Vol 9|Issue 1| 2019 | 1-3.

e-ISSN 2248 - 9169
Print ISSN 2248 - 9150

International Journal of
Experimental Pharmacology

www.ijepjournal.com

ANTI-ARTHRITIC ACTIVITY OF THE PLANT *ARISTOLOCHIA INDICA* (LINN.) ROOT

Suresh Vadivelu * [1], K L Senthilkumar [2]

[1] Sun Rise University, Bagdad Rajput, Teh. Ramgarh. Distt. Alwar 301030, Rajasthan, India.
[2] Sri Vijay Vidyalaya College of Pharmacy, Nallampalli, Dharmapuri, Tamilnadu -636807, India.

ABSTRACT

Rheumatoid arthritis is a major ailment among rheumatoid disorders. The present study is aimed to evaluate the root extract of *Aristolochia indica* L. of the root for acclaimed anti-arthritic activity using in-vitro inhibition of protein denaturation model. Aspirin was used as a standard drug. Results revealed that the ethanolic extract of *Aristolochia Indica* L. at two different concentrations (400mcg/ml and 800mcg/ml) possessed significant anti-arthritic activity as compared to standard used drug aspirin. The plant extract showed dose dependent activity.

Keywords: *Aristolochia Indica* L, Anti-arthritic activity.

INTRODUCTION

Arthritis is an auto immune disorder characterized by pain, swelling and stiffness; it is a form of joint disorder that involves inflammation in one or more joints[1, 2]. It is a common disease having peak incidence in 3rd to 4th decade of life with 3-5 times higher preponderance in female[3]. Its prevalence depends upon age[4]. Herbal drugs constitute a major part in all the traditional system of medicine. Herbal medicine is a triumph of popular therapeutic diversity. The factors responsible for the continued and extensive use of herbal remedies in India are their effectiveness, easy availability, low cost, comparatively less toxic effects and shortage of practitioners of modern medicine in rural areas. Number of synthetic medicines has been derived from medicinal herbs[5]. *Aristolochia Indica* L is distributed throughout India at low elevations. The young roots are light brown and fairly smooth, whereas the older ones are comparatively rough due to the development of cork, lenticles and the presence of scars of rootlets. The major chemical constituients are phenanthrene derivaties, terpenes, alkaloids, flavonoids, quinones and lactones[6]. The roots are bitter, acrid, astringent, thermogenic, purgative, anthelmintic, stomachic, cardio tonic and anti-inflammatory[7]. The crushed roots are claimed to expel bladder and kidney stones, dieresis and effective in rheumatism. Hence, the present study was undertaken to evaluate in vitro anti-arthritic activity of plant extract.

Corresponding Author

V Suresh
Email id: vsureshsph@gmail.com

MATERIALS AND METHODS:

Collection of plant and authentification

The species for the proposed study that is *Aristolochia Indica* (Linn.) root parts were collected in the month of July 2014 from the town of Uthangarai, Krishnagiri (DT). The species for the proposed study was identified and authenticated as *Aristolochia Indica* (Linn.) By Joint Director, Government of India, Ministry of Environment & Forest, Botanical Survey of India, Southern Circle, T.N.A.U. Campus, Lawley Road, Coimbatore.

Extraction:

Air-dried & coarsely powdered (500 gm) of *Aristolochia indica* root was extraction in a soxhlet extractor using ethanol. The extract was carried out until the solvent found to be colourless. Then the solvent was

filtered and distilled off. Final traces of ethanol were removed under pressure by using rotary vacuum flask evaporator and they were preserved in desiccators.

Preliminary Phytochemical Screening:

The crude ethanolic extract of root *Aristolochia Indica* L. were tested for its different chemical groups such as alkaloids, flavonoids, tannins, steroids, saponins, fixed oils, tri-terpenoids, carbohydrayes and glycosides.

Experimental In-vitro method:

ASSESMENT OF IN VITRO ANTIARTHRIC ACTIVITY

Anti - denaturation studies is performed by using bovine serum albumin (BSA). When BSA is heated it undergoes denaturation and express antigens associated with type-III hypersensitivity reaction and that is related to disease such as serum sickness, glolmeralonephritis, rheumatoid arthritis and system lupus erythematosus.

INHIBITION OF PROTEIN DENATURATION METHOD[8]

Preparation of Reagents

- **5% bovine serum albumin (BSA)-**
  5g of BSA was dissolved in 100ml of water.
- **Phosphate buffer saline pH 6.3**

8g of sodium chloride (Nacl), 0.2g of potassium chloride (KCl), 1.44g of disodium hydrogen phosphate (Na_2HPO_4) and 0.24g of potassium dihydrogen phosphate (KH_2PO_4) were dissolved in 800ml of distilled water. The pH was adjusted to 6.3 using 1N HCL and makes the volume to 1000ml with distilled water.

- **Test solution**

0.45 ml of bovine serum albumin and 0.05ml of test solution of various concentrations were prepared.

- **Test control solution**

0.45 ml of bovine serum albumin and 0.05ml of distilled water were prepared.

- **Product control solution**

0.45 ml of distilled water and 0.05ml of test solution.

- **Standard solution**

0.45 ml of serum albumin and 0.05ml of Aspirin of various concentrations.

METHOD

- 0.5ml of test solution, test control solution, product control solution, standard solution were prepared
- Various concentrations (100,200,400,800ug/ml) of test drug. (petroleum ether, aqueous and methanol extracts)/lapachol and standard drug aspirin, (100,200,400,800ug/ml) where prepared.
- 1N HCL was used to adjust the pH to 6.3 for all the above solutions. The samples were incubated at 37^0c for 20min and the temperature was increased to keep the samples at 57^0c for 3min.
- After cooling, 2.5ml of phosphate buffer was added to the above solution. The absorbance was measured at 4.6nm.
- The control represents 100% protein denaturation. The percentage inhibition of protein denaturation can calculated as

Percentage inhibition =100-[{(optical density of test control – optical density of product)/optical density of test solution}*100.

- The control represents 100% protein denaturation.

MEMBRANE STABILIZAION TEST:

PREPARATION OF RBC SUSPENTION:

Fresh whole human blood (10ml) was collected and transferred to the heparinized centrifuged tube. The tubes were centrifuged at 3000 rpm for 10 min and were washed three times with equal volume of normal saline. The volume of the blood was measured and reconstituted at 10%v/v suspension with normal saline.

HEAT INDUCED HEMOLYSIS:

The reaction mixture (2ml) consisted of 1ml of test drug solution and 1ml of 10% HRBC suspension. Instead of drug only saline was added to the control test tube.

Aspirin was taken as a standard drug. All the centrifuge tubes containing mixture were incubated in a water bath at 56^o C for 30 mints. At the end of the incubation, the tubes were cooled under running tap water[9].

The reaction mixture was centrifuged at 2500 rpm for 5 mints and the absorbance of the supernatants was taken at 560nm.The experiment was performed in triplicates.

Percent membrane stabilization activity was calculated by using the formula,

$$100 \times (V_t / V_c - 1)$$

Where,

V_t = **Absorbance of test sample**

V_c = **Absorbance of control**

RESULTS AND DISCUSSION:

Anti arthritic effects of ethanol, petroleum ether and aqueous extract of *Aristolochia Indica* L. root was studied significantly by using in-vitro inhibition of protein denaturation model. Maximum percentage of protein denaturation inhibition 66% was observed from ethanol extracts followed by Petroleum ether 52% at the maximum concentration of 800µg/ml. Aspirin, a standard anti-inflammatory drug showed the maximum inhibition 90% at the concentration of 800µg/ml. Most of the investigators have reported that denaturation of protein is one of the cause of arthritis. Production of auto-antigens in certain rheumatic disease may be due to in vivo denaturation of proteins. Literature suggest that, the anti-denaturation property of BSA was due to the presence of two interesting binding sites in the aromatic tyrosine and aliphatic threonine and lysine residue regions of the BSA. They have also reported that therapeutic molecules could be activating

the tyrosine motif rich receptor dually with threonine that regulates signal transduction biological pathways for their overall biological action. Obtained data stated that *Aristolochia Indica* L. root could be used as potent anti-arthritic agent.

Table 1. *Invitro* Anti arthritic activity by membrane stabilization method

Test sample	Conc. (µg/ml)	% protection
Ethanol	100	30.4%
	200	42.6%
	400	55.3%
	800	68.7%
Petroleum ether	100	20.9%
	200	31.4%
	400	40.6%
	800	56.8%
Aqueous	100	17.3%
	200	20.6%
	400	29.7%
	800	38.4%
Aspirin (Standard drug)	800	88.2%

CONCLUSION:

From the results obtained in the present study, it may be concluded that *Aristolochia Indica* L. root possess significant anti-arthritic activity. Hence, it could be beneficial for further work as active anti-arthritic agent.

ACKNOWLEDGEMENT:

My sincere thanks to Thiru. D.N.C.Manivannan, Chairman, Thiru. D.N.C.Deepak, Director, Sri Vijay Vidyalaya College of Pharmacy, Nallampalli, Dharmapuri, Tamilnadu-636807 and Thiru K.L.Senthilkumar, Principal, Sri Vijay Vidyalaya College of Pharmacy, Nallampalli, Dharmapuri, Tamilnadu-636807 for providing very excellent facilities for the completion of my research work.

REFERENCES

1. Vignais, Paulette M.; Pierre Vignais, Discovering Life, Manufacturing Life: How the Experimental Method Shaped Life Sciences. Berlin: Springer, 2010, ISBN 90-481-3766-7.
2. Jacqueline Nairn; Price, Nicholas C, Exploring Proteins: A student's Guide to Experimental Skills and Methods. Oxford [Oxfordshire]: Oxford University Press, 2009, ISBN 0-19-920570-1.
3. Mohan H. text book of pathology, JPPublisher, New Delhi, 2000, pp.1648.
4. Pandey S. Various techniques for the evaluation of anti arthritic activity in animal models, J. adv. Pharm. tech., Res. 1(2), 2010, 164-170.
5. Payel Bhattacharjee Characterization of the aqeous extract of the root of *Aristolochia Indica*: Evaluation of its traditional use as an antidote for snake bites. *J.Ethanopharmacolgy,* 145, 2013, 220-226.
6. J.Marlin Cynthia effect of aqueous root extract of *Aristolochia Indica* Linn on diabetic induced rats; *AJPSKY*, 2 (4), 2012, 464-467.
7. Bal, S.N. and B.Gupta, Morphology of roots of *Aristolochia Indica* Linn. Sci. Cult., 22, 1956, 276-277.
8. Deshpande V VJ. In vitro anti arthritic activity of *Abutilon Indicum* (Linn.) Sweet. *Journal of Pharm., Res*. 2(4), 2009, 644-645.
9. Williams LAD, Corner AO, The in vitro anti denaturation effects induced by natural products and non steroidal compounds in heat treated (immunogenic bovine serum albumin, *West Indian Med* J, 57, 2008, 327-331.