Available online **www.ijpras.com**

International Journal of Pharmaceutical Research & Allied Sciences, 2016, 5(3):132-135

Research Article

ISSN : 2277-3657
CODEN(USA) : IJPRPM

Investigation of anti-inflammatory activity of *Aristolochia krisagathra* Sivarajan and Pradeep

Koilpitchai Paulpriya, Pious Soris Tresina and Veerabahu Ramasamy Mohan*

Ethnopharmacology Unit, Research Department of Botany, V.O.Chidambaram College, Tuticorin – 628008, Tamil Nadu, India
** Email: vrmohanvoc@gmail.com*

ABSTRACT

In the present study, Aristolochia krisagathra whole plant was extracted with ethanol and evaluated for anti-inflammatory activity in rats using a carrageenan induced paw edema method. Ethanol extract exhibited potent anti-inflammatory activity at the dose of 400 mg/kg at 3hr administration (87.19%). The study was compared with standard drug indomethacin 85.11% (10mg/kg). Observed pharmacological activity in the present study provides scientific validation of ethnomedicinal uses of this plant in treating acute inflammation.

Keywords: *Aristolochia krisagathra*, paw edema, carrageenan, phytol.

INTRODUCTION

Inflammation is the complex biological response of vascular tissues to harmful stimuli including pathogens, irritants or damaged cells. It is a protective attempt by the organism to remove the injurious stimuli as well as initiate the healing process for the tissue [1]. The process of inflammation is necessary in healing of wounds, inflammation however, if runs unchecked, lead to onset of disease and like vasomotor rhinorrhoea, rheumatoid arthritis and atherosclerosis [2]. Inflammation is either acute or chronic inflammation. Acute inflammation is characterized by classical signs edema, erythrema, pain, heat and above all, loss of function. The classical signs are triggered by the infiltration of the tissues by serum and white blood corpuscles (leucocytes). Chronic inflammation results in a progressive shift in type of cells, present at site of inflammation. It is characterized by simultaneous destruction and healing of the injured tissue from incidence of inflammation. Cyclooxygenase (COX) is the key enzymes in the synthesis of prostaglandins, prostacyclins and thromboxanes which are involved in inflammation, pain and platelet aggregation [3]. Inflammatory diseases are major worldwide problem.

Now there is a need for the new safe, potent, nontoxic or less toxic anti-inflammatory drug. Plant medicines are great importance in the primary health care in many developing countries. Plant based drugs used in the traditional medicine have paid great attention because it is easily available, less expensive and also have no side effects [4]. Plants have the ability to synthesize a wide variety of phytochemical compounds such as secondary metabolites. Many of the phytochemicals have been used effectively to treat various ailments for mankind. The phytomedicine are more important in the treatment of inflammation. Many medicinal plants have shown to exhibit potent anti-inflammatory effect in the treatment of inflammation by using various models [5].

The genus *Aristolochia* finds a prominent place in different Indian Systems of Medicine. The different ethnic communities in India have used different species of *Aristolochia* in the treatment of various human ailments [6]. Kanikkar tribals of Kalakad – Mundanthurai Tiger Reserve Sanctuary, Tamil Nadu, boiled the equal quantity of

fresh root and leaves of *Aristolochia krisagathra* in coconut oil for about 15 – 20 minutes over a flow flame. The oil is filtered after cooling and applied on the head once in a day as the treatment of rheumatism. The therapy is used to reduce excessive heat of the body [7]. However, no data are available in the literature on the anti-inflammatory activity of whole plant of *A. krisagathra.* This study was therefore undertaken to evaluate the effect of ethanol extract of the whole plant of *A.krisagathra* on anti-inflammatory activity in carrageenan induced rat paw edema.

MATERIALS AND METHODS

2.1 Plant Material

The whole plant of *Aristolochia krisagathra* Sivarajan and Pradeep were collected from the natural forest of Agasthiarmalai Biosphere Reserve, Western Ghats, Tirunelveli, Tamil Nadu, India. The plant was identified with help of local flora and authenticated in Botanical survey of India, Southern Circle, Coimbatore, Tamil Nadu. A voucher specimen was deposited in Ethnopharmacology unit, Research Department of Botany, V.O.Chidambaram College, Tuticorin, Tamil Nadu.

2.2 Preparation of plant extract for anti-inflammatory activity

The dried whole plant material of *A.krisagathra* was powdered in a Wiley mill. Hundred grams of whole plant powder was packed in a Soxhlet apparatus and extracted with ethanol. The ethanol extract was concentrated in a rotary evaporator. The concentrated ethanol extract was used for phytochemical screening [8] and anti-inflammatory activity.

2.3 Animals

Adult Wistar Albino rats of either sex (150-200g) were used for the present investigation. Animals were housed under standard environmental conditions at temperature (25±20C) and light and dark (12:12 h). Rats were fed with standard pellet diet (Goldmohur brand, MS Hindustan lever Ltd., Mumbai, India) and water *ad libitum*. Study was carried out as per IAFC approval No: 82 /PHARMA/SCRI, 2010.

2.4 Acute toxicity study

Acute oral toxicity was performed by following OECD-423 guidelines (acute toxic class method), albino rats (n=6) of either sex selected by random sampling were used for acute toxicity study [9]. The animals were kept fasting for overnight and provided only with water, after which the extracts were administrated orally at 5mg/kg body weight by gastric intubations and observed for 14 days. If mortality was observed in two out of three animals, then the dose administrated was assigned as toxic dose. If mortality was observed in one animal, then the same dose repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for higher doses such as 50,100 upto 2000 mg/kg body weight.

2.5 Anti-inflammatory activity of carrageenan induced hind paw edema

Albino rats of either sex weighing 150-200 grams were divided into four groups of six animals each. The dosage of the drugs administered to the different groups was as follows. Group I - Control (normal saline), Group - II and III – Ethanol extract of *A.krisagathra* whole plant (200 and 400 mg/kg, p.o.), Group IV – Indomethacin (10 mg/kg, p.o). All the drugs were administered orally. Indomethacin served as the reference standard anti-inflammatory drug.

After one hour of the administration of the drugs, 0.1 ml of 1% W/V carrageenan solution in normal saline was injected into the sub plantar tissue of the left hind paw of the rat and the right hind paw was served as the control. The paw volume of the rats were measured in the digital plethysmograph (Ugo basile, Italy), at the end of 0 min, 60min, 120min, 180min, 240min, 360min, and 480min. The percentage increase in paw edema of the treated groups was compared with that of the control and the inhibitory effect of the drugs was studied. The relative potency of the drugs under investigation was calculated based upon the percentage inhibition of the inflammation. Percentage inhibition was calculated using the formula;

Percentage inhibition = $[(Vc-Vt)/Vc] \times 100$

Where, Vt the percentage represents the percentage difference in increased paw volume after the administration of test drugs to the rats and Vc represents difference of increased volume in the control groups.

2.6 Statistical analysis

The data were analyzed using student's t-test statistical methods. For the statistical tests a *p* values of less than 0.001, 0.01 and 0.05 was taken as significant.

RESULTS AND DISCUSSION

The phytochemical screening of ethanol extract of whole plant of *A.krisagathra* revealed the presence of alkaloid, anthraquinone, coumarin, flavonoid, phenol, quinone, saponin, steroid, tannin, terpenoid, sugar, glycoside and xanthoprotein. Acute toxicity study revealed the non-toxic nature of the ethanol extract of whole plant of *A.krisagathra.*

In the present study, the anti-inflammatory activity of ethanol extract of whole plant of *A.krisagathra* was assayed in albino rats using carrageenan induced rat paw edema model. Table 1 shows the anti-inflammatory activity of ethanol extract of whole plant of *A.krisagathra* which significantly inhibited the rat paw edema at 3rd hour post carrageenan were 76.19% and 87.19% for 200mg/kg and 400mg/kg respectively. The results were compared with indomethacin at 10mg/kg which shows paw reduction of 85.11%.

Table 1: Effect of the whole plant ethanol extract of *A.krisagathra* on the Percentage inhibition of carrageenan induced paw oedema

Treatment Groups	Oedema volume (ml)					% Inhibition after 180 minutes
	Dose mg/kg	0 minute	60 minutes	120 minutes	180 minutes	
Group I	Normal saline	30.28±1.65	73.92±1.68	102.26±1.65	129.56±2.14	-
Group II	200 mg/kg	34.22±1.31	48.31±2.04*	40.31±0.98***	30.84±1.86***	76.19
Group III	400 mg/kg	30.16±1.78	36.91±0.64**	31.62±1.55***	16.59±0.86***	87.19
Group IV	10 mg/kg	27.28±1.13	36.93±1.39**	26.62±0.87***	19.29±0.77***	85.11

*Each Value is SEM ±5 individual observations * p < 0.05; ** p<0.01; *** p<0.001 Compared paw edema induced control vs drug treated*

Inflammation is a common phenomenon and it is a reaction of living tissues towards injury [10]. Carrageenan induced inflammation is a useful model for the estimation of anti-inflammatory effect [11]. The time course of development in carrageenan induced paw edema model in rats is generally represented by a biphasic curve. The first phase occurs within an hour of injection and partly due to the trauma of injection and partly due to the release of histamine 5-HT and kinins [12]. Platelet activating factor and arachidonic acid metabolites also play a role during this phase [13]. Prostaglandins (PGs) play a major role in the development of the second phase of reaction which is measured around 3h times [14, 15]. The presence of PGE_2 in the inflammatory exudates from the injected foot can be demonstrated at 3h and period thereafter. It has been reported that the second phase of edema is sensitive to the most clinically effective anti-inflammatory agents [16]. The carrageenan induced paw edema model in rats is known to be sensitive to cyclooxygenase inhibitors and has been used to evaluate the effect of non-steroidal anti-inflammatory agents which primarily inhibit the cyclooxygenase involved prostaglandins synthesis [17].

In the present study, ethanol extract of *A.krisagathra* whole plant possess significant anti-inflammatory activity as compared to control group. This result indicates that the ethanol extract can prevent the release of inflammatory mediators like histamine, kinin and prostaglandin or may be antagonizing the activity of the mediators after their release.

3, 7, 11, 15 – Tetramethyl 1-2-hexadecan-1 0l, phytol, 9, 12- Octadecadienoic acid (Z, Z) – 9, 12, 15 – Octadecatrienoic acid, methyl ester, (Z, Z, Z) -, Stigmasterol and β-sitosterol were reported in the ethanol extract of *A.krisagathra* whole plant by GC-MS analysis. These compounds may have the role in anti-inflammatory effect [18]. Further study will be carried out to isolate and characterize other anti-inflammatory chemical constituents present in the extract of this part.

REFERENCES

[1] Denko, C.W. A role of neuropeptides effects in inflammation, In: Biochemistry of inflammation. Whicker, J.T., Evans, S.W. **1992**, 177 – 181.
[2] Henson, P.M., Murphy, R.C. Mediators of the inflammatory process. Elsevier, Amsterdam, **1989**, 404.
[3] Pilotto, A., Sancarlo, D., Addante, F., Scarcelli, C., Franceschi, M. Non steroidal antiinflammatory drug use in the elderly, *Surgi. Oncol*. **2010**, 19, 167 – 172.
[4] Cathrine, L., Prabavathi, N. Preliminary phytochemical analysis and antibacterial activity of leaf extracts of *Vitex leucoxylon* L.F., *Int. J. Curr. Pharm. Res*. **2011**, 13, 71 – 73.
[5] Murugesan, D., Deviponnuswamy, R. Potential antiinflammatory medicinal plants – a review, *Int. J. Pharma Pharmaceu. Sci*. **2014**, 6, 43 – 49.
[6] Jegadeeswari, P. Nishanthini, A., Muthukumarasamy, S., Mohan, V.R. Evaluation of antioxidant activity of *Aristolochia krisagathra* (Aristolochiaceae) an important medicinal herb, *Int. J. Pharmacy*. **2014**, 4, 410 – 416.
[7] Sutha, S., Mohan, V.R., Kumaresan, S., Murugan, C., Athiperumalsami, T. Ethnomedicinal plants used by the tribals of Kalakad-Mundanthurai Tiger Reserve (KMTR), Western Ghats, Tamil Nadu for the treatment of rheumatism, *Ind. J. Trad. Knowl.* **2010**, 10, 502 – 509.

[8] Brinda. P, Sasikala. P, Purushothaman, K.K. Pharmacognostic studies on Murugan kizhangu, *Bull. Med. Ethnobot. Res*. **1988**, 3, 84 - 96.
[9] OECD (Organization for Economic Cooperation and Development): OECD guidelines for the testing of chemicals / section 4: Health Effects Test No. 423: Acute Oral Toxicity – Acute Toxic Class Method, OECD. **2002**
[10] Jeong, W, Su H.S, Kim N, Yang YT, Su SY, Lee C et al. Bioactive triterpenoids from *Callistemon lanceolatus*, *Arch. Pharm. Res*. **2009**, 32, 845 – 849.
[11] Ratheesh, M. Helen, A. Antiinflammatory activity of *Ruta graveolens* Linn on carrageenan induced paw edema in wistar male rats, *Afr. J. Biotechnol*. **2007**, 6, 1209 – 1211.
[12] Crunkhorn, P., Meacock, SCR. Mediators of the inflammation induced in the rat paw by carrageenan, *Br. J. Pharmacol*. **1971**, 42, 96 – 103.
[13] Boughton-Smith, N.K., Deckin, A.M., Follenfant, R.L., Whittle, B.J., Garland, L.G. Role of oxygen radicals and arachidonic acid metabolites in the reverse passive arthus reaction and carrageenan paw edema in the rat, *Br. J. Pharmacol*. **1993**, 110, 896 – 902.
[14] DiRosa, M. Biological properties of carrageenan. *J. Pharm. Pharmacol*. **1972**, 24, 89.
[15] Jin, D.Z., Yin, L.L., Ji, X.Q., Zhn, X.Z. Cryptotanshinone inhibits cyclooxygenase – 2 – enzyme activity but not its expression, *Eu. J. Pharmacol*. **2006**, 549, 166 – 172.
[16] Smucker, E., Arrhenous, E., Hilton, T. Alteration in microsomal electron transport induced by liver injury, *Biochem. J*. **1967**, 103, 55 – 64.
[17] Channa, S., Dar, A., Anjum, S., Yaqoob, M., Atta-Ur-Rahman. Antiinflammatory activity of *Bacopa monniera* in rodents, *J.Ethnopharmacol*. **2006**, 104, 286 – 289.
[18] Jegadeeswari, P., Nishanthini, A., Muthukumarasamy, S., Mohan, V.R. GC-MS analysis of bioactive components of *Aristolochia krisagathna* (Aristolochiaceae), *J. Curr. Chem. Pharmaceu. Sci.* **2012**, 2, 226 – 232.