Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage:www.elsevier.com/locate/apjtb

Document heading doi:10.1016/S2221-1691(12)60342-6

In vivo anti-inflamatory potential of various extracts of *Sida tiagii Bhandari*

Ramkumar Kumawat, Sunil Sharma, Neeru Vasudeva and Suresh Kumar*

Department of Pharmaceutical Sciences,Guru Jambheshwar University of Science and Technology, Hisar-125001, India

ARTICLE INFO

Article history:
Received 5 June 2012
Received in revised from 5 July 2012
Accepted 17 August 2012
Available online 28 August 2012

Keywords:
Anti-inflammation
Sida tiagii B
Carrageenan
Paw edema
Cotton wool granuloma.

ABSTRACT

Objective: The present study is an attempt to explore the anti-inflammatory activity of n-Hexane extract (HS), Ethyl acetate extract (EAS) and Residual ethanolic extract (RES) of fruits of *Sida tiagii* Bhandari by using carrageenan and egg-albumin induced paw edema, xylene induced ear edema and cotton wool granuloma animal models. **Methods:** The biochemical markers like or lysosomal enzymes viz. serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) and alkaline phosphatase (ALP) were also found out in blood serum. **Results:** There was decrease in edema volume in EAS and RES administered animals in carrageenan and egg-albumin induced edema models. The percentage inhibition of inflammation in EAS (34.15%) and RES (39.66%) was found comparable with that of the standard drug, diclophenac sodium (46.69%). The two extracts EAS and RES was found to have good anti-inflammatory activity as compared to standard drug. **Conclusions:** Thus the plant can be used as a potential anti-inflammatory candidate in animals.

1. Introduction

Inflammation (Latin, *inflammare*, to set on fire) is part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. Inflammation is a protective attempt by the organism to remove the injurious stimuli and to initiate the healing process. Inflammation is one of the responses of the organism to the pathogen as infection is caused by various microorganisms [1]. Inflammation is some of the most common manifestations of many diseases afflicting millions of people worldwide [2]. Although there are number treatment available for inflammation, but yet is not satisfying the need of patients suffering from disease. Recently there is vast prevalence of the disease due to the continuous change in life style of people. Hence it is necessary to introduce such therapy which would be more effective and reliable. Since many centuries drugs from plant origin have served through the ages of mainstay for treatment of human diseases and ailments.

The plant *Sida tiagii* Bhandari *(S. tiagii* B.) or Sida pakistanica *B. belonging* to family Malvaceae is a bushy gregarious shrub, a native species of the India and Pakistan desert area, popularly known as "Kharinti" in India. It is used in the folk medicine as blood purifier, tonic and muscle strengthener [3]. The reported activities of *Sida tiagii* have anxiolytic, antiseizure [3], spasmogenic, spasmolytic [4], and anti platelets activities [5], but a few works is still not reported on fruit. While the traditional use of herbal medicines is more or less obsolete today, it is still used for the remedy of diseases in a large number of patients throughout the world. The present study was done for finding the effects of *Sida tiagii* fruit extract in anti-inflammatory animal models.

Despite the progress made in medical research during the past decades, the treatment of many serious diseases is still problematic. Inflammatory diseases remain one of the world's major health problems [6]. It involves a complex array of enzyme activation, mediator release, and extravasations of fluid, cell migration, tissue breakdown and repair [7]. Inflammation has become the focus of global scientific research because of its implication in virtually all human and animal diseases. The efficacy of plant-based drugs used in the traditional medicine have been paid great attention because they are cheap, have little side effects and according to WHO still about 80% of the world population rely mainly on plant-based drugs [8]. In view of the above problems the study was performed for the evaluation of

*Corresponding author: Suresh Kumar Pharmacology Division, Department of Pharmaceutical Sciences, Guru Jambeshwar University of Science and Technology, Hisar, Haryana-125001,
E-mail : pharm.vermasuresh@gmail.com
Contact No. 91-9255577435
Fax Number 01662278181

anti-inflammatory activity of various fruit extracts of *Sida tiagii* plant.

2. Methods and materials

2.1 Drugs and Chemicals

For phytochemical study, the required chemicals procured from college chemical store supplied by Hi-media and Loba chemicals. The standard drugs Diclofenac sodium was obtained as gift samples from Novartis and Ranbaxy respectively. Xylene, hexane, ethyl acetic acid and ethanol were taken from SD Fine Chem. Ltd, Mumbai.

2.2 Plant Material and Preparation of extract

Sida tiagii was collected from the local field of Rajasthan (India) and identified by Dr. H.B. Singh, Head, Raw materials Herbarium and Museum, National Institute of Science Communication and Information Resource, (NISCAIR), New Delhi; specimen was deposited to Herbarium, Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hisar. The fruits were dried at 40± 1 ℃, grounded into coarse size powder and defatted with petroleum ether. The ethanolic extract was obtained by extracting 4 kg of defatted seed powder with ethanol (95%) at 50 ℃ for 72 hr in soxhelet apparatus followed by filtration and concentrated in Rota-evaporator at 50± 5 ℃ to its one third volume. The filtrate was partitioned with n-hexane (n-Hexane Extract of *Sida tiagii* B.; HS) and ethyl acetate (Ethyl acetate Extract of *Sida tiagii* B; EAS) and the respective layers were separated out and dried on water bath at 50 ℃ till dryness (HS 32.23 g, EAS 26.68 g). The residual ethanolic fraction (Residual Ethanolic Extract of *Sida tiagii* B) was dried on water bath separately (104.10 g) and the extracts were stored at temperature below 10 ℃. In the present study we hereby explored the anti-inflammatory potential of different extracts of fruits of *Sida tiagii*. The extracts were freshly prepared with 2% Tween 80 for pharmacological experiments [10].

2.3 Experimental Animals

Male Swiss mice (weighing 25-35 g, 90 days old) and Albino rats (weighing 180-220 g, 90 days old) were obtained from Disease Free Small Animals House, Lala Lajpat Rai University of Veterinary and Sciences, Hisar, India. The animals were maintained at controlled room temperature (21 ± 2 ℃) on a 12 hr light/dark cycle with free access to food and water. All experiments were conducted between 8:00AM and 17:00PM. The experimental protocol was approved by the Institutional Animal Ethical Committee, GJUS&T, Hisar, Haryana, India (Registration no. 0436).

2.4 Experimental Design for different experimental Models

Carrageenan and egg-albumin paw edema

Group I: Control group: 2% Tween 80 solution was administered orally

Group II: Standard group: Diclofenac sodium (10 mg/kg) was administered i. p

Group III-VIII: Test groups: HS, EAS and RES (300 mg/kg and 500 mg/kg of each extract)

Cotton wool granuloma

Group IX: Control group: 2% Tween 80 solution was administered orally

Group X: Standard group: Diclofenac sodium (10 mg/kg) was administered orally

Group XI-XVI: Test groups: HS, EAS and REA (300mg/kg and 500 mg/kg of each extract)

Xylene induced ear edema

Group XVII: Control group: Distilled water was administered orally

Group XVIII: Standard group: Diclofenac sodium (10 mg/kg) was administered i. p

Group IXX-XIV: Test groups: HS, EAS and RES (300 mg/kg and 500 mg/kg of each extract)

2.5 Phytochemical Analysis

Freshly prepared organic extracts were tested for the presence of alkaloids, steroids, triterpenoids, glycosides, tannins, flavonoids, carbohydrates and cardiac glycosides using standard procedures [3].

2.6 Acute toxicity and lethality study

Sida tiagii was administered in different doses 10, 100, or 1000 mg/kg p.o. (n = 5) and 1600, 2900 and 5000 mg/kg doses of the extract were administered to a fresh batch of animals (n = 5) in pilot study to determine sub-maximal effective concentrations for further studies. LD_{50} of various extracts of *Sida tiagii* in mice (n = 30) was estimated using the method described by Lorke. In stage one, animals received oral administration of 10, 100, or 1000 mg/kg (n = 5) of extracts (HS, EAS and RES) and observed for 24 hr for number of deaths. Since no death occurred in any of the group in the first stage of the test, dose was increased (1600, 2900 & 5000 mg/kg) and administered to a fresh batch of animals (n = 5). No death was recorded within 24 h. same test was repeated with different route of administration (i.p.) showing same results [11].

2.7 Carrageenan induced hind paw edema in rat

In this test 0.1 ml of 1% (w/v) carrageenan was injected, s. c. into the planter region of the hind paws of rat [12].

2.8 Egg-albumin induced hind paw edema in rat

Albumin edema was induced by injecting 0.1 ml of 2% w/v of bovine albumin prepared in normal saline into the planter region of the hind paws of rats subcutaneously. Diclofenac sodium was used as standard anti-inflammatory drugs for comparison in both carrageenan and egg-albumin induced paw edema anti-inflammatory model. The extracts of *Sida*

tiagii (doses viz., 300mg/kg, 500mg/kg, orally) and standard drug diclofenac sodium (15mg/kg, p.o.) was administered one hour prior to carrageenan and egg albumin injection. The paw thickness was measured at hourly interval for 5 h using a digital vernier caliper [13]. The anti-inflammatory activity of extracts was studied at different doses along with various relevant biochemical changes.

2.9 Cotton wool granuloma

Male Wistar rats with an average weight of 200 g were anaesthetized with ether. The back skin was shaved and disinfected with 70% ethanol. An incision was made in the lumbar region. The sterilized cotton pellet is placed in both subcutaneous tunnels of scapular region with the help of a blunted forceps. The pellets were either standardized for use in dentistry weighing 20 mg or pellets formed from raw cotton which produced a more pronounced inflammation than bleached cotton. Different group of rats were treated with extracts of *Sida tiagii* at various doses (300 and 500mg/kg) and Diclofenac sodium (15mg/kg) orally for 7 days [14]. On the 8th day, cotton pellets were removed and dried at 60 ℃ for 6 hr [15]. The dry weight was calculated after deducting cotton pellet weight and taken as a measure of granuloma tissue formation and the blood sample were collected for the estimation of biochemical markers like SGOT, SGPT and ALP by using UV kinetic method [16].

2.10 Xylene-induced Ear edema in mice

Edema was induced in each mouse by applying a drop of xylene to the inner surface of the right ear. After 15 minutes, the animals were surgical by decapitate and 7 mm diameter section of the right and left ears were cut and weighed [17].
The level of inhibition (%) was calculated according to the following equation:
Inhibition (%) = [1-Et/Ecke] 100
Where Et = average edema in the treated group and Ec = average edema of the control group.

2.11 Statistical analysis

All experimental data are expressed as Mean± SEM. Statistical analysis were carried out by using one way ANOVA followed by Dunnett's test. The values at P<0.01 were considered as significant.

3. Results

3.1 Preliminary phytochemical screening of Sida tiagii

Preliminary phytochemical screening of *Sida tiagii* was done by using standard procedures of alkaloids, glycosides, carbohydrates, tannins, resins, sterols, proteins, phenolic compounds, saponin and flavanoids and the results are shown as in Table 1.

Table 1
Preliminary phytochemical screening

S.No.	Test	HS	EAS	RES
1	Test for alkaloids			
1.1	Mayer's reagent	+	−	+
1.2	Dragendroff's reagent	−	−	+
1.3	Wagner reagent	+	−	+
1.4	Hagner reagent	+	−	+
2.	Test for Glycosides			
2.1	Borntrager's test	−	+	+
2.2	Keller-killiani test	−	+	+
3	Test for Carbohydrates			
3.1	Molish's test	−	+	+
3.2	Fehling's test	−	+	+
3.2	Benedict's test	−	+	+
4	Test for Sterols			
4.1	Liebermann-Burchard's test	−	+	+
4.2	Salkowski reaction	−	+	+
4.3	Hesse's reaction	−	+	+
4.4	Herch-Sohn's reaction	−	+	+
5	Test for Phenolic compounds and Tannins			
5.1	Ferric chloride test	−	+	+
5.2	Lead acetate test	−	+	+
6	Test for Proteins and amino acids			
6.1	Million's test	−	+	+
6.2	Ninhydrin reagent	−	+	+
7	Test for saponins			
7.1	Foam test	−	−	−
7.2	Sodium bicarbonate test	−	−	−
8	Test for Flavanoids			
8.1	Shinoda/Pew test	−	+	+
8.2	Ammonia test	−	+	+

(+) Present; (−) absent; (HS) n-Hexane extract of *Sida tiagii*; (EAS) Ethyl acetate extract of *Sida tiagii*; (RES) Residual ethanol extract of *Sida tiagii*

3.2 Carrageenan and Egg-albumin induce Paw edema

Sub-plantar injection of carrageenan and egg-albumin in mice resulted in a time-dependent increase in paw thickness (Table 2 and 3 respectively). The EAS and RES of *Sida tiagii* showed the significant ($p<0.01$) decrease in inflammation when compared to control groups but no significant ($p>0.05$) result was shown with HS of *Sida tiagii* The test preparation elicited greater anti-inflammatory activity in egg-albumin induced paw edema as compared to the Carrageenan induced paw edema as shown in Table 2 and 3.

3.3 Cotton wool granuloma

In cotton wool granuloma, the EAS and RES of *Sida tiagii* treatment orally inhibited both exudatory as well as granulatory phases of inflammation. The potency of anti-inflammatory activity observed by RES was found more than that of EAS at 500mg/kg. The standard drug, Diclofenac sodium also elicited inhibitory effect on both exudatory and granulatory phases of inflammation. HS has no significant

(P>0.05) result as anti-inflammatory effect as shown in Table 4.

3.4 Xylene-induced ear edema

In xylene-induced ear edema, EAS and RES of *Sida tiagii* have significantly (p<0.01) inhibited ear edema by xylene induced at both doses 300 mg/kg and 500 mg/kg but with HS do not show significant (p>0.05) result as indicated in Table 5.

3.5 Biochemical estimations

Biochemical parameters SGOT, SGPT and ALP in blood

Table 2

Effect of various extracts of *Sida tiagii* on carrageenan induced paw edema in rats.

Group (Dose of drug)	Edema volume in ml						
	0 hr	30 min	1hr	2hr	3hr	4hr	5hr
Control (2%Tween 80)	3.170±0.09	4.06±0.87	5.11±0.09	4.52±0.087	4.03±0.823	3.85±0.193	3.55±0.093
Diclofenac Sodium(15mg/kg)	2.62 ±0.093	3.26±0.08*(19.70%)	3.60±0.065*(29.55%)	2.78±0.098*(38.49%)	2.72±0.39*(33.47%)	2.708±0.094*(29.71%)	2.63±0.079*(29.7%)
HS (300mg/kg)	3.11 ±0.87	3.99±0.64(1.72%)	4.84±0.98(5.47%)	4.39±0.068(2.8%)	4.27±0.621(0.69)%	3.8±0.931(1.4%)	3.22±0.219(9.2%)
HS (500mg/kg)	3.28±0.045	4.01±0.39(1.23%)	4.84±0.067(5.36%)	4.50±0.231(0.44%)	4.27±0.233(0.20%)	3.85±0.139(0.2%)	3.38±0.931(4.78%)
EAS (300mg/kg)	2.85±0.38	3.82±0.13*(6.15%)	4.37±0.21*(14.48%)	3.57±0.067*(21.02%)	3.26±0.212*(24.35%)	3.13±0.394*(18.78%)	2.94±0.251*(17.21%)
EAS (500mg/kg)	2.76±0.68	4.03±0.40*(0.59%)	4.24±0.087*(17.02%)	4.11±0.821*(9.07%)	3.5±0.911*(18.68%)	2.93±0.939* (24.02%)	2.79±0.930*(21.4%)
RES (300mg/kg)	2.35 ±0.98	3.39±0.076*(16.5%)	4.28±0.087*17.8%	4.08±0.122*(9.73%)	3.35±0.293*22.16%	3.27±0.093*2.17%	3.15±0.041*11.29%
RES (500mg/kg)	2.67 ±0.39	3.51±0.11*(13.55%)	4.568±0.321*(10.76%)	4.14±0.972*(8.45%)	3.798±0.098*(13.10%)	2.74±0.921*(28.9%)	2.69±0.491*(24.25%)

Values are expressed as Mean± SEM, n =6 in each group. Data was analyzed by one way ANOVA followed by Dunnett's Test. * P<0.01 as compared to control group.

Table 3

Effect of various extracts of *Sida tiagii* on egg-albumin induced paw edema rats.

Group (Dose of drug)	Edema volume in ml						
	0 hr	30 min	1hr	2hr	3hr	4hr	5hr
Control (2%Tween 80)	3.412±0.07	4.056±0.029	5.24±0.024	5.058±0.029	4.86 ±0.049	4.018±0.66	3.818±0.047
Diclofenac Sod(15mg/kg)	2.116±0.102	3.134±0.021* (22.73%)	3.81±0.058* (27.48%)	2.85±0.025* (43.69%)	2.70±0.041*(44.44%)	2.648±0.509* (34.24%)	2.56±0.024* (32.94%)
HS (300mg/kg)	2.964±0.160	4.02±0.049 (0.88%)	5.126 ±0.056 (2.2%)	4.924±0.0375 (2.65%)	4.81 ±0.038 (1.02%)	3.856±0.06 (4.03%)	3.736±0.026 (2.14%)
HS (500mg/kg)	3.174±0.06	3.952±0.02 (2.56%)	5.18 ±0.37 (1.15%)	4.94±0.04 (233%)	4.75±0.505 (2.26%)	3.91±0.045 (2.68%)	3.714±0.038 (2.72%)
EAS (300mg/kg)	2.196±0.06	3.728±0.019* (8.28%)	4.04 ±0.219* (22.9%)	3.45±0.025* (31.79%)	3.19 ±0.018* (34.36%)	2.538±0.073* (36.83%)	2.4±0.032* (37.14%)
EAS (500mg/kg)	2.466±0.048	3.88±0.032* (4.33%)	4.086 ±0.014* (22.02%)	3.872±0.038* (23.44%)	3.18±0.058* (34.56%)	2.73±0.056* (32.05%)	2.49±0.012* (34.78%)
RES (300mg/kg)	2.34±0.043	3.84±0.035* (5.27%)	4.026±0.018*(23.16%)	3.931±0.044* (22.28%)	3.048±0.024* (37.28%)	2.658±0.015* (33.84%)	2.402±0.018* (37.08%)
RES(500mg/kg)	2.173±0.038	3.736±.048* (7.88%)	4.012±0.065* (22.43%)	3.82±0.08* (24.47%)	3.03±0.025*(37.65%)	2.36±0.025* (41.26%)	2.26±0.027* (40.75%)

Values are expressed as Mean±S.E.M, n =6 in each group. Data was analyzed by one way ANOVA followed by Dunnett's Test. * P<0.01 as compared to control group.

Table 4

Effect of various extract of *Sida tiagii* on cotton wool induced granuloma in rats.

Group & Dose of drug	Weight of wet cotton wool (mg)	% Inhibition	Weight of dry cotton wool(mg)	% Inhibition
Control	299.17±5.69		86.5±0.86	
Diclofenac sod(15 mg/kg)	159.5±5.76*	46.69	44.33±1.4*	48.75
HS (500 mg/kg)	285.5±2.53*	4.569	85.33±1.69*	1.70
EAS (500mg/kg)	187.11±2.98*	34.15	55.33±1.26*	36.03
RES (500 mg/kg)	180.5±2.85*	39.66	53.33±1.2*	38.34

Values are expressed as Mean±S.E.M, n =6 in each group. Data was analyzed by one way ANOVA followed by Dunnett's Test. * P<0.01 as compared to control group.

Table 5

Effect of various extracts of *Sida tiagii* on xylene induced ear edema in mice

Group (Dose of drug)	Weight of right ear (mg)	Weight of left ear (mg)	Difference(mg)	Inhibition%
Control	41.22±0.43	23.51±0.21	17.71±0.23	
Diclofenac sod	29.76±0.14	24.83±0.11*	4.94±0.04	72.11
HS(300mg/kg)	42.09±1.12	24.93±0.89	17.16±1.02	3.16
HS (500mg/kg)	44.02±0.94	24.07±0.29	16.95±0.79	4.29
EAS (300mg/kg)	28.45±0.20	23.46±0.37*	4.99±0.37	71.82
EAS (500mg/kg)	28.35±0.19	23.71*±0.22*	4.82±0.09	72.78
RES (300mg/kg)	27.69±0.15	22.70*±0.15*	5.26±0.18	70.30
RES (500mg/kg)	28.77±0.12	24.58*±0.17*	4.2±0.10	76.28

Values are expressed as Mean±S.E.M, n =6 in each group. Data was analyzed by one way ANOVA followed by Dunnett's Test. * P<0.01 as compared to control group.

serum were elevated significantly (P<0.01) in egg albumin induced hind paw edema. Pretreatment with EAS, RES of *Sida tiagii* and Diclofenac sodium, prevented the increase in level of these biochemical parameters significantly (P<0.01). But the level of SGOT, SGPT and ALP was not prevented (P>0.05) by HS of *Sida tiagii* B. (Table 6).

In cotton wool granuloma experimental model, the increased SGOT, SGPT and ALP in serum were significantly (p<0.01) prevented with the concomitant administration of EAS and RES of *Sida tiagii* as well as Diclofenac sodium but was not prevented by HS of *Sida tiagii* (Table 7).

Table 6

Effect of extract of *Sida tiagii* on various Biochemical changes in egg-albumin induced paw edema

Group(Dose of drug)	SGOT(U/ml)	SGPT(U/ml)	ALP(U/ml)
Control	105.67±0.98	60.75±0.32	112.67±0.92
Diclofenac sod	62.48±0.23*	23.37±0.32*	71.13±0.39*
HS (300mg/kg)	102.83±1.85	60.09±0.26	111.08±0.52
HS (500mg/kg)	102.33±1.78	60.83±0.31	114.44±0.40
EAS (300mg/kg)	64.5 0±0.22*	24.33±0.49*	72.49±0.26*
EAS (500mg/kg)	63.60±0.23*	24.00±0.44*	72.49±0.26*
RES (300mg/kg)	60.50±0.43*	21.67±0.42*	68.71±0.28*
RES (500mg/kg)	59.50±0.43*	20.17±0.40*	68.29±0.35*

Values are expressed as Mean±S.E.M, n =6 in each group. Data was analyzed by one way ANOVA followed by Dunnett's Test. * P<0.01 as compared to control group.

Table 7

Effect of various extract of *Sida tiagii* on various biochemical changes on Cotton wool induced Granuloma in rats

Dose Group (Dose of drug)	SGOT(U/ml)	SGPT(U/ml)	ALP(U/ml)
Control	47.33± 0.41	118.17± 0.31	73.5±0.85
Diclofenac sod	27.01±0.365*	6733±0.56*	55.27±0.40*
HS (500 mg/kg)	47.83±0.48	116.83±0.30	75.03±0.36
EAS (500mg/kg)	27.19±0.45*	66.67±0.56*	56.5±1.76*
RES (500mg/kg)	22.67±0.58*	64.33±0.34*	54.5±0.22*

Values are expressed as Mean±S.E.M, n =6 in each group. Data was analyzed by one way ANOVA followed by Dunnett's Test. * P<0.01 as compared to control group.

4. Discussion

In spite of tremendous development in the field of synthetic drugs during recent era, they are found to have one or the other side effect, whereas plants still hold their own unique 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. The enzyme, Phospholipase A2, is known to be responsible for the formation of mediators of inflammation such as prostaglandins and leukotrienes. Phospholipids in the cell membrane are converted into arachidonic acid with the help of Phospholipase A2, which is highly reactive and is rapidly metabolized by cyclooxygenase (prostaglandin synthesis) to prostaglandins, which are responsible for the induction of pain and inflammation [18].

It is well known that carrageenan induced paw edema is characterized by biphasic event with involvement of different inflammatory mediators. In the first phase (during the first 2 h after carrageenan injection), chemical mediators such as histamine and serotonin play role, which in second phase (3, 4 h after carrageenan injection) Kinins and prostaglandins are involved [19]. Our results revealed that administration of EAS & RES extracts of *Sida tiagii* inhibits inflammation which is caused by chemical or other mediators of inflammation.

The cotton wool granuloma is widely used to evaluate the transudative and proliferative components of chronic inflammation. The moist weight of the wool correlates with transude, the dry weight of the wool correlates with the amount of granulumatous tissues [20-21]. Chronic inflammation occurs by means of the developments of proliferated cells. These cells can be spread in granuloma form. Non-steroidal anti-inflammatory drugs decrease the size of granuloma which results from cellular reaction by inhibiting granulocyte infiltration, preventing generation of collagen fibers and suppressing mucopolysaccharides [22]. The extracts (EAS & RES) of S. tiagii showed significant (p<0.01) anti-inflammatory activity in cotton wool induced granuloma and thus found to be effective in chronic inflammatory condition, which reflected its efficacy in inhibiting the increase in the number of fibroblasts and synthesis of collagen and mucopolysaccharides during granuloma tissue formation.

The xylene-induced ear edema method has certain advantages for natural product testing and has good predictive values for the screening of anti-inflammatory agents [23]. Xylene causes instant irritation in the mouse ear, which leads to fluid accumulation and edema characteristic of an acute inflammatory response. Suppression of this response is a likely indication of antiphlogistic effect [24]. In the present study, both the extracts, RES (300 & 500 mg/kg) and EAS (300 & 500 mg/kg), of S. tiagii showed significant (P<0.01) antiphlogistic effect against xylene-induced ear edema.

There is an evidence that lysosomal enzyme played an important role in the development of acute and chronic inflammation [25-26]. Most of the anti-inflammatory drugs exert their beneficial effect by inhibiting either release of lysosomal enzyme or by stabilizing lysosomal membrane which is one of the major events responsible for the inflammatory process [30]. Administration of EAS, RES and Standard drug decrease the serum levels of SGOT, SGPT and ALP in both carrageenan induced paw edema and cotton wool granuloma model which was found significant (p<0.01) as compared to control groups. The HS extract of S.tiagii B. did not show any significant (p>0.05) effect in all the animal

models.

Acknowledgement

The authors are highly grateful to the All India Council for Technical Education, New Delhi for providing research fellowship during research work.

Conclusion

From the above results it was found that EAS and RES extracts have significant anti-inflammatory activity. So these extracts of S. tiagii can be used as a potential natural source of inflammation disorders by preventing or slowing the progress of inflammation. Again, no mortality was recorded up to 5000 mg/kg of the extracts in the acute toxicity test; it showed that the plant is safe for use. Thus the plant can be used as a potential anti-inflammatory candidate in animals. In future study the main constituent can be separated out which is responsible for anti-inflammatory activity.

References

[1] Ferrero-Miliani L, Nielsen OH, Andersen PS, Girardin SE. Chronic inflammation: importance of NOD2 and NALP3 in interleukin-1beta generation. *Clin. Exp. Immunol* 2007; **147** (2): 227-35.

[2] Amabeoku JG and Kabatende J. Antinociceptive and anti-inflammatory activities of leaf methanol extract of cotyledon orbiculata L. (crassulaceae). Advances in Pharmacological Sciences 2012, Article ID 862625, 6 pages. doi:10.1155/2012/862625

[3] Datusalia AK. Anxiolytic antiseizure effect of *Sida tiagii* Bhandari. *J. Health Science* 2008; **54**: 544-554.

[4] Hassan GAU et al. Spasmogenic and spasmolytic constituents in Sida pakistanica, *International journal of Pharmacognosy* 1999; **37**: 173-178.

[5] Sahid TM et al. Inhibition of Adrenalin-induced aggregation of human platelets by Pakistani medicinal plants. *Pakistani journal of pharmaceutical sciences* 1991; **4**: 145-152.

[6] Hossain H, Shahid-Ud-Daula AFM, Hasan K, Mansur AA and Haq MM. Anti-inflammatory activity, total flavonoids and tannins content from the ethanolic extract of spilanthes paniculata leaf growing in bangladesh. *Int J Pharm* 2012; **2**(2): 271-277.

[7] Perianayagam JB, Sharma SK, Pillai KK. Antiinflammatory activity of Trichodesma indicum root extract in experimental animals. *J Ethnopharmacol* 2006; **104**: 410-414.

[8] 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.

[9] Dharmasiri JR, Jayakody AC, Galhena G, Liyanage SSP, Ratnasooriya WD. Antiinflammatory and analgesic activities of mature fresh leaves of Vitex negundo. *J Ethnopharmacol* 2003; **87**: 199-206.

[10] Badole SL, Anand A. Kumar ZA, Ghosh P et al. Analgesic and anti-inflammatory activity of alcoholic extract of stem bark of Pongamia pinnata (L.) Pierre. *Biomedicine & Aging Pathology*. 2012; **2** (1); 19-23.

[11] Sastry1 AVS. Chemical and pharmacological evaluation of aqueous extract of seeds of Cassia occidentalis. *J. Chem. Pharm Res* 2011; **3**(2): 566-575.

[12] Winter CA, Risely EA, Nuss GW. Carrageenan induced edema in the hind paw of the rat as an assay for anti-inflammatory drug. Proc. *Soc. Expel. Boil. Med* 1962; **111**: 544.

[13] Thambi PT, Kuzhivelil B, Sabu MC, Jolly CI. Antioxidant and anti-inflammatory activities of the flowers of Tabernaemontana coronaria (l) R.BR. *Indian J Pharm Sci* 2006; **68**: 352-5.

[14] Vogel HG. Drug Discovery and Evaluation Pharmacological Assays, 2nd ed., Springer, New York, 2002, pp 57 .

[15] Winter CA, Portar CC. Effect of alternation in side chain on anti-inflammatory and liver glycogen activities of hydrocortisone esters. *J Am Pham Sci* 1957; **46**: 515.

[16] Kalpan A, Lavernel LS. Clinical chemistry, Interpretation and techniques, 2nd ed, Lea and Febiger, Philandelphia, 1983, pp 34.

[17] Delporte C, Backhouse N, Erazo S, Negrete R, Vidal P, Silva X, Lopez Perez JL, Feliciano AS, Munoz O. Analgesic-antiinflammatory properties of Proustia pyrifolia. *J Ethnopharmacol* 2005; **99**: 119-124.

[18] Hernandez PM, Rabanal GR. Evaluation of the anti-inflammatory and analgesic activity of Sideritis anariensis var. pannosa in mice. *J Ethnopharmacol* 2002; **8**: 43-47.

[19] Vane JR. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature New Biol* 1971; 231-235.

[20] Castro J, Sasme H, Susman H, Bullette P. Diverse effect of SKF 52 and anti-oxidants on CCL4 induced changes in liver microsomal P-450 content and ethyl morphine metabolism. *Life Sci* 1968; **7**: 129-136.

[21] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. *J Biol Chem* 1951; **193**:265-75.

[22] Alcaraz MJ, Jimenez MJ. Flavonoids an anti-inflammatory agent. *Fitoterapia* 1988; **59**: 25-38.

[23] Lu HM, Liang YZ, Yi LZ, Wu XJ. Anti-inflammatory effect of Houttuynia cordata injection. *J Ethanophrmacol* 2006; **104**: 245-249.

[24] Okoli CO, Akah P, Nwafor S, Anisiobi A, Ibegbunam I and Erojikwe O. Anti-inflammatory activity of hexane leaf extract of Aspilia africana C.D Adams. *J. Ethnopharmacol* 2006; **109**(2): 219-225.

[25] Plaa G, Charbonnean M. Detection and evaluation of chemically induced liver injury, In: Hayes, A.W., Principals and methods of Toxicology, Raven press, New York, 1994, pp 34.

[26] Nair RB, Ravishankar B, Vijayan NP, Sasikala CK, Saraswathy VN. Anti-inflammatory effect of Stribilanthus heyneanus leaves, A biomedical study. *J. Res Ay Slid* 1988; **9**(1-2): 46.