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Evaluation of anti-inflammatory potential of various parts of *Pterospermum canescens*, Roxb., (Sterculiaceae) extracts in experimental animals.

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ABSTRACT

Inflammation and oxidative stress are features of many degenerative diseases. In this present study, the petroleum ether, chloroform and methanol extracts (100 mg/kg, 200 mg/kg) of *Pterospermum canescens*, Roxb., (Sterculiaceae) plant extracts (leaves, stem and stem bark) was investigated for its anti-inflammatory activity. Carrageenan induced paw oedema method in Wistar Albino rats were used in this study to assess anti-inflammatory potential of the plant using Indomethacin as standard (10 µg/kg). Petroleum ether, chloroform and methanol extracts of leaf, stem and stem bark were exhibited significant ($P < 0.001$) anti-inflammatory activity at 100 mg/kg and 200 mg/kg doses when compared with the standard, Indomethacin.

Key words: *Pterospermum canescens*, Anti-inflammatory activity, Carrageenan, Plethysmometer.

INTRODUCTION

Inflammation is a complex pathophysiologic response of vascularised tissue to injury [1]. It is the body's attempt at self protection and the aim is to remove harmful stimuli, including damaged cells, irritants or pathogens and then begin the process of healing [2]. Inflammation is our body's natural reaction to invasion by an infectious agent, burn, toxin or physical, chemical or traumatic damage. One purpose of inflammation is to protect the site of an injury [3]. The mechanism of inflammation injury is attributed, in part, to release of ROS from activated neutrophils and macrophages. This over production leads to tissue injury by damaging macromolecules and lipid peroxidation of membranes. In addition, ROS propagate inflammation by stimulating release of cytokines such as interleukin-1, tumor necrosis factor- α , and interferon- γ , which stimulate recruitment of additional neutrophils and macrophages [4]. Moreover, these reactive species are involved in the biosynthesis of prostaglandins and cyclooxygenase in the cyclooxygenase- and lipoxygenase-mediated conversion of arachidonic acid into proinflammatory intermediates [5].

The side effects of the currently available anti-inflammatory drugs pose a major problem during their clinical uses. Therefore, the development of newer and more potent anti-inflammatory drugs with lesser side effects is necessary [6]. Since ROS, NO production, related enzymes, proinflammatory cytokines might cause inflammatory damage, many studies about inflammation focused to find materials which selective modulate these free radicals and inflammatory mediators from traditional plant-derived medicines [7].

Although non-steroidal anti-inflammatory drugs (NSAIDs) and opiates have been used classically in these conditions, but some adverse reactions occur with these drugs such as gastrointestinal disturbances, renal damage, respiratory depression, and possible dependence [8, 9]. In recent years, there has been an increasing interest to find new anti-inflammatory drugs with possibly fewer side effects from natural sources and medicinal plants. The need for anti-inflammatory drugs arises when the inflammatory response becomes inappropriate, aberrant or sustained, and when it causes tissue destruction [10]. A great number of anti-inflammatory drugs (both steroids and non-

steroidal anti-inflammatory drugs) are extensively used for the treatment of acute and chronic inflammatory conditions [11]. Among these drugs, none have proved to be curative. They suppress rather than abolish the inflammatory reactions thereby providing symptomatic relief and are usually accompanied by severe adverse effects such as gastrointestinal irritations, ulcers, bone marrow depression, hypertension, myocardial infarction and muscular degenerations among others [12, 13].

There is currently a worldwide upsurge in the use of herbal preparation and the active ingredient isolated from medicinal plants in health care. Plant based drugs have been used worldwide in traditional medicines for the treatment of various diseases. Approximately 60% of worldwide population still relies on medicinal plants for their primary health care [14].

With this background, this study was conducted with an objective of evaluation of the acute anti-inflammatory activity of *Pterospermum canescens*, Roxb., in Wistar albino rats.

The genus *Pterospermum* Schreb., (Sterculiaceae) represents of about 40 species in the world, of which 12 species were reported from India [15] and 8 species has been reported from TamilNadu state [16]. An ethnomedicinal plant species *Pterospermum canescens* Roxb., (Syn. *Pterospermum suberifolium* Lam.) locally known as *Sempulavu* was distributed in all districts of Tamil Nadu. Ethnomedicinally, the leaves are used for headache [17], treatment of fractured bones [18] small pox [19] and antimicrobial properties [20]. The plant has been reported to contain β -amyirin, betulin, kaempferol, lupeol, quercetin, scopoletin and β - sitosterol [21] and α -sitosterol, 3, 7, 11, 15-tetramethyl-2-hexa decane-1-ol, ricinoleic acid, vitamin-E, phytol, α -tocopherol, diethyl phthalate, squalene, benzhydrazide-3-mthoxy-N2-(4-henylcyclo hexylideno, benzoic acid, 4- heptyl-4-cyanophenyl ester and n-hexa decanoic acid [22]. After the scrutiny of literatures, it was confirmed that so far no other work has been carried out on this plant. Hence, the present study aims to develop an antimicrobial lead of therapeutic interest from this selected ethnomedicinal plant.

MATERIALS AND METHODS

Collection of plant material

The bark of *Pterospermum canescens* Roxb., were collected from the Kalapet vicinity of Pondicherry and the collected plant material was botanically identified and confirmed by the Plant Taxonomist Dr.A.C.Tangavelou and the herbarium specimen (KPJ 42) was prepared and deposited at the department for future reference.

Preparation of extracts

The collected plant material (leaf, stem, stem bark) were chopped into small pieces, shade dried and coarsely powdered by using a pulverizer. Then, the bark powder was subjected to successive solvent extraction with organic solvents of increasing polarity such as petroleum ether, chloroform and methanol by continuous hot percolation method using soxhlet apparatus [23, 24]. The extracts were collected and distilled off on a water bath at atmospheric pressure and the last trace of the solvent was removed *in vacuo*. The resulted extracts were used for screening of anti-inflammatory activity.

Animals

Wistar albino rats (180 – 230 g) were used for the pharmacological studies. They were kept in polypropylene cages at $25 \pm 2^\circ\text{C}$, with relative humidity 45-55% under 12 h light and dark cycles. All the animals were acclimatized to the laboratory conditions for a week before use. They were fed with standard animal feed (Kamadhenu agencies, Bangalore, India) and water *ad libitum*. The experimental protocols were carried out at C.L. Baid Metha College of Pharmacy, Thoarpakkam, Chennai (IAEC/ 34/ 22/ CLBMCP/ 2011, dated on 7/2/2011) approved by the Institutional Animal Ethics Committee.

Anti-inflammatory activity

Anti-inflammatory activity of *Pterospermum canescens* Roxb., was studied by carrageenan induced rat hind paw edema method [25, 26]. Wistar albino rats were divided into eight groups of six animals each respectively at doses of 100 and 200mg/kg body weight and they were fasted overnight, during the experiment free access of water *ad libitum*. The dose of the extracts was selected on the basis of folkloric uses of the plant. Group I served as control (0.9% Normal saline with 3% Tween, 2 ml/kg), Group II, III (PETL, PETH - 100, 200 mg/kg); Group IV, V (CHL, CHH - 100, 200 mg/kg) and Group VI, VII (MEL, MEH - 100, 200 mg/kg) were administered with petroleum ether, chloroform and methanol extracts of *Pterospermum canescens*, Roxb., (leaf, stem, stem bark) respectively and Group VIII served as standard (Indomethacin, 10 mg/kg, orally).

Acute inflammation was produced by the sub-plantar administration of 0.1 ml of 1% carrageenan in normal saline that contains Tween-80 in the right hind paw of rats, 30 minutes after the administration of test extract, saline and standard drug. Paw volumes of all the rats were measured at 0, 30, 60, 120 and 180 minutes respectively by using plethysmometer [27]. A significant reduction in the paw volume compared to control group was considered as inflammatory response.

For the measurement of paw volume, the inflamed paw was immersed into mercury contained in a U- tube, which consisted of a right cylindrical glass tube (8 × 2.2 cm) connected to a narrow side-arm (10 × 0.72 cm) having a wall of uniform cross-section and open upper end. The volume of mercury displaced was recorded with a travelling microscope (ELFO Scientific Apparatus, India). Prior to immersion into mercury, each inflamed right hind paw was labeled with permanent marker pen so that the immersion would be uniform in each episode. The average percentage increase in paw volume with time was calculated and compared against the control group.

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

Where, V_c = average paw volume of control group

V_t = average paw volume of test group

Statistical calculation

The results of the study were expressed as mean ± SEM. Statistical significance was analyzed by one way ANOVA followed by Dunnet's method. $P < 0.05$ are significant [28].

RESULTS AND DISCUSSION

Leaf

Standard group of animals were exhibited significant ($P < 0.001$), decrease of paw volume (odema) when compared with the control group of animals. Petroleum ether (200 mg/kg), chloroform (200 mg/kg) and methanol (100, 200 mg/kg) leaf extracts were exhibited significant ($P < 0.001$), decrease of paw volume (odema) when compared with the control group of animals. Chloroform (100 mg/kg) leaf extract exhibited significant ($P < 0.01$), but petroleum ether (100 mg/kg) leaf extract did not show significant when compared with the control group of animals (Figure 1; Table 1).

Stem

Standard group of animals were exhibited significant ($P < 0.001$), decrease of paw volume (odema) when compared with the control group of animals. Petroleum ether, chloroform and methanol (200 mg/kg) stem extracts were exhibited significant ($P < 0.001$), decrease of paw volume (odema) when compared with the control group of animals. Chloroform and methanol (100 mg/kg) stem extracts were exhibited significant ($P < 0.01$ and $P < 0.05$), decrease of paw volume (odema) when compared with the control group of animals respectively. Petroleum ether (100 mg/kg) stem extract did not show significant (Figure 2; Table 2).

Stem bark

Standard group of animals were exhibited significant ($P < 0.001$), decrease of paw volume (odema) when compared with the control group of animals. Petroleum ether (200 mg/kg), chloroform (200 mg/kg) and methanol (100, 200 mg/kg) bark extracts were exhibited significant ($P < 0.001$), decrease of paw volume (odema); chloroform and petroleum ether (100 mg/kg) bark extract was exhibited significant ($P < 0.01$ and $P < 0.05$), decrease of paw volume (odema) when compared with the control group of animals (Figure 3; Table 3).

In the present study, petroleum ether (200 mg/kg), chloroform (200 mg/kg) and methanol (100, 200 mg/kg) leaf extracts were exhibited significant ($P < 0.001$), decrease of paw volume (odema) when compared with the control group of animals while in stem, petroleum ether, chloroform and methanol (200 mg/kg each) extracts were exhibited significant. Similarly, petroleum ether (200 mg/kg), chloroform (200 mg/kg) and methanol (100, 200 mg/kg) extracts of stem bark were also exhibited significant ($P < 0.001$). In leaves, methanol extracts were observed to significantly reduce the edema induced by carrageenan in a dose dependent manner. This is an indication of anti-inflammatory effect of the extracts.

Inflammatory processes are the physiological response of the organism to different stimuli such as trauma, infections or immunological mechanisms [29]. The presence of edema is one of the prime signs of inflammation [30]. The method was chosen for this study since edema induced by carrageenan is the most prominent acute experimental model in search for new anti-inflammatory drugs [31]. It is known that carrageenan induced paw edema involves many mediators which induce inflammatory reaction in two different phases [32]. The initial phase

is attributed to the release of mediators such as histamine, serotonin and bradykinin. The second phase of oedema is due to the release of prostaglandins, protease and lysosome in tissues [33]. There was significant differences observed throughout the experiment between control and indomethacin treated groups. The inflammatory granuloma is a typical feature of established chronic inflammatory reaction. The dry weight of the pellet correlates with the amount of granulomatous tissue. Methanol extracts at the dose level 200 mg/kg were effectively and significantly reduced. These data showed the ability of the extracts in reducing the number of fibroblasts, and synthesis of collagen and mucopolysaccharides, which are natural proliferative events of granulation tissue formation [34]. Phenolic compounds [35] inhibit cyclooxygenase, lipoxigenase and eicosanoid biosynthesis thereby diminishing the formation of inflammatory metabolites. Flavonoid, quercetin ameliorates the inflammatory response induced by carrageenan [36]. Various flavonoids (i.e.) quercetin, apigenin, tea catechins have also been shown to have anti-inflammatory activity by inhibiting cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase, which is related to antioxidant activity. Flavonoids also inhibit cytosolic and tyrosine kinase and also inhibit neutrophil degranulation [37]. Thus, the results of this study confirmed the traditional uses, claiming that *Pterospermum canescens* leaves, stem and stem bark have significant anti-inflammatory activity.

Table 1: Anti-inflammatory activity of leaf extracts

Treatment	Paw oedema volume in ml				
	0 min	30 min	60 min	120 min	180 min
Control	1.67 ± 0.15	1.80 ± 0.04	2.10 ± 0.10	2.58 ± 0.05	2.58 ± 0.06
PETL	2.05 ± 0.07	1.82 ± 0.13	1.80 ± 0.07	1.92 ± 0.09	1.97 ± 0.03
PETH	1.43 ± 0.08***	1.40 ± 0.07***	1.35 ± 0.10***	1.50 ± 0.04***	1.78 ± 0.10***
CHL	1.70 ± 0.08**	1.73 ± 0.12**	1.52 ± 0.03**	1.50 ± 0.17**	1.80 ± 0.10**
CHH	1.28 ± 0.08***	1.37 ± 0.19***	1.68 ± 0.17***	1.57 ± 0.14***	1.60 ± 0.16***
MEL	1.42 ± 0.14***	1.47 ± 0.11***	1.58 ± 0.11***	1.57 ± 0.12***	1.87 ± 0.07***
MEH	1.20 ± 0.04***	1.08 ± 0.06***	1.13 ± 0.10***	1.03 ± 0.06***	1.48 ± 0.09***
STD	1.03 ± 0.08***	1.13 ± 0.11***	1.28 ± 0.03***	1.20 ± 0.12***	0.95 ± 0.03***

Values shown are mean ± SEM (n = 6). *** P < 0.001, ** P < 0.01 experimental groups were compared with control

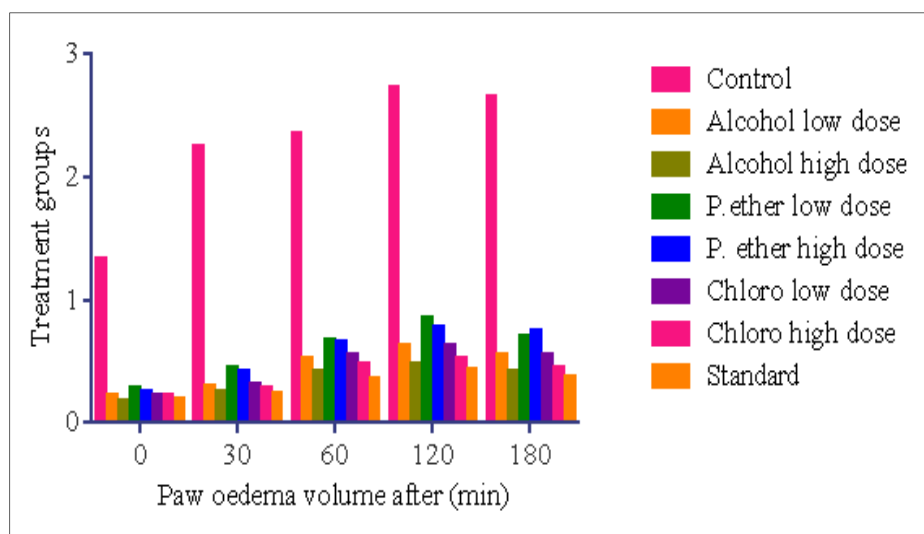


Figure 1: Anti-inflammatory activity of leaf extracts

Table 2: Anti-inflammatory activity of stem extracts

Treatment	Paw oedema volume in ml				
	0 min	30 min	60 min	120 min	180 min
Control	1.67 ± 0.15	1.80 ± 0.04	2.10 ± 0.10	2.58 ± 0.05	2.58 ± 0.06
PETL	1.98 ± 0.10	1.70 ± 0.11	1.80 ± 0.07	1.87 ± 0.10	1.90 ± 0.04
PETH	1.47 ± 0.10***	1.37 ± 0.10***	1.30 ± 0.10***	1.47 ± 0.03***	1.60 ± 0.10***
CHL	1.82 ± 0.10*	1.98 ± 0.02*	1.55 ± 0.04*	1.63 ± 0.13*	1.73 ± 0.12*
CHH	1.27 ± 0.07***	1.27 ± 0.19***	1.53 ± 0.15***	1.53 ± 0.15***	1.50 ± 0.17***
MEL	1.58 ± 0.14**	1.92 ± 0.04**	1.72 ± 0.07**	1.48 ± 0.12**	1.80 ± 0.07**
MEH	1.27 ± 0.06***	1.22 ± 0.07***	1.20 ± 0.10***	1.03 ± 0.06***	1.38 ± 0.06***
STD	1.03 ± 0.08***	1.13 ± 0.11***	1.28 ± 0.03***	1.20 ± 0.12***	0.95 ± 0.03***

Values shown are mean ± SEM (n = 6). *** P < 0.001, ** P < 0.01, * P < 0.05 experimental groups were compared with control

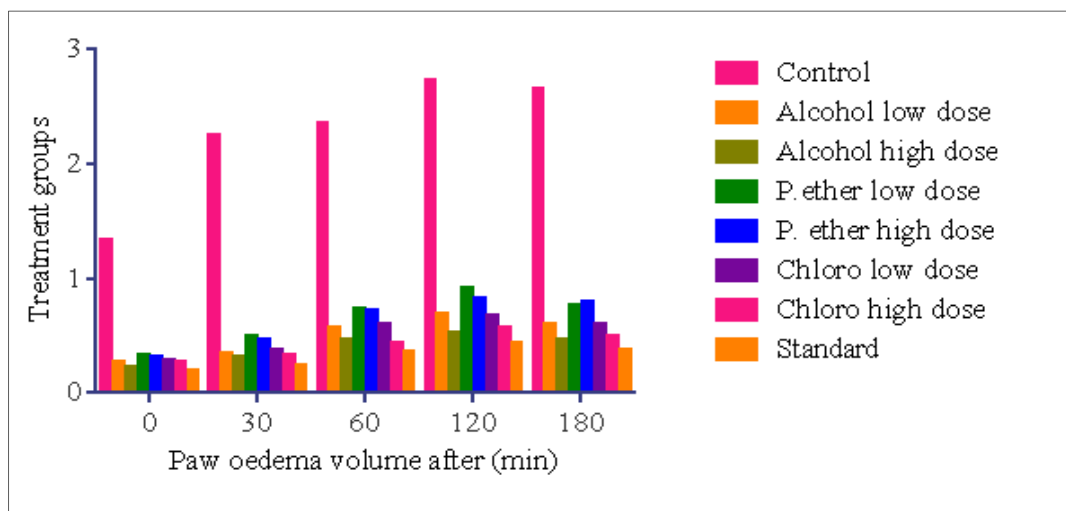


Figure 2: Anti-inflammatory activity of stem extracts

Table 3: Anti-inflammatory activity of stem bark extracts

Treatment	Paw oedema volume in ml				
	0 min	30 min	60 min	120 min	180 min
Control	1.67 ± 0.15	1.80 ± 0.04	2.10 ± 0.10	2.58 ± 0.05	2.58 ± 0.06
PETL	1.88 ± 0.10*	1.63 ± 0.06*	1.60 ± 0.13*	1.77 ± 0.08*	1.70 ± 0.05*
PETH	1.58 ± 0.10***	1.43 ± 0.08***	1.13 ± 0.05***	1.35 ± 0.08***	1.43 ± 0.12***
CHL	1.72 ± 0.14**	1.92 ± 0.03**	1.55 ± 0.04**	1.33 ± 0.10**	1.62 ± 0.08**
CHH	1.30 ± 0.07***	1.16 ± 0.13***	1.26 ± 0.07***	1.32 ± 0.05***	1.22 ± 0.11***
MEL	1.27 ± 0.06***	1.58 ± 0.11***	1.48 ± 0.11***	1.20 ± 0.04***	1.50 ± 0.03***
MEH	1.27 ± 0.08***	1.38 ± 0.04***	1.32 ± 0.08***	1.02 ± 0.05***	1.23 ± 0.06***
STD	1.03 ± 0.08***	1.13 ± 0.11***	1.28 ± 0.03***	1.20 ± 0.12***	0.95 ± 0.03***

Values shown are mean ± SEM (n = 6). *** P < 0.001, ** P < 0.01, * P < 0.05 experimental groups were compared with control

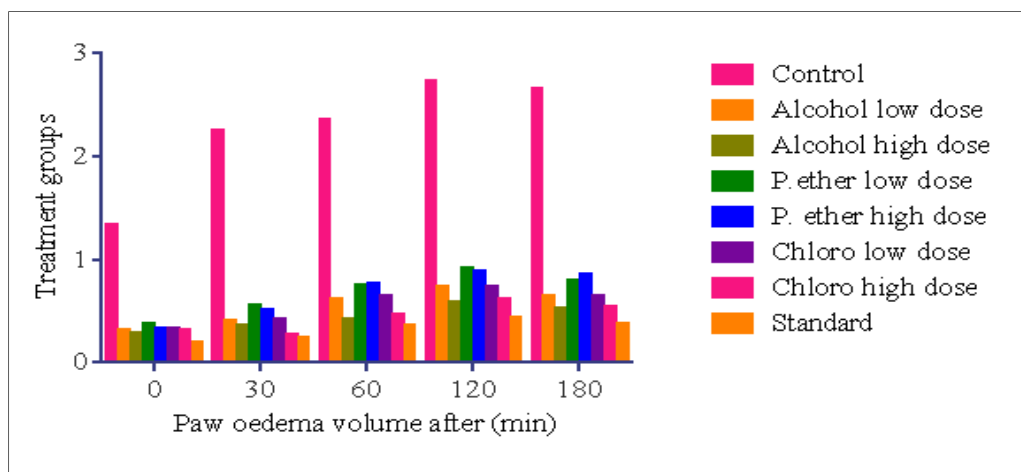


Figure 3: Anti-inflammatory activity of stem bark extracts

CONCLUSION

Furthermore, the results of this study confirmed the traditional uses claiming that *P. canescens* leaves, stem and stem bark have significant anti-inflammatory activity. However, to know the exact mechanism of action of all the extracts of *Pterospermum canescens* further study with purified fractions is warranted. Hence the anti-inflammatory activity of *Pterospermum canescens* reported here for the first time in Pharmaceutical science.

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