

Anti-inflammatory activity of flavonoid fraction of *Pisonia grandis* R.Br leaves

S. Jayakumari, V. Ravichandiran, S. Nirmala, P.Divya*, Malarkodi Velraj, A. Vijayalakshmi and Arthanareswar

Department of Pharmacognosy, School of Pharmaceutical Sciences, Vels University, Pallavaram, Chennai-600117, Tamilnadu, India

*St. Pauls College of Pharmacy, Turkyajamjal, Hayatnagar, Hyderabad-500501. A.P., India

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Abstract

In the past decade, there has been a resurgence of interest in bioactive components of natural origin. Many of the inflammatory activities of plants of ISM were attributed to their bioactive markers. It has been reviewed that natural flavonoids have been found to exert their anti-inflammatory effects either by scavenging free radicals or by decreasing the formation of reactive oxygen species. Reviewing the folk medicine as well as the literature, some species of the genus *Pisonia grandis* have been shown to possess anti-inflammatory activity. Leaves contain flavonoids, glycosides and steroids viz., octacosanol, beta sitosterol, alpha spinosterol and dulcitol. A flavonoid glycoside 4-o-methyl-5'-o-acetyl myricetin-3-o-glucoside 2 to 1 rhamnoside was reported. So the present study reports the anti-inflammatory activity of *Pisonia grandis* methanolic extract and its flavonoid rich (ethylacetate) fraction. The results obtained from the models showed that methanolic extract and the ethyl acetate fraction (flavonoid fraction) separated from the leaf of *Pisonia grandis* significantly reduce the inflammation in both acute and chronic phases

Key words: Anti-inflammatory activity, flavonoid, Carageenan induced paw edema, Cotton pellet granuloma, *Pisonia grandis* Leaves

Introduction

Inflammation is the complex biological response of vascular tissues to harmful stimuli such as pathogens, damaged cells or irritants. Inflammation is a protective attempt by the organism to remove the injurious stimuli as well as to initiate the healing process for the tissue. In the absence of inflammation, wounds and infections would never heal and progressive destruction of the tissue would compromise the survival of the organism. However, chronic inflammation can also lead to a host of diseases such as hay fever, atherosclerosis and rheumatoid arthritis. It is for that reason the inflammation is normally closely regulated by the body. It has been reviewed that natural polyphenols have been found

to exert its anti-inflammatory effects either by scavenging free radicals and decreasing formation of reactive oxygen species (Ostrakhovitch and Afanas, 2001) or by modulation of proinflammatory gene expression of cyclooxygenase, lipoxygenase and cytokines mainly by acting through Nuclearfactor - kappa B and mitogen activated protein kinase signaling (Bonedeppo *et al.*, 2007). The side effects of steroidal and non-steroidal anti-inflammatory drugs currently used for management of chronic inflammatory diseases may be more difficult to manage than the disease itself. Therefore, there is a need for new safe approaches for such diseases (Tripathi, 2011). The natural botanical sources in Asia may supply us with natural anti-inflammatory agents which may have minimal draw backs. Reviewing the folk medicine as well as the literature, some species of the genus *Pisonia grandis* have been shown to possess anti-inflammatory activity.

Pisonia grandis R.Br. (Nyctaginaceae) is an evergreen tree, all parts are glabrous and the young shoots are minutely

Author for correspondence: Dr. S. Jayakumari

Professor, Department of Pharmacognosy, School of Pharmaceutical Sciences, Vels University, Pallavaram, Chennai-600117, Tamilnadu, India

E-mail address: nisajayaa@gmail.com.

Tel.: +91- 09840282617

puberulous. The leaves of *Pisonia grandis* is used as analgesic, anti-inflammatory, diuretic and hypoglycaemic agent. Leaves contain flavonoids, glycosides and steroids viz., octacosanol, beta sitosterol, alpha spinosterol and dulcitol. A flavonoid glycoside 4-o-methyl-5'-o-acetyl myricetin-3-o-gluco (2to1) rhamnoside was reported by Manogaran, (2003). Analgesic, anti-inflammatory and diuretic (Anbalagan *et al.*, 2002), hypoglycemic activity (Manogaran, 2003) have been reported. So the present study reports the anti-inflammatory activity of *Pisonia grandis* methanolic extract and its flavonoid rich (ethylacetate) fraction.

Materials and Methods

Collection and authentication

The plant specimen for the proposed study was collected from Kodambakkam, Chennai, Tamil Nadu and authenticated by Dr. P. Jayaraman, Director, Plant Anatomy Research Center, (PARC) Tambaram, Chennai (PARC/ 2009/ 357).

Extraction method

The leaves of *Pisonia grandis* R.Br. were shade dried and coarsely powdered. About 500 gms of powder was extracted with methanol (70% v/v) by cold maceration method. The solvent was filtered and distilled off. Final traces of solvent were removed under vacuum. The total extract, thus, obtained was fractionated successively with the solvents of increasing polarity viz., petroleum ether, ethyl acetate and ethanol (Kokate, 1994). The percentage yield of methanolic extract is 10.53% w/w and the ethylacetate fraction was 3.435% w/w.

Preliminary phytochemical analysis (Kokate *et al.*, 1997)

The total methanol extract and its fractions such as petroleum ether, ethyl acetate and ethanol fractions of the leaf of *Pisonia grandis* were subjected to the following chemical tests for identification of various phytoconstituents present.

Chemicals and instruments

Carrageenan(Sigma),1,1-diphenyl-2-picrylhydrazyl (DPPH) (Aldrich), Naphthylethylenediamine dihydrochloride (Loba chemie), Thiobarbituric acid (TBA) and Trichloro acetic acid(Sd fine chemicals Ltd). All other reagents used were of analytical grade. UV spectra were recorded in Shimadzu 1601 UV-Visible spectrophotometer.

Animals

Albino Swiss mice of either sex (20-30g) were obtained from Institutional Animal Breeding House, Vels College of Pharmacy, Pallavaram, Chennai-117. Animals were housed in plastic cages at an ambient temperature (25±2°C) and relative humidity of 45-55%. A 12:12 hr light- dark cycle was maintained during the experiments. They were fed with balanced rodent pellet diet from Poultry Research Station, Nandanam, Chennai-35 and water *ad libitum* throughout the

experimental period. Animals were acclimatized to their environment for atleast one week before experimentation. The animals were randomly divided into different groups. Each animal was housed separately after recording its body weight and had kept separate marks for identifying the dose level, group and individual number. The Institutional Animal Ethics Committee (IAEC) approved the protocol of the study on Registration no. 190 CPCSEA dated 8.11.2009.

Acute toxicity study

The toxicity effect of drug is evaluated and the LD₅₀, ED₅₀ and the therapeutic index was determined for the drug under investigation (Lipinic *et al.*, 1995).

Procedure

Acute toxicity study - up and down procedure - was carried out as per the guidelines by Organization for Economic Co-operation and Development (OECD) 423. In this experiment, three groups of albino Swiss mice n=6 were used. Animals were fasted overnight with water *ad libitum* and food was withheld for 3-4 h after oral administration of the EF of *Pisonia grandis*. First group of animal were treated with starting dose of 1000 mg/kg body weight of Ethyl acetate fraction (EF) or EAF orally. Second group of animals were treated with a maximum dose of 2000 mg/kg body weight of EF. Control group was treated with normal saline. Animals were observed individually after dosing. Observation included mortality and gross behaviors *e.g.*, body positions, locomotion, rearing, tremors were observed. The effect of EF on passivity, grip strength, pain response, stereotypy, righting reflex, and mortality were assessed (Lipinic *et al.*, 1995). No abnormality in the gross behavioral studies also no mortality were noted. Based on these observations three different doses (100, 250 and 500 mg/kg) were selected for the pharmacological studies.

Anti-inflammatory activity

Carageenan induced paw edema model (Victor B. Owoyele *et al.*, 2005)

Animals : Albino swiss mice of either sex (20-30 gm)
 Test drug : Methanolic extract of the leaf of *Pisonia grandis* R.Br (MEPG) Ethyl acetate fraction (EF)
 Standard drug : Aspirin

Procedure

Albino Swiss mice were divided into 8 groups each consisting of 3 mice. The treatment schedules of animals belonging to different groups were shown below,

Group I (Vehicle) - 10 ml/kg of CMC (2%)
 Group II (Standard) - Aspirin (2 mg/kg, orally) in 2% w/v CMC

- Group III (Test) - MEPG (100 mg/kg, orally) in 2% w/v CMC
- Group IV (Test) - MEPG (250 mg/kg, orally) in 2% w/v CMC
- Group V (Test) - MEPG (500 mg/kg, orally) in 2% w/v CMC
- Group VI (Test) - EAF (100 mg/kg, orally) in 2% w/v CMC
- Group VII (Test) - EAF (250 mg/kg, orally) in 2% w/v CMC
- Group VIII (Test) - EAF (500 mg/kg, orally) in 2% w/v CMC

Inflammation was induced by injecting 0.1 ml of 1% w/v carrageenan in 2% w/v CMC into the right hind paw of the mice. Paw volume was measured by using plethysmometer at 3rd, 5th and 24th hr after the carrageenan injection. Activity was expressed in terms of percentage inhibition of paw edema untreated control. Aspirin 2mg/kg suspended in 2% CMC was used as positive control.

Cotton pellet granuloma method (Victor B. Owoyele et al., 2005)

- Animals : Albino wistar rats of either sex (20-30 gm)
- Test drug : Methanolic extract of the leaf of *Pisonia grandis* R.Br (MEPG),
Ethyl acetate fraction (EAF)
- Standard drug : Indomethacin

Procedure

Albino wistar rats were divided into 8 groups each consisting of 3 animals. The treatment schedules of animals belonging to different groups were shown below,

- Group I (Vehicle) - 10 ml/kg of CMC (2%)
- Group II (Standard) - Indomethacin (5 mg/kg, p.o.) in 2% w/v CMC
- Group III (Test) - MEPG (100 mg/kg, orally) in 2% w/v CMC
- Group IV (Test) - MEPG (250 mg/kg, orally) in 2% w/v CMC
- Group V (Test) - MEPG (500 mg/kg, orally) in 2% w/v CMC
- Group VI (Test) - EAF (100 mg/kg, orally) in 2% w/v CMC
- Group VII (Test) - EAF (250 mg/kg, orally) in 2% w/v CMC
- Group VIII (Test) - EAF (500 mg/kg, orally) in 2% w/v CMC

Inflammation was produced by cotton pellet granuloma in rats. Sterile cotton (50±1mg) soaked in 0.2 ml of distilled water was implanted subcutaneously under ether anaesthesia. The treatment was given upto 7 days after cotton implantation. The animals were sacrificed on the 8th day and the granulation tissue with cotton pellet was dried at 60°C overnight and the dry weight was noted. The weight of the cotton pellet before implantation is subtracted from the weight of the dried dissected pellets. Statistical analysis was done by one way ANOVA and by unpaired Student's 't' test. p values <0.05 were considered as significant.

Results

Preliminary phytochemical analysis

Preliminary phytochemical screening of all tested fractions, ethyl acetate and methanol fraction revealed the presence of flavonoids, tannins and phenolic compounds.

Toxicity study

Acute toxicity study was carried out for the extract and ethanol fraction upto 2000mg/kg in mice. No mortality and abnormality was observed in all the groups.

Effect on carrageenan induced paw edema model

The rats footpad became edematous soon after injection of carrageenan. Edema value of the injected footpad reached its peak at 5 h administration of methanol extract (100,200 & 500 mg/kg) and ethyl acetate fraction (100,200 & 500 mg/kg) significantly inhibited the development of pad swelling after 1h to 18 h after carrageenan injection (p<0.01, Table 1). The reference drug aspirin (100,200 & 500 mg/kg) had significant anti inflammatory effect (p<0.01) compared to the treatment groups.

Effect on cotton pellet granuloma model

Methanol and ethylacetate fraction of *Pisonia grandis* leaf at the dose level of 100,200&500 mg/kg/p.o., significantly (p < 0.01) reduced the weight of the cotton pellet granuloma in rats. The percentage inhibition of methanol extract and ethylacetate fractions was shown in the Table 2. The effect was compared with the standard drug Aspirin 100 mg/kg.

Discussion

The present study was undertaken for the evaluation of anti-inflammatory activity of methanolic extract and flavonoid rich fraction separated from the leaves of *Pisonia grandis*.

For the anti-inflammatory effect it is important to estimate the activity in the acute phase as well as the chronic phase of inflammation. So the inflammatory condition induced by

carrageenan induced paw edema method and Cotton pellet granuloma model. The edema formation is a biphasic event, the early phase (during the first hour) is due to the hyperemia being due to the release of histamine and serotonin (Vinegar *et al.*, 1969) and the delayed edema due to the release of bradykinin and prostaglandins (Flower *et al.*, 1985). Accordingly the carrageenan model was selected because of its sensitivity in detecting orally active anti-inflammatory agents particularly in the acute phase of inflammation. Histamine, serotonin, bradykinin and prostaglandins are established mediators of acute phase of inflammation causing increase in vascular permeability and vasodilatation. These inflammatory mediators are released endogenously and contribute to the various phases of paw edema. The inhibitory effect of EAF on these mediators was well observed in the 3rd hour. The administration of methanolic extract and ethyl acetate fraction of *Pisonia grandis* shown the significant effect on inflammation in carrageenan induced paw edema model (Table 1).

The cotton pellet granuloma was used as a model of chronic inflammation. Cytokines, such as IL-1 and TNF as well as growth factors influence proliferation of smooth muscle cells and fibroblasts and thus production of granuloma. The dry weight of the pellets correlates with the amount of granulomatous tissue. Administration of methanolic extract (100, 200 & 500 mg/kg) and ethyl acetate fraction (100, 200 & 500 mg/kg) appears to be effective in inhibiting the dry weight of cotton pellet that was almost comparable to that of standard drug aspirin (Table 2). The activity was found to be dose dependant and significant reduction in granular tissue formation also recorded.

The results obtained from the models showed that methanol extract and the ethyl acetate fraction separated from the leaf of *Pisonia grandis* significantly reduce the inflammation in both acute and chronic phases. Among the tested samples flavonoid rich ethyl acetate fraction was shown maximum inhibition by both the tested models. This inhibition may be due to reduction in the release of arachidonic acid, by which flavonoids inhibit the cyclooxygenase and 5-lipoxygenase enzymes (Yoshimoto, 1983). Quercetin, in particular, inhibits both cyclooxygenase and lipoxygenase activities, which was already reported in the leaves of the plant. Anti-inflammatory feature of flavonoids is the ability to inhibit eicosanoid biosynthesis (Formica and Regelson, 1995 and Damas *et al.*, 1985). Eicosanoids, such as prostaglandins, are involved in various immunologic responses (Moroney, 1988) and are the end products of the cyclooxygenase and lipoxygenase pathways. Flavonoids also inhibit both cytosolic and membranal tyrosine kinase (Formica and Regelson, 1995). Integral membrane proteins, such as tyrosine 3-monooxygenase kinase, are involved in a variety of functions, such as enzyme catalysis, transport across membranes, transduction of signals that function as receptors of hormones and growth factors, and energy transfer in ATP synthesis. Inhibition of these proteins results in inhibition of uncontrolled cell growth and proliferation. Tyrosine kinase substrates seem to play key roles to inhibit eicosanoid biosynthesis (Formica and Regelson, 1983 and Damas *et al.*, 1985). All the above effects were contributed to the presented work to show that bioflavonoid plays a vital role in inflammation. These observations strongly suggest that the flavonoids may cause a defective effect of inflammatory responses.

Table 1. Anti-inflammatory activity of *Pisonia grandis* leaves by Carrageenan induced paw edema model

S.NO	Group	Treatment	Dose (mg/kg)	% of activity		
				3 rd hr	5 th hr	24 th hr
1	I	Control	1ml	11.25±3.59**	23.54±6.25	8.13±1.99*
2	II	MEPG	100	32.48±1.67**	44.12±3.67	12.39±1.36*
3	III	MEPG	250	39.46±2.35**	50.31±1.12**	21.09±2.51**
4	IV	MEPG	500	47.52±3.33**	57.44±1.44**	27.36±2.73*
5	V	EAF	100	40.71±0.92*	47.12±2.67**	18.28±0.75**
6	VI	EAF	250	46.08±4.97**	54.01±1.79*	25.06±1.27**
7	VII	EAF	500	52.57±2.39*	62.12±3.29**	30.10±2.91*
8	VIII	STD	100	65.13±1.76**	82.25±0.49**	44.02±2.00**

MEPG - Methanolic extract of *Pisonia grandis*

EAF - Ethyl acetate fraction

Values are mean \pm SEM of 6 parallel measurement.

Statistical significant test for comparison was done by ANOVA, followed by Dunnet's 't' test (n=6)

All the values are significant **P<0.01 when compared against control.

All the values are significant ^aP<0.01 when compared against standard

Table 2. Anti-inflammatory activity of *Pisonia grandis* leaves by Cotton pellet granuloma method

S.No	Group	Treatment	Dose (mg/kg)	Dry weight of granuloma (mg)	% activity
1	I	Control	-	54.2	8.6 \pm 3.59**
2	II	MEPG	100	65.3	32.6 \pm 4.97*
3	III	MEPG	250	69.7	39.56 \pm 1.72*
4	IV	MEPG	500	72.6	45.2 \pm 5.02*
5	V	EAF	100	68.1	36.2 \pm 3.28**
6	VI	EAF	250	72.4	46.8 \pm 2.91**
7	VII	EAF	500	75.8	51.6 \pm 1.76**
8	VIII	STD	100	88.3	78.6 \pm 4.27**

MEPG - Methanolic extract of *Pisonia grandis*

EAF - Ethyl acetate fraction

Values are mean \pm SEM of 6 parallel measurement.

Statistical significant test for comparison was done by ANOVA, followed by Dunnet's 't' test (n=6)

All the values are significant **P<0.01 when compared against control.

All the values are significant ^aP<0.01 when compared against standard

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