ANTI-INFLAMMATORY ACTIVITY OF CEIBA PENTANDRA L. SEED EXTRACTS

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Abstract: Present study was carried out to evaluate the anti-inflammatory effects of petroleum ether and ethanolic extract of the seeds of Ceiba pentandra (L). Gaertn. (Bombacaceae) commonly known as silk cotton tree. The plant has been used as antibacterial, anti-inflammatory, antipyretic, for rheumatism, diabetes and in headache in Indian system of traditional medicine. Anti-inflammatory activity was assessed by induction of carrageenan in left hind paw. CPE and CPO at both administered doses of 200 mg/kg and 400 mg/kg reduced paw edema volume significantly. These results clearly show anti-inflammatory effect of seed extracts. Further studies are needed to isolate phytoconstituent(s) responsible for anti-inflammatory effect.

Key words: Ceiba pentandra, Anti-inflammatory effects

INTRODUCTION

India is a rich source of medicinal plants and a number of plant derived oils and extracts are used against diseases in various systems of medicine such as Ayurveda, Unani and Siddha. Only a few of them have been scientifically explored. Plants have been known to synthesize a variety of chemical substances such as alkaloids, glycoside, steroids, phenolic compounds, triterpenoids, tannins, fats and oils. These secondary metabolites have profound effects on animals along with therapeutic properties [1].

Inflammation is a pathophysiological response of living tissue to injuries that leads to the local accumulation of plasmic fluid and blood cells. The complex events and mediators involved in the inflammatory reaction can induce, maintain or aggravate many diseases. However, studies have been continuing on inflammatory diseases and the side effects of currently available anti-inflammatory drugs pose a major problem during their clinical uses. Therefore development of newer and more substantial anti-inflammatory drugs with lesser side effects is necessary [2].

Ceiba pentandra L. Gaertn. (Bombacaceae) commonly called silk-cotton, is a tropical tree and the capsules are known as Kapok. It is widely spread around the world ranging from tropical America to Asia through Africa and cultivated in Southeast Asia, Western South India, Sri Lanka, other parts of East Asia and Africa. This plant has been used in traditional medicine for the treatment of several ailments like headache, dizziness, diabetes, diuretic, fever, hypertension, constipation, mental troubles, peptic ulcer, rheumatism and leprosy [3-6].

Reports on plant parts have shown that stem bark of the plant possesses chronic hypoglycemic activity [7]; fresh bark has antibacterial and antifungal activity [8]; dried bark used in chest pains and as diuretic [9] and leaves extract for treatment of fever [10]. Previous work on C. pentandra described the isolation of number of sesquiterpenoids naphthoquinones [11]; two new isoflavones, pentandrin and pentandrin 5’-O-β-D-glucoside [12] and vavain and its glucoside have been isolated [13]. In the present study, we examined the anti-inflammatory activity of C. pentandra seed oil and ethanolic extract.
MATERIALS AND METHODS

Collection and authentication of plant material: The *C. pentandra* seeds were collected from the campus of Sitabai Thite College of Pharmacy, Shirur, Dist. Pune. The plant was identified taxonomically by Prof. T. Chakraborty, Scientist D, Botanical Survey of India, Pune. A voucher specimen was deposited in herbarium as AMSCP2.

Preparation of petroleum ether and ethanolic extracts: The collected seeds were sun dried in open air for 5 days and reduced to coarse powder (200 g). The powder was subjected to successive extraction in soxhlet extractor as per standard American Oil Chemical Society procedure using petroleum ether (40-60°C) and ethanol at their boiling points for 24 h and 48 h respectively. The extracts were filtered and filtrate was evaporated by distillation under reduced pressure using rotary vacuum evaporator at 30°C and stored in the dark at 4°C. The extraction yielded 24.39% w/w of petroleum ether extract (CPO) and 5.97% w/w of dried ethanolic extract (CPE).

Phytochemical screening: Phytochemical screening of CPO and CPE was carried out employing standard procedures and tests [14,15] to assess the presence of chemical constituents such as alkaloids, flavonoids, tannins, saponins, carbohydrates, steroids, fats and oils, glycosides, proteins etc.

Animals: Female Wistar albino rats (Sri Venkateshwara Traders, Bangalore) weighing between 150-200 g were selected for this study. They were housed in acryl fiber cages at 24 ± 2°C, humidity 50 ±1.0% and were kept on a 12 h light/dark cycle. They were fed with standard pellet diet (Amrut laboratories, Sangli) and water ad libitum and acclimated for 7 days before experimentation. Rats were fasted for 12 h before each test. Experimental protocols were approved by the Institutional Animal Ethical Committee of CPCSEA, Govt. of India (IAEC-Resolution No.13, 31-7-2010) were carried out in accordance with local IAEC guidelines.

Chemicals and drugs: Pure chemicals carrageenan and Tween 80 were purchased from Himedia lab. Mumbai. and Loba Chemie, Mumbai, respectively. Reference drugs aspirin (Aspin-100, Cipla pharmaceuticals), Sterile water for injection (Core Health Care Ltd.) and all other chemicals and solvents used are of analytical grades purchased from local market.

Drug administration: The reference drug and extracts used were orally administered with the aid of stainless metallic feeding canula in equivalent volume of 0.5 ml/100 g body weight of animal. A 1% tween 80 was used for preparation of doses of extracts.

Acute toxicity assay: Acute toxicity assay was performed as per OECD guidelines 423 (limit test). Six female Wistar albino rats (three animals in each step) were randomly selected. The animals were kept fasting overnight providing only water. Then the extracts CPO and CPE were administered orally at one dose level of 2000 mg/kg b.w. In further, rats were observed continuously for the first 4 h and then periodically up to 24 h for toxic symptoms and mortality.

Evaluation of carrageenan induced inflammation: Acute inflammation was produced by injecting 0.1 ml of 1% carrageenan in sterile WFI into sub plantar region of rat left hind paw [16,17]. The extracts CPO, CPE (200 mg/kg and 400 mg/kg) were administered orally 1 h before carrageenan injection. The control group received equivalent volume of vehicle. The paw volume was measured at 0, 1, 2, 3, 4 and 5 h, using plethysmometer. The mean changes in injected paw edema volume with respect to initial paw volume, were calculated. Percentage inhibition of paw edema volume with respect to control group was calculated using following formula.\( I = \left(1 - \frac{D}{C}\right) \times 1, \) where \( I = \% \) inhibition of paw edema, \( D = \) mean change in paw volume of treated rats, \( C = \) mean change in paw volume of control rats.

Statistical analysis: The results were expressed as mean ± SEM. Statistical significance was determined using one way analysis of variance test (ANOVA), followed by Dunnet’s \( t \)-test, value with \( p<0.05 \) considered as statistically significant.

RESULTS

Phytochemical screening: The phytochemical screening of the CPO showed presence of protein, fats and oil, steroids and triterpenoids. CPE showed presence of carbohydrates, proteins, alkaloids, tannins and phenolic compounds.
Acute oral toxicity: Acute oral toxicity study was carried out according to OECD guidelines. The tested extracts did not exhibit any acute toxicity symptoms and mortality in all groups when given orally at dose of 2000 mg/kg bw. Hence, both the extracts were safe up to dose of 2000 mg/kg bw orally. Two doses (200 and 400 mg/kg, p.o.) i.e 1/10th and 1/5th of LD50 were selected for pharmacological studies.

Carrageenan induced inflammation: The anti-inflammatory activity of CPO and CPE was evaluated at the dose of 200 and 400 mg/kg body weight on carrageenan induced paw edema on experimental rats (Table 1). There was gradual increase in edema paw volume in rats in disease control group showing its maximum value at 4 h. The result showed significant anti-inflammatory activity (p<0.001) by treated extracts. CPO at higher and CPE at lower dose showed significant reduction in paw edema 2-5 h. Maximum percent inhibition of paw edema volume in all extracts was found at 5 h. Amongst tested extracts CPO at higher dose showed maximum inhibition of paw edema (44.39) whereas aspirin as reference drug showed inhibition (67.69) at 5 h.

DISCUSSION

Anti-inflammatory activity of petroleum ether and ethanolic extract of C. pentandra seeds revealed its traditional claim. This plant has long been used for its anti-inflammatory activity but work on seed extracts lacked a scientific support. The results of present study reveal the anti-inflammatory activity of tested extracts in acute phase of inflammation. Carrageenan induced rat paw edema is a suitable test for evaluating anti-inflammatory drugs and has frequently been used to assess the anti-edematous effects of natural products [18].

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 release of histamine and serotonin. The second phase of edema is due to the release of prostaglandins, protease and lysosome. 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 second phase may be explained by an inhibition of cyclo-oxigenase [19]. Our results indicate that CPO and CPE at (200 mg/kg and 400 mg/kg, p.o.) and aspirin play a crucial role as a protective factor against carrageenan induced acute inflammation. Thus it can be estimated that the extracts derived from the seeds of C. pentandra may exerts anti-inflammatory effects in dose dependent manner by inhibiting the synthesis, release or action of inflammatory mediator’s viz. histamine, serotonin and prostaglandin involved in inflammation.

The variation in order of activity for CPO and CPE indicated that the different constituents present in both extracts may be responsible for these activities. Presence of secondary metabolites in medicinal plants like steroids, tannins, phenolic compounds, triterpenoids, have been previously reported to be responsible for anti-inflammatory activity [20-22]. The presence of these constituents in C. pentandra seed extracts may be responsible for the observed activities.

CONCLUSION

Based on the results of present study, it can be concluded that the C. pentandra seeds have anti-inflammatory activity in studied animal model. Hence, our research supports traditional use of C. pentandra on a scientific basis. Further investigation is advocated especially for establishing structural components of the responsible phytoconstituents.
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