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In-vitro and *In-vivo* Anti-Inflammatory Activity of *Sapindus emarginatus* Leaf Extract

R Ch Sekhara Reddy D*, Seetharam T, Amrutha Sonia P, Maharshi V, Presley KA, and Swathi M

Acharya Nagarjuna University College of Pharmaceutical Sciences, Nagarjuna Nagar, Guntur 522510, India.

ABSTRACT

To appraise the Anti-inflammatory activity of *Sapindus emarginatus* (*S. emarginatus*), leaf aqueous extract (SEAE) by using *in-vitro* membrane stabilization of human RBC and *in-vivo* formalin induced paw edema models. The acute toxicity study was performed by administering 2g/kg aqueous extract of *S. emarginatus* leaves in rats by oral gavage. The anti-inflammatory activity was evaluated by using both *in-vitro* membrane stabilization of human RBC and *in-vivo* formalin induced paw edema model in rats. The dose of aqueous extract of *S. emarginatus* leaves was given at a dose of 200and 400 mg/kg *p.o.* In acute toxicity study of the plant extract, at a dose of 2g/kg *p.o.* was not shown any toxic events in rats. It was observed that *Sapindus emarginatus* leaf aqueous extract have demonstrated dose dependent increase in the % membrane stabilization property significantly. The indomethacine had shown significant ($p<0.001$) reduction in paw edema at 1st, 3rd and 6th hr when compared with normal control. SEAE-200 had shown significant ($p<0.05$) action at 6th hr but not 1st and 3rd hr. SEAE- 400 had shown significant ($p<0.05$, $p<0.001$) actions at 3rd and 6th hr but not 1st hr. In the present study the aqueous extract of *S. emarginatus* leaves poses anti-inflammatory activity.

Keywords: Formalin, *Sapindus emarginatus,* Anti-inflammatory activity

Corresponding author

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INTRODUCTION

Inflammation is a common defensive mechanism for various noxious stimuli like irritants, cell damage and infections. So, inflammation is a protective mechanism for the organism in order to remove the noxious stimuli and to commence the healing process. Inflammation is one of the manifestations of pathogenic microorganisms [1]. In many diseases inflammation is a most common manifestation which is affecting most of the people worldwide [2]. At present non-steroidal anti-inflammatory (NSAIDS) drugs occupies the major share for the treatment of pain and inflammation but these drugs are showing lot more complications. These NSAIDS are usually producing gastrointestinal problems, so there is lot of limitations to use these allopathic drugs [3]. So, there is a need for alternative medicine with less unwanted effects and most reliable medicine. Since time immemorial plants has been used as medicaments because of their less unwanted effect. The plant *Sapindus emarginatus* (*S. emarginatus)* belonging to the family sapindaceae is a tropical tree species sparsely distributed throughout India [4].The plant is known for their surfactant and detergent properties, due to the presence of saponins [5]. In folk medicine it is used as anti-inflammatory and antipyretic. Traditionally *S. emarginatus* seed oil used to purify the blood [6]. Phytochemical constituents like triterpene saponins has been found in the pericarp of the fruit which, are commonly used as antifertility, antipruritic and anti-inflammatory agents in traditional Thai and Indian medicines [5]. In plant review we had found that *S. emarginatus* is having antifertility and antiandrogenic [7], antihyperlipidemic [8], CNS depressant activity [9], Anti-mosquito [10], antidibetic [11], antimicrobial [12] and antioxidant [13] activities. Inflammation is one of the world's foremost health problem [14]. It involves a complex enzymatic activation, extravasations of fluid, mediators release, cell migration, tissue breakdown and repair [15]. Now a day's plant based traditional medicine have been gained more importance because they are inexpensive, have little side effects and according to WHO still about 80% of the world population rely on plant-based drugs [16]. So, the present work was intended to evaluate the anti-inflammatory activity of *S. emarginatus* leaf aqueous extract (SEAE) since, there is no reported anti-inflammatory activity on leaves.

MATERIALS AND METHODS

Preparation of whole plant extract:

The plant leaves of *S. emarginatus* used for the present study was collected from A.N.U. campus, Guntur district of Andhra Pradesh. The plant was identified, conformed and authenticated by field Botanist Dr. SK. Kasim, Assistant professor, Acharya Nagarjuna University, Guntur. The plant leaves were shade dried and pulverized into coarse powder by a mechanical grinder. The resulting powder was used for the aqueous extraction process. The powder was extracted directly with water, which was used for in-vitro and in-vivo anti-inflammatory investigations and, after subjecting it to preliminary qualitative phytochemical studies [17]. The extract was concentrated under reduced pressure and stored in vacuum desiccators.

Chemicals:

The entire chemicals were analytical grade. Formalin, standard drugs diclofenac and indomethcin were obtained from E. Merck Indi Ltd.

Preliminary phytochemical investigation:

The preliminary phytochemical screening was carried out for qualitative identification of phytoconstituents [18].

Experimental animals:

Albino wistar rats weighing 150-250g was procured from Biogen, Bangalore. They were maintained in the animal house of Acharya Nagarjuna University College of Pharmaceutical Sciences. Animals were maintained under controlled condition of temperature at 27° ± 2° C and 12-h light-dark cycles. They were housed in polypropylene cages and a free access to standard pellets and ad libitum. All the studies conducted were approved by the Institutional Animal Ethical Committee (IAEC) according to prescribed guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (Reg No: 1725/GO/a/13/CPCSEA), Govt. of India.

Determination of acute toxicity:

Experimental animals and procedure:

Foe the determination of the dose $LD_{50,}$ albino rats of weighing 160-220g were used for the study. They were nulliparous and non-pregnant. These were acclimatized to laboratory conditions for one week prior to start of dosing. The *S. emarginatus* extract was suspended in distilled water by using tween 80, to prepare a dose of 2g/kg. The doses were selected according to the OECD guideline no.425. The procedure was divided into two phases. Phase I (observation made on day one) and phase II (observed the animals for next 14 days of drug administration). Two sets of healthy rats (each set of 3 rats) were used for this experiment. First set of animals were divided into three groups, each of one in a group. Animal were divided into three groups, each of one in a group. Animals were fasted overnight with water ad libitum. Animals received a single dose of 2g/kg was selected for the test, as the test item was a source from herb. After administration of extract, food was withheld for 3-4 hrs. if the animal dies, conduct the main test to determine the $LD_{50.}$ If the animal survives, dose four additional animals sequentially so that a total of five animals are tested. However, if three animals die, the limit test is terminated and the main test is performed. The LD_{50} is greater than 2g/kg, if three or more animals survive. If an animal unexpectedly dies late in the study, and there are other survivors, it is appropriate to stop dosing and observe all animals to see if other animals will also die during a similar observation period. Late deaths should be counted the same as other deaths. The same procedure was repeated with another set of animals to nullify the errors.

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***In -Vitro* Human Rbc Membrane Stabilization Bioassay:** [19]

Standardisation of *In-vitro* Human RBC Membrane Stabilization Bioassay.

In-vitro Human RBC membrane stabilization inhibition bioassay was standardized using diclofenac sodium as standard.

Preparation of Standard solution:

Diclofenac sodium injection is a sterile solution of Diclofenac sodium in water for injection. I.P containing 25mg/ml was used as standard. It was diluted suitably to get a concentration of 10 and 20 µg/ml.

Procedure:

The method described by Chatterjee S et al was used to study the membrane stabilization. The bioassay was standardised by using Diclofenac sodium as standard. The reaction mixture 4.5ml consisted of 2ml hyposaline (0.25% NaCl), 1ml of 0.15M phosphate buffer (pH 7.4) and 4, 8 & 20µl of Diclofenac sodium 25 µg/ml injection, 0.5ml of 10% human RBC in normal saline was added. The mixtures were incubated at 56^0C for 30 minutes. The tubes were cooled under tap water for 20 minutes. The mixtures were centrifuged and the absorbance of the supernatants read at 560 nm. A control was also maintained where drug was replaced by buffer solution.

Calculation:

The % Membrane stabilization was calculated from control where no drug was added

% Membrane stabilization= (Abs of control-Abs of treated) / Abs of control ×100

***In-Vitro* Human RBC Membrane Stabilization Activity Bioassay of *Sapindus Emarginatus* Extracts.**

Preparation of sample solution

Stocks solutions of 10,000 µg/ml were prepared by using normal saline as a solvent. From this stock solution 3 different concentrations of 100, 200, 400 µg/ml were prepared by using normal saline as a solvent.

Procedure:

The method described by Chatterjee S et al was used to study the membrane stabilization assay of the drug extract. The reaction mixture 4.5ml consisted of 2ml hypo saline (0.25% NaCl), 1ml of 0.15M phosphate buffer (pH 7.4) and 1ml of test solution (100,200,400µg/ml of final volume) in normal saline, 0.5ml of 10% human RBC in normal saline was added. For control tests, 1ml of Isosaline was used instead of test solution while product control tests lacked red blood cells. The mixtures were incubated at 56^0C for 30 minutes. The tubes were cooled under tap water for 20 minutes. The mixtures were centrifuged and the absorbance of the supernatants read at 560 nm. Percentage membrane stabilization was calculated.

Evaluation of *In-vivo* Anti-inflammatory Activity by Formalin Induced Rat Paw Edema Model.[20]

In the dose response experiment, albino rats were randomly assigned into 4 groups of 6 animals each.

Group I: 2% tween 80 sollution was administered daily once throughout the experiment.
Group II: This group of animals received indomethacin (10 mg/kg, *p.o*.) on the second day, one hour before the induction of paw edema by using 0.2 ml of formalin (1% w/v).
Group III: The animals of this group received 200 mg/kg, *p.o*. of SEAE for two days.
Group IV: The animals of this group received 400 mg/kg, *p.o.* of SEAE for two days.

On the second day, all the groups of animals were given with 0.2 ml of formalin (1% w/v) injected into the rat hind paw, one hour after the treatment of normal saline, Indomethacin, SEAE low dose and SEAE high dose for groups I,II,III and IV respectively.

Before formalin injection, the paw volumes for each rat were measured separately by means of plethysmometer. Edema caused by formalin was measured at 0, 3, 6 and 24 hours. The anti-inflammatory potency of the extract was determined by comparing it with a group in which a 10 mg/kg dose of indomethacin was administered orally. Then percentage of inhibition of edema was calculated for each group with respect to the control group as follows.

$$\text{Percentage of inhibition of paw edema} = (1-V_t/V_c) \times 100$$

Where V_c and V_t represent average paw volume of control and drug treated animals respectively.

Statistical analysis

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The values were expressed as Mean ± SEM. The data analysed by using one way ANOVA followed by Dunnett's test using Graph pad prism software. Statistical significance was set at P ≤ 0.05.

RESULTS

Phytochemical investigation:

Phytochemical investigation revealed that the plant extract contains alkaloids, flavanoids, carbohydrates, glycosides, proteins, tannins and saponins.

Acute toxicity study:

In both phase I and II procedures, none of the animals did not show any toxicity upon single administration of *S. emarginatus* aqueous extract (2000 mg/kg, *p.o*). Thus, a low dose 200 mg/kg, *p.o* and a high dose 400 mg/kg, *p.o* were selected for the present study.

In-vitro Human RBC Membrane Stabilization Activity:

It was observed that *S. emarginatus* aqueous extract have demonstrated dose dependent increase in the % membrane stabilization property (Tble 1, Fig 1).

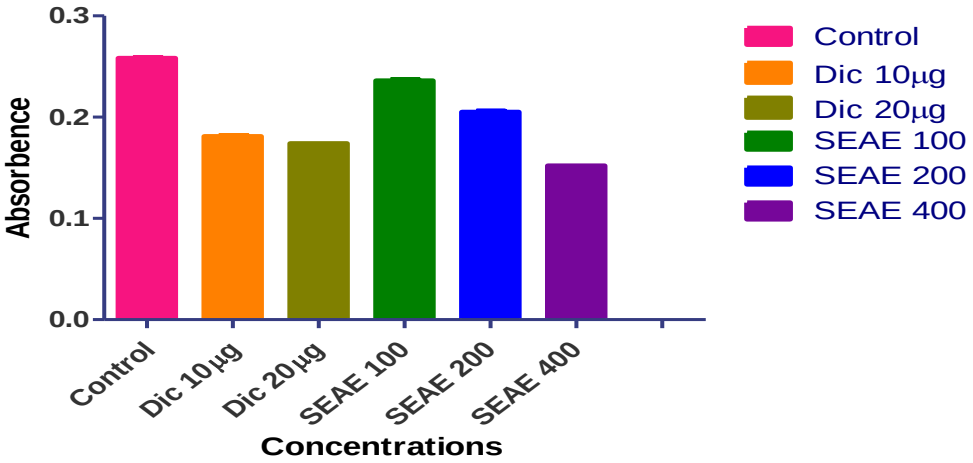

Figure 1: In-Vitro Human RBC Membrane Stabilization Activity Bioassay

Table 1: In-Vitro Human RBC Membrane Stabilization Activity Bioassay of SEAE

Groups	Absorbance	% Membrane Stabilization

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	Mean ± SEM	
Control	0.258 ± 0.0006***	-
Diclofenac 10µg	0.181 ±0.001***	29.84
Diclofenac 25µg	0.174 ±0.002***	32.55
SEAE 100 µg	0.236 ±0.001***	8.52
SEAE 200 µg	0.205 ±0.001***	20.54
SEAE 400 µg	0.152 ± 0.001***	41.08

Values are the mean ± S.E.M., n=3, ***Significant at p<0.001 compared to control.
SEAE: Sapindus emarginatus aqueous extract

***In-vivo* Anti-inflammatory Activity of *S. Emarginatus* aqueous extract by Formalin Induced Rat Paw Edema Model.**

The indomethacine had shown significant (p<0.001) reduction in paw edema at 1st, 3 rd and 6th hr when compared with normal control. SEAE-200 had shown significant (p<0.05) action at 6th hr but not 1st and 3rd hr. SEAE- 400 had shown significant (p<0.05, p<0.001) actions at 3 rd and 6th hr but not 1st hr. (able 2,Fig 2)

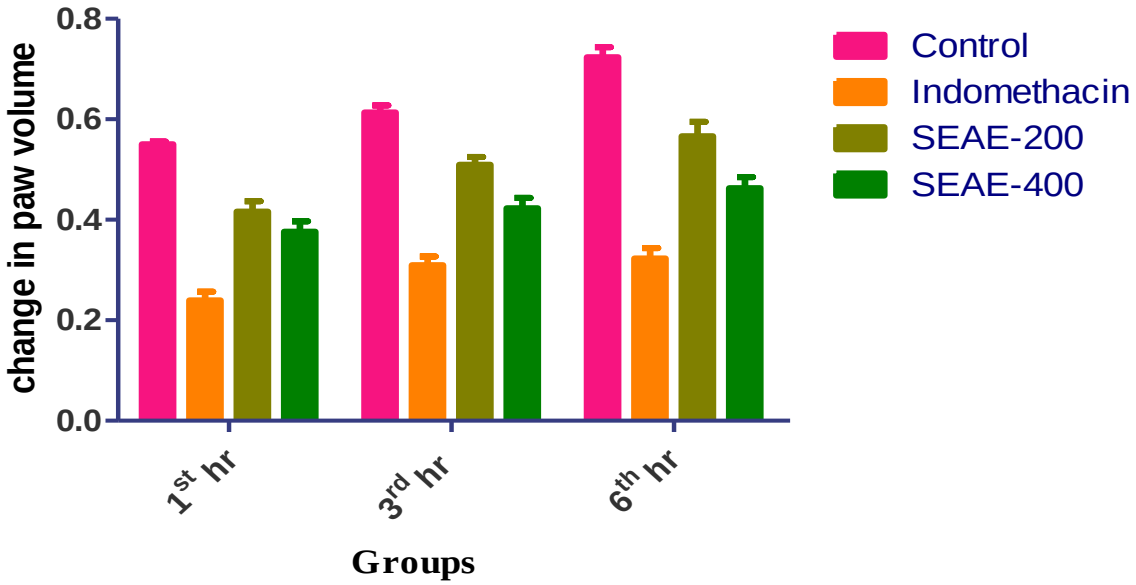

Figure 2: Effect of SEAE on Formalin Induced Rat Paw Edema.

Table 2: Effect of SEAE on Formalin Induced Rat Paw Edema.

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Group	Treatment	Change in paw volume (ml) and % Inhibition		
		1hr	3hr	6hr
Control	saline	0.607 ± 0.03	0.663 ± 0.04	0.703 ± 0.02
Standard	Indomethacin (10mg/kg)	0.280 ± 0.02*** (53.8)	0.330 ± 0.02*** (50)	0.333 ± 0.02*** (52.6)
SEAE-200	Extract -200 mg/kg	0.490 ± 0.03^{ns} (19.2)	0.527 ± 0.03^{ns} (20.5)	0.580 ± 0.03* (17.4)
SEAE-400	Extract – 400 mg/kg	0.483 ± 0.03^{ns} (20.4)	0.487 ± 0.04* (26.5)	0.490 ±0.017*** (30.2)

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett's test. Where, ***P<0.001, ** P<0.01, *p<0.05 and ns . All values are compared with control.
SEAE: Sapindus emarginatus aqueous extract

DISCUSSION

In spite of remarkable development in the field of synthetic drugs during recent years, they are found to have one or the other side effect, whereas plants still hold their own exceptional place, by the way of having no side effects. Therefore, a systematic approach should be made to find out the efficacy of plants against inflammation so as to exploit them as herbal anti-inflammatory agents [21]. The extract of *S. emarginatus* was prepared by aqueous extraction procedure and was subjected to preliminary phytochemical screening. It was observed that alkaloids, carbohydrates, flavanoids, glycosides, terpenoids, proteins, tannins and saponins present in the SEAE.

In order to evaluate anti-inflammatory activity of *S. emarginatus* extract*; in-vitro* bioassay involving HRBC membrane stabilization was performed. This bioassay is widely reported for investigations on anti-inflammatory activity of plant drugs.[19]

In the *in-vitro* membrane stabilization activity, diclofenac was used as standard anti-inflammatory drug and it had showed significant membrane stabilization by preventing haemolysis. SEAE had shown significant dose dependent % membrane stabilization activity in HRBC. Thus *S.emarginatus* extract may have anti-inflammatory activity by preventing release of inflammatory mediators from lysosomal granules whose membrane is structurally similar to erythrocyte membrane.

Formalin induced rat paw edema model was used to evaluate the *in-vivo* anti-inflammatory activity. Formalin-induced paw edema model, this model based upon the ability of test drug to inhibit the edema produced in the hind paw of the rat after injection of formalin. The nociceptive effect of formalin is biphasic, an early neurogenic component followed by a later tissue mediated response. In the first phase there is release of histamine, 5-HT and kinin, while the second phase is related to the release of prostaglandins.

Administration of *S. emrginatus* inhibits inflammation which, is caused by chemical induced paw edema model.

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REFERENCES

[1] Ferrero-Miliani L, Nielsen OH, Andersen PS, Girardin SE. Clin Ex pImmunol 2007; 147 (2): 227-35.
[2] Ramkumar K, Sunil S, Neeru V, and Suresh K. Asia Pac J Tropical Biomed 2012; S947-S952
[3] Rang H P, Dale M M, Ritter J M, Flower R J. Rang and Dale's pharmacology, 6th ed. Elsevier Publiction; 2008. P. 226-245.
[4] Kamalesh S M,Tikam S R, Shirish A R, Baleshwar M. J Gene 2011; 485(1): 32-39.(15)R
[5] Bharti A, Preeti B, Deepak T, Alok S. Indo Global J Pharm Sci 2012; 2(3): 250-257.(16)R
[6] Nair R, Kalariya T, Chanda S. Turk J Biol 2005; 29: 41-47.(17)R
[7] Venkatesh V, Sharma J D, Kamal R. Asian J Exp Sci 2002; 16 (1 & 2): 51-63.(18)
[8] Otway S and Robinson DS. J Physiol 1967; 190: 309-319.(19)
[9] Srikanth J and Muralidharan P. J Sci Res 2009; 1 (3): 583-593.(21)
[10] Surendran SN, Kumaran V, Sivarajah R, Krishnarajah SR, Rikaran RS, Raghavendra K. J Natn Sci Foundation Sr iLanka 2009;37 (3): 215-217.(23)
[11] Srikanth J and Muralidharan P. Asian Biomed 2009; 3(3): 313-318(27)
[12] Nair R, Kalariya T, Chanda S. Turk J Biol 2005; 29: 41-47.(28)
[13] Arvind K, Rajbir K, Saroj A. J Med Plants Res 2010; 4(19): 2034-2042.(30)
[14] Hossain H, Shahid-Ud-Daul AFM, Hasan K, Mansur AA and Haq MM. Int J Pharm 2012; 2(2): 271-277.
[15] Perianayagam JB, Sharm SK, Pillai KK. J Ethnopharmacol 2006; 104: 410-414.
[16] Kumara NKVMR. Identification of strategies to improve research on medicinal plants used in Sri Lanka, In WHO Symposium. University of Ruhuna, Galle, Lanka, 2001, pp 23.
[17] Friday E U, Iniobong E O, Moses B E. Gastroenterol Res 2013; 3(1):32-38.
[18] Kokate CK, Khandelwal KR, Pawar AP, Gohale SB. Practical Pharmacognosy. 3rd ed. Pune: Nirali Prakashan; 1995, p. 137-139.
[19] Rajurkar R, Jain R, Matake N, Aswar P, Khadbadi SS. Res J Pharm Tech 2009; 2(2):415-416
[20] Gupta R, Lohani M, Arora S. Int J Pharm Sci Rev Res 2010;3(1): 16-18.
[21] Hernandez PM, Rabanal GR. J Ethnopharmacol 2002; 8: 43-47.