

## Invivo Anti-inflammatory Activity Potential of Ethanolic extract of Stem Bark of *Sapindus trifoliatus* Linn.

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### Abstract

*Sapindus trifoliatus* Linn (*Sapindaceae*) is commonly known as reetha, which is used in Indian traditional medicine to treat various ailments. The present study is aimed for the invivo anti-inflammatory activity of ethanolic extract of stem bark of *Sapindus trifoliatus* by carrageen paw edema and cotton pellet granuloma induced inflammation models in wistar albino rats. Dried and powdered stem barks were extracted using soxhlet extractor and screened for the various phytoconstituents present in the ethanolic extract of stem bark. Acute toxicity of the test extract was carried out by OECD guidelines 425. The test extract was administered orally with three different dose levels (100, 200 and 400 mg/kg bodyweight) and anti-inflammatory activity potential was compared with Indomethacin as a standard drug. Preliminary phytoconstituents analysis showed the presence of steroids, triterpenoids, saponins, glycosides. No mortality was observed for the test compound up to 2000 mg/kg body weight. The stem bark extract showed significant dose-dependent reduction in paw edema when compared to the control at all time intervals, which was also comparable to that of produced by Indomethacin. The oral administration of test extract possessed significant level of anti-inflammatory activities possibly due to inhibition of prostaglandin production, supporting the evidence to the use of the plant stem bark as an anti-inflammatory agent.

Key words: Anti-inflammatory, *Sapindus trifoliatus*, cotton granuloma method, paw edema, carrageenan

### Introduction:

Herbal medicines are the oldest remedies known to mankind. Herbs had been used by all cultures throughout history but India has one of the oldest, richest & most diverse cultures living traditions associated with the use of medicinal plants [1]. Herbal medicines form a major part of remedies in traditional medical systems such as Ayurvedic, Rasa Sidha, Unani & Naturopathy. The science of using herbal medicines to cure different illnesses is known as herbal medicine, or phytotherapy. It includes all facets of herbal medicine, from strong to gentle plants. People are becoming more conscious of the harmful side effects of synthetic medications, which has resulted in a surge in the production of natural products as treatments for a range of ailments [2]. As a result, both animal and clinical reports on herbal drugs were reviewed, with data from 1981 to 1983 serving as a benchmark for evaluating recent herbal drug research patterns. The present study showed that interest has increased in herbal drug research in India, which supported the finding of Adithan (1996), with maximum utilization of the phytotherapeutic approach where in crude plant preparations were used. The maximum work was observed with poly herbal preparations [3].

*Sapindus trifoliatus* Linn. (Fam: *Sapindaceae*) is known in Hindi as "Reetha." It is a lovely medium to large deciduous tree with a height of up to 18 metres. Usually found in peninsular

India, especially Andhra Pradesh and Karnataka[4]. Many seed oils are well known for their anti-inflammatory and antioxidant properties. Pai et al.(2014) examined the n-hexane and ethyl acetate extracts of *S. trifoliatum* seeds and discovered that the seed extracts had antibacterial, antifungal, and antioxidant properties[5]. The roots of *Sapindus trifoliatum* used as expectorant, collyrium in sore eyes and ophthalmic. The fruit is used as stomachic, alexeteric, aphrodisiac, useful in chronic dysentery, diarrhoea, cholera, hemicrania, tubercular glands, paralysis of the limbs, lumbago, epileptic fits of children, allays uterine pain, fumigation good in melancholy, and used as emetic. Both the root and the fruit are given as anthelmintic. The main active principle of the plant is saponin. It also contains beta glucose and pectin.

Inflammation is a phenomenon that plays a role in the onset and development of a variety of diseases. It's a natural defence mechanism that protects our bodies from tissue damage and microorganisms[6]. As a result of tissue damage and genomic changes caused by constant low-grade inflammation in and around the affected tissue or organ, a variety of chronic conditions such as cancer, diabetes, cardiovascular disorders, autoimmune diseases, and neurodegenerative disorders arise[7]. Anti-inflammatory drugs are medications that prevent or minimise inflammation, which can be caused by physical trauma, infection, heat, or antigen-antibody interactions[8]. Present anti-inflammatory medications only offer symptomatic relief and have side effects[9]. Natural plants have been identified as potential anti-inflammatory agents that can safely protect the human body from inflammation, avoiding diseases and disorders associated with inflammation. The study's aim is to discover new and safe natural agents with anti-inflammatory properties[10].

### **Materials and Methods:**

#### **Plant collection:**

The plant of *Sapindus trifoliatum* Linn was collected during the month of August to September from local areas of Dakshina Kannada District(Karnataka,India). The plant material was authenticated by Dr.Noeline J. Pinto, Professor and Head,Dept. of Botany, St. Agnes College, Mangalore.

#### **Extraction:**

The collected stem barks of *Sapindus trifoliatum* were cleaned, dried and powdered. And then extracted using ethanol in Soxhlet extractor. After the completion of the extraction process, the solvent was distilled out by the simple distillation method. The recovered solvent has been used for the further extraction procedure. The finally obtained ethanolic extract was concentrated under a vacuum and stored in a desiccators [11,12]

#### **Phytochemical screening:**

To determine the group of organic compounds and secondary metabolites present in the crude ethanolic extract of *Sapindus trifoliatum*, preliminary phytochemical tests were performed. Carbohydrates, steroids, flavonoids, and glycosides were among the metabolites screened, and they were all qualitatively analysed[13]

#### **Animal Selection:**

Wistar rats weighing between 100-150gms of either sex were selected for the study and obtained from KSHEMA, Deralakatte Mangalore. The experiments were carried out as per the guidelines and regulations of institutional animal ethics committee. (Ref. No. KSHEMA/AEC/28/2010).

#### **Acute toxicity studies:**

Acute toxicity study was performed to determine the median lethal dose (LD<sub>50</sub>) of the ethanolic extract. It was carried out by “up and down” method [14] and as per OECD guidelines 425 [15]. The animals were then observed for general behavioural, autonomic, neurological profiles and finally death after 24 hours.

#### **Assessment of anti-inflammatory effect of the extract:**

##### **Carrageenan induced acute paw edema [16,17]**

Edema was induced in the left hind paw by injecting 0.1 ml carrageenan (1% w/v) in the subplanter tissue of hind paw of each rat after 30 minutes of treatment. The standard drug and the extracts of stem bark of *Sapindus trifoliatus* (100, 200 and 400 mg/kg) were administered orally 30 min prior to carrageenan administration. At the time interval of 30, 60, 90, and 120 minutes the paw volume was measured by the mercury displacement method using a plethysmograph. Increase in the paw volume in animals which treated with standard drug and the different doses levels of ethanolic extract were compared with the volume of untreated control animals after 2 h. And the percentage inhibition of edema volume was calculated.

##### **Cotton pellet granuloma method [17, 18, 19]**

Wistar rats with an average weight of 200 g were selected for the study. Animals were divided into five different groups which consists of six rats in each. The group I served as control. Group two referred as standard drug treated. Third, fourth and fifth groups were treated with 100, 200, 400 mg/kg body weight (p.o.) ethanolic extract of stem bark of *Sapindus trifoliatus* (suspended in 0.5% NaCMC). The rats anaesthetized, and the dorsal skin was shaved and disinfected with 70% ethanol. At the lumbar region an incision was made and presterilized cotton pellets weighing about 20 mg ± 0.05 was placed on both axillae and groin regions. The animals were then treated for 0-9 days with standard and test drugs to the different group respectively.

Animals were anaesthetized on 10<sup>th</sup> day and pellets were removed, cleaned and dried at 60-70° C for 6 h. Granuloma weight was calculated by deducting the weight of the cotton pellets on 0 day (i.e., before start of the experiment) from the weight of the cotton pellet on 9th day (i.e., at the end of the experiment). The average weight of the pellets of the control group as well as the test group were recorded. The percent change of the granuloma weight relative to vehicle control group was determined.

#### **Statistical Analysis:**

The results were obtained with mean ± SEM. Statistical significance of the data was assessed by one way analysis of variance (ANOVA) followed by Dunnett's test [20].

#### **Results**

##### **Phytochemical investigation:**

The phytochemical investigations of ethanolic extract of stem bark of *Sapindus trifoliatus* Linn indicated the presence of steroids, triterpenoids, flavonoids, saponins and glycosides.

##### **Pharmacological investigation**

##### **Acute toxicity studies and selection of doses**

The stem bark extract of *Sapindus trifoliatus* was found to be safe up to 2000 mg /kg body weight. Hence a dose of 100, 200 and 400 mg/kg body weight were chosen for the study.

##### **Anti-inflammatory activity:**

##### **Carrageenan induced paw edema method**

The wistar rats treated with ethanolic stem bark extract of *Sapindus trifoliatus* by oral administration, reduced acute paw edema volume as compared with that control group. The rats treated test extract at a dose of 100, 200 and 400 mg/kg body weight revealed 33.7, 60.95

and 76.12 % inhibition of paw edema volume while compared with control at 120 minutes respectively. The % inhibition of paw edema was increased with time and gave maximum effect at 2 h and stem bark extract displayed significant results ( $P < 0.05$ ) while compared with control. The ethanolic extracts of stem bark of *Sapindus trifoliatus* possessed dose dependent anti-inflammatory activity.

**Table 1: Effect of ethanolic stem extract of *Sapindus trifoliatus* on carrageenan induced paw edema**

Treatment	Dose (mg/kg)	Increase in paw volume (min)				% decrease in paw volume
		30	60	90	120	
Control	5ml/kg	0.198±0.01	0.35±0.027	0.64±0.021	0.77±0.005	-
Indomethacine	10	0.13±0.021**	0.17±0.016**	0.35±0.021**	0.27±0.016*	80.63
Ethanolic extract of <i>Sapindus trifoliatus</i> L(EEST)	100	0.21±0.005**	0.29±0.005**	0.48±0.005**	0.57±0.005*	33.7
	200	0.19±0.01*	0.22±0.01*	0.4±0.01**	0.45±0.01**	60.95
	400	0.17±0.01*	0.2±0.01**	0.34±0.05*	0.37±0.01**	76.12

\*\* The mean difference is significant at the 0.05 level, when compared to the control group

#### Cotton pellet granuloma method

Significant reduced ( $P < 0.05$ ) the granuloma mass formation in the animals treated with test extract at a dose of 100, 200, 400 mg/kg body weight when compared to control. The rats displayed 33, 43 and 50% inhibition of granuloma mass formation after the nine days treatment with 100, 200 and 400 mg/kg body weight of test extract while compared with that of control.

**Table 2: Effect of ethanolic stem bark extract of *Sapindus trifoliatus* on the weight of cotton pellet granuloma.**

Treatment	Dose (mg/kg)	Granulation weight in mg	% inhibition
Control	0.5% NaCMC	76.03±1.89**	-
Indomethacine	10	34.00±2.31**	55
Ethanolic extract of <i>Sapindus trifoliatus</i> L(EEST)	100	50.83±0.89**	33
	200	43.43±1.57**	43
	400	38.10±3.07**	50

\*\* The mean difference is significant at the 0.05 level, when compared to the control group

#### Discussion

Many of the plant extracts represent as a valuable bioactive resources for the development of anti-inflammatory agents, which could be helpful in the management of inflammation and pain. The ethanolic extract of stem bark of *Sapindus trifoliatus* reported to possess anti-inflammatory activity [21,22]. Also the preliminary phytochemical studies shows the presence steroids, terpenoids, flavonoids.

Carrageenan model was used to assess the anti-inflammatory activity is a well established model for the development of edema inflicted by carrageenan is a three-stage process: The

early stage (the first 90min) during which histamine and serotonin are released and the second stage (90-150min) which is driven by kinin, and at the third stage (after 180min); which is mediated by prostaglandin (reference) [23]. The above data indicates that the test extract was likely to act by hindering the release and/or action of prostaglandin. The development of granuloma in rodents by cotton pellet illustrates a chronic inflammation model extensively used to study the transductive and proliferative components of the inflammation. The weight of the cotton pellets corroborates with the amount of the granulomatous tissue. Based on the study, administration of the test extracts in graded dose manner has suggested presence of the anti-inflammatory effect.

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