www.sphinxsai.com

PharmTech

International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.3, No.1, pp 337-341, Jan-Mar 2011

Analgesic and anti-inflammatory activities of leaf extract of *Pithecellobium dulce* Benth.

S.Arul Selvan[1], P.Muthukumaran[2]*

[1]Department of Chemistry, Anna University, Guindy-600 025,Tamil nadu,India

[2]Department of Biochemistry, Meenakshi
Chandra Sekaran College of Arts and Science, Pattukkottai -614 626,Tamil nadu,India

Corres.author:kumaran.bio82@yahoo.com

Abstract: The methanol extract of leaves of *Pithecellobium dulce* Benth. was screened for the analgesic(using hot plate test and acetic acid-induced writhing test in mice) and anti-inflammatory(using rat paw edema test) activity at the doses of 200 and 400mg/kg body weight. A significant ($p<0.0005$) analgesic effect was observed with200 mg/kg and 400 mg/kg in both tests. The maximum anti-inflammatory response was produced at 3 hour with extract doses of 200 and 400 mg/kg. These results suggest that the methanol extract of *Pithecellobium dulce a* has exhibited significant analgesic and anti-inflammatory effects, which were comparable with standard drugs
Key words: *Pithecellobium dulce* Benth, analgesic and anti-inflammatory activity, Pentazocine, Diclofenac sodium.

Introduction

Pithecellobium dulce Benth. (Leguminosae)[1] is a small to medium sized, evergreen, spiny tree up to 18 m height, native of tropical America and cultivated throughout the plains of India and in the Andamans. It is known as 'Vilayati babul' in Hindi and 'Kodukkapuli' in Tamil. The bark of the plant is reported to be used as astringent in dysentery, febrifuge and it is also useful in dermatitis and eye inflammation. The leaves have been reported to possess astringent, emollient, abortifiacient and antidiabetic properties. The presence of steroids, saponins, lipids, phospholipids, glycosides, glycolipids and polysaccharides have been reported in the seeds. [2-5] The bark contains 37% of tannins of catechol type. Quericitin, kaempferol, dulcitol and afezilin have been reported from the leaves. [6, 7] Roots have been reported to possess estrogenic activity.[8] Studies on alkylated resins from seed oil have been reported recently .[9] It is evident that the plant has great potentials in treating a number of ailments where the free radicals have been reported to be the major factors contributing to the disorders.[10]So far no information is available for the analgesic and anti inflammatory activity of the methanol extract of *Pithecellobium dulce* . So, the present study has been undertaken to evaluate the analgesic and anti inflammatory activity of the methanol extract of *Pithecellobium dulce using* hot plate, writhing and rat paw edema methods.

Materials and Methods

Plant material

The leaves of *Pithecellobium dulce* were collected from Kancheepuram District, Tamilnadu, India .The leaves were examined carefully and old, infected, and fungus damaged leaves were removed. Extracts were prepared from dried and also from fresh leaves. Healthy leaves were spread out and dried at room temperature for about ten days and ground into fine powder using electric blender.

Preparation of extract: The leaves were cut into small pieces and shed dry and then ground into coarse powder for the maceration process with methanol at room temperature. After exhaustive extraction, the methanol extract was concentrated under reduced

pressure at 50-55 0 C and stored in a vaccum desiccator.The suspension of the extract prepared in 2% gum acacia was used in the entire experimental studies.

Drugs and chemicals: The drugs and chemicals used were carrageenan and acetic acid (SD fine chemicals Limited, Mumbai), gum acacia and diclofenac sodium (Dr. Reddy's Labs, Hyderabad), Pentazocine (PurePharma Ltd., Mumbai) and methanol (Merck, Mumbai).

Animals: Wister rats (175-250 g) and albino mice (25-30g) of either sex were selected and maintained under standard husbandry conditions and had free access to food and water *ad libitum.* The animals were allowed to acclimatize to the environment for 7 days prior to the experimental session. The animals were divided into different groups each consist of six animals were fasted overnight prior to the experiments. Experiments on animals were performed in accordance with guidelines of the Institutional Animal Ethical Committee.

Hot-plate test: The hot plate test was used to measure analgesic activity by the method described by Eddy[11] with minor modifications. In this experiment, the hot plate was maintained at 55 ± 0.50 C. All animals were selected 24 hour prior to experimentation and the animals were selected on the basis of their normal reaction time i.e., pain response to the hot plate to the minimum and maximum of 2-15 sec respectively. In order to avoid the damage to the paws of the animals, the time standing on the plate was limited to 20 sec. Pentazocine 10 mg/kg was administered intraperitoneally as a reference standard.30 min after administration of vehicle (2% gum acacia)/methanol extract (200 and 400 mg/kg)/standard drug, animals were placed individually on to the hot plate and the time from placing the animal on the hot plate to jumping of the animal from the hot plate was recorded as the reaction time or latency of the pain response.

Writhing test: Abdominal construction induced by intraperitoneal injection of acetic acid was carried out according to the procedures described previously[12]**.** The leaf extract of *Pithecellobium dulce* was tested at 200 and 400 mg/kg. Diclofenac sodium, a reference anti-inflammatory and analgesic compound, was used at 20 mg/kg. The extract and reference drug were administered orally 30 min before the administration of 0.7% acetic acid in a volume of 10mg/kg i.p. Control animals received 2% of gum acacia under the same experimental condition. Immediately after injection of the acetic acid, each animal was isolated in an individual cage and the normal construction was cumulatively counted for a period of20 min, beginning 3 min after acetic acid injection. The number of writhing and stretching was recorded and the % was calculated using the following ratio: % of protection= (Control mean—Treated mean)/Control mean * 100

Screening for anti-inflammatory activity by rat paw edema method: The normal paw volumes of all the rats were measured initially and were divided into four groups each consists of six animals treated orally with the vehicle as control (2% gum acacia), standard diclofenac sodium (20 mg/kg) and methanol extract (200 and 400mg/kg) respectively. Carrageenan (0.1 mL of a 1%suspension in saline) was injected sub plantar region of the right hind paw of each rat. The vehicle, drug and extract were administered 30 min prior to the injection of Carrageenan. The paw volumes of all the rats were recorded at 1, 2, 3 and 4 hours after Carrageenan treatment by using plethysmometer[13]. A significant reduction in the paw volume compared to vehicle treated control animals was considered as inflammatory response.

% Inhibition=
[(VT –V0) control—(VT –V0) treated groups] / (VT – V0) control *100

V0 = paw volume of the rat before administration of Carrageenan

VT = paw volume of the rat after administration ofCarrageenan at different time intervals

Statistical analysis: All the results were expressed as Mean ± Standard deviation (SD). Data was analyzed using one-way ANOVA followed by Dunnett's t-test. Pvalues <0.05 were considered as statistically significant.

Table I; Effect of methonal extract of *Pithecellobium dulce* on the Hot plate Method test in mice

Group	Reaction time after administration of control/ standard/extract in sec			
	0 min	60 min	120 min	240 min
Control	2.17 ± 0.75	2.33 ± 0.52	2.17 ± 0.41	1.97 ± 0.52
Pentazocine 10 mg/kg	2.83 ± 0.75	6.83 ± 0.75[b]	6.33 ± 1.63[b]	2.33 ± 0.52[a]
Pithecellobium dulce 200 mg/ kg	2.83 ± 0.75	7.17 ± 0.75[b]	7.67 ± 1.03[b]	2.00 ± 0.63
Pithecellobium dulce 400 mg/kg	3.00 ± 0.63	9.00 ± 0.89[b]	9.00 ± 0.89[b]	2.17 ± 0.75[a]

Values are in mean ± SD; (n =6), a= $p < 0.05$, b= $p < 0.0005$ Vs control

Table II: Effect of methonal extract from *Pithecellobium dulce* on acetic acid induced Writhing test in mice

Group	Number of writhes	% Inhibition
Control	79.5 ± 5.96	-
Diclofenac sodium 20 mg/kg	17.17 ± 3.46a	78.46 ± 3.57
Pithecellobium dulce 200 mg/ kg	40.83 ± 3.97a	48.13 ± 8.69
Pithecellobium dulce 400 mg/kg	32.5 ± 2.81a	58.76 ± 6.26

Values are in mean ± SD; (n =6); a= $p < 0.00001$ Vs control

Table III: Effect of methonal extract from *Pithecellobium dulce* on paw edema test in Rats

Group	1 hour	2 hour	3 hour	4 hour
Control	0.18 ± 0.02	0.20 ± 0.03	0.22 ± 0.03	0.18 ± 0.02
Diclofenac sodium 20 mg/kg	0.14 ± 0.01[a]	0.13 ± 0.02[b]	0.12 ± 0.02[c]	0.12 ± 0.01[c]
Pithecellobium dulce 200 mg/ kg	0.15 ± 0.02[a]	0.14 ± 0.02[b]	0.11 ± 0.01[c]	0.11 ± 0.01[c]
Pithecellobium dulce 400 mg/kg	0.15 ± 0.01[a]	0.13 ± 0.02[b]	0.11 ± 0.01[c]	0.10 ± 0.02[c]

Values are in mean ± SD; (n =6); a= $p < 0.05$, b=$p<0.001$, c=$p<0.00005$ Vs control

Results and Discussion

In this study, we have demonstrated the effect of *Pithecellobium dulce* extract (200 and 400 mg/kg; p.o.) on hot plate test and acetic acid induced writhing in mice. The results of hotplate test and acetic acid induced writhing test were shown in Table I and II. The extract (200 and 400 mg/kg) showed the significant increase in reaction time and reduction in the number of writhes induced by acetic acid in a dose dependent manner which were comparable with reference compounds, diclofenac and pentazocine respectively. A significant ($p<0.0005$) analgesic effect to the thermal stimulus was observed at 60 min with 200 and 400 mg/kg of *Pithecellobium dulce* which is comparable to the effect of standard pentazocine. The mouse writhing assay is useful test to evaluate mild analgesic agents. This method is not only simple and reliable but also affords rapid evaluation of peripheral

type of analgesic action. In this test the animals' react with characteristic stretching behavior, which is called writhing. Acetic acid causes algesia by liberating endogenous substances including serotonin, histamine, PGs, bradykinin and substance P which stimulate pain nerve endings[14]. Local peritoneal receptors are postulated to be partly involved in the abdominal constriction (writhing) response.

The method has been associated with prostanoids in general, i.e. increases levels of PGE2 and PGF2α in peritoneal fluids as well as lipoxygenase products[14] . It was found that extract significantly inhibited the acetic acid induced writhing response. The abdominal constriction is related to the sensitization of nociceptive receptors to prostaglandins. It is therefore possible that extract produced analgesic effect may be probably due to the inhibition of synthesis or action of prostaglandin.

Carrageenan-induced rat paw edema is a suitable test for evaluating anti-inflammatory drugs which has frequently been used to assess the anti-edematous effect of natural products[15] .Development of edema in the paw of the rat after injection of carrageenan is a biphasic event. The initial phase observed during the first hour is attributed to the release of histamine and serotonin. The second phase of edema is due to the release of prostaglandins, protease and lysosome[16,17] .Based on this, it could be argued that the suppression of the first phase may be due to inhibition of the release of early mediators, such as histamine and serotonin, and the action in the second phase may be explained by an inhibition of cyclooxygenase.Sub plantar injection of carrageenan in rats showed to a time-dependent increase in paw thickness(Table III); this increase was observed at 1 hour and was maximal at 3 hour after administration of carrageenan injection in the vehicle treated groups. The results methanol extract of *Pithecellobium dulce* against Carrageenan induced paw edema is shown in Table III. There was a dose dependent inhibitory activity in Carrageenan induced paw inflammation at all assessment times. Diclofenacsodium, a COX-inhibitor at the dose of 20 mg/kg, p.o.significantly reduced the paw edema. This indicates action against release of histamine, serotonin and kininsin early phase, while later phases are suspected to be arachidinate metabolites producing an edema dependent on mobilization of neutrophils[18].The result of the present study indicates that *Pithecellobium dulce* (200 and 400 mg/kg, p.o.) and indomethacin play a crucial role as protective factors against the carrageenan-induced acute inflammation.

In conclusion, this study demonstrated that the methanol extract of *Pithecellobium dulce* have a significant analgesic and anti inflammatory activity.

References

1. Anonymous. The Wealth of India: Raw materials, Vol.VIII. New Delhi: Publications and Information Directorate, CSIR, 1969: 140.
2. Nigam Shyam K, Mitra. and Chittranjan R. Fette 1971; 73(2): 75.
3. Bhargva Krishna P, Gupta M B.and Chittranjan R. *Indian J Med Res* 1970; 58:724.
4. Nigam S K, Misra G, Uddin R, Yoshikawa K, Kawamoto M.and Arihara S. *Phytochemistry* 1997; 44 (7): 1329.
5. Misra G, Nigam S K, Singh S, Nigam S G and Saxena R C. *Indian Drugs* 1979; 17(1):6.
6. Adinarayana D. and Ramachandraiah chetty P. *Indian J Chem.* 1985; 24B: 453.
7. Zapesochnaya G G, Yarosh E A, Syanidze N V.and Yarosh G I. Khim Prir Soed in, 1980**;** 2:252.
8. Saxena V K. and Singal M. *Fitoterapia* 1998; *69*(4):305.
9. Anup Banarjee. *J Indian Chem Soc* 2005; 82: 186.
10. Aruoma O I. *J Am Oil Chem Soc* 1998; 75:199. 11.
11. Eddy NB, LeimbackD.Synthetic analgesic. II. Dithienylbutenyland dithienylbutylamines. *J Pharmacol Exp Ther*.1953; 107: 385.
12. 12.Koster R, Anderson M, De-Beer EJ. Acetic acid analgesic screen. *Federation Proc.* 1959; 18: 418.
13. 13.Winter CA, Risley EA, Nuss GW. Carrageenan induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Proc. Soc. Exp. Biol. Med 1962; 111: 544.
14. 14. Ochi T, Motoyama Y, Goto T. The analgesic effect profile of FR122047, a selective cyclooxygenase-1 inhibitor, in chemical nociceptive models. *Eur J Pharmacol*. 2000; 391: 49.
15. 15.Panthong A, Kanjanapothi D, Taesotikul T, Wongcome T,Reutrakul V. Anti-inflammatory and antipyretic properties of *Clerodendrum petasites* S. Moore. *J Ethnopharmaco*l. 2003; 85:151.

16. 16. Vinegar R, Schreiber W, Hugo R. Biphasic development ofcarrageenan edema in rats. *J Pharmacol Exp Ther*. 1969; 166:96

17. 17.Crunkhon P, Meacock SER. Mediators of the inflammation induced in the rat paw by carrageenan. *Bri J Pharmacol*.1971; 42: 392.

18. 18.Just MJ, Recio MC, Giner RM, Cullar MJ, Manez S, Bilia AR. Anti-inflammatory activity of unusual lupine saponins from Bupleurum fruticescens. *Planta Medica* 1998; 64: 404.

*****