.ajmb.org

and macrophages colony stimulating factors [17, 18]. In the present study, the migration of leucocytes into the inflamed area is significantly suppressed by ethanol extract when compared to standard drug indomethacin, as seen from the significant reduction in the total WBC count.

Erythrocyte Sedimentation Rate (ESR) is an estimate of the suspension stability of RBC's in plasma. It is related to the number and size of the red cells and to the relative concentration of plasma proteins, especially fibrinogen, alpha and beta globulins. Increase in the rate, is an indication of active but obscure disease processes. The acute phase proteins in ESR and C-Reactive Proteins (CRP) share the property of showing elevations in the concentration in response to stress or inflammations like injection, injury, surgery and tissue necrosis. The ESR count significantly increased in arthritic control group, whereas these counts were remarkably counteracted in the standard, indomethacin and ethanol extract groups and thus justifying its significant role in the arthritic conditions [18].

Changes in body weight have also been used to assess the course of the disease and the response to therapy of anti-inflammatory drugs [19]. As the incidence and severity of arthritis increased, the changes in the body weights of the rats also occurred during the course of the experimental period. The loss of the body weight during arthritic condition was also supported by earlier observations, [20] on alterations in the metabolic activities of diseased rats.

Earlier findings suggest that absorption of ^{14}C-glucose and ^{14}C-leucine in rat's intestine was reduced in the case of inflamed rats [21]. Treatment with anti-inflammatory drugs, the decrease in absorption was nullified [22] and it shows that the anti-inflammatory drugs have corrected the decreased absorption capacity of intestine during inflammation. The increased body weight during the treatment of standard drug and the ethanol extract may be due to the restoration of the absorption capacity of the intestine.

From the results observed in the current investigation, it may be concluded that the ethanol extract of *Premna serratifolia* Linn., wood at the dose of 300 *mg/kg* body weight displays a significant anti-arthritic activity which may due to the presence of phytoconstituents such as alkaloids, steroids, flavonoids, phenolic compounds and glycosides specifically iridoid glycosides. Several studies indicate that aforementioned phytoconstituents possess significant anti-arthritic activity [23 - 25].

The study is further extended to identify and characterize the exact active phytoconstituents and to elucidate the exact mechanism of action, which is responsible for the observed significant anti-arthritic activity against adjuvant induced arthritis in rats.

References

1. The Wealth of India, Dictionary of Indian raw materials and industrial products, vol. 8, New Delhi: Council of Scientific and Industrial Research; 1972,240.
2. Kapoor LD. CRC Handbook of Ayurvedic Medicinal Plants, Herbal Reference Library. Boca Raton London, New York Washington: CRC Press; 2001, 70.
3. Rajendran R, Suseela L, Meenakshi Sundaram R , Saleem Basha N. Cardiac stimulant activity of bark and wood of Premna serratifolia Linn. Bangladesh J Pharmacol 2008;3(2):107-113.
4. Gopal RH, Purushothaman KK. Effect of plant isolates on coagulation of blood: An in-vitro study. Bull Med Ethnobot Res 1984;5:171-177
5. Rathore RS, Prakash A, Singh PP. Premna integrefolia Linn, A preliminary study of anti-inflammatory activity. Rheumatism 1977;12:130-134.
6. Dash GK, Patrolm CP, Maiti AK. A study on the anti-hyperglycaemic effect of roots of Premna corymbosa Rottl. J Nat Rem 2005;5:31-34.
7. Desrivot J, Waikedre J, Cabalion P, Herrenknecht C, Bories C, Hocquemiller R, et al. Anti-parasitic activity of some New Caledonian medicinal plants. J Ethnopharmacol2007;112(1):7-12.
8. Rajendran R, Saleem Basha N, Ruby S. Evaluation of in vitro antioxidant activity of stem-bark and wood of Premna serratifolia Linn., (Verbenaceae). Phytochemistry 2009;1(1):11-14.

Downloaded from http://www.ajmb.org

9. Rajendran R, Saleem Basha N. Antimicrobial activity of crude extracts and fractions of Premna serratifolia Linn., root. Medicinal Plants-Int J Phytomed Rel Indus 2010;2(1):[1p.].

10. Hazeena BV, Sadique J. Long term effect of herbal drug Withania somnifera on adjuvant induced arthritis in rats. Indian J Exp Biol 1988;26(11):877-882.

11. Henry AN, Kumari GR, Chitra V. Flora of Tamil Nadu. Botanical survey of India southern circle (Vol 2). Coimbatore;1987,167.

12. Harbone JB. Phytochemical methods, a guide to modern techniques of plant analysis. 2nd ed. London, New York: Chapman and Hal;1973,90.

13. Ecobichon DJ. The basis of toxicology testing. 2nd ed. New York: CRC press; 1999,43-46.

14. Newbould BB. Chemotherapy of arthritis induced in rats by mycobacterial adjuvant. Br J Pharmacol Chemother 1963;21(1):127-136.

15. Carl MP. Experimental joint disease observations on adjuvant induced arthritis. J Chronic Dis 1963; 16(8):863-864.

16. Singh S, Majumdar DK. Effect of fixed oils of Ocimum sanctum against experimentally induced arthritis and joint edema in laboratory animals. J Pharmacogn 1996;34(3):218-222.

17. Eric GB, Lawrence JL. Rheumatoid arthritis and its therapy, The Textbook of therapeutic drugs and disease management. 6th ed. Baltimore: Williams and Wilkins Company; 1996,579-595.

18. William JK. Arthritis and allied condition, A Textbook of rheumatology (Vol. 1). 3rd ed. Baltimore: Tokyo: A Waverly Company; 1996,207-226.

19. Winder CV, Lembke LA, Stephens MD. Comparative bioassay of drugs in adjuvant induced arthritis in rats: flufenamic acid, mefenamic acid and phenylbutazone. Arthritis Rheum 2005;12(5):472-482.

20. Walz DT, Dimartino MJ, Misher A. Adjuvant induced arthritis in rats.II. Drug effects on physiologic, biochemical and immunologic parameters. J Pharmacol Exp Ther 1971;178(1):223-231.

21. Somasundaran S, Sadique J, Subramoniam A. Influence of extra-intestinal inflammation on the in vitro absorption of 14C-glucose and the effects of anti-inflammatory drugs in the jejunum of rats. Clin Exp Pharmacol Physiol 1983;10(2):147-152.

22. Somasundaran S, Sadique J, Subramoniam A. In vitro absorption of [14C] leucine during inflam-mation and the effect of anti-inflammatory drugs in the jejunum of rats. Biochem Med 1983;29(2):259-264.

23. Elmali N, Esenkaya I, Harma A, Ertem K, Turkoz Y, Mizrak B. Effect of resveratrol in experimental osteoarthritis in rabbits. Inflamm Res 2005;54(4): 158-162.

24. Choi EJ, Bae SC, Yu R, Youn J, Sung MK. Dietary vitamin E and quercetin modulate inflammatory responses of collagen-induced arthritis in mice. J Med Food 2009;12(4):770-775.

25. Amresh G, Singh PN, Rao ChV. Antinociceptive and antiarthritic activity of Cissampelos pareira roots. J Ethnopharmacol 2007;111(3):531-536.

Downloaded from http://www.ajmb.org