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Anti-Inflammatory activity of *sapindus laurifolius* leaf extract in wistar rats

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Objectives: The present work was aimed to study the anti-inflammatory activity of *Sapindus laurifolius* leaf extract in a systematic way using Wistar albino rats as a model animal. **Methods and Material**: To evaluate the anti-inflammatory activity of methanolic leaf extract of *S. laurifolius*,inflammation was induced by injecting formalin into the right hind paw of the rats. Paw volume was measured in term of water displacement periodically after formalin injection using a plethysmometer. The difference in the paw volumes indicated the degree of inflammation. Analgesic activity was tested in rats using the hot plate method, the time (sec) of discomfort reaction (licking hind paws or jumping) was taken to identify the analgesic activity. **Results**: There was a significant reduction in paw volume in a dose dependant manner, significant increase in the analgesic effect in comparison to the control group. The maximum analgesic effect of leaf extract was observed at the dose of 400 mg/kg at 120 min, which showed a reaction time of 11.54±0.28seconds compared to the reference drug diclofenacwithreaction time 13.29±0.13 seconds. **Conclusion**: The methanolic extract showed significant anti-inflammatory and analgesic effect comparative to the standard drug diclofenac. Further studies are required to extract the particular active constituents responsible for the action.

Keyword: *Sapindus laurifolius*, Anti-Inflammatory, Analgesic, Diclofenac.

1. Introduction

Plants have played a significant role in maintaining human health and improving the quality of human life for thousands of years and have served humans well as valuable components of medicines, seasonings, beverages, cosmetics and dyes. Herbal medicine is based on the premise that plants contain natural substances that can promote health and alleviate illness. In recent times, focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems [1]. One such plant *Sapindus laurifolius* (Indian soap nut) belongs to the family Sapindaceae widely distributed in Western Ghats region of the Karnataka (India). The plant is widely used for both human and animal treatment for various purposes especially as antibacterial, exfoliant, expectorant, emetic and it clears the skin problems like eczema, psoriasis, itchy skin [2, 3]. Seeds of *Sapindus emarginatus* contain anti-inflammatory oil which is traditionally used to purify the blood [4], pericarp contains triterpene

saponins, commonly used as antifertility, antipruritic and anti-inflammatory agents in traditional Indian and Thai medicines. In folklore practice, fruits and leaves of *Sapindus laurifolia* are used in baths to relieve joint pain and the roots are used in the treatment of gout and rheumatism. Sridhar *et al.,* [5] reported the traditional use of *S. laurifolius* leaves and fruits in the alleviation of joint pain and yolk gall in cattle. However, there are no such scientific literatures are available to prove its anti-inflammatory activity, and hence the present work is aimed towards the anti-inflammatory activity of *S. laurifolius* in wistar rats.

2. Materials and Methods

2.1 Plant Extract

S. laurifolius fresh leaves were collected from Western Ghats of Karnataka State and dried under the shade. The dried powdered material was subjected to repeated extraction by maceration at room temperature with methanol as a solvent. Extract was filtered and concentrated and dried at reduced pressure and controlled temperature (40-60 °C) in a rotary evaporator. The residual methanol from the extract was evaporated after keeping the extracts in a in a vacuum oven at 60 ^{o}C at the pressure of 25 psi (Murophy, India). The residues were weighed after drying and the final residual leaf extract was used to evaluate the anti-inflammatory and analgesic activity.

2.2 Experimental design

Healthy Wistar albino rats aged around 8-9 weeks weighing 160 ±20 g were obtained from the stock of the animal house, Indian Institute of Sciences, Bangalore, India. Animals were acclimatized to the laboratory conditions for 7 days prior to the study and maintained on normal diet and *ad libitum* water. Experimental protocol was approved by Institutional Animal Ethical Committee (IAEC).

3. Anti-inflammatory Study

The study was conducted on Wistar albino rats to test the anti-inflammatory activity of methanolic leaf extract of *S. laurifolius.* Oedema was induced by injecting 0.1 ml of 2% (w/v) formalin into the sub plantar region of the right hind paw of the rats according to the method described by Chau, [6]. Rats were divided into five groups consisting of six animals in each group. The rats were fasted with free access to water for 12 h prior to the test. The methanolic leaf extract of *S. laurifolius* in graded doses were administered to animals as a single dose by oral gavage. The volume of administration was maintained to 2 ml/200g through proper dilution of methanolic leaf extract in distilled water. Santhosh *et al.,* [7] reported the nephrotoxic activity of *S. laurifolius* leaf extract with biochemical and histological alterations in the wistar rats at the dose rate of 800 mg/kg body weight, hence the dose which doesn't cause any abnormalities were selected for the evaluation of anti-inflammatory activity. Group I served as control which was gavaged with distilled water where as group II, III and IV were gavaged with *S. laurifolius* methanolic leaf extract at the dose level of 100, 250 and 500 mg/kg body weight respectively and the Group V received Diclofenac sodium at the dose of 10 mg/kg body weight (Diclofam® MAX), 1 h before formalin injection. Paw volume was measured in term of water displacement at 0, 1 and 3 h after formalin injection using a plethysmometer (IITC Life Science, USA). The difference in the paw volumes indicated the degree of inflammation [8, 9].

Percentage inhibition was calculated as:

$$\text{Percent inhibition} = (1 - Vt/Vc) \times 100$$

Vc = volume of control, Vt = volume of test

4. Analgesic study

4.1. Eddy's hot-plate test in rat

The study was conducted on Wistar albino rats (six per group) to test the analgesic activity of *S. laurifolius* leaf extract. The paws of rat are very sensitive to temperature at 55 + 0.5 ^{o}C, which are not damaging to the skin. The responses were recorded in the form of jumping,

withdrawal or the licking of the paws [10]. Rats were grouped in four consisting of six animals in each group. Group I served as control which was gavaged with distilled water where as group II and III were gavaged with *S. laurifolius* methanolic leaf extract at the dose level of 200 and 400 mg/kg body weight respectively and the Group V received Diclofenac sodium at the dose of 10 mg/kg body weight (Diclofam® MAX).

After 0, 30, 60, 90 and 120 min of extract administration, animals were lowered onto the surface of a hot plate. The Eddys hot-plate (MKM Industries, Chennai) was maintained at 55.0°C and the animals were placed on the heated surface. Cut of period of 15 seconds was observed to avoid damage to the paw. Reaction time and the type of response were noted using a stop watch. Response latency was recorded at 0, 30, 60, 90 and 120 min after administration of the test drugs [11].

5. Results

5.1 Anti-inflammatory study

In the formaldeyde-induced paw oedema method, the oral administration of methanolic leaf extract of *S. laurifolius* at graded dose (100, 250 and 500 mg/kg) produced a significant decrease (P <0.001) in volume of water displacement in turn there was reduction in paw volume in a dose dependant manner in comparison to control. The maximum anti-inflammatory effect was observed in 3 h in all the doses of tested drug (Table 1).

Table 1: Effect of *S. laurifolius* leaf extract on formalin induced paw oedema in rats (Volume of water displaced expressed in ml).

Hour	0 h	1 h	3 h	Paw volume (diff) after 3 h (in ml)	% inhibition
Group I Control	0.68±0.01	1.17±0.01	1.25±0.02	0.57±0.02	0
Group II (100mg/kg)	0.70±0.02	1.12±0.01***	1.10+±0.01***	0.40±0.01	29.82%
Group III (250 mg/kg)	0.68±0.02	1.05±0.01***	1.00±0.01***	0.32±0.01	43.86%
Group IV (500 mg/kg)	0.64±0.01	0.97±0.01***	0.86±0.01***	0.22±0.01	61.40
Group V Diclofenac (10 mg/kg)	0.63±0.02	0.82±0.01***	0.77±0.01***	0.14±0.01	75.44

P <0.001*** n = 6, Compared with the control group values of respective hour**,** values are Mean ±SEM

5.2 Analgesic study

In this present study, the analgesic effects of methanolic leaf extract of *S. laurifolius* increased significantly (P<0.001) in comparison to the control group. The maximum effect of the test drug was observed at the dose of 400 mg/kg of methanolic leaf extract of *S. laurifolius* at 120 min, which showed a reaction time 11.54±0.28 seconds as compared to the reference drug diclofenac which showed a reaction time 13.29±0.13 seconds (Table 2).

Table 2: Analgesic activity of leaf extracts of *S. laurifolius* by Eddy's hot plate method

Group	Treatment	Observation interval (Min)				
		0	30	60	90	120
I	Control	3.05±0.15	3.10±0.12	3.10±0.13	3.17±0.08	3.28±0.11
II	Leaf Extract 200 mg/kg	3.32±0.17	6.25±0.40***	7.96±0.17***	9.69±0.28***	11.06±0.16***
III	Leaf Extract 400 mg/kg	3.25±0.10	7.22±0.76***	9.66±0.30***	10.47±0.28***	11.54±0.28***
IV	Standard Diclofenac 10 mg/kg	3.22±0.13	11.48±0.19***	12.32±0.13***	12.66±0.12***	13.29±0.13***

P <0.001*** n = 6, Reaction time in second**.** Values are Mean ±SEM

6. Discussion

6.1 Anti-inflammatory Activity Study

The present study demonstrated that the methanolic extract of *S. laurifolius* was effective in rat model of acute inflammation, leaf extract at a dose of 500 mg/kg produced maximum inhibition of formalin induced paw oedema, these findings were in accordance with Jennifer *et al.*, [12] who reported the anti inflammatory activity of *S. trifoliatus* in carrageenan-induced acute paw oedema in rats. The *S. laurifolius* leaf extract consist of saponins, glycosides, flavonoids and bitter principles, these phytochemical constituents might be responsible for the biological activity of the *S. laurifolius* leaves. Among the chemical constituents, saponins are most important biologically [13,14,15]. The development of formalin induced edema is believed to be biphasic. The early phase is attributed to the release of histamine and serotonin and the delayed phase is sustained by the leucotrienes and prostaglandins [16], Saponins and flavanoids and tannins are reported to inhibit prostaglandin synthesis [17, 18]. Takagi *et al.* [19] reported that crude saponin isolated from *Sapindus mukorossi* inhibited the development of carrageen-induced edema in the rat hind paw as well as on granuloma and exudates formations induced by croton oil in rats. The ethanolic extract of *S. trifoliatus* produced the inhibition of carrageenan-induced rat paw edema in wistar rats and showed an inhibitory effect on leukocyte migration, reduction on the pleural exudates as well as reduction on the granuloma weight in the cotton pellet granuloma method [20]. Arulmozhi *et al*. [21] showed the anti inflammatory activity of *Sapindus trifoliatus* possibly mediated through 5-lipoxygenase (5-LOX) and cyclo-oxygenase (COX) pathways, evaluation of extract revealed the inhibitory activity of *S. trifoliatus* against major inflammatory mediators 5-LOX, COX, leukotriene B4 and nitric oxide synthase.

6.2 Analgesic activity study

Any injury or tissue damage is associated with pain and inflammation. Analgesics can act on peripheral or central nervous system. Peripherally acting analgesics act by blocking the generation of impulses at chemoreceptors site of pain while centrally acting analgesics not only raise the threshold for pain but also alter the physiological response to pain and suppress the animals anxiety and apprehension. Pain and inflammation are an essential prelude to the repair process [22]. The hot plate method is considered to be selective for screening of the compound acting through the opoid receptor [23], the extract of *S. laurifolius* increased the mean basal latency which shows that extract act through centrally acting analgesic.

In the present study, the maximum effect of the test drug was observed at the dose of 400 mg/kg of methanolic leaf extract of *S. laurifolius* at 120 min. The above findings are in accordance with the observations of Srikanth *et al.* [24], who reported the analgesic activity of methanolic extract of *Sapindus emarginatus* in rats at the dose rate of 400 mg/kg body weight.

From the above discussion the methanolic extract from the leaves of *S. laurifolius* exhibited significant analgesic and anti inflammatory activity. Further detailed investigation is underway to determine the exact phytoconstituents that are responsible for these activities.

7. Conclusion

The methanolic extract showed significant anti-inflammatory and analgesic effect comparative to the standard drug. It is concluded that the pharmacological screening of the extract showed significant analgesic and anti-inflammatory profile. Further studies are anticipated to extract the particular active constituents responsible for the action.

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