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IN-VIVO ANTI-INFLAMMATORY EFFECT OF AQUEOUS AND ETHANOLIC EXTRACT OF *SIDA RHOMBIFOLIA* L. ROOT

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ABSTRACT

Inflammation plays an imperative role in pathophysiology of various diseases. *Sida rhombifolia* L. (Malvaceae), commonly known as Atibala is traditionally used for numerous medicinal purposes in India. The present study was carried out to demonstrate the *in vivo* anti-inflammatory effect of aqueous and ethanolic extract of *Sida rhombifolia* L. root using Wistar rats by carrageenan induced acute inflammatory paw edema method. Preliminary phytochemical screening and acute toxicity test were performed for aqueous and ethanolic extracts. Both aqueous and ethanolic extracts of *Sida rhombifolia* L. root at the dose rate of 200, 400 and 600 mg/ kg body weight were evaluated for anti-inflammatory property. Indomethacin at the rate of 5 mg/ kg body weight was employed as reference drug. Phytochemical screening of aqueous and ethanolic extracts revealed the presence of phenolic compounds, flavonoids, tannins and glycoside. Ethanolic extract was also indicated for the presence of steroids, alkaloids and terpenes. Acute toxicity testing upto 2000 mg/ kg body weight did not show any toxicity in rats. *Sida rhombifolia* L. root extract produced significant ($P< 0.05$) and a dose dependent anti-inflammatory activity. The time dependent inhibition of oedema was observed starting from 2 hours. The ethanolic extract produced most effective inhibition of edema. Our findings revealed that *Sida rhombifolia* L. root extract contribute to the reduction of the inflammatory response which validates the use of the extract in traditional medicine for treating inflammatory conditions

INTRODUCTION: Inflammation is a part of the complex biological local response of living tissue to injurious stimuli. It is a body defense attempt in order to get rid of stimuli [1].

An augmentation in release of pro-inflammatory cytokines, including interleukins, tumor necrosis factor, transcription factor, interferon, are entail in peripheral inflammation which is alienated by anti-inflammatory cytokines and transforming growth factor [2].

Currently available steroidal and non-steroidal anti-inflammatory (NSAID) drugs are exhibit undesired side effects and have their own limitations. Hence, it has become necessary to revisit the importance of the herbal remedies.

The anti-inflammatory activity of extracts from fruits, herbs and spices and isolated compounds has already been reported [3 and 4].

Sida rhombifolia L. (Family: Malvaceae), a semi woody, erect annual or perennial subshrub, found as a weed throughout India. Other names included Atibala, Hastibala (Sanskrit); Wild mallow (English); Aanakurun-thotti (Malayalam) and Chithamutti (Tamil). Previous phytochemical investigations of *Sida* species revealed the presence of cyclopropenoid fatty acids in seed oil [5], alkaloids such as β-phenethylamine, N-methyl- β - phenethylamine, vasicinol, vasicine, choline, betaine, ephedrine and c-ephedrine in root and aerial parts [6] and n-alkanes, long chain alcohols, sterols in aerial parts [7].

Stem and root of *Sida rhombifolia* L. are used to treat rheumatism, asthma, tuberculosis and sexual disorders [8]. The root and leaves are known to be stomachic, demulcent, diaphoretic, diuretic, tonic and sudorific agents and used in various inflammatory conditions [9 and 10]. Extracts of various parts of this plant were reported to have anti-nociceptive and anti-inflammatory [11 and 12], hepatoprotective [13], antibacterial [14 and 15], antifungal [14], antipyretic [15], cytotoxic [12 and 16], antigout [17] and anti- diarrhoeal [18] properties.

As a part of continuing search for naturally occurring cure, the current investigation was aimed to assess the anti-inflammatory effect of root of *Sida rhombifolia* L. in carrageenan induced acute inflammatory rat model.

MATERIALS AND METHODS:

Plant materials/ Drugs: The plant *Sida rhombifolia* L. was collected locally from Thrissur district, Kerala and authentication was done by Kerala Agriculture University, Thrissur. The root of *Sida rhombifolia* L. was separated and air dried at room temperature and powdered using an electrical pulverizer. Indomethacin was used as reference drug.

Preparation of Aqueous Extract: Aqueous extract was prepared by decoction process. One part of powdered root of *Sida rhombifolia* L. and four parts of sterilized water were taken in a boiling apparatus and boiled for 15 minutes. After cooling, the extract was filtered through a Whatmann filter paper No. 1 and autoclaved at 121˚c for 15 minutes. Sterilized aqueous extract was stored at 4˚c till further use.

Preparation of Ethanolic Extract: The powder obtained were extracted using Soxhlet apparatus with 95% ethanol. The ethanolic extract was then concentrated in a rotary vacuum evaporator under reduced pressure, temperature and preserved for further study. Solvents used were of analytical grade.

Experimental Animals: The study was conducted in 48 adult Wistar rats weighing 150-200 g. The rats were procured from Small Animal Breeding Station, College of Veterinary and Animal Sciences, Thrissur. The animals were housed in appropriate clean cages in a well ventilated room with temperature (21-24° C), relative humidity (65-68 %) with 12 hours daylight and 12 hours dark cycle. They were maintained under identical feeding and management practices in the laboratory. All procedures involving the use of experimental animals were in accordance to the committee for the purpose of control and supervision of experiments on Animals (CPCSEA) and were approved by the Institutional Animal Ethics Committee (No. Acad [3] 6554/ 04).

Phytochemical Screening: The aqueous and ethanolic extract of root of *Sida rhombifolia* L. were tested for the presence of various active chemical constituents namely steroids, alkaloids, tannins, phenolic compounds, flavonoids, glycosides, diterpenes, triterpenes and saponins by standard procedures [19].

Acute Toxicity Study: The acute toxicity testing of *Sida rhombifolia* L. root extract was carried out according to The Organization of Economic Co-operation and Development (OECD) Test Guidelines (OECD 423- Limit test procedure). Five animals and the dose level of 2000 mg/ kg body weight were used and the critical observations were made on mortality and signs of toxicity.

Evaluation of Anti-inflammatory activity of *Sida rhombifloia* L. root:

Experimental Design: The animals were randomly divided into eight groups comprising six animals each. Group I served as carrageenan control. Rats of group II, III and IV were treated orally with the single dose of

aqueous extract of *Sida rhombifolia* L. root at the dose rate of 200, 400 and 600 mg/kg body weight respectively. Group V, VI and VII animals were treated with ethanolic extract of *Sida rhombifolia* L. root at the rate of 200, 400 and 600 mg/kg body weight respectively. Group VIII administered with indomethacin at the dose rate of 5 mg/kg body weight.

Carrageenan-induced Acute Inflammatory Model: Anti-inflammatory activity was measured using carrageenan induced rat hind paw edema method [20]. Paw edema was induced by subplantar injection of 0.1 ml of 1% w/ v freshly prepared solution of carrageenan in sterilized water into the right hind paw of each rat of all the groups except the group I. Animals from all the groups were treated orally with above mentioned compounds at prescribed dose levels respectively, 30 minutes prior to carrageenan injection. Paw thickness of all animals were measured initially just before the carrageenan injection and then at 1, 2, 3, 4 and 5th hour after carrageenan injection. Increase in footpad thickness was measured as the difference in paw thickness initially and paw thickness at respective hours.

The per cent (%) inhibition was computed from the following formula.

$$\% \text{ Inhibition} = [1- T_t / T_c] \times 100$$

T_t – increase in paw thickness of animals with test compounds; T_c – increase in paw thickness of control animals

Statistical analysis: The data obtained were analyzed using SPSS 17.0 software (Statistical Package for Social Sciences) by one-way ANOVA followed by student's t test to determine the significant difference between the control and experimental groups. Results were expressed as mean ± standard error. The value of $P \leq 0.05$ was considered statistically significant.

RESULTS:

Phytochemical Screening: Results of phytochemical screening of aqueous and ethanolic extracts of *Sida rhombifolia* L. root are listed in **Table 1**.

TABLE 1: PHYTOCHEMICAL SCREENING OF *SIDA RHOMBIFOLIA* L. ROOT

Chemical Constituent	Aqueous Extract	Ethanolic Extract
Steroids	-	+
Alkaloids	-	+
Phenolic compounds	+	+
Tannins	+	+
Flavonoids	+	+
Glycosides	+	+
Diterpenes	-	+
Triterpenes	-	+
Saponins	-	-

Acute Toxicity Study: There were no changes in behavior pattern and no signs and symptoms of toxicity and mortality were observed in animals treated with *Sida rhombifolia* L. root extracts. It was found that the animals were safe upto a maximum dose of 2000 mg/kg body weight indicating the safety of the extract studied.

Carrageenan-induced Acute Inflammatory Assay: Carrageenan administered at the dose level of 0.1 ml of 1% w/v as subplantar injection in right hind paw of rats showed a time-dependent increase in paw thickness (**Figure 1 and 2**). This increase in inflammatory response was observed at 1h and was maximal at 3 h after administration of carrageenan injection in the carrageenan control group. However, carrageenan induced acute inflammation was significantly ($P \leq 0.05$) reduced in extracts and reference drug treated groups.

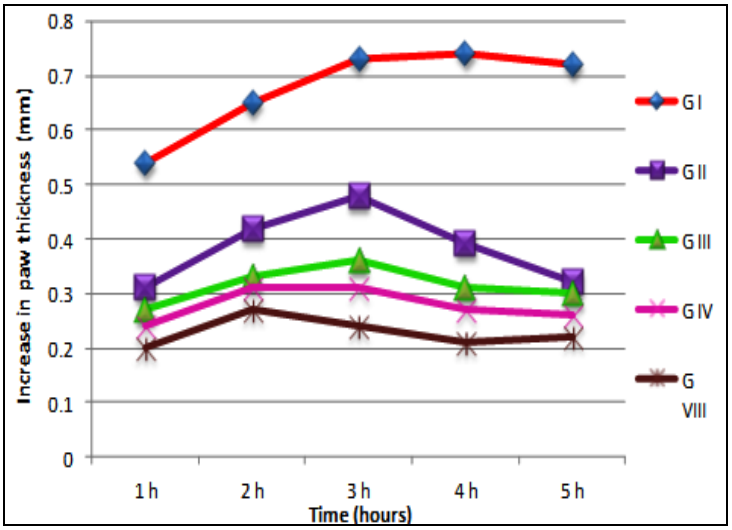

FIGURE 1: EFFECT OF AQUEOUS EXTRACT OF *SIDA RHOMBIFOLIA* L. ROOT ON CARRAGEENAN INDUCED CHANGE IN PAW THICKNESS (mm) IN RATS

Group I: Carrageenan control; Group VIII: Indomethacin at 5 mg/ kg body weight.

Group II, III and IV: Aqueous extract at 200, 400 and 600 mg/ kg body weight

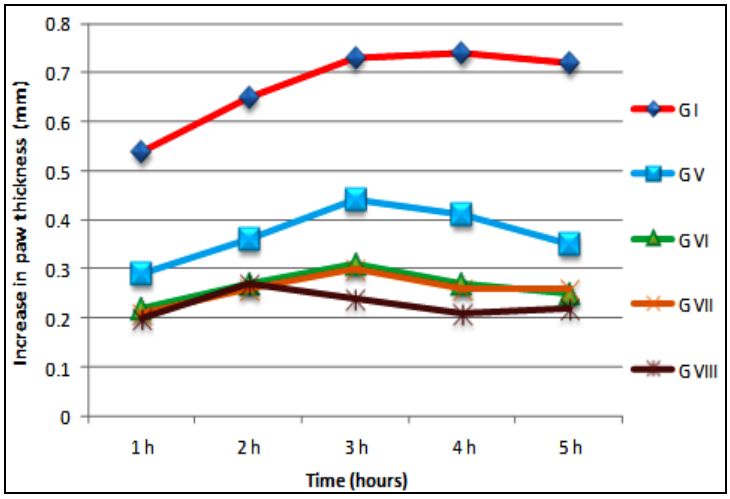

FIGURE 2: EFFECT OF ETHANOLIC EXTRACT OF *SIDA RHOMBIFOLIA* L. ROOT ON CARRAGEENAN INDUCED CHANGE IN PAW THICKNESS (mm) IN RATS

Group I: Carrageenan control; Group VIII: Indomethacin at 5 mg/ kg body weight.

Group V, VI and VII: Ethanolic extract at 200, 400 and 600 mg/ kg body weight.

Aqueous extract at the rate of 200 and 400 mg/ kg body weight shown inhibition at 3 h. Aqueous extract at rate of 600 mg/ kg body weight shown inhibition from 2nd h onwards (Figure 1). Ethanolic extract of *Sida rhombifolia* L. root at the dose rate of 400 and 600 mg/ kg body weight shown inhibition from 3rd hr (Figure 2) which was comparable to reference drug indomethacin treated group. The per cent inhibition of aqueous and ethanolic extracts was dose dependent (**Table 2 and 3**). Though both extract shown significant ($P \leq 0.05$) anti-inflammatory activity from 3rd hr, the peak anti-inflammatory activity (per cent inhibition) was observed in ethanolic extract treated groups (Table 2).

TABLE 2: EFFECT OF AQUEOUS EXTRACT OF *SIDA RHOMBIFOLIA* L. ROOT ON CARRAGEENAN INDUCED PAW EDEMA IN RATS

Groups	Dose	Change in paw thickness (mm/time interval) and (% inhibition)				
		1 h	2 h	3 h	4 h	5 h
Carrageenan (mL)	0.1	0.54±0.01	0.65±0.02	0.73±0.01	0.74±0.02	0.72±0.01
AE (mg/kg)	200	0.31±0.06 **(42.59 %)**	0.42±0.05 **(35.38%)**	0.48±0.04 **(34.25%)**	0.39±0.01 **(47.30%)**	0.32±0.05 **(55.55%)**
AE (mg/kg)	400	0.27±0.04 **(50.00%)**	0.33±0.07* **(49.23%)**	0.36±0.04 **(50.68%)**	0.31±0.06 **(58.10%)**	0.30±0.03 **(58.33%)**
AE (mg/kg)	600	0.24±0.05 **(55.50 %)**	0.31±0.04* **(52.31 %)**	0.31±0.04 **(57.53%)**	0.27±0.06* **(63.51%)**	0.26±0.04[a] **(63.89%)**
Indomethacin (mg/kg)	5	0.20±0.07 **(63.00%)**	0.27±0.07* **(58.50%)**	0.24±0.01* **(67.12 %)**	0.21±0.04* **(71.62 %)**	0.22±0.01 **(69.50%)**

Values bearing superscript * differ significantly ($P \leq 0.05$) from carrageenan control group. Values bearing superscript [a] differ significantly ($P \leq 0.05$) from reference drug groups, AE- Aqueous extract

TABLE 3: EFFECT OF ETHANOLIC EXTRACT OF *SIDA RHOMBIFOLIA* L. ROOT ON CARRAGEENAN INDUCED PAW EDEMA IN RATS

Groups	Dose	Change in paw thickness (mm/time interval) and (% inhibition)				
		1 h	2 h	3 h	4 h	5 h
Carrageenan (mL)	0.1	0.54±0.01	0.65±0.02	0.73±0.01	0.74±0.01	0.72±0.01
EE (mg/ kg)	200	0.29±0.07 **(46.30%)**	0.36±0.08 **(44.62%)**	0.44±0.06 **(40.003%)**	0.41±0.09 **(44.60%)**	0.35±0.04 **(51.39%)**
EE (mg/ kg)	400	0.22±0.03 **(59.30%)**	0.27±0.05* **(58.50%)**	0.31±0.03* **(57.53%)**	0.27±0.04 **(63.51%)**	0.25±0.08 **(65.28%)**
EE(mg/ kg)	600	0.21±0.04 **(61.11%)**	0.26±0.05*a **(60.77%)**	0.30±0.04* **(59.00%)**	0.26±0.03 **(64.86%)**	0.26±0.04 **(63.89%)**
Indomethacin (mg/kg)	5	0.20±0.07 **(63.00%)**	0.27±0.07* **(58.50%)**	0.24±0.01* **(67.12 %)**	0.21±0.04* **(71.62 %)**	0.22±0.01 **(69.50%)**

Values bearing superscript * differ significantly ($P \leq 0.05$) from carrageenan control group. Values bearing superscript [a] differ significantly ($P \leq 0.05$) from reference drug groups, EE- Ethanolic extract

DISCUSSION: Nature has long been a major source for medicinal elements. There is a growing interest in the pharmacological evaluation of various herbal plants used in Indian traditional systems of medicine. In the present investigation, we have studied the anti-inflammatory property of aqueous and ethanolic extracts of root *Sida rhombifolia* L.

Phytochemical screening of aqueous and ethanolic extracts revealed the presence of phenolic compounds, flavonoids, tannins, glycosides etc. (Table 1). The current data is in accordance with earlier reported studies [11 and 21].

In single dose toxicity studies, the time and mode of death of study animals are important indication of toxic response. Rats treated with *Sida rhombifolia* L. root extracts did not produce any toxicity indicating the safety of the extract. The current findings were in agreement with the previous safety reports on *Sida rhombifolia* L. extract [21 and 22].

Carrageenan, sulfated polysaccharides that are extracted from red seaweeds, is a potent chemical known to release inflammatory and proinflammatory mediators. Carrageenan induced hind paw oedema model in rats is acknowledged to be the acute inflammatory model responsive to cyclooxygenase (COX) inhibitors and has often been used to screen the effect of non steroidal anti-inflammatory drugs (NSAID), which primarily involve in inhibition of the cyclooxygenase pathway mediated prostaglandin synthesis.

Cellular and molecular mechanism of carrageenan induced acute inflammation is well exemplified. The time course of edema formation is known to be biphasic. The first phase, occurs in first few hours of injection of the phlogistic agent which has been attributed to the release of histamine, kinins and serotonin [23].

The peak edema volume occur around three hours after the injection and this delayed phase is mainly continued through release of bradykinin, protease, lysosome, leukotrienes and prostaglandin like substances [24 and 25]. Prostaglandin-E2, a potent vasodilator, synergizes with other inflammatory vasodilators such as histamine and bradykinin and contributes to the redness in carrageenan induced acute inflammation [26]. The prostaglandin mediated phase is sensitive to both steroidal and non-steroidal anti-inflammatory agents [25].

The current investigation demonstrated that aqueous and ethanolic extracts from root of *Sida rhombifolia* L. hold significant anti-inflammatory effect in the acute inflammatory model in rats. It is well known that phenolic compounds, flavonoids and glycosides are potent antioxidants. The protective effect of root extract of *Sida rhombifolia* L. under this investigation may be attributed to the anti-inflammatory property of steroids [7] and antioxidant property of flavonoids and phenolic compounds [27].

The observed significant anti-inflammatory activity of both extract may be attributed to the inhibition of the inflammatory mediators such as histamine, serotonin, kinin and prostaglandin. Ethanolic extract of root was found to be the most effective at medium concentration (400 mg/kg body weight) and aqueous extract shown peak activity when higher concentration (600 mg/ kg body weight) employed.

The edema inhibitory effect of extracts was highest in the delayed phase (at 3 hours) of carrageenan induced acute inflammation which opines that *Sida rhombifolia* L. root extract involve mainly in the cyclooxygenase pathway mediated prostaglandin synthesis. Eventhough, lipoxygenase pathway take part in the inflammatory course of action, the inhibition of cyclooxygenase pathway is more efficient in inhibiting carrageenan induced acute inflammation than lipoxygenase inhibitors [28]. Previous Studies on aerial parts, flower buds, leaves and stems of *Sida rhombifolia* L. confirmed the anti-inflammatory activities of its fractions [11 and 13].

CONCLUSION: The results of the current study demonstrate that the aqueous and ethanolic extracts obtained from root of *Sida rhombifolia* L. exhibit dose dependent significant acute anti-inflammatory activity which might be due to a number of bioactive anti-inflammatory elements. Nonetheless, a profound study is necessary for isolation, structural elucidation of bioactive elements to explicate the exact mechanism of action.

These findings uphold the use of *Sida rhombifolia* L. plant in traditional medicine for the management of inflammatory conditions.

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