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Study of anti inflammatory and analgesic activity of methanolic extracts of *Cedrus deodara*

Hemalatha S.Manne*, B.Basavaraju B, V.Bindhu, S.Keerthi, Namratha N.Vernekar, Varsha Laturkar

GM Institute of Technology, Department of Biotechnology, Davangere-577006.

Tel: +9109448979918

E-mail: hemalathamanne@yahoo.co.in

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ABSTRACT

Methanolic extract of stem bark of *Cedrus deodara,* an Indian medicinal plant was screened for its anti-inflammatory and analgesic activity against carrageenan induced rat paw edema and acetic acid induced writhing in Albino rats was compared with Aspirin and Diclofenac sodium. The tests were conducted in laboratory maintained albino rats at doses of 50mg/Kg and 100 mg/kg. It showed significant anti-inflammatory activity at dose of 100mg/Kg i.e 43.47% inhibition. It also showed analgesic activity of 55.80% protection at dose of 100mg/Kg.

KEYWORDS

Cedrus deodara;
Methanolic extract;
Anti-inflammatory;
Analgesic.

INTRODUCTION

Cedrus deodara (Pianaceae) has traditionally been used in many parts of India for its diuretic, anti-inflammatory, anticonvulsant, antibacterial, antistress and antihepatotoxic properties[1,2,3]; it is also used in treatment of fevers, flatulence, pulmonary and urinary disorders, rheumatism, piles, kidney stones, insomnia, diabetes etc.[4].It has been used as an antidote to snakebites[5]. It is a large evergreen coniferous tree reaching 40-50m tall, exceptionally 60m, with a trunk up to 3m diameter. It has a conic crown with level branches and drooping branch lets[6]. The leaves are needle-like, mostly 2.5-5cm long, occasionally up to 7cm long, slender (1mm thick), borne singly on long shoots, and in dense clusters of 20-30 on short shoots; they vary from bright green to blue-green in colour. The female cones are barrel-shaped, 7-13cm long and 5-8 cm broad, and disintegrate when mature (in 12months) to release the winged seeds. The male cones are 4-6cm long, and shed their pollen in autumn.

Phytochemical investigation showed that Cedrus deodara contains a large number of compounds such as flavonoids (deodarin, taxifolin, quercetin1, Aleoresin, Turpentine, Atlantone) and Sequiterpenes like himachalol. The plant contains heavy metals like Arsenic, Cadmium, Lead and Mercury not more than 1ppm. Older literature suggests that the characteristic odour of C. deodora is due to the presence of *p*-methyl-δ-3- tetrahydroacetophenone, but the oil also contains p-methyl acetophenone, cis- and trans- atlantones, α- and β-himalchenes, ar-dihydroturmerone as well as (+)-himachalol and (+)-allohimachalol, amongst others. The oil sold to aromatherapists is usually rectified the

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unrectified oil is dirtier and more crudely resinous and urinic, also possessing a slight cassis note[6]. We are interested in searching the most effective and safe indigenous analgesic and anti-inflammatory preparation and to provide an experimental basis for the efficacy of analgesic and anti-inflammatory preparation which were already used in Ayurveda. We are also interested in comparing the methanolic bark extract of *C. deodora* with standard drugs Aspirin and Diclofenac sodium.

MATERIALS AND METHODS

Extraction of powder using soxhlet apparatus

The Cedrus Deodara powder was collected from Ayurvedic hospital, Hubli. About 10g of powder was extracted using soxhlet apparatus for 16 hours in 100ml of solvent (i.e. 98.22% Methanol).This process was carried out for successive days and the thick crude extract was dried for 4-5 days in open air. 250g of powder yields around 13-14g crude extract.

Confirmatory tests

To confirm the crude extract some qualitative tests like test for Carbohydrates, Proteins, Amino acids, Flavonoids and Alkaloids were carried out[7].

Major tests

Animals: The animals used in the present experimental work are mainly colony breed albino rats of either sex weighing between 150-200gms were obtained from the animal house of SCS Pharmacy College, Harapanahalli. The animals are maintained under standard laboratory conditions (light period of 12hrs/day and temperature $27^0C \pm 2^0C$), with free access to food, water and libitum. Each group consisted of 6 animals of either sex randomly selected.

Drugs: The following drugs were used in our experimental work

1. Aspirin –for testing peripheral activity
2. Diclofenac Sodium- for testing anti-inflammatory activity
3. Carrageenan- as a phlogistic agent
4. Gum acacia- as suspending agent.

Drug preparation & doses of drugs

1. Standard drugs were suspended in 2% gum acacia in water & administered dose of 10mg/Kg.
2. *Cedrus deodara* methanolic stem bark extract was suspended in 2% gum acacia in water and administered in a dose of 100mg/Kg.
3. 3.Carrageenan (1%) was suspended in normal saline and injected in a dose of 0.1 ml 1%w/v suspension of carrageenan was injected into the subplantar region of the left hind paw to all group of animals.
4. Doses of the drugs were calculated for each animal based on the body weight and respective volumes were administered.

Plethysmograph

Mercury Plethysmograph is used to measure paw edema in albino rats. Fabrication of this instrument consists vertical glass cylinder which is 8cm in length and 14mm inside diameter. The cylinder is connected to a graduated pipette by means of connecting rubber tube of 3mm diameter. The pipette capacity is 2ml and divided into 200 divisions. The pipette is connected to a glass syringe with pressure tubing. The zero mark is so adjusted that mercury level was adjusted zero mark by the help of syringe. The hind paw of the rat was made to dip in the mercury in cylinder. As a result the levels of mercury go little higher keeping the paw inside the cylinder. The level of mercury was brought down by drawing piston of syringe. This result in elevation of mercury level in pipette and the amount of increase from 0 marks on pipette is taken as actual increase in paw volume. This procedure needs two operators 1 to dip the rat paw and the other to adjust mercury levels.

Exclusion criteria

1. Weight of the rats below 150gms and above
2. More than 4 months of age
3. within 21days of life
4. Any visible disease

Evaluation of anti-inflammatory activity

The extract (suspended in 2 % aqueous gum acacia) was screened for anti-inflammatory activity by carrageenan induced hind paw edema method in albino rats (Wistar strain of either sex, 150-200g)[8]. Rats were divided into four groups of six animals each. Group I served as control received 0.2ml 2% aqueous gum acacia, Group II served as standard received 10mg/kg, p.o. of diclofenac sodium and groups III & group

IV were treated with 50 & 100mg/kg, p.o. of test extract in 2% aqueous gum acacia.

After 1hr, 0.1ml of 1%w/v suspension of carrageenan was injected into the subplantar region of the left hind paw to all group of animals. The paw volume was measured plethysmographically after 3 hrs of carrageenan administration and the % inhibition of edema was calculated[9].

The percentage inhibition of paw volume was calculated by using the formula

% Inhibition =(1-Vt/Vc)×100

Where, Vt=Mean increase in the paw volume in test animals group; Vc=Mean increase in the paw volume in control group.

Statistical significance was analysed using one way ANOVA followed by Turkey-Krammer multiple comparison test and P<0.001 was considered significant.

Evaluation of analgesic activity

Acetic acid induced writhing model was used to evaluate analgesic activity of the compounds. After an over night fast, mice(20-25g) were divided into four groups of six each and 0.6%v/v acetic acid (dose 1ml/100g body weight) was administered ip. . Group I (Control) received 0.2ml of. 2% aqueous gum acacia, group II(standard) received aspirin 100mg/kg body weight & groups III & IV were administered orally with the suspension of test extract in 2% aqueous gum acacia at doses of 50 & 100mg/Kg body weight of the animals 1 hr before the injection of acetic acid. The numbers of writhes [constriction of abdomen, turning of trunk (twist) and extension of hind legs] were recorded 10 min after administering acetic acid for next 10min. The percentage protection for each group was calculated and compared with the control[10,11]. Statistical significance was analysed using one way ANOVA followed by Turkey-Krammer Multiple Comparison Test and P<0.001 was considered significant.

The percentage protection was calculated using the formula

%protection= (Mean no. of writhings in control group–Mean no. of writhings in treated group/Mean no. of writhings in control group)×100

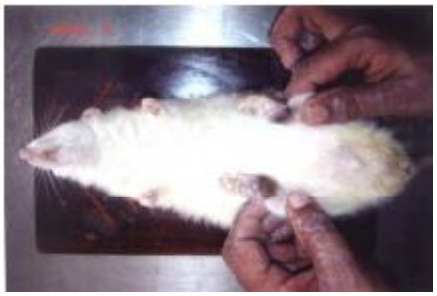

Figure 1: Carrageenan induced rat paw edema

RESULTS

Test extract exhibited dose dependent significant analgesic & anti-inflammatory activity compared to control groups. In the present study analgesic & anti-inflammatory activity of the extract was found less effective than the respective reference standard drugs.

DISCUSSION

The Phytochemical research has demonstrated the presence of flavonoids in *Cedrus deodara*, which may have role in its anti-inflammatory and analgesic activity and the results were displayed in TABLE 1.

The complete process of inflammation generally consists of three phases, viz. (1) dilatation and increased permeability of small blood vessels resulting in edema and swelling, (2) immigration of leukocytes from venules and capillary, cellular infiltration and general mopping of reaction (3) proliferation of fibroblasts and synthesis of new connective tissue to repair the injury .

Pain is produced when there is physiological alteration due to diverse conditions of noxious stimuli and also produced by inflammation. No single animal model

TABLE 1: Qualitative tests

Qualitative tests	Confirmatory tests	Results
Test for carbohydrates	Molish test	-
	Barfoed's test	-
	Benedict's test	-
Test for proteins	Millon's test	-
	Xanthoproteic test	-
	Biuret test	-
	Ninhydrin test	-
Test for flavonoids	Shinoda test	+
	Alkaline reagent test	+
	Zinc-hydrochloric test	+
	Ferric chloride test	+
Test for alkaloids	Drangendroff's test	-
	Mayer's test	-
	Hager's test	-
	Wagner's test	-

+Positive result; -Negative result

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TABLE 2: Results of anti-inflammatory activity

Group	Paw volume after 3hrs±SEM	% Inhibition
Control	0.92±0.06	-
Standard diclofenac	0.29±0.02*	68.47
Test extract 50mg/kg	0.67± 0.04*	27.17
Test extract 100mg/kg	0.52 ± 0.04*	43.47

Results are mean ±SEM (n=6); *P<0.001 vs control

TABLE 3: Results of analgesic activity

Group	Mean no. of writhings	% Protection
Control	34.28 ± 4.26	-
Standard aspirin	08.46± 1.20*	75.32
Test extract 50mg/kg	22.80 ± 3.18*	33.48
Test extract 100mg/kg	15.15± 1.80*	55.80

Results are mean ±SEM (n=6), *P<0.001 vs. control

can thus be sufficient to assess, the analgesic and anti-inflammatory effect of drugs. Therefore in the present study , the analgesic and anti-inflammatory effect of *Cedrus deodara* was studied in two models, viz Carrageenan induced paw edema and acetic acid induced writhing method were employed.

In this study the anti-inflammatory and analgesic effect of *Cedrus deodara* was compared with control(group I) and standard drug(group II). The inhibition of Carrageenan induced inflammation in rats is an established model to screen compounds for potential anti-inflammatory activity. The development of Carrageenan induced inflammation is bi-phasic; the first phase occurs within one hour of Carrageenan administration and attributed to the release of serotonin and histamine from the mast cell, the second phase(occur after more than one hour) is mediated by an increased release of prostaglandins in the inflammatory area and continuity between the two phases is provided by Kinins.

In our present study, in Carrageenan induced paw edema, the *Cedrus deodara* aqueous stem bark extract treated group showed highly significant anti-inflammatory activity(inhibition of paw edema), when compared to control and standard drug treated groups($p<0.001$) and the results were displayed in TABLE 2.

The analgesic activity of *Cedrus deodara* methanolic bark extract was studied in acetic acid induced writhing method showed significant analgesic effect ($p<0.001$) ad the results were displayed in TABLE 3.

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