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## Effect Of Saponins From Of *Sesbania sesban* L.(Merr) On Acute And Chronic Inflammation in Experimental Induced Animals

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**Abstract:** *Sesbania sesban* L. Merr (Leguminosae) is an important medicinal plant in India, used by indigenous people to relieve rheumatic pains. The anti-inflammatory activity of *Sesbania sesban* L. Merr. (Leguminosae) crude saponin (SAP) was investigated for the first time. The SAP was tested for the presence of triterpenoid and steroids. The diosgenin and oleanolic acid were isolated from saponin fraction and characterized by using HPTLC and GC-MS. The anti-inflammatory activity of crude saponin was studied by *in vivo* animal models like carrageenan and histamine induced rat paw edema, cotton pellet granuloma, acetic acid induced vascular permeability and oxazolone induced delayed type hypersensitivity. Mechanism action of inflammation was also observed by *in vitro* heat induced haemolysis and inhibition of protein denaturation assay. The crude saponin (SAP) extract showed significant activity in the *in vivo* and *in vitro* model at the dose of 500 mg kg<sup>-1</sup> body weight orally, when compared to control and standard drug. These findings showed that the crude saponin contains triterpenoid and steroidal moiety which may be contributes to the observed anti-inflammatory activity.

**Key words:** Anti-inflammatory, crude saponin, delayed type hypersensitivity, HPTLC, *Sesbania sesban*

### INTRODUCTION

Studies in the last decades have demonstrated that inflammation plays an essential role in the pathogenesis and progression of atherosclerosis. Anti-inflammatory agents have been shown good effects in the prevention and treatment of atherosclerosis and coronary artery diseases (Wang *et al.*, 2008).

*Sesbania sesban* Merr. (Leguminosae), commonly known as Jayanti, is widely distributed in Africa and Asia. It is a small tree that grows up to 8 m tall. Leaves are claimed to possess wonderful pain relieving properties and are used as purgative and demulcent. The poultice of *S. sesban* leaves are used to cure boils abscesses and absorption of inflammatory rheumatic swellings (Kirtikar and Basu, 1996). Leaves are also clinically useful in vicharchika, skin disease like eczema (Anonymous, 1958). Plant are attributed with several medicinal activities like galactogogues, hypoglycemic (Khare, 2004), healing and antifertility (Bhaduri *et al.*, 1968). The fresh juice of leaves exhibited anti bacterial, antifungal and anthelmintic property (Sasikumar *et al.*, 2005). The leaves and flowers are used in medicinal poultices and teas. The phytochemical analysis of the plant has revealed the presence of  $\beta$ -sitosterol, lupeol, triterpenoidal saponins

(Haraguchi *et al.*, 2000; Dorsaz *et al.*, 1988), oleanolic acid (Varshney *et al.*, 1959) and diosgenin (Anonymous, 1958). The folklore claim of *Sesbania sesban* leaves are to relieve pain and inflammation, however, there is no supportive scientific evidence reported in the literature. Therefore an attempt has been made to investigate the possible anti-inflammatory activity and chromatographic pattern of crude saponins of *Sesbania sesban*.

### MATERIALS AND METHODS

**Plant collection:** *Sesbania sesban* leaves were collected in September (2009) from the village Khadakjam of Dist. Nashik, Maharashtra, India. The plant was identified by Dr.D.A. Patil, Taxonomist, Department of Botany, S.S.V.P.S College of Science, Dhule, Maharashtra, India and a voucher specimen was deposited at R.C. Patel Institute of Pharmaceutical Education and Research, Shirpur, Dhule, Maharashtra, India.

**Chemicals:** Carrageenan, histamine, oxazolone were procured from Sigma-Aldrich, USA. Indomethacin (Cadila Healthcare, India) and betamethasone valerate (Glaxo Smithclem Bechem, Mumbai). All chemicals used were of analytical grade.

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**Preparation of extract:** The dried leaves were powdered using pulverizer and passed through sieve no. 40. The powder (200 g) was extracted methanol. Filter the extract with whatman filter paper no. 1 and concentrated using rotary vacuum evaporator (Buchi, Switzerland). The methanol extract was further fractionated in butanol: water (1:1), concentrated the extracts and saponins were precipitated by adding solvent ether to get crude saponin (SAP). It was then preserved in desiccator for further use (Rajpal, 2002).

**Experimental animals:** Male wistar rats and swiss albino mice of either sex weighing between 150-200 g and 15-22 g, respectively were obtained from the institutional animal house. They were housed in standard cages, six animal in each cage and kept at temperature  $22\pm 2^\circ\text{C}$  and 12:12 h light dark cycle. The animals were provided with pelleted diet and water *ad libitum*. This study was proceeding after the approval obtained from Institutional Animal Ethical Committee (IAEC) of R. C. Patel Institute of Pharmaceutical Education and Research, Shirpur, India (RCP/IAEC/2007-08/12), registered under CPCSEA, Chennai, India.

**Phytochemical screening:** The preliminary phytochemical test of SAP was performed according to the method described by Trease and Evans (1989). The crude saponins were subjected to column chromatography by gradient elution using ethyl acetate followed by methanol in increasing proportion, for separation and isolation of natural compound. Isolated compound were characterized by using chemical test, UV, IR, HPTLC and GC-MS.

**Gas chromatography-mass spectrometry (GC-MS):** The GC-MS analysis of SAP and isolated compound was performed using Agilent model 6890 interfaced to a 5973 mass selective detector. HP-5MS capillary columns (30 m $\times$ 0.25  $\mu\text{m}$  film thickness). The column temperature was programmed at  $60^\circ\text{C}$  (6 min), increasing to  $240^\circ\text{C}$  at a rate of  $5^\circ\text{C min}^{-1}$ ; carrier gas (helium) was set at a flow rate of  $0.9 \mu\text{L min}^{-1}$ ; ionization energy 70 eV and scan mode of electro-ionization (EI). One  $\mu\text{L}$  of sample was injected and the compounds were identified by matching their mass fragmentation pattern and retention time with the standard.

**HPTLC analysis:** The High Performance Thin Layer Chromatography (HPTLC) of crude saponins was performed on CAMAG (Switzerland) model equipped with Linomat V applicator and scanner III (CAMAG). It was performed on TLC plate precoated with silica gel 60 GF<sub>254</sub> as stationary phase and hexane: acetic acid (4:1) used as mobile phase. The plate was visualized by spraying with 25% methanolic  $\text{H}_2\text{SO}_4$  followed by heating

at  $110^\circ\text{C}$  and were scanned at 366 nm densitometrically. The Quantitative analysis of diosgenin and oleanolic acid ( $1 \text{ mg mL}^{-1}$  in chloroform) was calculated in crude saponin.

#### Pharmacological activity

**Acute toxicity study:** Rats and mice were divided into seven groups of ten animals each. One group served as control and received saline solution ( $10 \text{ mL kg}^{-1}$ ), while the remaining six groups were treated with increasing dose of SAP  $100\text{-}5000 \text{ mg kg}^{-1}$  p.o. The mortality rate and toxicity parameters were observed during 24 h for next seven days period and  $\text{LD}_{50}$  was determined according to the method described by Miller and Tainter (1944). According to the result of acute toxicity test, the dose of 250 and  $500 \text{ mg kg}^{-1}$  were chosen for pharmacological experiments.

#### Anti-inflammatory activity

**Carrageenan-induced rat paw edema test:** The method of Winter *et al.* (1962) was adopted for the carrageenan-induced edema test in rats. Control group received saline, the standard group received indomethacin ( $10 \text{ mg kg}^{-1}$ ) and the test groups received extract of *S. sesban* (SAP) at the dose of 250 and  $500 \text{ mg kg}^{-1}$  p.o. The paw volume, up to tibiotarsal articulation, was measured at 0, 60, 120 and 180 min using the plethysmometer (Ugo Basile, Italy). The percentage inhibition was calculated using the expression:

$$\text{Inhibition of inflammation (\%)} = \frac{V_c - V_t}{V_c} \times 100$$

where,  $V_c$  was the average degree of inflammation in the control group and  $V_t$  was the average degree of inflammation in the test groups. The percentage inhibition of paw volume in treated groups was compared with the control group (treated with vehicle).

**Histamine-induced rat paw edema:** Paw edema was produced by sub-plantar administration of 0.1 mL of 1% freshly prepared solution of histamine into the right hind paw of the rats according to the method described by Winter *et al.* (1962). The animals were pretreated as follows: negative and positive control group received normal saline and the standard indomethacin ( $10 \text{ mg kg}^{-1}$ ) by p.o. route, respectively. The test groups received extract of *S. sesban* (SAP). The right hind paw volume was measured at 0, 1, 2 and 3 h using the plethysmometer (Ugo Basile, Italy). The percentage inhibition of the inflammatory activity was calculated.

**Cotton pellet granuloma test:** The method of D'Arcy *et al.* (1960) was adopted for the cotton pellet granuloma test in

rats. Control group received the normal saline (5 mL kg<sup>-1</sup>) by p.o. SAP at dose of 250 and 500 mg kg<sup>-1</sup> was administered orally for 7 days. Standard group received indomethacin (10 mg kg<sup>-1</sup>) by p.o. for the same period. On the 8th day, the animals were sacrificed and the pellets along with the granuloma tissue were carefully removed, freed of tissue attachments and dried in an oven at 60°C. The percent change of granuloma weight relative to vehicle control group was determined.

**Acetic acid induced vascular permeability:** Acetic acid induced vascular permeability was performed according to Whittle (1964) with slight modification in swiss albino mice. The positive control group received dexamethasone (50 mg kg<sup>-1</sup>) i.p and negative control group received normal saline, respectively. One hour after oral administration of vehicle (saline), the test groups received SAP. After 1 h, 1% solution of Evan's blue (0.1 mL 10 g<sup>-1</sup> body weight) was injected intravenously. Thirty minute later, 0.7% of acetic acid (0.1 mL 10 g<sup>-1</sup>) was administered by i.p route. Half an hour later the animals were sacrificed by cervical dislocation. The peritoneal cavity was washed with normal saline and solutions were collected in the test tubes. Sodium hydroxide (1 N NaOH) is added to clear the turbidity caused due to proteins. The solution was subjected to micro titer plate ELIZA reader (BIO-Tek Power wave TM XS, Model-96 well micro plate) and absorbance measured at 610 nm. The vascular permeability was represented in terms of the absorbance (A<sub>610</sub>) which leaked into the cavity.

**Oxazolone induced delayed type hypersensitivity test:** The study was carried out to investigate whether plant extracts exhibited antiphlogistic activity. The method of Moodley *et al.* (1995) was adopted for oxazolone induced delayed type hypersensitivity test. Animal groups (six animals per group) were fed on a standard diet ad libitum.

**Sensitization:** The immunological reaction was measured by comparing thickness of ears of all treated animals. Ear swelling was measured using a digimatic caliper (Mitutoyo Model No.CD-6 CSX series 293, Japan). The percentage of edema inhibition was expressed as the reduction in thickness with respect to the control group.

**In-vitro heat induced haemolysis:** The rat erythrocyte haemolysis was assayed by Olajide *et al.* (2000). Twenty microlitre fresh rat blood was added to the vials containing 1 mL of 0.1 M phosphate buffer saline (PBS pH 7.4). The percentage inhibition of heat induced haemolysis with respect to control was calculated as follows:

$$\text{Inhibition (\%)} = \frac{A_{540} \text{ control} - A_{540} \text{ sample}}{A_{540} \text{ control}} \times 100$$

**In-vitro inhibition of protein denaturation:** The method of Chatterjee and Das (1996) adapted for inhibition of protein denaturation. The percentage inhibition of protein denaturation was calculated as follows:

$$\text{Inhibition (\%)} = \frac{\text{Absorbance of test} - \text{Absorbance of control}}{\text{Absorbance of control}} \times 100$$

**Statistical analysis:** All data were represented as Mean±SEM. Data obtained were subjected to one way analysis of Variance (ANOVA) followed by multiple comparison Dunnett's test. p<0.05 was considered significant.

## RESULTS

**Phytochemical analysis:** The percentage yield of crude saponin was found to be 10% w/w. Preliminary phytochemical analysis of *S. sesban* revealed the presence of triterpenoid and saponins. SAP was subjected to column chromatography with varying proportions of solvents to obtained compound 1 λ max was found to be 349.5 nm which resembles to diene keto and spiro-ketal steroids. The results of chemical test viz. Lieberman Burchard, Salkowaskii, Whitby and trichloro acetic acid test performed for compound 1 showed positive for the presence of steroidal saponin (Mohammad, 2005). The hydrolyzed product of crude saponin (sapogenin) was subjected to GCMS analysis. Results obtained from GC analysis showed the presence of 7 major peaks with their retention time (Table 1). Mass fragmentation pattern of selected peaks R<sub>f</sub> 58.59 and 44.62 represents compound having molecular ion peak at m z<sup>-1</sup> 464 and m z<sup>-1</sup> 425, respectively. Both the compound has base peak at m z<sup>-1</sup> 43. According to its molecular fragmentation pattern both the peaks are similar to that of molecular fragmentation pattern diosgenin and oleanolic acid. Similarly compound 1 was also resembled with diosgenin.

HPTLC study of crude saponin showed six spots with R<sub>f</sub> 0.04, 0.07, 0.19, 0.23, 0.26 and 0.33. The R<sub>f</sub> values were found to be 0.20 and 0.26 (Fig. 1) which

Table 1: Results of major peaks, retention time and area for GC analysis of sapogenin

Major peak	Retention time (min)	Area (%)
1	18.576	7.91
2	20.354	12.13
3	39.238	2.33
4	43.858	2.94
5 (Oleanolic acid)	44.629	17.39
6	55.537	4.78
7 (Diosgenin)	58.599	52.53

**Table 2: Effect of *S. sesban* saponin on Carrageenan and histamine induced paw edema**

Treatment/group	Dose (mg kg <sup>-1</sup> )	Mean increase in paw volume (mm)			
		Carrageenan		Histamine	
		1 h	3 h	1 h	2 h
Control	---	0.38±0.06	1.00±0.025	0.27±0.005	0.41±0.012
Indomethacin	10	0.18±0.024** (52.63)	0.31±0.023** (62.00)	0.21±0.004 (21.34)	0.23±0.003** (44.15)
SAP	250	0.25±0.019** (34.21)	0.63±0.031** (56.00)	0.22±0.004 (17.60)	0.25±0.004** (38.41)
SAP	500	0.23±0.021** (39.47)	0.59±0.031** (59.00)	0.21±0.005 (22.00)	0.23±0.006** (43.02)

Values in brackets denote percentage inhibition of the edema paw volume. Values are expressed as Mean±SEM, n = 6; \*\*p<0.01, \*p<0.05 as per control by one way ANOVA followed by Dunnett's multiple comparison test. SAP-crude saponin

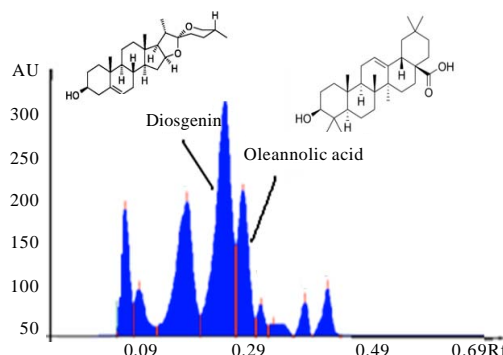
corresponding with the standard diosgenin and oleanolic acid, respectively. The amount of diosgenin and oleanolic acid present in the plant *S. sesban* was estimated and found to be 0.084 and 0.053 % w/w, respectively.

**Animal study:** The *in-vivo* anti-inflammatory effects of SAP were assessed using carrageenan and histamine-induced paw edema, cotton pellet granuloma, acetic acid induced vascular permeability, oxazolone induced delayed type hypersensitivity test. Mechanism based *in-vitro* assay was also evaluated by heat induced haemolysis and inhibition of protein denaturation.

**Carrageenan induced paw edema:** In the carrageenan-induced paw edema, a maximum edema paw volume of 1.00±0.025 mm was observed in the control rats, 3 h after the carrageenan injection. Rats pre-treated with SAP significantly decreased (p<0.01) the carrageenan-induced edema paw edema by 59% at a higher dose of 500 mg kg<sup>-1</sup>, 3 h after the injection of noxious agent. It was observed that the crude saponins (SAP) at the higher dose of 500 mg kg<sup>-1</sup> exhibited comparable results with that of standard indomethacin which inhibited the rat paw edema by 62% (Table 2).

**Histamine induced rat paw edema:** Histamine induced rat paw edema was dose dependant (250 and 500 mg kg<sup>-1</sup>) and found to be statistically significant (p<0.01). Size of paw volume increased to maximum of 0.41±0.012 mm was observed in the control group. Rats pre-treated with SAP significantly inhibited (p<0.01) the histamine induced rat paw edema by 38.41 and 43.02% at the dose of 250 and 500 mg kg<sup>-1</sup>, respectively. The low dose of SAP did not show significant inhibition at early phase (at 1 h) of inflammation but they showed significant activity at the later phase (after 3 h) of inflammation (Table 2).

**Cotton pellet granuloma test:** Anti-inflammatory activity of SAP (500 mg kg<sup>-1</sup>) in cotton pellet granuloma test was significantly inhibiting the formation of fibroblast by 38.17% which was comparable with that of standard Diclofenac sodium which showed an inhibition of 44.32% (Table 3).



**Fig. 1:** HPTLC chromatogram of extract showing the presence of diosgenin (R<sub>f</sub> 0.20) and oleanolic acid (R<sub>f</sub> 0.26) under 366 nm

**Table 3: Effect of *Sesbania sesban* saponin on percent inhibition in cotton pellet-induced granuloma in rats**

Group	Dose (mg kg <sup>-1</sup> )	Weight of dried cotton pellet (mg)
Control	Normal saline (10 mL kg <sup>-1</sup> )	64.58±2.8980
Diclofenac sodium	5	35.96±2.149 (44.32)
SAP	250	40.99±1.912** (36.53)
SAP	500	39.93±2.186** (38.17)

Values in brackets denote percentage inhibition of the edema paw volume. Values are expressed as Mean±SEM, n = 6, \*\* p<0.01, as per control by one way ANOVA followed by Dunnett's multiple comparison Test. SAP-crude saponin

**Acetic acid induced vascular permeability:** In acetic acid induced vascular permeability, the test groups as well as the standard were compared with control group. Crude saponin from *S. sesban* (SAP) at the dose 250 and 500 mg kg<sup>-1</sup> significantly (p<0.01) reduced vascular permeability by 68.28 and 83.22%, respectively as compared to control group (Fig. 2). SAP showed dose dependent inhibition to increased vascular permeability comparable to the standard drug (89.74%).

**Oxazolone induced delayed type hypersensitivity test:** Crude saponins (500 mg kg<sup>-1</sup>) showed maximum inhibition (69.68% on 22 day) of ear edema in oxazolone induced delayed type hypersensitivity test which was comparable to the standard drug which gave 73% inhibition on 22 day (Table 4).

Table 4: Effect of *Sesbania sesban* saponin on Oxazolone-induced ear edema

Groups (n = 6)	Dose	Oxazolone induced ear oedema (Thickness in mm)					
		7th Day	10th Day	13th Day	16th Day	19th Day	22 Day
Control	2% (300 µL)	0.46±0.012	0.65±0.014**	0.95±0.012**	1.35±0.004**	1.57±0.006**	1.88±0.004**
Betamethasone	5 mg/ear	0.47±0.012	0.49±0.009** (24.16)	0.53±0.012** (44.00)	0.56±0.015** (58.01)	0.48±0.007** (69.30)	0.50±0.009** (73.00)
SAP	250 mg kg <sup>-1</sup> , p.o	0.47±0.012	0.54±0.013** (16.30)	0.64±0.013** (32.00)	0.78±0.015** (42.00)	0.79±0.005** (49.10)	0.75±0.009** (60.00)
SAP	500 mg kg <sup>-1</sup> , p.o	0.41±0.010	0.43±0.013** (33.85)	0.49±0.011** (48.42)	0.59±0.011** (56.29)	0.51±0.001** (67.51)	0.57±0.004** (69.68)

Values in brackets denote percentage inhibition of the edema paw volume. Values are expressed as Mean±SEM, n = 6; \*\* p<0.01, as per control by one way ANOVA followed by Dunnett's multiple comparison test. SAP-crude saponin

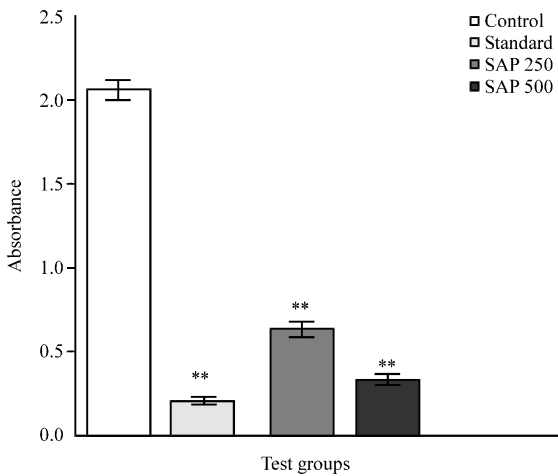


Fig. 2: Effect of crude saponin extract and standard drug on vascular permeability. \*\*p<0.01 (ANOVA). Values of absorbance shown are Mean±S.E.M. (n = 6)

Table 5: Effects of *Sesbania sesban* saponin on heat induced erythrocytes haemolysis and protein denaturation

Concentration (µg mL <sup>-1</sup> )	Heat hemolysis <sup>a</sup> absorbance	Protein denaturation <sup>b</sup> absorbance
150	0.139 (27.22)	0.127 (36.81)
200	0.127 (33.50)	0.118 (41.29)*
250	0.111 (41.88)*	0.110 (45.27)**
300	0.108 (43.45)**	0.101 (49.75)**
350	0.100 (47.64)**	0.098 (51.24)**

Control absorbance of a and b: 0.191 and 0.201, respectively. Values in brackets denote percentage inhibition. Values are expressed as Mean±SEM, n=6. \*\*p<0.01, \*p<0.05 as per control by one way ANOVA followed by Dunnett's multiple

**Heat-induced haemolysis:** SAP inhibited heat-induced haemolysis of RBCs to varying degrees. Test samples evoked conc. related inhibition of haemolysis. The inhibitory effect produced by SAP at 350 µg mL<sup>-1</sup> concentration was 47.64%. Denaturation of protein is one of cause of inflammation. Effect of SAP on inhibition of protein denaturation, is shown in Table 5 at different concentrations (150 -350 µg mL<sup>-1</sup>) provided significant protection against denaturation of proteins. The

percentage inhibition of protein denaturation was observed in SAP was 51.24% (Table 5).

## DISCUSSION

The overall results showed that the crude saponins (SAP) from *Sesbania sesban* leaves exhibited a significant anti-inflammatory activity against experimental models. Since carrageenan induced rat paw edema is commonly used as an experimental animal model for acute inflammation. It is biphasic reaction; of which first phase is mediated by the release of histamine and 5-HT followed by kinin release and then prostaglandin in the latter phase (Mazumder *et al.*, 2003). SAP significantly (p<0.01) inhibited carrageenan and histamine induced rat paw edema. These results suggested that saponins play an important role in acute phase of inflammation and its action was partly related to histamine. The significant ameliorative activity of the extracts observed in the present study may be due to inhibition of inflammatory mediators such as histamine, serotonin and prostaglandin. Contact hypersensitivity is characterized by an antigen-specific T cell-mediated skin inflammation that occurs at the site of challenge with a hapten in individuals who have already generated an immune response to that hapten. Ear edema test is regularly used for topical testing of anti-inflammatory activity. As histamine play an important role in the effectors phase of delayed-type hypersensitivity (Kou *et al.*, 2006) which is known to be involved in many inflammation disorders such as hepatitis and arthritis. The significant activity shown by *Sesbania sesban* saponin in the oxazolone induced contact type delayed hypersensitivity may be due to its action on the 5-HT.

In vascular permeability assay, of inflammatory mediators, released following stimulation, leads to dilation of arterioles and venules leading to increased vascular permeability (Vogel and Vogel, 1997). A compound diminishing the dye leakage caused by acetic acid that increase vascular permeability, might well be found to have anti-inflammatory due to an action on

the vessel wall and on the exudative phase of inflammation (Spector and Willoughby, 1998). The decrease in vascular permeability by SAP indicates the suppression of vascular response in the process of acute inflammation. From these findings it is assumed that an anti-inflammatory activity of *Sesbania sesban* may be from its protection on the release of inflammatory mediators at the first stage.

The repair phase of the inflammatory process begins with the proliferation of fibroblasts as well as multiplication of small blood vessels. Such proliferating cells penetrate and the exudate producing a highly vascularised and reddened mass known as granulation tissue (Fang *et al.*, 2007). Granuloma of chronic inflammation comprises an accumulation of modified macrophages arranged in small clusters or nodular collections or surrounded by a cuff of lymphocyte and is a consequence of cell mediated immunity (Okoli and Akah, 2004). Investigation revealed that the crude saponins exerted significant ( $p < 0.01$ ) effect on the granulomatous inflammation (Mazumder *et al.*, 2003).

Lysosome plays a major role in the inflammatory reaction. Stabilization of lysosomal membrane thus decreases the release of tissue destroying enzyme and phospholipase A<sub>2</sub>. steroids and non steroidal anti inflammatory agent exert their anti inflammatory action (Dingle, 1961; Shen, 1967). Since there is close similarity of the red blood cell membrane system to the lysosomal membrane system, protection against heat induced hemolysis of red blood cell is often extra plotted to stabilization of lysosomal membrane can used as a biochemical index of anti inflammatory activity (Saso *et al.*, 2001). To confirm the membrane stabilizing action of *Sesbania sesban*, experiment was performed on the erythrocyte membrane. The SAP was found to inhibit the haemolysis of erythrocyte induced by heat. A possible explanation for the stabilizing activity of test sample could be an increase in the surface volume ratio of the cells which could be brought about by an expansion of membrane or shrinkage of the cell and an interaction with membrane proteins (Shinde *et al.*, 1999).

Moreover, it has also been shown that the deformity and cell volume of erythrocytes is closely related to the intracellular content of calcium. Hence it may be speculated that the cytoprotective effect of erythrocyte membrane may be due to the ability of the SAP to alter the influx of calcium into the erythrocytes. The present study suggests that the membrane stabilizing activity of *Sesbania sesban* may be playing significant role in its anti-inflammatory activity. Production of auto antigens in

inflammation disease may be due to *in vivo* denaturation of protein. Their effect on heat-induced denaturation Human Serum Albumin (HSA) in comparison with several fatty acids which are known to be potent stabilizers of this protein (Saso *et al.*, 2001).

Phytochemical investigation of crude saponins (SAP) revealed the presence of both steroidal and triterpenoidal aglycone likes oleanolic acid and diosgenin (Varshney *et al.*, 1959). The GC-MS technique used to evaluate the mass fragmentation pattern of hydrolyzed product of crude saponin. Saponin and triterpenoid acid eg. diosgenin and oleanolic acid has been associated with various biological activity including anti-inflammatory action (Giner-Larza *et al.*, 2001; Kim *et al.*, 2004; Kaskiw *et al.*, 2008). Thus the significant anti-inflammatory activity may be due to saponins and sapogenin present in the *Sesbania sesban*.

## CONCLUSION

Based on the results it can be concluded that the anti-inflammatory potentials of leaves of *Sesbania sesban* is may be due to the presence of saponins, which protect inflammatory mediators. The results helped in giving pharmacological evidence and scientific explanation to the ethno medical claims of *Sesbania sesban* leaves. Besides it also demonstrated that *Sesbania sesban* leaves could be a good candidate for the development of new anti-inflammatory drug.

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