ANTI-INFLAMMATORY ACTIVITY OF ETHANOLIC EXTRACT OF PLUMBAGO ZEYLANICA LINN LEAVES

[1]M. Hema Mani, [2]A. Jayachitra,
[1]Department of Biochemistry and Biotechnology, Sourashtra college, Madurai
[1]Research Scholar, Department of Biochemistry, Bharathiyar University
[2]School of Biotechnology, Madurai Kamaraj University, Madurai

Abstract: **Objective:** To determine the anti-inflammatory activity of ethanolic extract of Plumbago zeylanica Linn leaves (EEPZ), and its possible mechanism of action. **Methods:** Wistar albino female rats (150-200 g) were used in this study. The animals were housed in poly propylene cages in the standard environmental conditions of 20-250C temperature, 12 h light: 12 h dark cycle, and fed with standard rodent pellet diet and water ad libitum. The groups were divided into 5 groups (n=6/group) and assigned as positive control, negative control, and standard and two different test dose groups of P. zeylanica. Paw edema induced by phlogistic agent of 0.05 mL of carrageenan (suspended in 1% carboxymethyl cellulose) into the left hind paw in all groups except negative control group. Granuloma induced by cotton pellets (10±1 mg) were implanted into groin region of each rat. The groups were divided into 4 groups (n=6/group) and assigned as possitive control, two different test dose groups of P. zeylanica and standard. **Results:** Oral administration of EEPZ shown a significant ($p<0.05$) dose-dependent protection against carrageenan-induced paw edema. At 1st hr, P. zeylanica shown an inhibition effect of edema in the different doses of 100 mg/kg and 150 mg/kg were found and got the significant result ($p<.001$). **Conclusion:** Thus, the present study revealed that the EEPZ offered significant protection against inflammation.

IndexTerms **– Anti-inflammatory, EEPZ, Plumbago Zeylanica, paw edema.**

I. INTRODUCTION

Plumbago zeylanica is a family of Plumbaginaceae and commonly known as “chittiramulam or vellai” in Tamil and widely distributed in southern parts of India. In the traditional system of medicine, different parts of the plant used a variety of diseases (Tabassum and Hamdani 2014; Shankar et.al 2012). P. zeylanica is widely used as a gastrointestinal disease (Maurya et. al 2015), respiratory disease (Kumar et. al 2014), gonorrhea and syphilis (Kishore et. al 2012), inflammatory diseases (Parekar et.al 2015), scabies (Arivanathan, Saraswathy and Rajamanickam 2010), blood coagulation profile activity (Shukla and Singh 2015), antiallergic activity (Tabassum and Hamdani 2014), central nervous system (CNS) stimulant activity (Ittivavirah and Ruby 2014), antioxidant (Day et.al 2004), anti-infertility activity (Bopaiah and Pradhan 2001), lipid metabolism activity (Jain et.al 2014), and cytotoxicity activity (Kumar et.al 2015). There is no documentary evidence of contraindication and interaction. Subcutaneous injection of the carrageenan is to promote hyperalgesia and to develop erythema. This response due to proinflammatory mediators such as bradykinin, histamine, tachykinins, reactive oxygen, and nitrogen species (Zholos 2015). These mediators readily migrate to sites of inflammation and proven with current study. After administration of the carrageenan showed significant inflammatory response in paw edema model (Ma et.al 2013). Inflammation is a disorder involving swelling associated with multiple complex mediators (Ricciotti and Fitzgerald 2011). Inflammation is a pathological state and characterized by concurrent active inflammation, tissue destruction, and attempts at repairing stage (Wilgus, Roy and McDaniel 2013). The natural system of medicines is believed that one of the important source of health-care field (Ameni et.al 2015). However, we investigated the protective effect of ethanolic extract of P. zeylanica (EEPZ) influence on regulating complex mediators in inflammatory rats to provide a definite experimental basis for the clinical medication.

II. MATERIALS AND METHODS

2.1 Experimental design and drug treatments

2.1.1 Animals

Wistar albino female rats (150-200 g) were used in this study as shown in figure 1. All animals were obtained were from agricultural university, Mannuthy, Trissur, Kerala and housed for at least one week in the laboratory animal room prior before testing. The animals were housed in poly propylene cages in the standard environmental conditions of 20-250C temperature, 12 h light: 12 h dark cycle, and fed with standard rodent pellet diet and water ad libitum. The experiments were conducted in accordance with the internationally accepted principles for laboratory animal use and the experimental protocols duly approved by the institutional animal ethical committee (IAEC) of KMCH College of Pharmacy, Coimbatore – 48. The ethical committee approval number was KMCRET/ 01/2011-12.

Fig.1(a) Left hind paw of rat

Fig.1(b) Closure Left hind paw of rat

Fig 1. Anti-inflammatory Left hind paw of rat

2.1.2 Preparation of Extract

The Plumbago zeylanica. Linn plant was purchased from the local market. The leaves were collected and they were washed to remove the surface contaminants in running water and desiccated between folds of soft tissue paper. Then the leaves were dried under shade and finely powdered. The powder was weighed to 50 gm and soaked overnight in petroleum ether to remove chlorophyll and the PET ether was drained. Then the remaining residue was refluxed using sox let apparatus using ethanol for 48 hrs. The extract was concentrated and used for analysis.

2.1.3 General consideration

The inflammatory technique entails a chain of occasions that may be elicited via several stimuli, e.g., infectious agents, ischemia, antigen-antibody interactions, chemical, thermal or mechanical injury. The reaction is accompanied by means of the scientific signs and symptoms of erythema, edema, hyperalgesia and ache.

2.2 Paw edema method

2.2.1 Purpose and Rational

Among the many strategies used for screening of anti-inflammatory drugs, one of the maximum commonly employed techniques is based totally upon the ability of such sellers to inhibit the edema produced inside the hind paw of the rat after injection of a phlogistic agent. Many phlogistic agents (irritants) have been used, together with brewer's yeast, formaldehyde, dextran, egg albumin, kaolin, Aerosil®, sulfated polysaccharides like carrageenan or naphthoyl heparamine. The effect may be measured in several ways. The hind limb can be dissected on the talocrural joint and weighed. Usually, the quantity of the injected paw is measured before and after software of the irritant and the paw volume of the dealt with animals is compared to the controls. Many methods had been described the way to measure the paw volume with the aid of easy and much less correct and by means of greater sophisticated electronically devised strategies. The price of the assessment is less depending on the equipment however tons more on the irritant being chosen. Some irritants result in simplest a short lasting irritation while different irritants reason the paw edema to continue over extra than 24 hours.

2.2.2 Procedure

Male or female Sprague-Dawley rats with a body weight between 100 and 150 g are used. The animals are starved overnight. To insure uniform hydration, the rats obtain 5 ml of water with the aid of stomach tube (controls) or the check drug dissolved or suspended inside the identical volume. 30mins later, the rats are challenged by means of a subcutaneous injection of 0.05 ml of 1% solution of carrageenan into the plantar aspect of the left hind paw. The paw is marked with ink at the extent of the lateral malleolus and immersed in mercury as much as this mark. The paw volume is measured plethysmographically right away after injection, once more three and six h, and in the end 24h after mission.

2.2.3 Apparatus

Various tools had been evolved for plethysmography of the paw. (Winter et al., 1963) used mercury for immersion of the paw. A greater sophisticated equipment has been described by means of (Hofrichter et al., 1969). (Alpermann and Magerkurth, 1972) defined an equipment primarily based on the precept of reworking the quantity being improved through immersion of the paw into a proportional voltage the usage of a pressure transducer. (Webb and Griswold, 1984) suggested a sensitive approach of measuring mouse paw extent by means of interfacing a Mettler Delta Range pinnacle-loading stability with a microcomputer.

2.2.4 Evaluation

The increase of paw extent after three or 6h is calculated as percentage in comparison with the quantity measure right away after injection of the irritant for each animal. Effectively handled animals display an awful lot less edema. The difference of average values among handled animals and control groups is calculated for on every occasion c program language period and statistically evaluated. The difference on the various time intervals offers a few recommendations all through the anti-inflammatory effect. A dose- reaction curve is run for energetic pills and ED50 values may be determined.

2.2.5 Standards

Depending at the irritant steroidal and non-steroidal anti-inflammatory tablets have a mentioned impact in the paw edema take a look at. With carrageenan as irritant doses of 50 to 150 mg/kg phenylbutazone p.o. Had been found to be effective.

2.2.6 Critical assessment of the method

The paw edema technique has been utilized by many investigators and has been demonstrated to be appropriate for screening functions in addition to for more intensive evaluations. Dependent on the irritant steroidal and nonsteroidal anti-inflammatory drugs, antihistaminics and also, to a lesser degree, serotonin antagonists are energetic in the paw edema tests.

III. RESULT AND DISCUSSION

Table: 1: Anti-inflammatory activity of ethanolic extract of Plumbago zeylanica Linn leaves

	Dose (mg/kg)	Paw Volume(ml) after induction of Carageenan						
		0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
Control	Vehicle (5 ml/kg)	1.053±0.04	1.545±0.05	1.737±0.02	1.757±0.03	1.822±0.04	1.822±0.027	1.805±0.029
Standard	Indomethacin 20	0.99±0.026	1.573±0.01^{ns}	1.858±0.03**	1.848±0.02*	1.732±0.02^{ns}	1.533±0.01***	1.248±0.02***
Ext S2	100	1.020±0.05	1.437±0.03^{ns}	1.897±0.02***	1.922±0.02***	1.778±0.02^{ns}	1.515±0.04***	1.388±0.007***
Ext S2	150	1.038±0.03	1.557±0.04^{ns}	1.897±0.02***	1.885±0.019**	1.797±0.03^{ns}	1.610±0.02***	1.385±0.01***

All data were expressed in mean ± SEM (n=6).
aP < 0.05, bP < 0.01, cP < 0.001 compared with 0 h of same group.
ns – No significance compared with 0 h of same group

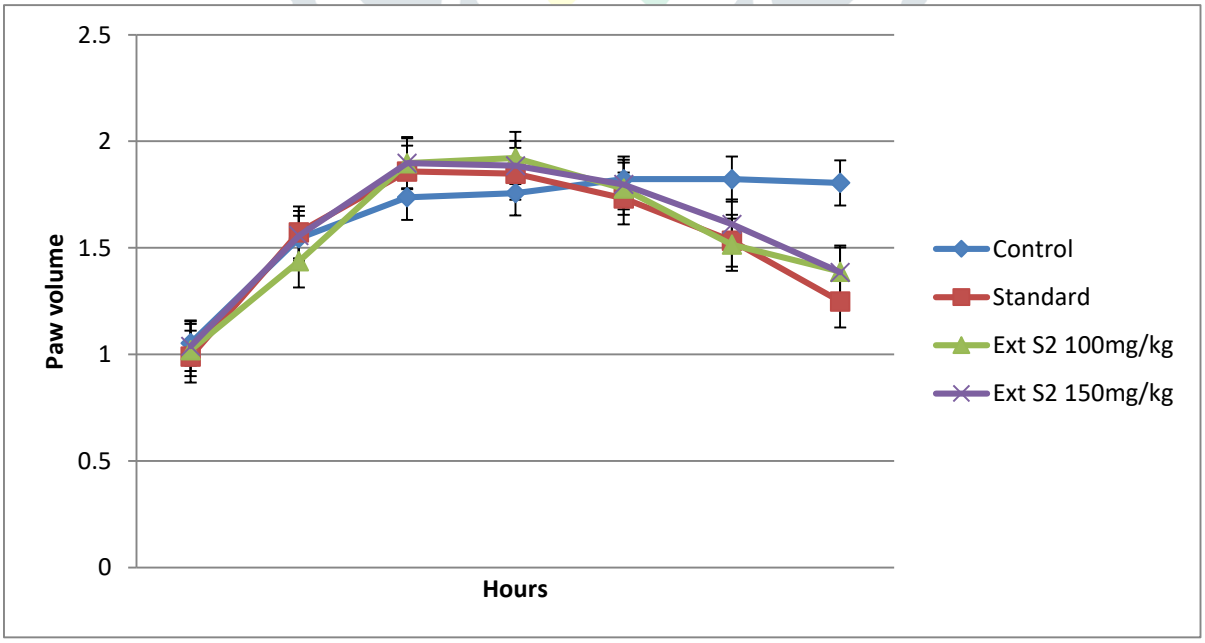

Fig. 2 Anti-inflammatory activity of Plumbago zeylanica Linn leaves

The result of anti-inflammatory interest of EEPZ turned into given in Table.1. The most extensively used primary take a look at for screening anti-inflammatory marketers is carrageenan-triggered edema within the mouse hind paw (Chen et al., 2008), which has regularly been used to assess the anti-edematogenic impact of natural merchandise (Thomazzi et al., 2010). The inflammatory reaction brought on via carrageenan is characterised via a biphasic response with marked edema formation because of the fast manufacturing of numerous inflammatory mediators (Mendes et al., 2010). Early section is attributed to a launch of histamine, serotonin and bradykinin, whereas past due section is due to the overproduction of prostaglandin (Ogonowski et al., 1997). The inflammatory reaction is commonly quantified by way of the increase in paw size (edema) and is modulated via inhibitors of precise molecules within the inflammatory cascade, together with nonsteroidal anti-inflammatory tablets (Morris, 2003). Pre-treatment of animals with ethanolic extract of Plumbago zeylanica. Linn become powerful in decreasing the edematogenic reaction caused by carrageenan, and these consequences were just like the ones exhibited by using the control institution dealt with with indomethacin. These consequences may be related to a discount in the liberation of mediators (histamine, serotonin, or bradykinin) in local tissue or be due to the blockage of receptors to the distinctive mediators stated before. The anti- inflammatory properties proven on this look at, similarly to the Plumbago zeylanica. Linn functions make this plant a capability goal for the improvement of new compounds that can be explored as alternatives to presently used drugs.

IV. CONCLUSION

P. Zeylanica extract showed lively in opposition to carrageenan-brought on rat paw edema in a dose-established manner. At 150 mg/kg P. Zeylanica become comparable to conventional (20 mg/kg) within the inhibition of paw edema. The impact of EEPZ may be attributed to its loose radical scavenger hobby and safety of apoptosis. In the experimental models, EEEPZ become observed to show off full-size ($p<0.001$) anti-inflammatory pastime, and the results were comparable to traditional drug of diclofenac. Thus, the prevailing has a look at discovered EEPZ phytoconstituents exerts the favored consequences against hypersensitive reaction and irritation.

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