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Research Article

Analgesic and Anti Inflammatory Activity of the Leaves of Caryota urens Linn.

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ABSTRACT

Caryota urens Linn (Palmae) commonly known as Sugar palm is used in traditional medicine to treat hemicrania and rheumatic swelling. Methanolic extract of plant leaves was used to perform analgesic and anti inflammatory activity. Analgesic study was performed by hot plate and tail flick method and anti-inflammatory effect analyzed by carrageenan induced paw edema. The methanol extract produced significant dose-dependent inhibition of carrageenan-induced rat paw edema. The extract also showed marked analgesic activity. In both the model of analgesic activity methanolic extract of Caryota urens at doses of 200 mg/kg and 400 mg/ kg significantly increase reaction time in rats when compared with control. The dose 400 mg/kg of Caryota urens was highly significant which is comparable with standard in both the studies.Phytochemical investigation of Caryota urens extracts shows presence of phytosterols, tepenoids, flavonoids , tannins and Phenolic compounds which has been responsible for analgesic and anti inflammatory activity.

Keywords: Caryota urens, Hot plate method, Tail flick method, Carageenan induced rat paw edema, Analgesic and anti inflammatory activity.

INTRODUCTION

The present study was undertaken to find out the analgesic and anti inflammatory activity of crude methanol extracts of Caryota urens leaves. Caryota urens is cultivated in Sub Himalaya tract from Nepal eastwards, Assam, Khasi Hills,Orissa, sub montane forests of Mysore,in Malabar, Coorg, Konkan, Cochin and Travancore, also in tropical Asia, Malaya, Singapore, Australia and Ceylon have been used in India for the treatment of urinary disorders , hemicranias, snake bite poisoning, rheumatic swelling and jaundice [1-4]. Preliminary phytochemical investigation of Caryota urens extracts indicated presence of proteins, fats, minerals, fibres, carbohydrates and vitamins and tannins identified were epicatechin, gallocatechin and epicatechin gallate. [5]

MATERIALS AND METHODS

Preparation of herbal powder

The plant Caryota urens was collected in the month of October-November from the area around New Vallabh Vidyanagar of Gujarat State. The collected plant was authentified by Dr. Bhanu Kakrani , Dept. of Botany , Tolani College of Arts & Science , Adipur (Kutch) and deposited this plant (voucher specimen no. MRP/CU-1/5/ARCP/11) to Department of Pharmacognosy of A.R. College of Pharmacy, Vallabh Vidyanagar. The plants were dried under shade and separate the leaf. Leaves were powdered to 60# separately and stored in airtight containers. About 200g of coarsely powered drug was successively extracted with petroleum ether, toluene, chloroform, acetone, methanol and water. All the extracts were filtered and dried at reduced pressure (40□C) and methanol extract was used for present investigation. The extracts and fraction were subjected to phytochemical analysis for constituent identification using standard procedures. [6, 7]

Animal used: Mice of either sex weighing between 250-300 grams from Cadia Pharmaceutical Research Centre, Changodar, Ahmedabad were divided in three groups. The animal experiment was approved by Animal Ethical Committee of Institute under the CPCSEA guideline no. as CPCSEA /IAES/ARCP/10-11/10.

Toxicity studies [8]

The acute oral toxicity studies were performed to study the acute toxic effects and to determine minimum lethal dose of the drug extracts. Swiss albino mice of either sex weighing 18-25 g were used for the study. The aqueous and alcohol extracts were administered orally to different groups of overnight fasted mice at the doses of 30, 100, 300, 1000 and 3000 mg/kg body weight. After administration of the extracts, animals were observed continuously for the first three hours for any toxic manifestation. Thereafter, observations were made at regular intervals for 24 hrs. Further the animals were under investigation up to a period of one week.

Analgesic activity

Hot plate method

The animals were divided into six groups of 6 animals each. Group I served as control. Group II served as standard and were injected Indomethacin (20 mg/kg) intraperitonially. Group III and IV were treated orally with alcoholic extract of 200 and 400 mg/kg body weight respectively. The animals were individually placed on the hot plate maintained at 55 ± 1°C, one hour after their respective treatments. The response time was noted as the time at which animals reacted to the pain stimulus either by paw licking or jump response, whichever appeared first. The cut off time for the reaction was 15 seconds to avoid damage to the paws.

Statistical analysis

Data were statistically analyzed by analysis of variance (ANOVA) with the level of significance set at $p < 0.05$. Critical differences between means were evaluated by Dunnett's multiple comparison test and Student's t-test at

p< 0.05 (Sokal and Rohlf, 1995)

Tail flick method

The animals were divided into six groups of 6 animals each. Group I served as control. Group II served as standard and were injected Indomethacin (20 mg/kg) intraperitonially. Group III and IV were treated orally with alcoholic extract of 100 and 200 mg/kg body weight respectively. After one hour, the tip of tail was kept at the radiant heat source. The response time was noted as the sudden withdrawal of the tail from the heat source. Cut off time of 10 seconds was maintained to avoid damage to the tail for all groups. The time required for flicking of the tail, was recorded, to assess response to noxious stimulus.

Statistical analysis

Data were statistically analyzed by analysis of variance (ANOVA) with the level of significance set at p< 0.05. Critical differences between means were evaluated by Dunnett's multiple comparison tests and Student's t-test at p < 0.05 (Sokal and Rohlf, 1995).

Anti inflammatory activity

Carageenan induced rat paw edema method

The methanol extract was evaluated for their anti-inflammatory activity on a carrageenan-induced rat paw edema model. Inflammation was produced in the rats (Female, Wistar, weighing 250- 400 g) using 100 µL of 1% carrageenan (wt/vol) in distilled water. This was injected into the plantar surface of the rat's left hind paw. To evaluate the topical anti-inflammatory activity of the formulation, 3 groups of animals (n = 6) with carrageenan-induced paw edema were examined. The test drug was given i.p on the paw. The increase in paw volume was measured before (time 0) and 1, 2 and 3 hours after carrageenan administration. The difference between the two readings was taken as the volume of the edema and % inhibition was calculated using the formula mentioned below:

% Inhibition in edema = (A-B/ A)* 100

Where,

A= Mean paw volume in untreated control group

B= Mean paw volume of treated

Table 1: Effect of extracts of Leaves using Hot plate method

Group	Dose in mg/kg	Basal reaction time in sec	Reaction time in sec at 1 hour
Control (Normal saline)	--	3.8	3.833 ± 0.567
Standard (Indomethacin)	20	3.7	9.983 ± 0.427**
Methanol extract	200	4.3	6.100 ± 0.304*
	400	4.2	9.283 ± 0.533**

n = 6 in each group number of rats in each group done by one way analysis of variance followed by Dunett's test. Control vs all group * *P< 0.001 highly significant as compare to control, *P< 0.05 more significant as compare to control.

Different groups used in the activity are mentioned below:
Group-1: Control,
Group-2: standard drug and
Group-3: test drug

All groups are treated with 0.1 ml of 1% Carrageenan solution. Results are compared to control group.

RESULT AND DISCUSSION

The hot plate method and tail flick method was used to evaluate analgesic activity of drugs by observing increase in reaction time of rats in 1 hr. In hot plate method rats in control group showed 3.8 sec reaction time at 1 hr. which was significantly increased to 9.9 sec by standard drug Indomethacin (20mg/kg) and to 6.1 sec and 9.2 sec by methanolic extract of Caryota urens at the doses of 200 mg/kg and 400 mg/kg same way in tail flick method rats in control group showed 3.9 sec reaction time at 1 hr. which was significantly increased to 10.8 sec by standard drug Indomethacin (20mg / kg) and to 5.45 sec and 9.9 sec by methanolic extract of Caryota urens at the doses of 200 mg/kg and 400 mg / kg. Thus both the standard drug Indomethacin and methanolic extract of Caryota urens significantly increase reaction time in rats when compared with control.The dose 400 mg / kg of Caryota urens was highly significant which is comparable with standard.

Table 2: Effect of extracts of leaves using Tail flick method

Group	Dose in mg/kg	Basal reaction time in sec	Reaction time in sec at 1 hour
Control (Normal saline)	--	3.9	3.90 ± 0.650
Standard (Indomethacin)	20	4.9	10.80± 0.404**
Methanol extract	200	4.9	5.45 ± 0.391*
	400	5.3	9.9 ± 1.224*

n=6 number of rats in each group done by one way analysis of variance followed by Dunett's test; Control vs all group **P< 0.001 Highly significant,*P< 0.05 more significant.

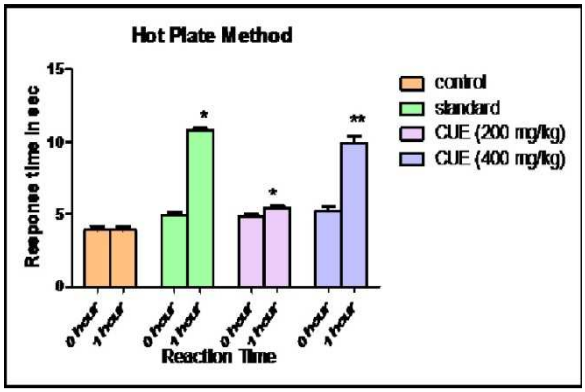

Figure 1 Histograph of Comparison of basal reaction time vs time by hot plate method (n = 6 in each group number of rats in each group done by one way analysis of variance followed by Dunett's test. Control vs all group* *P< 0.001 highly significant as compare to control, *P< 0.005 more significant as compare to control.)

The hot plate method and tail flick method have been found to be suitable for evaluation of centrally acting analgesics [9]. The nociceptors seem to be sensitized by sensory nerves. The involvement of endogenous substances such as PGs may be minimized in this model [10] .In centrally acting analgesic methods drug in 200 mg/kg and

400 mg/kg were found to be significantely effective which is comparable with standard drug Indomethacin. Hence from the study we can conclude that analgesic effect of methanolic extract of Caryota urens is may be due to effect on release of prostaglandin.

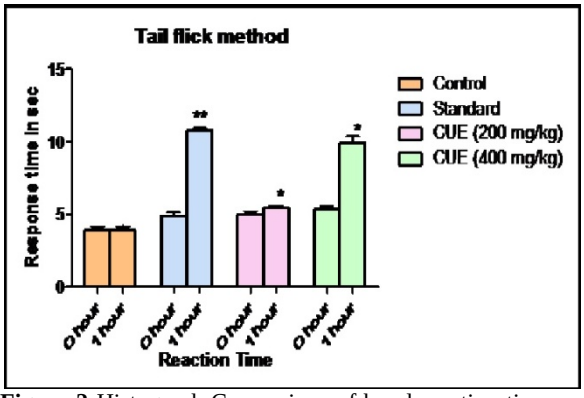

Figure 2 Histograph Comparison of basal reaction time vs time by tail flick method (n = 6 in each group number of rats in each group done by one way analysis of variance followed by Dunett's test. *P< 0.001 significant as compare to control, *P< 0.05 less significant.)

The rat paw edema model is used to evaluate anti inflammatory activity of drugs by observing change in thickness of rat paw for 3 hrs. Rats in standard group showed highly significant % inhibition in rat paw when compared with control. The methanolic extract of Caryota urens also showed more significant % inhibition which was comparable to standard. Carageenan induced rat paw edema has been a popular inflammatory model to investigate anti inflammatory effect of compounds. [11]

It has a biphasic effect .The first phase due to release of histamine and serotonin (0-2 hrs), plateu phase is maintainted by kinin like substance (3 hrs) and second accelerating phase of swelling is attributed to PG release (>4 hrs) [12]. In our study methanol extract of Caryota urens (200 mg/kg, 400 mg/kg) significanly reduced edema indused by carageenan in whole three phases.

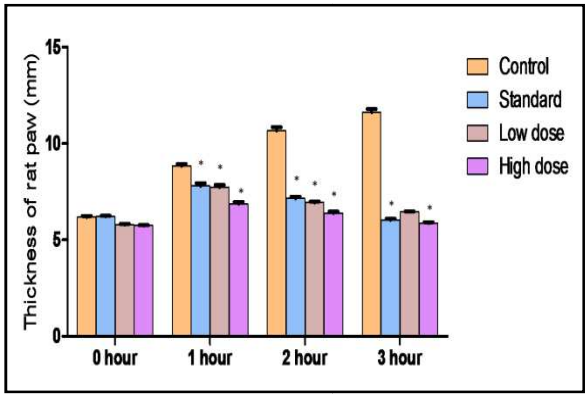

Figure 3: Histograph of Comparison of thickness of rat paw vs time by rat paw edema method
(n = 6 in each group number of rats in each group done by one way analysis of variance followed by Dunett's test. **P< 0.001 highly significant as compare to control, *P< 0.005 more significant)

Hence it can be concluded that methanolic extract of Caryota urens possesses analgesic and anti inflammatory propery that may be mediated by the presence of sterols or flavanoids [13] or it may be presence of terpenoids.[14]

Table 3: Effect of extracts of leaves using rat paw edema method

Animal group	Drug dose (mg/kg)	Changes in paw thickness (mm) % Inhibition			
		0 hr	1 hr	2 hr	3 hr
Control (Normal saline)	--	6.1783±0.0697	8.85±0.099	10.683± 0.172	11.625±0.173
Standard-Indomethacin	20	6.235±0.0270 *	7.8033±0.137*	7.158±0.084 *	6.05±0.0428 *
Low dose methanolic extract	200	5.79±0.0444*	7.742±0.117 *	6.95±0.044 *	6.468±0.0176*
High dose methanolic extract	400	5.75±0.0175 *	6.883±0.843 *	6.388±0.079 *	5.881±0.0341 *

n=6 number of rats in each group done by one way analysis of variance followed by Dunett's test; Control vs all group **P< 0.001 Highly significant,*P< 0.05 more significant.

CONCLUSION

Caryota urens methanol extract of leaves showed the analgesic and anti inflammatory effect. Analgesic activity of methanolic extract by Hot plate method and Tail flick method and Anti inflammatory activity of methanolic extract by carageenan induced rat paw edema method showed good activity .But as compared to standard methanol extract gave less analgesic and anti inflammatory effect.

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