ISSN: 0976-3376

Available Online at http://www.journalajst.com

ASIAN JOURNAL OF SCIENCE AND TECHNOLOGY

Asian Journal of Science and Technology
Vol. 09, Issue, 01, pp.7416-7418, January, 2018

RESEARCH ARTICLE

IN VITRO ANTI- INFLAMMATORY ACTIVITY OF CHLOROFORM, METHANOL AND AQUEOUS LEAF EXTRACTS OF *JUSTICIA GLAUCA*. ROTTL.

Gayatri Devi, B. and *Hemalatha, K. P. J.

Department of Biochemistry, Andhra University, Visakhapatnam -530 003, Andhra Pradesh, India

ARTICLE INFO

Article History:
Received 17th October, 2017
Received in revised form
26th November, 2017
Accepted 19th December, 2017
Published online 31st January, 2018

Key words:
Inflammation,
Protein denaturation,
HRBC, diclofenac.

ABSTRACT

Inflammation is a normal protective response to tissue injury caused by physical trauma, noxious chemical or microbial agents. *Justicia glauca* belongs to the family Acanthaceae. The aim of present study is to evaluate the *in vitro* anti-inflammatory activity of chloroform, methanol and aqueous leaf extracts of *J. glauca* against the denaturation of proteins and Human red blood cell membrane stabilization (HRBC). The study conferred that the aqueous leaf extract showed good result compared to methanol and chloroform extracts.

Copyright©2017, Gayatri Devi and Hemalatha. *This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.*

INTRODUCTION

Medicinal plants are believed to be an important source of new chemical substances with potent therapeutic effects (Franthworth, 1988). Inflammation is a disorder involving localized increase in the number of leukocytes and variety of complex mediator molecules (Mantri and Witiak, 1994). Inflammation is a physiological response of a body to stimuli, including infections in the body and tissue injury, causes a variety of pathological conditions, such as bacterial sepsis, rheumatoid arthritis and skin inflammation (Dinarello, 1997; Palladino *et al*., 2003). Histamines, Seratonin, leukotrienes, prostaglandins and oxygen derived free radicals ($O_2^{\,-}$, OH, $ONOO^-$) are a variety of chemical mediators or signaling molecules which are produced predominantly by inflammatory and phagocytic cells in the sequence which participate in the onset of inflammation (Safayhi and Sailer, 1997). Many bacterial components and products such as peptidoglycans, lipoteichoic acid, exotoxins, lipoproteins and glycolipids can initiate the local inflammatory process. Currently, inflammatory diseases are treated with steroidal and non – steroidal anti-inflammatory drugs ($NSAID_s$) (Paul, 2008). Unfortunately, both these widely prescribed or used drugs have significant side effects. Natural product based anti-inflammatory agents offer promising treatment in prevention of inflammation related conditions, which have transcriptional mode of action, good efficacy and lower risk of side effects (Langman, 1998; Juni *et al*., 2005).

***Corresponding author:* Hemalatha, K.P.J.**
Department of Biochemistry, Andhra University, Visakhapatnam -530 003, Andhra Pradesh, India.

The vitality of cells depends upon their membranes, exposure of RBC's to injurious substances such as hypotonic medium results in lysis of its membrane accompanied by haemolysis and oxidation of haemoglobin. An injury to RBC membrane further render the cell, more susceptible to secondary damage through free radical induced lipid peroxidation (Augusto *et al*., 1982; Ferrali *et al*., 1992). Compounds with membrane stabilizing properties are well known for their ability to interfere with the early phase of inflammatory reactions, namely the prevention of phospholipases that trigger the formation of inflammatory mediators (Aitadafoun *et al*., 1996).

MATERIALS AND METHODS

Plant Material

Fresh leaves of *Justicia glauca* were collected from the regional areas of Srikakulam, Andhra Pradesh. The plant was identified at the Taxonomy section of Department of Botany, Andhra University, Visakhapatnam, Andhra Pradesh, India.

Preparation of plant extract

Aqueous extract

One gram of fresh leaf tissue was weighed and ground in a chilled mortar and pestle with 10ml buffer solution (Phosphate buffer 0.1M, pH 7.4, 1mM EDTA). The extract was centrifuged at 4°C for 10 min at 5000 rpm and the supernatant obtained was used for the determination of *in vitro* anti-inflammatory activity.

Chloroform and Methanol extracts

The leaves were cleaned and dried under shade and powdered. The 10 g of dried powdered leaves of the plant material were extracted with chloroform and methanol (based on order of the polarity) using soxhlet apparatus for 48 hrs. The solvents were distilled separately at lower temperature under reduced pressure and concentrated in rotary evaporator to get the crude extract which is stored in desiccator for future use.

Assessment of in vitro anti-inflammatory activity

Inhibition of albumin denaturation

Method of Mizushima *et al* (1968) was followed with minor modifications. The reaction mixture was consisting of test extract at different concentrations and 1% aqueous solution of bovine albumin fraction. pH of the reaction mixture was adjusted using small amount of 1N HCl.The samples were incubated at 37°C for 20 min and then heated at 57°C for 20min. After cooling the samples, the absorbance was measured spectrophotometrically at 660 nm. The experiment was performed in triplicate. Percent inhibition of protein denaturation was calculated as follows:

$$\text{Percent inhibition} = \frac{\text{(Abs of control – Abs of sample)}}{\text{Abs control}} \times 100$$

Membrane stabilization test

Preparation of Red Blood cells

(RBC) suspension

This method was done according to Gandhidasan *et al* (1991) and Azeem *et al* (2010). Fresh whole human blood (10 ml) was collected and transferred to the heparinized centrifuge tubes. 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 as 10% v/v suspension with normal saline. The assay mixture contained 1ml of phosphate buffer (0.15 M, pH 7.4), 2ml of hyposaline (0.36%), 0.5ml of HRBC suspension and 1 ml of various concentrations of the extracts. Diclofenac sodium was used as reference drug. Control was distilled water. The mixtures were incubated for 37°C for 30 min and centrifuged. The absorbance of the supernatant solution was measured at 560nm.

Percentage membrane stabilizing activity was calculated as follows:

$$\text{Percentage Stabilization} = \frac{\text{100- (O.D. of test – O.D. of product control)}}{\text{O.D of control}} \times 100$$

Statistical analysis: The results are expressed as the mean±SD for three replicates.

RESULTS AND DISCUSSION

Protein Denaturation: In protein denaturation method aqueous extracts of *J. glauca* at 1000 µg/ml showed 83.6% of inhibition when compared to standard diclofenac which showed 93.6% at the same concentration. Leaf extracts of methanol and chloroform showed inhibition of 80.03% and 39.8% respectively. The results are presented in Table 1 and Fig 1. In protein denaturation method the maximum of 65.8% inhibition was reported in methanol extract of leaves of *Cocculus hirsutus* when compared to stem (46.15%) and callus (60.21%) (Arya *et al*., 2014). *Coffea arabica* aqueous extract exhibited a concentration dependent inhibition of protein denaturation in reference to standard drug diclofenac. The IC_{50} value of the extract was reported as 40µg/ml whereas that of diclofenac was reported to be 625µg/ml (Chandra *et al*., 2012). In *Abutilon indicum* for protein denaturation aqueous extract showed maximum inhibition of 86% at 1000 µg/ml when compared to ethanol extract which showed 80% at 1000 µg/ml (Kousalya *et al*., 2013). The whole plant methanol extract of *Ecinostemma axillare* exhibited 53% of inhibition in protein denaturation method (Leelaprakash and Mohan Dass, 2011). *Oxalis corniculata* showed maximum inhibition of protein denaturation of 85.9% at 800µg/ml (Sakat *et al*., 2010).

Out of all three extracts of *J. glauca*, aqueous extract was very effective in inhibiting hypotonic solution induced haemolysis at different concentrations which was followed by methanol and chloroform exhibited very less inhibition compared to the other two extracts. The aqueous extract showed maximum stabilization of 86.3% at 1000µg/ml when compared to Diclofenac which showed 95.7 % of stabilization at 1000µg ml. At same concentration methanol extract exhibited 79.1% and chloroform exhibited 47.2% of stabilization. The results are presented in Table 1 and Fig 2. The methanol leaf extract of *J. gendarussa* showed maximum activity at 1000 mg/ml. The percent of membrane protection observed was 76.2% (Nirmalraj *et al*., 2015). In *Cocculus hirsutus* the methanolic extracts of leaves showed 88.8% of stabilization at 1000 µg/ml when compared to stem and callus which showed 62.2% and 78.5% respectively (Arya *et al*., 2014). In *Abutilon indicum* HRBC stabilization method at 1000 µg/ml both aqueous and ethanol extracts exhibited 80% and 77% of stabilization respectively ((Kousalya *et al*., 2013). The whole plant methanol extract of *Ecinostemma axillare* exhibited 75% of stabilization in membrane stabilization studies at 500µg/ml (Leelaprakash and Mohan Dass, 2011). *Oxalis corniculata* showed maximum membrane stabilization of 75.7% at 800µg/ml (Sakat *et al*., 2010).

Table 1. Percentage inhibition and Stabilization of chloroform, methanol and aqueous leaf extracts of *J.glauca*

Extract	Concentration µg/ml	Percentage inhibition	
		Protein denaturation	Membrane stabilization
Chloroform	100	32.2±0.49	32.1±0.61
	250	34.5±0.6	36.6±0.4
	500	36.7±0.5	41.2±0.65
	1000	39.8±0.39	47.2±0.43
Methanol	100	61.2±0.4	60±0.25
	250	65.3±0.5	63.8±0.35
	500	71.03±0.15	70.2±0.65
	1000	80.03±0.45	79.1±0.26
Aqueous	100	68.6±0.6	70.8±0.61
	250	74.2±0.6	75.8±0.2
	500	75.8±0.7	80.5±0.7
	1000	83.6±0.25	86.3±0.41
Standard Diclofenac	100	70.2±0.3	73.9±0.25
	250	75.6±0.18	78.4±0.36
	500	87.3±0.2	89.6±0.45
	1000	93.6±0.01	95.7±0.27

Each value represents mean±SD of three experiments

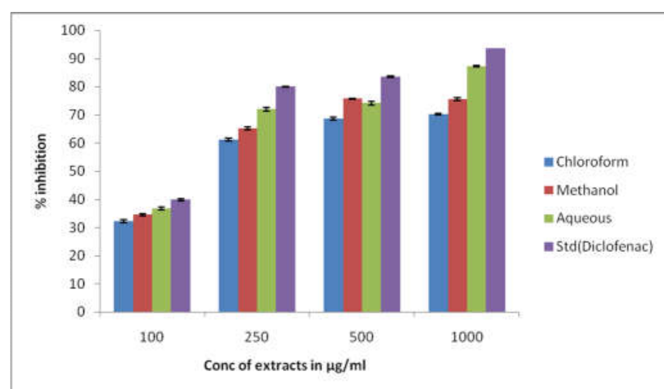

Figure 1. Protein Denaturation

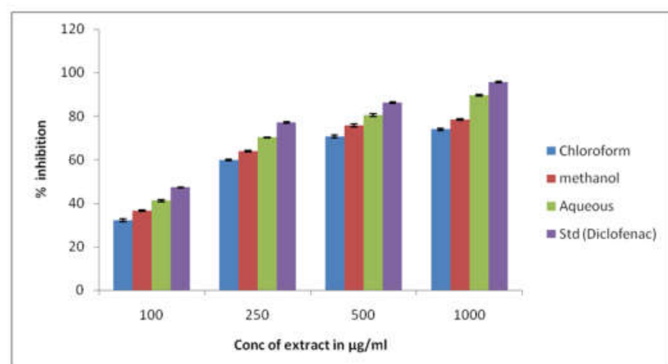

Figure 2. Membrane Stabilization

Conclusion

The present results reveal that the chloroform, methanol and aqueous extracts *of J. glauca* have anti-inflammatory activity. Methanol and aqueous extracts exhibited good potency. Isolation of active compounds would give us clear information about the compounds which possess anti-inflammatory activity.

REFERENCES

Aitadafoun, M., Mounieri, C., Heyman, S.F., Binistic, C., Bon, C. and Godhold, J. 1996. 4-Alkoxybenamides as new potent phospholipase A2 inhibitors. *Biochem Pharmacol.* 51: 737-742.

Arya, D., Meena, M., Grover, N. and Patni, V. 2014. *In vitro* anti--inflammatory and anti-arthritic activity in methanolic extracts of *Cocculus hirsutus* (I.) Diels. *In vivo and In vitro Int J Pharma Sci and Res.,* 5(5): 1957-1962.

Augusto, O., Kunze, K. L. and Montellano, P.R. 1982. *J Biol Chem.* 257:6231-6241.

Azeem, A.K., Dilip, C., Prasanth, S.S., Junise, V. and Hanan, Shahima. 2010. Anti-inflammatory activity of the glandular extracts of *Thunnus alalunga*. *Asia Pac Jfor Med.*, 3(10): 412-20.

Chandra, S., Chatterjee, P., Dey, P. and Bhattacharya, S. 2012. Evaluation of *in vitro* anti-inflammatory activity of coffee against denaturation of protein. *Asia pac J trop Biomed.*, 2(1): S178-180.

Dinarello, C. A. 1997. Proinflammatory and anti-inflammatory cytokines as mediators in pathogenesis of septic shock. *Chest.* 112:321-329.

Ferrali, M., Signormi, C., Ciccoli, L. and Comporti, M. 1992. Iron release and membrane damage in erythrocytes exposed to oxidizing agents phenylhydrazine, divicine and isouramil. *Biochem J.,* 285:295-301.

Franthworth, N.R. 1988. Screening plants from new medicines. In: Biodiversity, Wilson, E.D. (Ed.). National Academy Press, Washington, pp: 83-97.

Gandhidasan, R., Thamaraichelvan, A., Baburaj S. 1991. Anti-inflammatory action of *Lannea coromandelica* by HRBC membrane stabilization. *Fitoterapia.* Vol LXII; No1: 81-83.

Juni, P., Reichenbach, S. and Egger, M. 2005. COX2 inhibitors, traditional NSAIDs and heart. *Br MED J.* 330:1342-1343.

Kousalya, M., Kokila, N., Sudha, K. M. and Kalaiselvi, B. 2013. *In vitro* Anti inflammatory activity of *Abutilon indicum* Root. *Int J Front Sci and Tech.*, 1(4):63-73.

Langman, M.J.S. 1998. Ulcer compilations and NSAIDs. *Am J Med (Suppl.).* 84:53-63

Leelaprakash, G., Mohan Dass, S. 2011. *In vitro* Anti Inflammatory activity of Methanol extract of *Enicostemma axillare*. *Int. J. Drug Dev. & Res.* 3(3):189-196.

Mantri, P. and Witiak, D.T. 1994. Inhibition of cyclooxygenase and 5-lipoxygenase. *Curr. Med. Chem.* 1: 328-355.

Mizushima, Y. and Kobayashi, M. 1968. Interaction of anti□inflammatory drugs with serum proteins, especially with some biologically active proteins. *J Pharma Pharmacol.*, 20:169□ 173.

Nirmalraj, S., Ravikumar, M., Mahendrakumar, M., Bharath, B. and Perinbam, K. 2015. Antibacterial and Anti-Inflammatory Activity of *Justicia gendarussa* Burm. F. Leaves, *J. of Plant Sci.,* 10(2): 70-74.

Palladino, M. A., Bahjat, F.R., Theodorakis, E.A. and Moldwe, L.L. 2003. Anti-TNF-alpha therapies: The next generation. *Natl Rev Drug Discov.*, 2:736-746.

Paul, W. E. 2008. Fundamental Immunology 6th edition. Philadelphia: Lippincott Williams and Wilkins.

Safayhi, H. and Sailer, E. R. 1997. Anti-inflammatory actions of pentacyclic triterpenes. *Plant Med.* 63: 487-493.

Sakat, S., Juvekar, A.R., Gambhire, M.N. 2010. *In vitro* antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn. *International Journal of Pharma and Pharmacological Sciences;* 2(1):146-155.

****