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Phytochemical analysis and anti-inflammatory activity of *Pisonia grandis* R.Br.

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ABSTRACT

Alcohol and aqueous extract of *Pisonia grandis* (leaf) was taken and analyzed for anti-inflammatory activity. Preliminary phytochemical screening was performed for both the extracts. In the present study alcohol extract showed significant anti-inflammatory activity in carrageenan induced paw edema rats. Phytochemical analysis of alcohol extract revealed the presence of flavanoids, steroids, furan, alkaloids, anthraquinone, tannins and saponins but negative result was observed in aqueous extract except tannins. This study showed vital information regarding pharmacological and phytochemical activities of *P. grandis*.

Key words: Acute toxicity, anti-inflammatory, diclofenac sodium, *Pisonia grandis*.

INTRODUCTION

The World Health Organization (WHO) estimated that 80% of the population of developing countries still relies on traditional medicines, mostly plant drugs, for their primary health care (Fransworth *et al.,* 1985). Plant products also play an important role in the healthcare systems of the remaining 20% population, mainly residents of developed countries. The scientific data generated by research on the plants serves as a valuable tool for identifying plant species and for characterization of the pharmacological active constituent for their biological activities. In the search for new plant it is always important to screen for its activity as first step. Once the plant is identified for beneficial biological activity it is imperative to collect supporting scientific data generated through pharmacognostic and phytochemical properties of plant under investigation.

Based on this we have worked on the leaves of *Pisonia grandis* R.Br. (Nyctaginaceae) to find out anti-inflammatory activity. *P. grandis* is an herb claimed to be used for treatment of wound healing, algesia, ulcer and antibacterial activity (Kirithikar and Basu 1990; Prabu et al., 2008). Alcohol and aqueous extracts were taken and analyzed for anti-inflammatory activity. This study was aimed giving vital information regarding pharmacological and phytochemical activities. As a positive step is taken in this direction for achieving betterment of mankind. A small step has been taken through this research.

MATERIAL AND METHODS

Plant collection

The leaves of *Pisonia grandis* were collected in Chengalpattu (Tamil Nadu) in June 2006 and authenticated by a botanist Dr. P. Jayaraman at the Plant Anatomy Research Centre (PARC), Chennai, Tamil Nadu, India. A voucher specimen

has been deposited at the museum of the department of pharmacognosy, Madras Medical College, Chennai, India.

Preparation of extracts

The freshly collected leaves of *Pisonia grandis* were shade dried and then coarsely powdered in a blender. The coarse powder was successively extracted in an aspirated bottle with ethanol, water by cold maceration for 3-7 days. After decantation and filtering through What Mann filter paper no.41 nearly 81% of the solvent was removed by distillation over boiling water bath and remaining under reduced pressure. The extracts so obtained were further dried in vacuum desiccators and the residue obtained from various extracts was used for further studies by preserving it in refrigerator.

Phytochemical studies

The presence of phytochemicals, triterpenoids (Noller's test), flavones (Shinadow's test), steroids (Libermann-Burchard test), proteins (Biuret test), furans (Ehrlich's test), alkaloids (Dragendroff's reagent), anthraquinones (Borntrager's test), gums, tannins (5% ferric chloride), saponins (Frothing test), phenols and sugars were evaluated according to the method described by Edeogal *et al*., 2005.

Animals

Inbred Male and female Wistar albino rats (160-200 g) were procured from the animal experimental laboratory of Madras Medical College and used throughout the study. The study was conducted after obtaining Institutional animal ethical committee's clearance (20/236/Aug' 2006). The animals were maintained in colony cages at 25±2°C, relative humidity of 45-55% maintained under 12 h light and dark cycles. The animals were fed with standard animals feed (Hindustan Lever Ltd.) and water adlibitum. All the animals were acclimatized for a week before use and they were maintained in hygienic environment in our animal house.

Acute toxicity study (Ecobian, 1997)

The procedure was followed by using OECD (Organization of Economic Cooperation and Development) guidelines 423 (Acute toxic class method). Male wistar rats weighing 160-200 gm were used for the study. The starting dose level of poly herbal formulation was 2000 mg/kg body weight p.o. Dose volume was administered 0.1 ml/10 gm body weight to the rate which was fated overnight with water adlibitum. Food was with held for the further three to four hours after administration of the drug. Body weight of the rats before and after termination were noted and any changes in skin and fur, eyes and mucous membrane and also respiratory, circulatory, autonomic and central nervous system and locomotors activity and behavior pattern were observed and also sign of tremors, conversion, salivations, diarrhea, lethargy, sleep and coma were noted. The onset of toxicity and signs of toxicity are also noted.

Anti-inflammatory evaluation

The following experimental protocol was used to access anti-inflammatory activity on carrageenan induced paw edema rats (Wister *et al.,* 1962). The animals were divided into six groups. Each group composed of six animals.

Group Animals

I Animals received 1% carboxy methylcellulose 10 ml/kg p.o

II Animals received aqueous extract of *Pisonia grandis* 200 mg/kg p.o

III Animals received aqueous extract of *Pisonia grandis* 400 mg/kg p.o

IV Animals received alcoholic extract of *Pisonia grandis* 200 mg/kg p.o

V Animals received alcoholic extract of *Pisonia grandis* 400 mg/kg p.o

VI Animals administered reference standard diclofenac sodium 5 mg/kg p.o

Paw edema was induced by injecting 0.1 ml of carrageenan in physiological saline into sub-plantar tissue of rat right hind paw of each rat. The aqueous and alcoholic leaf extract of *Pisonia grandis* was administrated orally 30 minutes prior to carrageenan administration. The paw volume was measured at intervals of 1st hour, 2nd hour, 3rd hour and 4th hour by the mercury displacement method using plethysymograph. The percentage inhibition of paw volume in drug treated group was compared with the carrageenan control group (Group I). The diclofenac sodium was used as a reference standard.

Statistical analysis

All treated groups were compared with the control group (Group I) and the results were analyzed statistically using ANOVA and followed by Dunnet's test to identify the difference between treated groups and control. The data were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Phytochemical analysis

The result of phytochemicals is listed in the table 1.

Anti-inflammatory activity

Carrageenan induced paw edema is the most widely used method to screen anti-inflammatory agents. The development of carrageenan induced edema is biphasic, the initial phase is attributed to release of histamine, 5-hydrozytryptamine and kinins in the first hour after injection of carrageenan and the most pronounced second phase is related to release of prostaglandin like substances in 2-3 hours.

The aqueous and alcoholic extract of *Pisonia grandis* shows significant anti-inflammatory activity against carrageenan injection (Table 2), suggesting that it predominantly inhibits the release of inflammatory mediators form phylogenic stimuli. However, further studies are necessary to identify and isolate the active constituents responsible for its anti-inflammatory activity and also there is a need to elucidate its mechanism of anti-inflammatory action. The present study clearly demonstrated the

Table 1: Phytochemical constitutes of *Pisonia grandis*

Chemical constituents	Alcohol extract	Aqueous extract
Terpenoids	+	-
Flavones	+	-
Steroids	+	-
Proteins	-	-
Furan	+	-
Alkaloids	-	-
Anthraquinone	+	-
Gum	-	-
Tannins	+	+
Saponins	+	-
Phenols	-	-
Sugars	-	-

Table 2: Anti-inflammatory activity of alcohol and aqueous extract of *P. grandis* on carrageenan induced paw edema rats

Groups	1st hour	2nd hour	3rd hour	4th hour
I	0.70±0.16	0.67±0.19	0.65±0.11	0.58±0.06
II	0.58±0.08* (17.14%)	0.52±0.03* (22.38%)	0.51±0.16* (21.53%)	0.44±0.05** (24.13%)
III	0.50±0.06** (28.57%)	0.34±0.06** (49.25%)	0.38±0.09** (41.53%)	0.27±0.06** (53.47%)
IV	0.39±0.06** (44.28%)	0.37±0.05** (44.77%)	0.36±0.07** (44.61%)	0.33±0.05** (43.1%)
V	0.38±0.05** (45.71%)	0.36±0.09** (46.26%)	0.35±0.05** (46.15%)	0.19±0.03** (67.24%)
VI	0.16±0.01** (77.14%)	0.14±0.02** (76.11%)	0.16±0.01** (75.38%)	0.13±0.01** (77.58%)

Values are mean ± S.D. Paw volume expressed in ml, comparison were made between Group I Vs Group II, III, IV, V and VI. Percentage protection given in parenthesis. * $p<0.05$, ** $p<0.001$ statistical evaluation done by ANOVA followed by dunnet's test.

anti-inflammatory property of *Pisonia grandis*. The effects may due to flavonoids, steroids and tannins observed in the extracts. A detailed investigation into the potential plant constituents responsible for the anti-inflammatory property may provide scope for lead molecules that may be useful in treating humans effectively for inflammation. The acute toxicity study did not show any mortality at the dose level of 2000 mg/kg body weight, so that the extract is characterized as "X" unclassified (OECD-423), this indicates the safety profile of the extracts. The anti-inflammatory activity of *Pisonia grandis* leaf extracts exhibited at the dose level of 200, 400 mg/kg p.o. exhibited significant ($P<0.05$) activity compared with the carrageenan treated animals. The reference standard diclofenac sodium also exhibited significant activity.

CONCLUSION

The present study indicates that the plant contains potential anti-inflammatory components such as flavonoids, terpenoids and steroids that may be of use for development of phytomedicine for the therapy of inflammations. Further research work is to needed to establish the exact anti-inflammatory mechanism of action of alcoholic extract of *Pisonia grandis*.

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