

Anti-inflammatory activity of ethanolic leaf extract of *dalbergia sissoo* in vitro and in vivo

Abstract

The anti-inflammatory activity of *Dalbergia sissoo* ethanolic leaf extract (DSELE) was evaluated from the tests of HRBC (human red blood cell) membrane stabilization in vitro and cotton pellet granuloma test in mice. Inhibition of protein denaturation by DSELE was studied to assess anti-arthritis activity. Extract of *D. sissoo* in different solvents were evaluated for presence of phytoconstituents in the plant. From the results DSELE showed appreciable HRBC membrane stabilization activity. DSELE at 100, 200, 400, 800 and 1000 µg/ml concentration inhibited protein denaturation 47.62±2.25, 55.15±2.04, 56.41±0.51, 77.66±2.88 and 78.39±3.39 per cent, respectively. In cotton pellet granuloma test in mice DSELE showed dose dependant and significant ($P<0.01$) anti-inflammatory activity in all DSELE treated group as compare to controls. In phytochemical analysis *D. sissoo* extracts revealed the presence of anthroquinone, amino acids, protein, saponin, sterols, tannin, glycosides, flavonoids and phenols.

Keywords: anti-inflammatory, hrbc, cotton pellet granuloma, *dalbergia sissoo*

Volume 2 Issue 3- 2017

Yogita Amrutkar, Hajare SW, Ingawale MV,
Bhojane NM, Madhuri Hedau, Ingole RS
Department of Pharmacology & Toxicology, Post Graduate
Institute of Veterinary & Animal Science, India

Correspondence: Hajare SW, Assistant Professor, Department
of Pharmacology & Toxicology, Post Graduate Institute of
Veterinary & Animal Science, Akola-444104, India,
Email sunilwhajare@rediffmail.com

Received: October 16, 2016 | **Published:** May 02, 2017

Introduction

Dalbergia sissoo Roxb (Indian rosewood) is an important tree species belonging to the family *papilionaceae*. It is a medium to large-sized, gregarious deciduous tree, attaining a height up to 30 m and a girth up to 2.5 m in a favorable climate (sub-tropical and tropical zones). According to Champion et al.¹ the *Shisham* tree is a characteristic species of *Khair-sissoo* (*Acacia catechu-Dalbergia sissoo*) primary serial type forest of Asian subcontinent. *Dalbergia sissoo* is native to the plains occurs naturally at elevation of about 900m but sometime occurring up to 1500m from the Kabul River in Afghanistan through northern Pakistan, northern India, Nepal, Bhutan and into Assam. Human cultivation has greatly expanded its distribution to Southeast Asia, Africa, the Middle East, the Caribbean, tropical America, and Arizona in the United States. *Shisham* is a very hardy species and produces valuable timber and can be propagated both by seeds and vegetative parts.²

Dalbergia sissoo is broadly used in folk medicine for several diseases.^{3,4} Several biologically active compounds such as flavones, isoflavones, quinines and coumarins have been isolated from *Dalbergia sissoo*. It also contains tectoridin, caviunin-7-O-glucoside, iso-caviunin, tectorigenin, dalbergin, bio-chanin-A, and 7-hydroxy-4-methylcoumarin. The heartwood gave 3,5-dihydroxy-trans-stibene biochanin A, dalbergichromene, dalbergenone and iso-dalbergin.⁵ From the earlier studies scanty literature is available to authenticate anti-inflammatory activity from hydroethanolic extract of *Dalbergia sissoo*. We report herein, the anti-inflammatory activity of its hydroethanolic extract.

Materials and methods

Collection and processing of plant material

The mature green leaves of *Dalbergia sissoo* (Roxb.) were collected in January-February from the campus of Post Graduate Institute of Veterinary and Animal Science (PGIVAS), Akola and identified from expert Botanist. The shade dried leaves were pulverized to get fine powder. Freshly prepared powder (100 g) was immersed in 500 ml

of 60% ethanol and kept in orbital shaker at 150 rpm for 48 hrs. The resultant solvent filtered through Whatman No.1 filter paper. The filtrate obtained was concentrated to semisolid mass at 45°C in hot air oven. The extract thus obtained is termed as *D. sissoo* ethanolic leaf extracts (DSELE) and was used for further studies.

Animals

Swiss mice of either sex were procured from the Animal House unit of Department of Veterinary Pharmacology and Toxicology, PGIVAS, Akola. The animals were maintained under standard laboratory conditions and had standard pellet diet with free access to clean drinking water.

Acute toxicity study

Acute toxicity was performed according to the OECD-423 guide lines.⁶ Eighteen Swiss albino female mice (20-25g) were equally divided into three groups and were given DSELE 500, 1000 and 2000mg/kg, p.o. The animals were observed for 24h to record toxic effects and mortality and were further observed for 14 days to record any mortality. Based on acute toxicity studies two safe dose levels of DSELE were selected for *in vivo* studies.

Hypotonic solution induced HRBC membrane stabilizing activity

Membrane stabilization of human red blood cell (HRBC) by DSELE was studied as per the method of Sadique et al.⁷ The blood was collected from healthy human volunteer not taken any NSAIDS for 2weeks prior to the experiment and collected in heparinized vacutainer. The blood was washed three times with 0.9% saline and centrifuged simultaneously for 10 minutes at 3000rpm. The packed cells were washed with 0.9% saline and a 10% v/v suspension was made using phosphate buffer (154mM NaCl in 10mM Sodium Phosphate Buffer at pH 7.4) as stock erythrocyte or RBC suspension. Various concentration (100, 200, 400, 800 and 1000 µg/ml) of DSELE was prepared using distilled water and to each concentration 1ml of phosphate buffer, 2ml hyposaline and 0.5 ml of HRBC suspension

were added. Test solution was incubated at 37°C for 30min and centrifuged at 3,000 rpm for 10min and the hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560nm. Dexamethasone was used as reference drug and a control was prepared using 1ml Phosphate buffer, 2ml distilled water and 0.5ml HRBC suspension.

Inhibition of protein denaturation

Inhibition of protein denaturation by DSELE was evaluated *in-vitro* with bovine serum albumin.^{8,9} The reaction mixture (0.5ml) consisted of 0.45ml bovine serum albumin (5% w/v aqueous solution) and 0.05ml of DSELE (100, 200, 400, 800 and 1000µg/ml) or Dexamethasone (100, 200, 400, 800 and 1000µg/ml) in distilled water. All the above solutions were adjusted to pH 6.3 using 1N HCl. The samples were incubated at 37°C for 20min and the temperature was increased to keep the samples at 57°C for 3minutes. After cooling the samples, 2.5ml phosphate buffer saline (pH 6.3) was added to each tube. Absorbance was measured spectrophotometrically at 416 nm and the per cent inhibition of protein denaturation was calculated.

Cotton pellet granuloma in mice

Mice were grouped in to four groups of six animals each. The pellets weighing exactly 10±1mg each were prepared from 5 mm section of cotton rolls. The cotton pellets were sterilized in an autoclave for 30 min. Autoclaved cotton pellets weighing 10±1mg was implanted S.C. along the axillae of mice under light ether anesthesia as per the method of Winter & Porter.¹⁰ DSELE (200 and 400mg/kg) suspended in distilled water and dexamethasone (1mg/kg) and control group

(normal saline) were administered orally consecutively from the day of cotton pellet insertion up to 7 days. After the insertion of the pellets, the skin was sutured. The mice were sacrificed on 8th day and pellets covered by the granulation tissue were dissected out and dried in hot air oven at 60°C till the constant weight was achieved.

Statistical analysis

The data of present research work was analysed by one way ANOVA followed by student t-test for comparison between test and control using standard statistical method. The t-value at **p<0.01 were considered for analysis of significance.¹¹

Results and discussion

HRBC membrane stabilization activity

Five different concentrations viz. 100, 200, 400, 800 and 1000µg/ml of both DSELE and dexamethasone were used in this study. The results of HRBC membrane stabilization activity of DSELE is tabulated in Table 1. The DSELE showed dose dependant HRBC membrane stabilization activity with consistent rise in per cent stabilization of HRBC membrane. Mean per cent stabilization of HRBC membrane by DSELE at 100, 200, 400, 800 and 1000µg/ml was found to be 65.44±6.05, 66.51±6.81, 68.65±5.11, 70.8±5.32 and 73.09±5.63, respectively, while mean per cent stabilization of HRBC membrane by dexamethasone at 100, 200, 400, 800 and 1000µg/ml was found to be 58.26±6.03, 65.14±1.49, 68.60±2.43, 76.15±0.63 and 76.61±0.75, respectively. Thus DSELE showed remarkable dose dependant HRBC membrane stabilization activity which is found be comparable to standard drug dexamethasone.

Table 1 Effect of DSELE on inhibition of HRBC membrane stabilization

Concentration (µg/ml)	O.D Value		Percent inhibition	
	DSELE	Dexamethasone	DSELE	Dexamethasone
100	0.075±0.013**	0.091±0.013**	65.44±6.05	58.26±6.03
200	0.073±0.014**	0.076±0.003**	66.51±6.81	65.14±1.49
400	0.068±0.011**	0.068±0.005**	68.65±5.11	68.60±2.43
800	0.063±0.011**	0.052±0.002**	70.8±5.32	76.15±0.63
1000	0.058±0.012**	0.051±0.001**	73.09±5.63	76.61±0.75
Control O.D :- 0.218±0.004				
One way Anova : Df: 5, 12				
F Value		F(DSELE)=89.636	F(Dexa)=321.225	

Values are expressed in mean±S.D.(n=3) Student's t-test(n=3), **P<0.01, compared to control.

Inhibition of protein denaturation method

The DSELE showed dose dependant inhibition of protein denaturation activity with consistent rise in per cent inhibition of protein denaturation (Figure 1). Inhibition of protein denaturation by DSELE at 100, 200, 400, 800 and 1000µg/ml was found to be 47.62±2.25, 55.15±2.04, 56.41±0.51, 77.66±2.88 and 78.39±3.39 per cent, respectively, while mean per cent inhibition of protein denaturation by dexamethasone at 100, 200, 400, 800 and 1000µg/ml was found to be 40.29±2.88, 46.89±2.07, 69.96±1.36, 84.25±3.40 and 89.38±1.36, respectively. The maximum per cent inhibition of protein denaturation by DSELE found to be lesser than dexamethasone.

Cotton pellet granuloma in mice

In cotton pellet granuloma test in mice, DSELE showed dose dependant significant (P<0.01) reduction in granuloma weights. Mean per cent inhibition of granuloma pellet in groups treated with DSELE at dose rates of 200 and 400mg/kg was 18.66 and 19.5, respectively, while per cent inhibition by dexamethasone was 26.5. Thus the DSELE showed significant (P<0.01) anti-inflammatory activity at 200 and 400mg/kg concentration as compared to control group mice (Table 2). In phytochemical analysis *D. sissoo* extracts revealed the presence of anthroquinone, amino acids, protein, saponin, sterols, tannin, glycosides, flavonoids and phenols.

The actual mechanism of action of DSELE showing HRBC membrane stabilization may be due to inhibition of release of lysosomal content of neutrophils at the site of inflammation. These lysosomal constituents are involved in bactericidal action by containing bactericidal enzyme and protease, when released extracellularly cause damage to tissue leads to inflammation. Some of the NSAIDs are known to possess membrane stabilization due to prevention of osmotic loss of intracellular electrolyte and fluid components.¹² Compounds with membrane stabilizing properties are well known for their ability to interfere with release of phospholipases that trigger the formation of inflammatory mediators.¹³ The DSELE due to membrane stabilization effect may prevent efflux of these intracellular components which might interference with the release of phospholipases and alteration with the influx of calcium, as the influx of calcium is important in release of inflammatory mediators.

Denaturation of proteins is a well documented cause of inflammation and rheumatoid arthritis. Several anti-inflammatory drugs have shown dose dependent ability to inhibit thermally induced protein denaturation. Mechanism of denaturation probably involves alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding.¹⁴ From the results it is speculated that DSELE may be capable of controlling the production of auto antigen and inhibits denaturation of protein in rheumatic disease.

Table 2 Effect of DSELE on wet and dry weight of cotton pellets in mice

Treatment	Dose	Mean wet weight	Percent inhibition	Mean dry weight	Percent inhibition
Control	N.S.	104±7.96		37.16±2.98	
Dexa mg/kg	1	65.16±2.34**	63.16	9.66±0.77**	27
DSELE mg/kg	200	68.5±6.44**	66	6.65±0.67**	19
	400	71.66±7.55**	69.33	6.96±1.14**	20
One way Anova: Df=4,25					
F value	7.728	8.617			

Values are expressed in Mean±S.E(n=6), Student's 't'-test,**P<0.01, compared to control

Cotton pellet granuloma is a model of non immunological types of inflammation and edema is mainly due to proliferative phase of inflammation.¹⁵ Efficacy of DSELE in this *in vivo* test may be possibly due to inhibition of monocytes infiltration and fibroblast proliferation. Activated monocyte can release a series of pro-inflammatory cytokines and TNF- α .¹⁶ From the study it is concluded that DSELE possess significant anti-inflammatory activity seen from its effect on HRBC membrane stabilization, inhibition of protein denaturation and from inhibition of granuloma formation in mice.

Acknowledgements

None.

Conflict of interest

The author declares no conflict of interest.

References

1. Champion HG, SK Seth. A revised survey of forest types of India. Manager of publications Delhi, Government of India press, India: Springer; 1968.

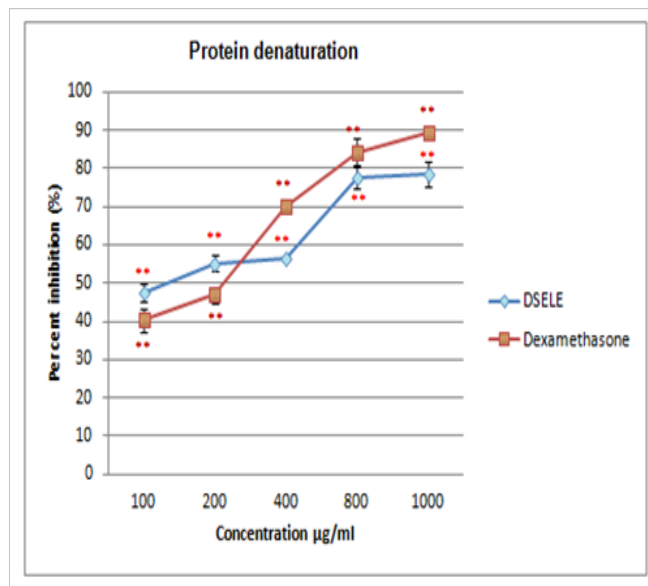


Figure 1 Effect of various concentrations of DSELE and dexamethasone on denaturation of protein.

- Lodhiyal Neelu, Lodhiyal LS, Pangtey YPS. Structure and function of Shisham forest in central Himalaya, India: dry matter dynamics. *Ann Bot.* 2002;89(1):41–54.
- Kirtikar KR, Basu BD. *Indian Medicinal Plants*. Dehra Dun, India: International book distributors and publishers; 1993. p. 818–910.
- Hajare SW, Chandra S, Sharma J, et al. Analgesic and antipyretic activities of *Dalbergia sissoo* leaves. *Indian Journal of Pharmacology.* 2000;32:357–360.
- Hugar Mallinath H, Kallappa HM, Liyakath Ahmed MD. Phytochemical and pharmacological studies of ethanol extract of *Dalbergia sissoo* seeds: an approach for the *in-vivo* analgesic and antipyretic activities. *International Journal of Pharma and Bio Sciences.* 2010;1(4):0975–6299.
- OECD. Guidance document on the recognition, assessment and use of clinical signs as humane end points for experimental animals used in safety evaluation environmental health and safety monograph series on testing and assessment No 19, France; 2000.
- Sadique J, Al Rqobab NA, Bughaith MF, et al. The bioactivity of certain medicinal plants on the stabilization of RBC membrane system. *Fitoterapia.* 1989;60:525–532.

8. Mizushima Y, Kobayashi M. Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins. *J Pharm Pharmacol*. 1968;20(3):169–173.
9. Aruoma OI. Free radicals, oxidative stress and antioxidants in human health and disease. *Journal of American Oil Chemists Society*. 1998;75(2):199–212.
10. Winter CA, Porter CC. Effect of alteration in side chain upon anti-inflammatory and liver glycogen activities of hydrocortisone esters. *J Am Pharm Assoc Am Pharm Assoc*. 1957;46(9):515–519.
11. Snedecor GW, Cochran WG. *Statistical methods*. 6th ed. New Delhi, India: Oxford and IBH; 1967. 593 p.
12. Khalid MM, Ansari AP, Rahman M, et al. Evaluation of *ex-vivo* cardio-protective and anti-inflammatory investigation of bangladeshi plants extract. *Journal of Scientific Research & Reports*. 2015;7(1):58–66.
13. Aitadafouri M, Mounnnieri C, Heyman SF, et al. 4-Alkoxybenzamides as new potent phospholipase A2 inhibitors. *Biochemical Pharmacology*. 1996;51(5):737–742.
14. Arya D, Patni V. Comparative analysis of *in vitro* anti-inflammatory and anti-arthritis activity in methanolic extract of *Pluchea lanceolata* Oliver & Hiern. *Int J Biol Pharm Res*. 2013;4(9):676–680.
15. Swindle KF, Shiederman FE. Phases of inflammatory response to subcutaneous implantation of cotton pellet and their modification by certain anti-inflammatory agents. *J Pharmacol Exp Ther*. 1972;183(1):226–234.
16. Laupattarakasem P, Wangsrimongkol T, Surarit R, et al. *In vitro* and *In vivo* anti-inflammatory potential of cryptoleisis buchanani. *J Ethnopharmacol*. 2006;108(3):349–354.