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IN VITRO ANTI-ARTHRITIC ACTIVITY ON LEAVES OF *SIMAROUBA GLAUCA* BY BOVINE SERUM ALBUMIN METHOD

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ABSTRACT

The aim of the study is to carry out the *in vivo* anti-inflammatory activity on leaves of *Simarouba glauca*. The *in-vitro* anti- arthritic activity of the ethanolic extract *Simarouba glauca* has been done by on bovine serum protein denaturation method and results are compared with standard. The results are at different concentration (10 to 1000µg/ml). *Simarouba glauca* showed significant activity at various concentrations. Significant activity was seen at the concentration 73.84% at 1000µg/ml when compared with the standard drug diclofenac sodium.

KEYWORDS

Anti-arthritic activity, Anti-inflammatory activity on leaves and *Simarouba glauca.*

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INTRODUCTION

Arthritis is a chronic auto immune disorder principally attacks the joints and characterized by pain, swelling, inflexibility and stiffness of the involved joints[1]. Arthritis is inflammation of one or more joints. Arthritis involves the breakdown of cartilage. Cartilage normally protects a joint, allowing it to move smoothly. Without the normal amount of cartilage, the bones rub together, causing pain, swelling (inflammation), and stiffness. The individuals of any age can be affected with Arthritis; the usual age of onset is between 25 and 50 with a peak in the 40s and 50s[2].

In India more than about 20% of total population is suffering from arthritis. Symptoms of arthritis can include reduced ability to move the joint, stiffness, especially in the morning, difficulty performing daily activities, disability, long-term (chronic) pain etc. The key risk factors of arthritis includes age, gender, excess weight, injury, dietary pattern, consumption of excess alcohol, life style, heredity, hormonal factors, environmental factors and lack of physical activity. There are four main groups of drugs used to treat arthritis: Pain killers (analgesics), non-steroidal anti-inflammatory drugs (NSAIDs), disease-modifying anti-rheumatic drugs (DMARDs) and corticosteroids (steroids)[3].

The production of auto antigens in certain arthritic diseases may be due to *invitro* denaturation of proteins[4]. The mechanism of denaturation probably involves alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding[5]. So, by controlling the production of auto antigen and inhibiting denaturation of protein and membrane lysis in rheumatic disease leads to anti-arthritic activity. Hence, inhibition of protein denaturation and membrane lysis were taken as a measure of the in vitro anti-arthritic activity[6].

Simarouba glauca also known as paradise tree has a long history of herbal medicine in many countries and belongs to the family *Simaroubaceae*. The *Simaroubaceae* family includes 32 genera and more than 170 species of trees and brushes of pan tropical distribution[7]. The bark and leaf extract of *Simarouba* is used as haemostatic, anthelmenthic, antiparasitic, antidysentric, antipyretic and anticancerous. The bark is used to cure fever, malaria, stomach and bowel disorders, haemorrhages, and ameobiasis. Leaf, fruit pulp and seeds are possessing medicinal properties such as analgesic, antimicrobial, antiviral, astringent, stomachic tonic and vermifuge. The crushed seeds are used as antigo against snake bites.

MATERIAL AND METHODS

Collection of plant material

The leaf of *Simarouba glauca* was collected from the local area of mid land of idukki in Kerala state. The plant was identified, confirmed and authenticated by Dr. Gurukar Mathew Botanist and Head of the Department of Botany, Bharathi College, Bharathinagara. The leaves were collected and shade dried under room temperature. The dried material was pulverized separately into coarse powder by mechanical grinder. The coarse powder was subjected to hot continuous extraction with ethanol in a Soxhlet extractor.

Extraction

Continuous hot extraction process- 1kg of dry coarse powder of the leaf of *Simarouba glauca* was extracted with ethanol by hot continuous using Soxhlet apparatus. The extraction was continued until the solvent in the thimble become clear. After complete extraction, the extract was filtered and solvent was distilled off. The extract was concentrated for drying in desiccators over anhydrous calcium chloride. A dark green residue was obtained. The extract was subjected to preliminary phytochemical screening and pharmacological activities.

Assessment of *in vitro* anti-arthritic activity

Phosphate buffer preparation

1N HCl preparation:- (85ml of HCl are dissolved in 1000ml distilled water). 8g Nacl, 0.2g kcl, 1.44g of disodium hydrogen phosphate (Na_2HPO_4), 0.24g of potassium dihydrogenphosphate (KH_2PO_4) are dissolved in 500ml of water. The PH was adjusted to 6.3 using 1N HCl and make up to volume to 1000ml with distilled water.

The test solution was prepared by taking 0.45ml of bovine serum albumin (BSA-5 % w/v aqueous solution) and 0.05 ml of ethanol extract of *Simarouba glauca* in various concentration (10-1000 µg/ml). The test control solution was prepared by adding 0.45ml of BSA and 0.05ml of DMSO. Product control consists of 0.45ml of distilled water and 0.05ml of ethanol extract of *Simarouba glauca* in various concentrations (10-1000 µg/ml). Standard solution consists of 0.45ml of BSA and 0.05ml of diclofenac sodium solution in various concentrations (10-1000 µg/ml). The pH of the above solutions is to be adjusted to 6.3 using 1N HCl and incubated at 37º C for 20 minutes. Then

the resultant solutions were heated at 57º C for 3 minutes. The solutions were cooled. 2.5 ml of phosphate buffer was added and the absorbance of resultant mixtures were read at 660 nm.

The percentage inhibition of protein denaturation was calculated as follows

Percent inhibition=100- [OD of test solution – OD of product control] / OD of test control × 100

The control represents 100% protein denaturation. The results are to be compared with the standard diclofenac sodium[8].

Statistical analysis

The data were expressed as Mean ± S.E.M.

RESULTS

Preliminary phytochemical screening

The qualitative analysis of extracts of *Simarouba glauca* were carried out and extracts showed the presence of various chemical constituents such as carbohydrates, saponins, terpenoids, glycosides, phenolics, flavonoids, and tannins. The results are shown in Table No.1

The *in-vitro* anti- arthritic activity of the ethanolic extract *Simarouba glauca* has been done by on bovine serum protein denaturation method and results are compared with standard. The results are summarized in table at different concentration (10 to 1000µg/ml). The percentage inhibition of protein denaturation was calculated and found for test at 10 to 1000 µg/ml showed 17.18±0.0404, 21.12 ± 0.0202 , 35.16± 0.0750, 45.16 ± 0.0288, 53.14± 0.0173, 64.15 ± 0.0519, 73.84 ± 0.0346.

Standard diclofenac (10 to 1000µg/ml) showed 26.27 ± 0.0115, 43.34 ± 0.0288, 55.36 ± 0.0692, 64.35 ±0.0288, 71.32 ± 0.0519, 77.28 ±0.0346, 84.95 ± 0.0230 % inhibition of protein denaturation respectively.

Simarouba glauca showed significant activity at various concentrations. Significant activity was seen at the concentration73.84% at 1000µg/ml when compared with the standard drug diclofenac sodium. The production of auto antigen in certain arthritic disease may be due to denaturation of protein. From the results of the present study it can be assumed that the plant extract is capable of controlling the production of auto antigen and inhibits denaturation of protein in arthritic conditions.

DISCUSSION

Many anti-inflammatory drugs have shown dose dependent ability to inhibit thermally induced protein denaturation. *In vitro* anti-arthritic activity was done for the extracts of *Simarouba glauca* by using bovine serum albumin method. The ethanolic extract fabricates significant activity at 73.84±0.0346 at 1000 µg/ml by inhibition of protein denaturation and its effect was compared with the standard drug diclofenac sodium. In present study, the ethanolic extract is capable of controlling the production of auto antigen and inhibits denaturation of protein in rheumatic disease.

Table No.1: Preliminary phytochemical screening

S. No	Tests	Crude ethanol Extract of *Simarouba glauca*
1	Alkaloids	-
2	Carbohydrates	+
3	Saponins	+
4	Terpenoids	+
5	Flavanoids	+
6	Proteins	-
7	Glycosides	+

(+) indicates present (–) indicates absent

Table No.2: *In Vitro* anti-arthritic activity of *Simarouba glauca by* bovine serum albumin method

	Concentration (µg/ml)	Test absorbance	Product control	%inhibition
Control	-	0.99	-	-
Test	10	0.84	0.02	17.18± 0.0404
	50	0.80	0.019	21.12±0.0202
	100	0.66	0.018	35.16±0.0750
	200	0.56	0.017	45.16± 0.0288
	400	0.48	0.016	53.14± 0.0173
	800	0.37	0.015	64.15± 0.0519
	1000	0.26	0.001	73.84± 0.0346
	Concentration (µg/ml)	**Test absorbance**	**Product control**	**%inhibition**
Control	-	0.99	-	-
Standard	10	0.75	0.02	26.27± 0.0115
	50	0.58	0.019	43.34± 0.0288
	100	0.46	0.018	55.36± 0.0692
	200	0.37	0.017	64.35± 0.0288
	400	0.30	0.016	71.32± 0.0519
	800	0.24	0.015	77.28±0.0346
	1000	0.17	0.001	84.95± 0.0230

The experiments were done in triplicate for concordant values.

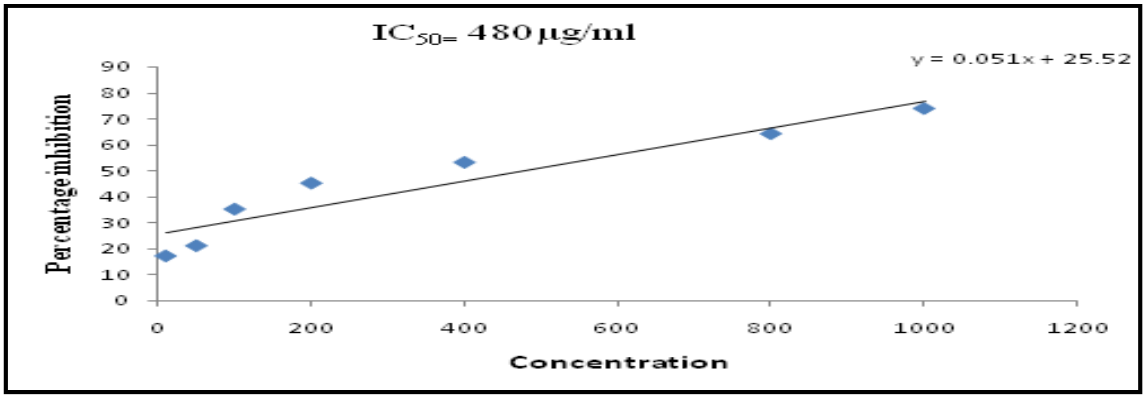

Figure No.1: The IC_{50} value of test by using bovine serum albumin method

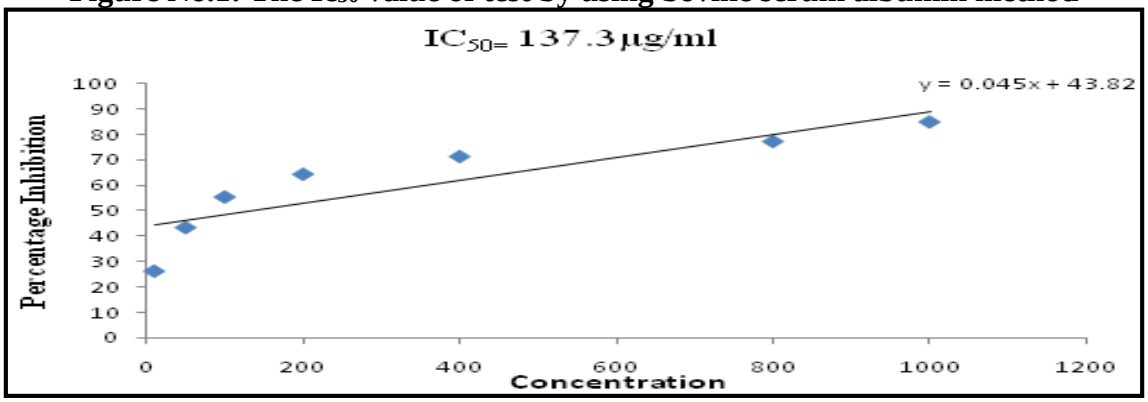

Figure No.2: The IC_{50} value of standard by using bovine serum albumin method

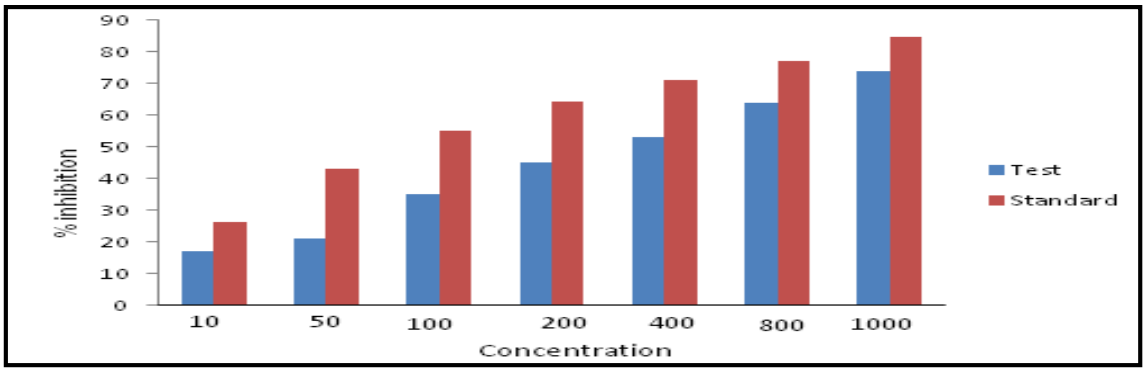

Figure No.3: Comparison between *in-vitro* anti-arthritic potential of test and standard by using bovine serum albumin denaturation method

CONCLUSION

Finally, it is concluded that the ethanolic extract of *Simarouba glauca* possess significant anti-arthritic activity. This may be due to presence of carbohydrates, flavonoids, glycosides, saponin, tannins and diterpenoids and triterpenoids, thus it proves the traditional information of the plant is scientifically validated and confirmed by anti-arthritic activity.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBLIOGRAPHY

1. Srikanth N, Elumalai A, Eswaraia M C, Veldi N. An updated review on anti-arthritic medicinal plants, *International Journal of Pharmacy Review and Research*, 2(1), 2012, 11-15.
2. Shivanand P. Arthritis an autoimmune disorder: Demonstration of *in vivo* anti-arthritic activity, *International Journal of pharmacy and life sciences*, 1(1), 2010, 38-43.
3. Sunetra K Patwardhan, Kaumundee S Bodas, Sameer S Gundewar. Copying with arthritics using safer herbal options, *International Journal of Pharmacy and Pharmaceutical Sciences*, 2(1), 2010, 1-11.
4. Brown J H and Mackey H K. Inhibition of heat-induced denaturation of serum proteins by mixture of non-steroidal anti-inflammatory agents and amino acids, *Proc Soc Exp Biol Med*, 128(1), 1968, 225-8.
5. Grant N H, Alburn H E and Kryzanauskas C. Stabilisation of serum albumin by anti-inflammatory drugs, *Biochem Pharmacol*, 19(3), 1970, 715.
6. Sharan, Suresh Volluri, Srinivasa Rao Bammidi, Seema Chaitanya Chippada and Meena Vangalapati. *In-Vitro* Anti-Arthritic Activity of Methanolic Extract of Bacopa Monniera, *Int J Chem Environ Pharma Research*, 2(2-3), 2011, 156-159.
7. A.B.S. *Alves* I, Mirandab H M, Luiz A L,Soaresa B, Karina P, Randaua B. Simaroubaceae family: botany, chemical composition and biological activities, *Rev Bras Farmacogn*, 24(4), 2014, 481-501.
8. Latha S, Seethalakshsmi S, Chamundeeswari D, Senthamarai R. Comparison of *in vitro* anti-arthritic activity of *Stereospermumcolalis* and *Stereospermumsuaveolen*, *International journal of phytopharmacy research*, 6(2), 2015, 59-60.

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