American Journal of Advanced Drug Delivery

www.ojadd.com

Original Article

Phytochemical Investigation and Anti-Inflammatory Activity of Ethanolic Extract of *Pulicaria Wightiana*

S. Nelson Kumar*, Rakhal Chandra Das, C. Rajaram, K. Ravindra Reddy

[1]Department of Pharmacology, P. Rami Reddy Memorial College of Pharmacy, Kadapa, Andhra Pradesh, India. Mail I.D: rakhal.das@gmail.com, Mobile No: 09391477664

Date of Receipt- 08/05/2013
Date of Revision- 11/05/2013
Date of Acceptance- 14/05/2013

Address for Correspondence
Department of pharmacology, P. Rami Reddy Memorial College of Pharmacy, Kadapa, Andhra Pradesh, India,
E-mail:
rakhal.das@gmail.com

ABSTRACT

Pulicaria wightiana (Asteraceae) is used traditionally for the health disorders. The present study was carried out to evaluate the anti inflammatory activity of ethanolic extract of *Pulicaria wightiana* which is used as traditional folk medicine in India for treatment of various diseases and disorders. On oral administration of the ethanolic extract of *Pulicaria wightiana* at a dose of 100, 200 and400 mg/kg body weight respectively. EEPW 100mg/kg produced a significant anti inflammatory effect in carragenan and cotton pellet induced inflammation. These findings support the traditional uses of *Pulicaria wightiana* as anti inflammatory agent.

Keywords: *Pulicaria wightiana,* Inflammation, Carrageenan, Plethismometer, Edema.

INTRODUCTION

Inflammation is a normal, protective response to tissue injury caused by physical trauma, noxious chemicals, or microbial agents and is the body's effort to inactivate or destroy invading organisms, remove irritants, and set the stage for tissue repair [1]. Recent studies indicate that interaction of foreign pathogens with innate immune cells like macrophage or monocytes, inflammatory immune response is trigger off. A series of pro-inflammatory mediators, specialized cytokines, prostaglandins, and chemokines are produced as a result in a way to amplify the inflammatory response[2, 3].

Various species of Pulicaria are distributed in different parts of India and are well known for their medicinal properties. Pulicaria wightiana belongs to the family Asteraceae. The leaves are sessile, and pubescent on both sides. The clerodane diterpenoids isolated from the aerial part of the plant Pulicaria wightiana, which shows moderate activity against Gram-positive organisms, Bacillus subtilis, Bacillus sphaeriusand Staphylococcus[4]. Clerodane diterpenoids are a family of secondary metabolites found to possess considerable biological activity, viz. antitumor,

antimicrobial, antibacterial and particularly antifeedant[5].

These drugs have serious limitations due to their side effects[1, 6, 7]. A natural agent with reduced or no toxicity is therefore, essential. In this context, the present investigation is under taken to evaluate the anti inflammatory activity of the plant *Pulicaria wightiana.*

MATERIALS AND METHODS

Plant Material

Whole plant of *Pulicaria wightiana* was collected during flowering season from village Utukur, Kadapa district, Andhra Pradesh, India. The plant was authenticated by Dr. Madhava chetty, Taxonomist, S.V. University, Tirupathi, India. The collected plant was cleaned immediately and shade-dried for a week, powdered mechanically, sieved (10/44) and stored in airtight containers.

Extraction

About 1000 grams of the powdered drug was extracted with ethanol (95%) for 48 hr. by soxhlation method. All the extracts were concentrated by using rota–vacuum evaporator (Buchi type, Mumbai, India). The semisolid extract was dried in an oven at less than 50^0C, comminuted in a ball mill and preserved in air tight containers kept in desiccators prior to its studies.

Chemicals

Carragenan was obtained from SD fine chemicals Ltd Mumbai. All other reagents used were of analytical grade.

Preliminary Phytochemical Investigation

A preliminary phytochemical investigation was carried out for all the extracts obtained from the

Pulicaria wightiana (4) using analytical grade chemicals, solvents and reagents. The respective yields and the preliminary phytochemical investigation results were given in Table 1.

Experimental Animals

Experimental animals of either sex weighing 150-170 gm were obtained used in this experiment. The animals were housed in stainless steel cages at a controlled room temperature of 25 ± 0.5^0C, under a 12 hr light and dark cycle. The animals were fed with standard pellet diet and water ad libitum. Institutional Animals Ethics Committee (IAEC) approved the experimental protocol and care of animals was taken as per guidelines of CPCSEA (NO: 1423/PO/a/11/CPCSEA), Department of Animal Welfare and Government of India.

Acute Toxicity Studies

Acute oral toxicity studies were conducted to determine the LD_{50} cut off value (mg/kg body weight) as per the OECD 2006 Guideline – 423 and OPPT Up and Down Procedure.

Anti-Inflammatory Activity

Anti-inflammatory activity of ethanolic extract of *Pulicaria wightiana* (EEPW) at doses 100, 200 and 400 mg/kg, P.O. was studied by two different methods. The results were given in Table 2.

A. Carrageenan – Induced Rat Paw Edema

The study was conducted according to the method of Winter *et al* [8]. Male albino Wistar rats weighing 100 – 250 gm were housed in wire netted cages in a controlled room temperature 25 ± 0.5ºC, relative humidity 60-70 % and with 12 hr light and dark cycle. The animals were maintained with pellet diet and water *ad libitum.* The animals were deprived of food for 24 hr before experimentation but allowed free access to tap water. All studies were carried out using six rats in each group. The chemicals, solvents and reagents used in the experiments were of

analytical grade. Five groups of six animals each were used for the experiment. Group I of animals were administered with 10 ml/kg, P.O. of 2% v/v aq. Tween 80, which served as control. ethanolic extract of *Pulicaria wightiana* (EEPW) 100, 200 and 400 mg/kg P.O. (suspended in 2% v/v aq, tween 80) was given to the II, III and IV groups of animals respectively. The group V was treated with Indomethacin 20 mg/kg, P.O. One hour after oral administration, edema was induced by sub plantar injection (left hind paw) of 0.1ml of 1% freshly prepared suspension of carragenan (Sigma Chemical Co., USA) in normal saline to all the animals. The volume of the injected and the contra lateral paws were measured at 3 hr after induction of inflammation using Plethismometer. The percent inhibitions of inflammation were calculated by using formula.

Percentage of inhibition inflammation= (A-B/A) X100

Where A and B denote mean increase in paw volume of control and drug treated animals respectively.

B. Cotton Pellet Method

Five groups of six animals each were used for the experiment. The rats were anaesthetized under ether anesthesia and 10 mg of sterile cotton pellets were inserted into the axilla of each rat. Group I animals was given 10 ml/kg, P.O. of 2% v/v aq. Tween 80, which served as control. Ethanolic extract of *Pulicaria wightiana* (EEPW) 100,200 and 400 mg/kg P.O. (suspended in 2% v/v aq, tween 80) was given to the II, III and IV groups of animals respectively. The group V was given with the standard drug Indomethacin (20 mg/kg, P.O.). The treatment was continued for seven consecutive days from the day of cotton pellets implantation. The animals were anaesthetized again on 8th day and the cotton pellets were surgically removed, freed from extraneous tissue; incubated at 37°C for 24 h and dried at 60°C to constant weight. The increment in the dry weight of the cotton pellets was taken as a measure of granuloma formation. [9]

Statistical Analysis

All results were expressed as the mean ± SEM. The results were analyzed for statistical significance by one way ANOVA test using computerized Graph Pad in Stat version 3.05, Graphpad software Inc., San Diego, U.S.A.

RESULTS

The results of anti-inflammatory studies for two different models were summarized in Table 2 and Graph no 1(a) and 1(b). Most of the investigators reported that inhibition of Carrageenan induced inflammation in rats is one of the most suitable test procedures to screen anti-inflammatory agents [10]. The sub planter injection of Carrageenan (1% w/v) developed edema of high intensity and persisted for 3 hr after injection in the control groups. The oral administration of EEPW at the doses of 100, 200 and 400 mg/kg P.O. showed significant and dose dependent inhibition (39.53, 44.86 and 51.71% respectively). The commercial anti-inflammatory drug, Indomethacin showed 58.39% of inhibition at the dose of 20mg/kg P.O. The development of Carrageenan induced oedema is bi-phasic. The first phase is attributed to the release of histamine, serotonin and kinins, whereas, the second phase is related to the release of prostaglandins [11]. The inhibitory action of the drug (EEPW) on Carrageenan induced paw edema in rats may be mediated through either any of the mediators alone or in combination.

The drug EEPW also exhibited significant anti–inflammatory effect in the cotton pellet induced granuloma test (60.60% for 400mg/kg, P.O.). This reflected its efficacy to a high extent to reduce an increase in the

number of fibroblasts and synthesis of collagen and mucopolysaccharide which are natural proliferative events of granulation tissue formation [12, 13]. It was observed that the gain in weight of the pellets was linear with the time. This linearity was continued for eight days and then leveled off.

DISCUSSION

Ethanolic extract of *Pulicaria wightiana* was systematically evaluated for its anti-inflammatory potential by following standard pharmacological screening methods. Results suggested that the EEPW found to possess comparable efficacy with that of standard anti-inflammatory drugs. *Pulicaria wightiana,* an abundantly available climbing perennial plant is certainly a nature's treasure for mankind for prevention and treatment of inflammation.

REFERENCES

1. Mary J. Mycek, Richard A. Harvey and Pamela C. Champe. 1997Lippincott's Illustrated Reviews: Pharmacology, Lippincott – Raven PUBLISHERS Philadelphia New York P-401.
2. Beg AA Endogenous ligands of Toll-like receptors: implications for regulating inflammatory and immune responses. *Trends Immunol*., (2002) 23: 509-512.
3. Heumann D and Roger T Initial responses to endotoxins and Gram-negative bacteria. *Clin. Chim. Acta,* (2002) 323: 59-72.
4. *Phytochemistry*, *Volume 66, Issue 6*, *March 2005*, *Pages 633-638* Biswanath Das, M. Ravinder Reddy, R. Ramu, N. Ravindranath, H. Harish, K.V.S. Ramakrishna, Y. Koteswar Rao, K. Harakishore, U.S.N. Murthy
5. Merritt, A. T. & Ley, S. V. (1992). *Nat. Prod. Rep.* 9, 243–287.
6. Rang HP, Dale MM, Ritter JM et al. Pharmacology. 5th ed., Churchill Livingstone. London; 1993: pp.562.
7. Sathoskar. R.S and Bhandarkar S. D. "Pharmacology and Pharmacotherapeutics" 1986, Tenth Edition, P-136.
8. Winter CA, Risley EA and Nuss GW. Carrageenin induced oedema in hind paw of rats as an assay for anti – inflammatory drugs. *Proc Soc Exp Bio Med.* 111: 544-7 (1962).
9. Mandal SC, Ashok Kumar CK, Mohana Lakshmi S, Murugesan T, Sinha S, Saha BP, and Pal M. Anti- tussive activity of *Asparagus racemosus* root against sulphur dioxide induced cough in mice, *Fitoterapia.* 71, 687-689 (2000).
10. Arrigoni-Maratellie E. Inflammation and anti-inflammatory. Spectrum publication Inc. New York. 1988:119.
11. Arrigoni-Martelli E..*Inflammation and Anti-Inflammatories,* Spectrum Publications Inc.: New York. 119–120 (1977).
12. Rahman MT, Shilpi JA, et al. Preliminary pharmacological studies on *Piper chaba* stem bark. *J. of Ethno pharmacology* 2005; 99:203.
13. Yashimoto T, Furukawa M, et al. Flavonoids: Potent inhibitors of arachidonate 5-lipoxygenase. Biochemical and Biophysical Research Communication 1983; 116:612.

Table 1. Qualitative chemical tests for phytoconstituents *Pulicaria wightiana*

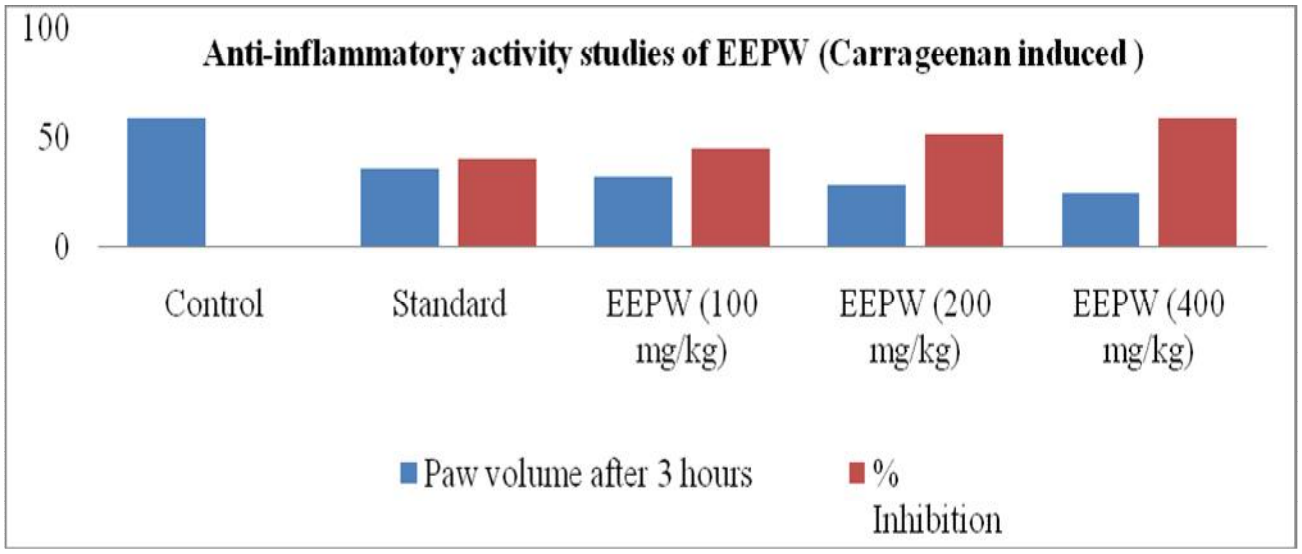
S.NO	Name of the Test	E.E.P.W
1	Tests for carbohydrates Molisch's test Fehling test Barfoed's test Benedicts test	 + + + +
2	Test for proteins and amino acids Biuret test Millon's test Ninhydrin test	 + + +
3	Test for steroids Salwski test Liebermann Burchard	 - -
4	Test for Alkaloids Dragendroff's test Wagner's test Mayer's test Hager's test	 + + + +
5	Test for cardiac glycosides Bal jets test Keller killiani test Test for Saponin glycosides Foam test Test for Anthraquinone glycosides Brontrager's test	 - - - +
6	Test for Tannins and Phenolic compounds	+
7	Test for Flavanoids (Sinoda test)	+
8	Terpenoids (Salkowski test)	+

Table 2. Anti-inflammatory activity studies of EEPW on male albino Wistar rats

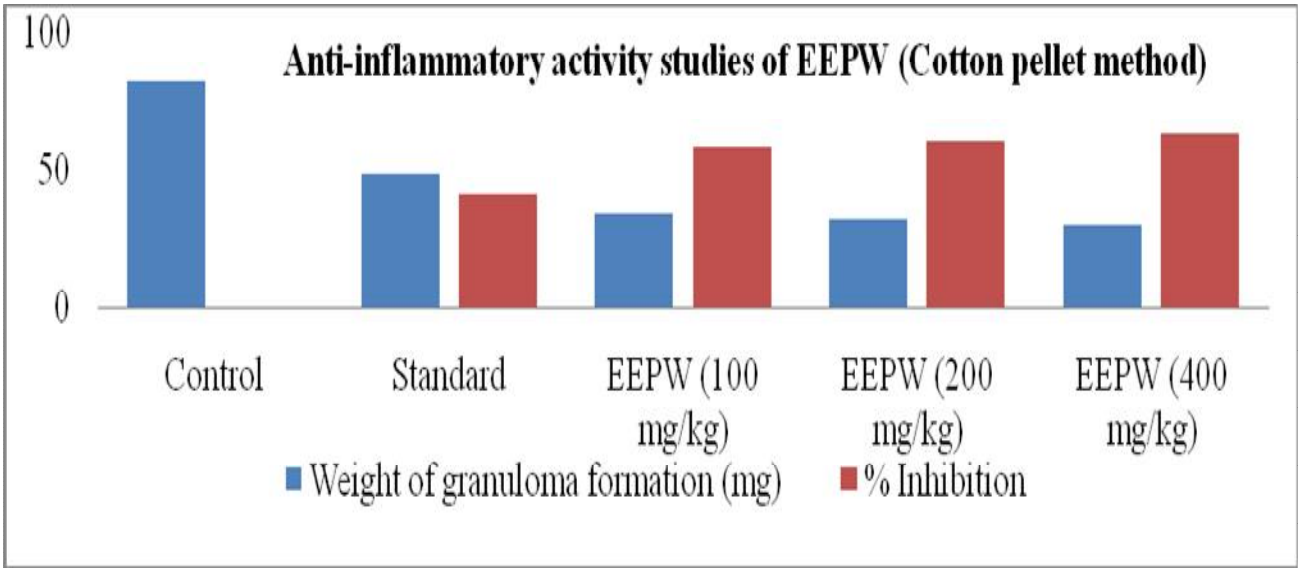
	Group I	Group II		Group III		Group IV		Group V	
Material administered	2%Tween 80	EEPW						Indomethacin	
Dose	10 ml/kg	100 mg/kg		200 mg/kg		400 mg/kg		20 mg/kg	
Route of administration	Oral								
Inducing agent	Paw volume after 3 hours (mean ± SEM)	Paw volume after 3 hours (mean ± SEM)	%	Paw volume after 3 hours (mean ± SEM)	%	Paw volume after 3 hours (mean ± SEM)	%	Paw volume after 3 hours (mean ± SEM)	%
Carrageenan	58.4 ± 2.78	35.34 ± 2.34 **	39.53	32.2 ± 2.65 *	44.86	28.2 ± 1.76 *	51.71	24.3 ± 1.84 *	58.39
	Weight of granuloma formation (mg)	Weight of granuloma formation (mg)		Weight of granuloma formation (mg)		Weight of granuloma formation (mg)		Weight of granuloma formation (mg)	
Cotton wool	82.04 ± 1.72	48.4 ± 3.54*	41.00	34.34 ± 4.65*	58.14	32.32 ± 3.45*	60.60	30.22 ± 3.68*	63.16

p-Value was calculated by comparing with the control by students t-test,*$p < 0.001$,

**$p < 0.05 > 0.02$: N=6



Graph no: 1(a)



Graph no: 1(b)