BJP

Bangladesh Journal of Pharmacology

Research Article

Analgesic and anti-inflammatory activities of leaf extract of *Kydia calycina* Roxb.

A Journal of the Bangladesh Pharmacological Society (BDPS)
Journal homepage: www.banglajol.info
Abstracted/indexed in Academic Search Complete, Agroforestry Abstracts, Asia Journals Online, Bangladesh Journals Online, Biological Abstracts, BIOSIS Previews, CAB Abstracts, Current Abstracts, Directory of Open Access Journals, EMBASE/Excerpta Medica, Global Health, Google Scholar, HINARI (WHO), International Pharmaceutical Abstracts, Open J-gate, Science Citation Index Expanded and Social Sciences Citation Index
Bangladesh J Pharmacol 2009; 4: 101-104
ISSN: 1991-0088

Analgesic and anti-inflammatory activities of leaf extract of *Kydia calycina* Roxb.

Baburao Bhukya[1], Rama Narsimha Reddy Anreddy[2], Carey M. William[1] and Krishna Mohan Gottumukkala[1]

[1]Department of Pharmacognosy and Ethnopharmacology; [2]Department of Pharmacology, University College of Pharmaceutical Sciences, Kakatiya University, Warangal 506009, India.

Article Info

Received: 2 March 2009
Accepted: 21 April 2009
Available Online: 1 May 2009

DOI: 10.3329/bjp.v4i2.2112

Cite this article:
Bhukya B, Anreddy RNR, William CM, Gottumukkala KM. Analgesic and anti-inflammatory activities of leaf extract of *Kydia calycina* Roxb. Bangladesh J Pharmacol. 2009; 4: 101-04.

Abstract

The methanol extract of leaves of *Kydia calycina* Roxb. 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 400 mg/kg body weight. A significant ($p<0.0005$) analgesic effect was observed with 200 and 400 mg/kg in both tests. The maximum anti-inflammatory response was produced at 3 hours with extract doses of 200 and 400 mg/kg. These results suggest that the methanol extract of *K. calycina* has exhibited significant analgesic and anti-inflammatory effects, which were comparable with standard drugs.

Introduction

Kydia calycina Roxb. (synonym: *Kydia fraternal)* is a herb of the family *Malvaceae* which is distributed in tropical Himalayas from the Indus eastwards to Myanmar (Burma) and in peninsular India from northern Maharastra and Madhya Pradesh south-wards, chiefly in mixed, moist, deciduous forests (Parrotta, 2001). The leaves of *K. calycina* were 7.5-15 cm long and wide, usually 3-7 lobed, apex angled or rounded, base cordate, palmately 7-nerved, hoary-tomentose beneath; petioles 2.5-5 cm. Among the Santalis, a paste of the pounded leaves is applied to relieve body pains, arthritis and lumbago; a poultice of the leaves is reportedly used to treat skin diseases (Parrotta, 2001). So far no information is available for the analgesic and anti inflammatory activity of the methanol extract of *K. calycina*. So, the present study has been undertaken to evaluate the analgesic and anti inflammatory activity of the methanol extract of *K. calycina* using hot plate, writhing and rat paw edema methods.

Materials and Methods

Plant material

The leaves of *K. calycina* were collected from Thirupathi hills, Andhara Pradesh, India. It was authenticated by Prof. V. Raju, Department of Botany, Kakatiya University, Warangal, India.

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°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 (Pure Pharma Ltd., Mumbai) and methanol (Merck, Mumbai).

Phytochemical screening

The methanol extract was screened for the presence of various phytoconstituents like steroids, alkaloids, terpenoids, glycosides, flavonoids, phenolic compounds and carbohydrates (Trease and Evan, 1983).

Animals

Wister rats (175-250 g) and albino mice (25-30 g) 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.

Hot-plate test

The hot plate test was used to measure analgesic activity by the method described by Eddy and Leimback (1953) with minor modifications. In this experiment, the hot plate was maintained at 55 ± 0.5°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 (Koster et al., 1959). The leaf extract of *K. calycina* 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 10 mg/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 of 20 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 x 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 400 mg/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 (Winter et al., 1962). A significant reduction in the paw volume compared to vehicle treated control animals was considered as inflammatory response.

%Inhibition= $[(V_T-V_0)$ control $-(V_T-V_0)$ treated groups$]$ / (V_T-V_0) control *100

V_0 = paw volume of the rat before administration of Carrageenan

V_T = paw volume of the rat after administration of Carrageenan 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. P values <0.05 were considered as statistically significant.

Results and Discussion

Preliminary phytochemical screening of the methanolic extract of *K. calycina* reveals the presence of steroids, terpenoids, carbohydrates and glycosides.

In this study, we have demonstrated the effect of extract (200 and 400 mg/kg; p.o.) on hot plate test and

Table I

Effect of methanol extract of *K. calycina* on the hot plate test in mice

Group	Reaction time after administration of control/ standard/extract in sec			
	0 min	60 min	120 min	240 min
Control	2.2 ± 0.8	2.3 ± 0.5	2.2 ± 0.4	2.0 ± 0.5
Pentazocine 10 mg/kg	2.8 ± 0.8	6.8 ± 0.8[b]	6.3 ± 1.6[b]	2.3 ± 0.5[a]
K. calycina 200 mg/kg	2.8 ± 0.8	7.2 ± 0.8[b]	7.7 ± 1.0[b]	2.0 ± 0.6
K. calycina 400 mg/kg	3.0 ± 0.6	9.0 ± 0.9[b]	9.0 ± 0.9[b]	2.2 ± 0.8[a]

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

Table II

Effect of methanol extract from *K. calycina* on ace-tic acid-induced writhing test in mice

Group	Number of writhes	% Inhibition
Control	79.5 ± 6.0	-
Diclofenac sodium 20 mg/kg	17.2 ± 3.5[a]	78.5 ± 3.6
K. calycina 200 mg/kg	40.8 ± 4.0[a]	48.1 ± 8.7
K. calycina 400 mg/kg	32.5 ± 2.8[a]	58.8 ± 6.3

Values are in mean ± SD; (n = 6); [a]p<0.00001 vs control

acetic acid induced writhing in mice. The results of hot plate 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 *K. calycina* 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 (Ochi et al., 2000). Local peritoneal receptors are postulated to be partly involved in the abdominal constriction (writhing) response.

Table III

Effect of methanol extract from *K. calycina* on the paw edema test in rats

Group	1 hour	2 hours	3 hours	4 hours
Control	0.2 ± 0.02	0.2 ± 0.03	0.2 ± 0.03	0.2 ± 0.02
Diclofenac sodium 20 mg/kg	0.1 ± 0.01[a]	0.1 ± 0.02[b]	0.1 ± 0.02[c]	0.1 ± 0.01[c]
K. calycina 200 mg/kg	0.2 ± 0.02[a]	0.1 ± 0.02[b]	0.1 ± 0.01[c]	0.1 ± 0.01[c]
K. calycina 400 mg/kg	0.2 ± 0.01[a]	0.1 ± 0.02[b]	0.1 ± 0.01[c]	0.1 ± 0.02[c]

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

The method has been associated with prostanoids in general, i.e. increases levels of PGE_2 and $PGF_{2\alpha}$ in peritoneal fluids as well as lipoxygenase products (Ochi et al., 2000). 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 (Panthong et al., 2003). 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 (Vinegar et al., 1969; Crunkhon and Meacock, 1971). 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 cyclo-oxygenase. 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 hours after administration of carrageenan injection in the vehicle treated groups. The results of MEKC 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. Diclofenac sodium, 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 kinins in early phase, while later phases are suspected to be arachidinate metabolites producing an edema-dependent on mobilization of neutrophils (Just et al., 1998). The result of the present study indicates that PG (200 and 400 mg/kg, p.o.) and indomethacin play a crucial role as protective factors against the carrageenan-induced acute inflammation.

Conclusion

This study demonstrates that the methanol extract of *K. calycina* has a significant analgesic and anti-inflammatory activity.

Financial Support

University Grants Commission, India

Ethical Issue

Experiments on animals were performed in accordance with guidelines of the Institutional Animal Ethical Committee.

Conflict of Interest

Authors declare no conflict of interest.

Acknowledgements

The authors wish to thank Principal, UCPSc, Kakatiya University, Warangal for providing the research facilities. The first author also wishes to thank University Grants Commission, India for providing Junior Research Fellowship.

References

Crunkhon P, Meacock SER. Mediators of the inflammation induced in the rat paw by carrageenan. Bri J Pharmacol. 1971; 42: 392-402.

Eddy NB, Leimback D. Synthetic analgesic. II. Dithienyl-butenyl and dithienylbutylamines. J Pharmacol Exp Ther. 1953; 107: 385-93.

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: 404-07.

Koster R, Anderson M, De-Beer EJ. Acetic acid analgesic screen. Federation Proc. 1959; 18: 418-20.

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: 49–54.

Panthong A, Kanjanapothi D, Taesotikul T, Wongcome T, Reutrakul V. Anti-inflammatory and antipyretic properties of *Clerodendrum petasites* S. Moore. J Ethnopharmacol. 2003; 85: 151-56.

Parrotta JA. Healing plants of peninsular India. New Delhi, CABI Publishing, 2001, pp 480-81.

Trease EG, Evan WC. Textbook of pharmacognosy. 12th ed, Oxford, Alden Press, 1983, pp 343-83.

Vinegar R, Schreiber W, Hugo R. Biphasic development of carrageenan edema in rats. J Pharmacol Exp Ther. 1969; 166: 96-103.

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: 544-47.

Author Info
Baburao Bhukya (Principal contact)
e-mail: babupharma79@gmail.com