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Anti-inflammatory activity of *Premna corymbosa* (Burm.f.) Rottl. & Willd. leaves extracts in Wistar albino rats

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ABSTRACT

Objective: To evaluate the acute toxicity and to investigate the effect of *Premna corymbosa* ethanolic extract (PCEE) at doses of 200 and 400 mg /kg body weight in acute and chronic models of inflammation in experimental animals. **Methods:** In the acute toxicity study, a single dose of PCEE of 2 000 mg/kg body weight, p.o. was administered and observed for 48 h. In acute models as egg albumin induced paw edema and chronic model as cotton pellet methods was followed. **Results:** In acute models, egg albumin induced paw edema PCEE significantly (P<0.01) inhibited the edema formation. In chronic model, cotton pellet induced granuloma formation in rats PCEE significantly (P<0.01) reduced the granuloma formation with percentage inhibition of 35.17% and 50.38 % respectively. **Conclusions:** The present study establishes the antiinflammatory activity of *Premna corymbosa* leaves.

1. Introduction

Inflammatory diseases including different types of rheumatic diseases are very common throughout the world. Rheumatic diseases is one of the oldest known diseases of man kind affecting the majority of the population in the active period of the life, no substantial progress has been made in achieving a permanent cure. The inflammatory process is a series of events that can be elicited by numerous stimuli such as infectious agents, ischaemia, antigen-antibody reaction interactions and thermal or physical injury through years of ingenious synthesis and structural modifications. New drugs, many non steroidal antiinflammatory agents (NSAIDs) and steroidal drugs have been available in the markets. A numerous immunosuppressant and pain killers have been developed based on the inhibition of cyclooxiginase -1(COX-1), but they cause detrimental side effects on long term administration. Accordingly selective inhibitors of cyclooxiginase -2 (COX-2) were developed to avoid the side effects of COX-1 inhibitors. However all among the selective and non selective inhibitors of cyclooxiginase enzyme and the steroidal antiinflammatory drugs has been reported to have severe side effect on long term administration. The synthetic drugs will not be suitable for the treatment of various inflammatory disorders including the rheumatic disorders, since there are no challenging drugs for these disorders in allopathic medicine.

Plant serves as the primary sources of medicine for man from the ancient period and has led to characterization, identification of novel lead molecules and isolation of active chemicals compound of therapeutic importance, which allows for structural modifications and origin of synthetic drugs. Plant derived drugs are widely used and believed to be safe, cost effective with fewer side effects. Synthetic drugs have more toxic and severe adverse side effects. Alternative medicine for treatments of various diseases is getting more and more popular. Many medicinal plants provide relief of symptoms comparable to that obtained from allopathic medicine; however, herbs and herbal medicine caused toxic adverse effects has been reported [1]. Therefore needs for agents of natural origin with little side effects are increasing since they can be substitute for chemical therapeutic agents.

Premna corymbosa (Burm.f.) Rottl. & Willd. is a small-sized tree or large shrub with a comparatively short trunk and numerous branches, bearing shortly acuminate and cymose panicles of small inconspicuous flowers. Its root is light brown or yellowish brown, woody aromatic. Leaves are elliptic ovate, sometimes pubescent; flowers greenish or

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greenish white, with a strong disagreeable odour, in terminal corymbose cymes, drupes globose[2]. It's widely distributed throughout India plains and is commonly known as munna in ayurvedic system of medicine in kerala[3]. The useful parts of the plants are whole plant, roots, root bark, stem and leaves. The leaves are stomachic, carminative, and galactagogue, and are useful in dyspepsia colic, flatulence, agalactia, cough, fever, rheumatalgia, neuralgia, heamorrhoids and tumors[4]. The plant is an ingredient of many ayurvedic preparation like dasamularistam, dhavantaramkasayam, agastyarasayanam, sukumaraghrtametc[5]. The phytochemical studies revealed the presence of premnine, ganiarine, resin, spermine, alphandrine, tannins, betulin and β-sitosterol in stem bark; β-sitosterol and luteolin in leaves; betulin and β-sitosterol in the stem[2,3,6]. Previous study showed the hydroalcaholic extract of the roots of *Premna corymbosa* Rottl. possess antihyperglycemiceffect on both normoglycemic and alloxan induced hyperglycemic rats[7].

The literature survey revealed that there is a lack of scientific report regarding to the antiinflammatory activity of *Premna corymbosa* leaves. The present study was designed based upon the traditional medicinal uses. Hence the present study was designed to investigate the antiinflammatory effects of *Premna corymbosa* in (Acute model) fresh egg albumin induced paw edema and (Chronic model) cotton pellets induced granuloma formation in rats.

2. Materials and methods

2.1. Plant material

Premna corymbosa (Burm. f.) Rottl. & Willd. were collected from Plant Anatomy Research Center (PARC), Chennai in December 2006. It was identified by Prof. P. Jayaraman, Director, PARC, Pharmacognosy Institute, West Tambaram, Chennai, Tamil nadu, India. A voucher specimen (No: PARC/07/SRM/31) was deposited in the herbarium of the institute.

2.2. Preparation of the ethanolic extract

The leaves of the tree were washed well with water, dried under shade and powdered to fine grade by using laboratory scale mill. A batch of 500 g of powdered material was subjected to extraction in a soxhlet apparatus at 50-60 ℃ for 36 hours of 6 cycles (6 hours per cycles) in 99 % (v/v) of 2 liters of absolute ethyl alcohol. The extracted material was concentrated over a heating mantel maintained at 50 ℃ until greenish semi solid masses were obtained. The yield of the product was approximately 18.39 % (w/w) of the dry leaves of *Premna corymbosa* (Burm.f.)Rottl. & Willd. The final product was stored in vacuum desiccators at room temperature until analysis. For administration, the extract was suspended in 2 % of tween 80, to required concentrations.

2.3. Animals

The rodents used in the experiments were Wistar albino rats of both sex (150-220 g) which were procured from the Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Madhavaram, Chennai Tamil Nadu, India. They were housed in well ventilated polypropylene cages at controlled temperature of (24±1) ℃, with a 12 h light / 12 h dark cycle and they are given standard pellet diet and water *ad libitum*. The mice were assimilated to laboratory conditions for 7 days. Animals were kept under fasting for over night, but allowed for free access of water before commencement of experiments. The experiments were conducted according to the ethical norms approved by ministry of social justice and empowerment, Government of India and the study got approved from the Institutional Animal Ethical Committee (IAEC) (Approval No.MPL/09/IAEC.9/2007) of Committee for the Purpose ad Control and Supervision of Experiments on Animal (CPCSEA).

2.4. Drugs and chemicals

The following drugs and chemical swereused for the study: Dexametasone (Cadila health care, Ahmedabad, India), Acetyl salicylic Acid (USV limited, Mumbai, India), Ethylalcohol (Changu & Hu,China), Tween 80 (Ranbaxy Fine Chemicals, Mumbai, India), Diethylether (TKMPharma, Hyderabad, India).

2.5. Acute toxicity studies

Acute toxicity studies were carried out using acute toxic class limit test dose guidelines 425 of Organization for Economic Co-operation and Development (OECD). Acute toxicity of the plant extract was carried out using groups of three Swiss albino mice by administering a dose of 2 000 mg/kg body weight, p.o., while control group received normal saline. The toxicological effects were assessed on the basis of mortality and behavioral changes during 48 hours.

2.6. Anti-inflammatory activity studies

2.6.1. Acute inflammation study: Egg albumin induced paw edema in rats

The paw edema method by Winter et al, 1962[8] was used. Young male Wistar albino adult rats were used. The acute inflammation of the hind paw was induced in each of the rats by injecting 0.1 mL/kg body weight of fresh egg albumin into the sub planter surface of the right hind paw. The paw volume is measured by using plethysmograph-apparatus. The test groups animals received 200 and 400 mg/kg body weight, p.o., of *Premna corymbosa* ethanolic extract (PCEE) 30 min before inducing inflammation with the injection of egg albumin. The positive control group received acetyl salicylic acid (ASA) of 100 mg/kg body weight orally. Negative control group received 1 mL/kg body weight, p.o.of 2 % Tween 80. The paw volume of all groups were measured before and 1, 2, 3 and 4 h after induction of edema. Inflammation was assessed as the difference between the zero time volume of the treated paw and the volume at the various times after the administration of the phlogistic agent. The odema rate and inhibition was calculated by using following ratio [9].

Edema rate (ER) % = Vt-Vo/Vo

Inhibition rate (IR) % = Ec-Et/Ec

Where Vo is the volume before egg albumin injection in mL; Vt is the volume day t after egg albumin injection (mL); Ec is the odema ratio of control group; Et is the odema rate of treated group.

2.6.2. Chronic inflammation study: Cotton pellet induced granuloma formation in rats

This study is performed in rats according to the method described by Okoli *et al* [10] and Sulaimana *et al* [11] with slight modifications. Wistar albino rats of either sex were divided into four groups of six animals. The test groups animals received PCEE of 200 and 400 mg/kg body weight, p.o. Positive control group animals received dexamethasone 5 mg/ kg body weight, i.p. Negative control group received 1 mL/kg body weight, p.o. of 2 % Tween 80. Thirty minutes later, autoclaved pellets of cotton wool weighing (30±1) mg were implanted subcutaneously on each sides of the abdomen of the rats anesthetized with anesthetic ether. The PCEE were administered once daily through out the experimental period for 7 days. On the 8th day the rats were killed by over dose of ether. The pellets were dissected out, freed of tissue attached and dried in oven at 60 ℃ for over night. The dry pellets were weighed and the mean weight of the granuloma tissue formed around pellets determined. The percentage of inhibition of granuloma tissue development was calculated using the relation:

$$\text{Percentage of granuloma inhibition} = \frac{(Tc-Tt)}{Tc} \times 100$$

Where Tc is weight of granuloma tissue of control group; Tt is weight of granuloma tissue of the treated group.

2.7. Statistical analysis

The statistical analysis of all the result was carried out using one- way ANOVA followed by Dunnett's multiple comparison using graph pad instat 3 software and all the results obtained in the study were compared with the control group. P value <0.05 were considered statistically significant.

3. Results

3.1. Acute toxicity studies

The behavior of the treated mice appears to be normal. No toxic effect was seen up to 10 times the effective dose of the PCEE and there was no mortality during 48 hours. Therefore an LD_{50}> 2 000 mg/kg body weight may be assumed.

3.2. Effect of PCEE on egg albumin induced paw edema in rats

Egg albumin induced rat paw edema was significantly (P<0.01) inhibited by oral pretreatment with PCEE of 200 and 400 mg/kg body weight in a dose dependent manner and ASA at a dose of 100 mg/kg body weight similarly produced significant inhibitory effect of the paw (Table 1).

3.3. Effect of PCEE on cotton pellet induced granuloma formation in rats

The study on PCEE at doses of 200 and 400 mg/kg body weight on proliferative phase of inflammation indicated slightly but significantly (P<0.01) reduced the granuloma formation with percentage inhibition of 35.17% and 50.38 % respectively. Dexamethasone at a dose 5 mg/kg body weight, i.p. showed a significant (P<0.01) inhibition on granuloma formation with a percentage inhibition of 66.72 (Table 2).

Table 1
Effect of *Premna corymbosa* ethanolic extract on egg albumin induced paw edema in rats.

Treatments	Dose mg/kg Body weight	[a]Edema rate in percentage			
		1 h	2 h	3 h	4 h
Negative control	–	79.08 ± 0.83	57.25 ± 0.61	36.16 ± 0.94	23.08 ± 0.52
Premna corymbosa extract	200	31.05 ± 0.38(60.735)*	26.52 ± 0.47(53.676)*	19.27 ± 0.38(46.709)*	14.44 ± 0.93(37.435)*
	400	29.33 ± 0.52(62.910)*	22.15 ± 0.77(61.310)*	14.60 ± 0.39(59.623)*	10.35 ± 0.57(55.155)*
ASA	100	30.29 ± 0.68(61.697)*	24.13 ± 0.40(57.851)*	16.19 ± 0.85(55.226)*	12.20 ± 0.58(47.140)*

aValues are expressed in Mean ± S.E.M. (n = 6); Values in parentheses indicate the percentage inhibition rate. Difference between groups were statistically analyzed by one-way ANOVA;*P<0.01, Dunnett's test as compared to control.

Table 2
Effect of PCEE on cotton pellet induced granuloma formation in rats.

Treatments	Dose mg/kg Body weight	[a]Weight of cotton pellet granuloma in mg	Percentage of granuloma inhibition
Control	–	140.080 ± 6.840	–
Premna corymbosa	200	90.810 ± 4.471*	35.17
extract	400	69.500 ± 3.768*	50.38
Dexamethsone	5	46.610 ± 1.848*	66.72

aValues are expressed in Mean ± S.E.M. (n = 6); Difference between groups were statistically analyzed by one-way ANOVA;*P<0.01, Dunnett's test as compared to control.

4. Discussion

In the present study, the PCEE have shown significant (P<0.01) anti-inflammatory activity in acute and chronic experimental animal models. A high dose of PCEE of 2 000 mg/kg body weight, p.o. caused neither any sign of mortality nor any observable negative symptoms over a period of 48 h and therefore an LD_{50}> 2 000 mg/kg body weight may be assumed.

It has been reported that egg-albumen induced inflammation is similar to that produced by carrageenin[12]. The egg albumin-induced edema peaked at 1 h and progressively decreased with time. The inhibition of the early acute phase of edema caused by egg albumin the

extract may suppress both the early and later phases of the acute inflammatory response. The PCEE may have inhibited the release or actions of prostanoids known to mediate acute inflammation[13,14].

Increased vascular permeability occurs as a result of contraction and separation of endothelial cells at their boundaries to expose the basement membrane, which is freely permeable to plasmaproteins and fluid[15]. Histamine and other mediators of inflammation increase vascular permeability at various times after injury. Chemical-induced vascular permeability (such as seen with acetic acid) causes an immediatesustained reaction that is prolonged over 24 h [16-19] and its inhibition suggests that the extract may effectively suppress the exudative phase of acute inflammation.

The migration of leukocytes to sites of inflammation, which certainly suppresses the inflammatory response[20], the PCEE may prevent the release of cytoplasmic pro-inflammatory mediators from these leukocytes by virtue of membrane stabilization effect[21] possibly also possessed by the constituents of this extract.

Efficacy of anti-inflammatory agents in chronic inflammatory states is indicated by their ability to inhibit the increase in the number of fibroblasts during granular tissue formation[22]. In order to assess its efficacy against proliferative phase of inflammation in which tissue degeneration and fibrosis occur, widely used cotton pellet granuloma test was employed. During the repair process of inflammation, there is proliferation of macrophages, neutrophils, fibroblasts and multiplication of small blood vessels, which are basic sources of forming a highly vascularised reddish mass, termed granulation tissue. This effect showed the ability of the PCEE in reducing the number of fibroblasts, and synthesis of collagen and mucopolysaccharide, which are natural proliferative events of granulation tissue formation. As a model of chronic inflammation, cotton pellet induced granuloma in rats was utilized in the present study. The observation showed that PCEE reduced the formation of granuloma tissue in a dose-dependent manner, which represented an ability of PCEE to inhibit the proliferationphase of inflammatory process. One possible mechanism involves the inhibitionof prostaglandin formation at the site of inflammation.

In conclusion, these present study reports showed that the PCEE significantly inhibited the egg albumin induced edema and reduced granuloma formation in cotton pellet method and have preferable anti-inflammatory effect with long term administration. The phytochemical studies revealed the presence of β-sitosterol and luteolin in leaves. The anti-inflammatory activity may be due to the presence of β-sitosterol or luteolin. Luteolin exerted the strongest blocking action on expression of this inflammatory mediator Luteolin, exerted inhibitory effects on TNF-alpha, IL-6 and IFN-gamma production Luteolin suppressed TNF-alpha-induced IL-8 production in dose-dependent manner. Luteolin inhibited TNF-alpha-induced phosphorylation of p38 MAPK andextracellular-regulated kinases (ERK), IkappaB degradation, and NF-kappaB activation Luteolin has significant anti-inflammatory action. It is more potent than ibuprofen. Further studies are required to isolate, characterize and finding the mechanism of action of the active compounds in ethanolic extract responsible for antiarthritic activity and to confirm the above possibilities of mechanism of actions.

Conflict of interest statement

We declare that we have no conflict of interest.

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