ANTI-INFLAMMATORY ACTIVITY OF *WRIGHTIA TINCTORIA* LEAVES BY MEMBRANE STABILIZATION

Rajalakshmi G.R. [1*], Jyoti Harindran[2]
1. Research Scholar, Karpagam University, Karpagam, Coimbatore, Tamil Nadu-641021, India.
2. Principal, University College of Pharmaceutical Sciences, Mahatma Gandhi University, Rubber Board (P.O), Kottayam , Kerala, India.
E mail: sijuellickal@rediffmail.com

ABSTRACT

Ethyl alcohol and aqueous extract of *Wrightia tinctoria* were investigated for anti-inflammatory activity by HRBC method. The preventation of hypo tonicity induced HRBC membrane lysis was taken a measure of anti-inflammatory activity and these extracts shows biphasic effects. Their activities are compared with standard drug diclofinac sodium.

Key Words: *Wrightia tinctoria*, anti-inflammatory, Phytoconstituents.

INTRODUCTION

The inflammatory process is the responses to an injurious stimulus. It can be evoked by a wide variety of noxious agents. The ability to mount an inflammatory response is essential for survival in the face of environmental pathogens and injury; in some situations and diseases, the inflammatory responses may be exaggerated and sustained without apparent benefit and even with severe adverse consequences [1]. So present study was undertaken to establish scientific evidence for anti-inflammatory activity of leaves extracts of *Wrightia tinctoria.*

Wrightia tinctoria is a member of the family Apocynaceae, is a small to medium-size deciduous tree [2]. Traditionally *Wrightia tinctoria* commonly called as "Jaundice curative tree" in south India and plant possesses high medicinal value [3]. Crushed fresh leaves when filled in the cavity of decayed tooth relieve toothache. In Siddha system of medicine, it is used for psoriasis and other skin diseases [4-6]. The plant has been assigned to analgesic, anti-inflammatory and antipyretic activities and to be effective in the treatment of psoriasis [7-8]. The literature survey revealed that no reports were found on the anti-inflammatory activity of the leaves extracts of *Wrightia tinctoria*. This prompted us to investigate the anti-inflammatory activity of *Wrightia tinctoria* leaves extracts.

MATERIALS AND METHODS

Plant Material

Fresh leaves of *Wrightia tinctoria* was collected from TBGRI, Thiruvananthapuram during the month of March 2007. The plant was identified by Mrs. Amina Ali, Associate Professor and Head, Department of Pharmacognosy, Govt. Medical College, Calicut, Kerala, India. Voucher specimen (AA-34/10) is preserved in institute herbarium for future reference.

Preparation of Extract

Ethyl alcohol extract: *The* shade dried powdered leaves (500g) were exhaustively extracted with 95% ethanol using a soxhlet apparatus. The extract was concentrated in vaccuo to a syrupy consistency. The percentage yield of extract was found to be 2.9 %.

Aqueous extract: The dried powders (24#) 100gm of the was taken in a 2000ml conical flask with 500ml of distilled water to which 10ml chloroform were added as a preservative. It was extracted up to 7 days with daily 2 hours stirring with the mechanical stirrer. After 7 days the extract was filtered through the muslin cloth and the marc was pressed and its filtrate dried in hot air oven at 45^0C to a semisolid mass. It was stored in airtight container in a refrigerator below 10^0C. The percentage yield of extract was found to be 3.1 %.

Membrane stabilization assay

The **HRBC** membrane stabilization has been used as method to study the anti-inflammatory activity [9] (Gandhisan etal. 1991). Blood was collected from healthy volunteer who was not taken any NSAIDS for two weeks prior to the experiment. The collected blood was mixed with equal volume of sterilized Alsever solution (2%dextrose,0.8% sodium citrate,0.5% citric acid and 0.42% sodium chloride in water). The blood was centrifuged at 3000 rpm and packed cell were washed with isosaline (0.85%.pH 7.2) and a 10 %(v/v) suspension was made with isosaline. The assay mixture contained the drug (concentration as mentioned in the table 2), 1 ml of phosphate buffer (0.15M, pH 7.4), 2 ml of hyposaline (0.36%) and 0.5ml of HRBC suspension.

Diclofenac was used as reference drug. Instead of hyposaline 2ml of distilled water was used in the control. All the assay mixture were incubated at 37^0C for 30 min and centrifuged. The hemoglobin content in the supernatant solution was estimated using spectrophotometer at 560 nm. The percentage hemolysis was calculated by following equation-% inhibition of hemolysis =100 x (OD_1-OD_2/OD_1).Where OD_1 = Optical density of hypotonic buffered saline solution alone (control) and OD_2 = Optical density of test sample in hypotonic solution.

RESULTS AND DISCUSSION

The ethyl alcohol and aqueous extracts of *Wrightia tinctoria* were studied for in vitro anti-inflammatory activity by HRBC membrane stabilization method. Phytochemical investigation reveals that ethyl alcohol extracts contains carbohydrates, steroids, alkaloids, terpenoids, flavanoids, tannins, polyphenols while aqueous extract contains carbohydrates, alkaloids, flavanoids, tannins, poly phenols. Both extracts showed significant anti-inflammatory activity in a concentration depended manner. Ethyl alcohol extract at a concentration of 1000 mcg/ml showed 70 % protection of HRBC in hypotonic solution and compared with standard diclofinac which showed 73% protection.

The extracts exhibited membrane stabilization effects by inhibiting hypo tonicity induced lyses of erythrocyte membrane [10]. The erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may well as stabilize lysosomal membranes. Stabilization of lysosomal membrane is important in limiting the inflammatory responses by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which further tissue inflammation and damage up on extra cellular release. Some of the NSAIDs are known to posse's membrane stabilization due to osmotic loss of intracellular electrolyte and fluid components [11]. The extract may inhibit the processes, which may stimulate or enhance the efflux of these intracellular components.

The study also provides a strong evidence for the use of the leaves *Wrightia tinctoria* in folkloric treatment as anti-inflammatory agent. The activity may be due to the presence of one or more phytochemical constuients.

Table 1: Phytochemical screening of plant material *Wrightia tinctoria*

Phytochemical constituents	Ethyl Alcohol Extract	Aqueous Extract
Carbohydrates	+	+
Steroids	+	-
Alkaloids	+	+
Saponins	-	-
Terpenoides	+	-
Flavonoids	+	+
Tannins	+	+
Polyphenols	+	+

(+): Present (-): Absent

Table 2: In vitro anti inflammatory activity of ethyl alcohol and aqueous extract of *Wrightia tinctoria*

Treatment	Conc. (mcg/ml)	% inhibition
Control	--------	----------
Ethyl alcohol Extract	1000	70.07
	500	63.96
	250	59.79
Aqueous Extract	1000	41.90
	500	35.77
	250	33.69
Diclofenac Sodium	50	72.99

REFERENCES

[1] Laurance L Brunton. Goodman & Gilman's The Pharmacological Basis of Therapeutics. Edn,Mc Graw Hill.,2009,11:671.

[2] M J Kothari, A N Londhe. Ethanobotany of Human Health Care of Chikhaldara, Amaravathi District in Maharashtra State: In J K maheshwari Ed,: Ethanobotany and Medicinal Plants of Indian subcontinent, Scientific Publisher.,2000,3:23.

[3] S.G. Joshi, Medicinal plants. Oxford and IBH publishing Co. Pvt. Ltd., 2000, 5:51- 52.

[4] A. K. Nadkarni, Indian Materia Medica, Popular Prakashan, Bombay, 1976.

[5] P.S. Varier, Indian Medicinal plants, Orient Longman Ltd., Madras, 1997.

[6] M.J.Kothari, K.M. Rao , Ethnobotanical studies of Thane District Maharashtra, In, J.K. Maheshwari Ed.: Ethnobotany and Medicinal plants of Indian Subcontinent, Scientific Publisher,2000,4:78-79.

[7] D.Ghosh, P.Thejmoorth, G.Veluchamy . Antiinflamatory, analgesic and antipyretic activities of 777 oil - a siddha medicine. Bull. Med. Ethanobot. Res., 1985, 6:141-154.

[8] J. R. Krishnamurthi, S. Kalaimani, G. Veluchamy. Clinical study of Vetapalai (*Wrightia tinctoria*) oil in the treatment of Kalanjagapadai (Psoriasis). Journ. Res. Ayur. Siddha. 1981, 2(1): 58-66.

[9] R. Gandhisan, A. Thamaraichelvan. Anti-inflammatory action of *Lannea coromandelica* HRBC membrane stabilization. Fitotherapia.1991, 62:82-83.

[10] C T Chou. The anti-inflammatory effects of *Tripterygium wilfordil* Hook on adjuvant induced paw edema in rats and inflammatory mediators release .Phytother Res., 1997, 11:152.

[11] A. V. Iwueke, O. F. Nwodo, C. O. Okoli. Evaluation of the anti-inflammatory and analgesic activities of *Vitex doinana* leaves.African J Biotech., 2006, 5:1929-35.