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 [1,2]. P. zeylanica is widely used as a gastrointestinal disease [3], respiratory disease [4], gonorrhea and syphilis [5], inflammatory diseases [6], scabies [7], blood coagulation profile activity [8], anti-allergic activity [1], central nervous system (CNS) stimulant activity [9], antioxidant [10], anti-infertility activity [11], lipid metabolism activity [12], and cytotoxicity activity [13]. 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 pro-inflammatory mediators such as bradykinin, histamine, tachykinins, reactive oxygen, and nitrogen species [14]. 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 [15]. Inflammation is a disorder involving swelling associated with multiple complex mediators [16]. Inflammation is a pathological state and characterized by concurrent active inflammation, tissue destruction, and attempts at repairing stage [17]. The natural system of medicines is believed that one of the important source of health-care field [18]. However, we investigated the protective effect of dichloromethane extract of P. zeylanica (DMEPZ) influence on regulating complex mediators in inflammatory rats to provide a definite experimental base for the clinical medication.

METHODS

Preparation of the extracts

The roots of P. zeylanica were collected in Nellore District, India. Botanical identification and voucher specimen No. RIP/2013/120 has been deposited in the museum of the Department of Pharmacognosy at Ratnam Institute of Pharmacy, Nellore, India. The roots were dried under shade, segregated, and pulverized by a mechanical grinder and passed through a 40 mesh sieve. The powdered 1 kg of the material was soaked in solvent dichloromethane (4000 mL) for 48 hrs and repeats the process for thrice to get complete extraction. The solvent was removed in a rotary vacuum and stored in an airtight container.

Drugs and chemicals

Carrageenan was obtained from SD. Fine Chemicals Limited, Bombay. All other chemicals were of analytical grade procured from reputed Indian manufacturers.

Experimental animals

The experimental design was approved by the Institutional ethical committee of Ratnam Institute of Pharmacy, Nellore (Ethical Approval No. 13/Institutional Animal Ethics Committee/Pharma/RIP/2013). Male albino Wistar rats weighing 180-200 g (6-8 weeks) were supplied from animal facility and housed six animals per cage at 23-25°C and relative humidity between 55 and 58%, respectively. They had access to food and water ad libitum and were exposed to alternate 12 hrs light and dark cycles.

Acute toxicity study

The acute toxicity study was carried out albino Wistar rats. The experiment made into six groups containing six animals in each group. DMEPZ was suspended in critical micelle concentration + dimethyl-sulphoxide and starting dose from 5, 50, 100, 200, and 400 to 2000 mg/kg body weight (bw) to all groups, respectively. These animals were observed for a 72 hrs period. The number of deaths was expressed as a percentile and the lethal dose 50 (LD50) was determined by probit a test using the death percentage versus the log dose [19].
The time at which signs of toxicity appear and disappear was observed systematically and recorded for each animal.

Carrageenan-induced rat paw oedema
A total of 30 animals were equally divided into 5 groups of six each. Before the experimental study allowed for overnight fasting in the rats. All the groups of rats, hindpaw volume measured by the plethysmograph instrument (Yukui et al.). All the groups were injected 0.1 mL of a suspension of 1% carrageenan under the subplantar aponeurosis of the right hind paw of rats except Group I. Group I is the positive control and injected 0.1 mL saline. Group II is a negative control and injected 0.1 mL of a suspension of 1% carrageenan under the subplantar region. Group V served as positive control and received diclofenac sodium was injected intraperitoneally at 25 mg/kg b.w 1 h before carrageenan injection. Group III and IV were orally administered with DMEPZ 250, 500 mg/kg b.w, respectively. After carrageenan injection, paw volume was measured at 1, 2, and 3 hrs to determine the inflammatory activity.

In the rats, percentage of inhibition of edema calculated using the following formula,

\[
\text{% of inhibition of oedema} = \frac{Vc - Vt}{Vc} \times 100
\]

Where, Vc is the edema in the disease control group and Vt is the edema in the treatment group.

Cotton pellet-induced granuloma
A total of 24 were equally divided into four groups of six each. The sterile cotton pellets in milligram of 10±1 were implanted to subcutaneously into both sides of the groins region of each rat, and before the pellets implantation rats were anesthetized. Group I received the vehicle (0.9% NaCl 10 mL/kg b.w) and served as control. The dose of 250 and 500 mg/kg b.w of DMEPZ was orally administered as Group II and III rats for seven consecutive days from the first day of cotton pellet implantation. Diclofenac at a dose of 25 mg/kg b.w received group IV.

Table 1: HPLC profiles of the DMEPZ

| Retention time (min) | Area (mV) | Height (mV) | Area (%) | Height (%) |
|----------------------|-----------|-------------|----------|-----------|
| 3.580                | 46.3701   | 2.7367      | 0.3292   | 0.3232    |
| 4.860                | 31.0003   | 3.9909      | 0.4240   | 0.4714    |
| 6.713                | 91.5804   | 5.8665      | 0.6725   | 0.6429    |
| 7.447                | 419.5180  | 22.7855     | 2.9192   | 2.6913    |
| 8.233                | 137.2056  | 13.0742     | 1.5443   | 1.4544    |
| 8.880                | 460.7721  | 316.7515    | 26.3048  | 25.3143   |
| 10.147               | 21.8959   | 1.5991      | 0.1452   | 0.1389    |
| 10.867               | 18.9115   | 0.8879      | 0.0827   | 0.0749    |
| 13.247               | 54.9761   | 3.3796      | 0.3825   | 0.3692    |
| 14.400               | 44.8306   | 2.3031      | 0.3119   | 0.2720    |
| 15.180               | 892.58227 | 461.7467   | 62.1094  | 58.5395   |
| 24.833               | 128.1171  | 3.6027      | 0.0891   | 0.0425    |
| 27.660               | 195.0064  | 5.6933      | 1.3569   | 0.6725    |
| 38.853               | 105.1888  | 2.2108      | 0.7320   | 0.6211    |

Values are expressed as mean±SE (n=6). Data were analyzed using one-way analysis of variance followed by Dunnett’s multiple comparison test.

Table 2: Effect of DMEPZ on cotton pellets-induced granuloma in rats

| Treatment | Dose (mg/kg) | Weight of wet cotton pellets (mg) | Percentage inhibition | Weight of dry cotton pellets (mg) | Percentage inhibition |
|-----------|--------------|-----------------------------------|----------------------|----------------------------------|----------------------|
| Control   | Saline 2 mL  | 102.34±1.44                       | 41.64                | 33.66±0.62                      | 30.35                |
| DMEPZ     | 250          | 106.40±1.77                       | 41.64                | 25.17±0.62                      | 47.92                |
| Diclofenac| 500          | 80.52±1.62                        | 55.84                | 23.54±0.58                      | 51.29                |

Values are expressed as mean±SE (n=6). Data were analyzed using one-way analysis of variance followed by Dunnett’s multiple comparison test. *p<0.001; p<0.05 considered as significant; NS: Non-significant; All groups are compared with normal control. SE: Standard error, DMEPZ: Dichloromethane extract of Plumbago zeylanica.

Table 3: Effect of DMEPZ on carrageenan-induced rat paw oedema

| Treatment | Dose (mg/kg) | Percentage inhibition | Weight of wet cotton pellets (mg) | Percentage inhibition | Weight of dry cotton pellets (mg) |
|-----------|--------------|-----------------------|-----------------------------------|----------------------|----------------------------------|
| Control   | Saline 2 mL  | 41.64                 | 102.34±1.44                       | 41.64                | 33.66±0.62                      |
| DMEPZ     | 250          | 41.64                 | 106.40±1.77                       | 41.64                | 25.17±0.62                      |
| Diclofenac| 500          | 55.84                 | 80.52±1.62                        | 55.84                | 23.54±0.58                      |

Values are expressed as mean±SE (n=6). Data were analyzed using one-way analysis of variance followed by Dunnett’s multiple comparison test. *p<0.001; p<0.05 considered as significant; NS: Non-significant; All groups are compared with normal control. SE: Standard error, DMEPZ: Dichloromethane extract of Plumbago zeylanica.
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Vetriselvan

Non-steroidal anti-inflammatory drug including indomethacin or aspirin is not inhibiting initial phase of edema and has been attributed to the release of chemical mediators. The second phase of swelling attributed to the production of cyclooxygenase-2 in the hind paw as revealed in previous study [22]. In the recent years, the biological effect of phytosterols emphasis on their in vitro and in vivo immune modulatory activity [23].

Some of the chemotactic and chemokinetic agents reported to be involved topical inflammation through arachidonic acid by lipoxygenase activity such as 12-hydroxy-6,8,11,14-eicosatetraenoic acid from platelets, leukotriene B4 from polymorphonuclear leukocytes, and 5-hydroxy-6,8,11,14-eicosatetraenoic acid [24]. Carrageenan-induced edema has been commonly used as an experimental animal model for acute inflammation. In the carrageenan-induced rat paw edema model, except control group, and all examined with DMEPZ administered orally. The results showed significant anti-inflammatory activity, where dose 500 mg/kg exhibited the highest effect. Initially, 1-2 h, carrageenan mainly mediated by histamine, serotonin, and increased synthesis of prostaglandins in the damaged tissue surroundings [25]. After sustained by prostaglandin release and mediated by Bradykinin, leukotrienes, and polymorphonuclear cells [26]. The findings of the present study confirmed carrageenan causes the production and release of nitric oxide (NO) at the injured site NO, which alerts pathological conditions of NO synthesis, this could be involved in tissue injury, including edema and hyperalgesia condition [27].

Treatment with P. zeylanica extract showed significant action against paw edema in a dose-dependent manner. At 500 mg/kg dose of DMEPZ was quite comparable to diclofenac (25 mg/kg). The present study results indicate that a dose of 250 and 500 mg/kg b.w influencing against the inflammatory process. The inflammation due to arachidonic cofactors also revealed a previous study [28]. Among groups, cotton pellet granuloma tissue compared with wet and dry weight of the cotton pellets. Different dose of 250 and 500 mg/kg b.w of DMEPZ showed curing effect of inflammation comparable to diclofenac treatment. The results demonstrated that herbal medicine has ability to treat inflammatory diseases. Hence, it needs further detailed pharmacological and clinical investigations to prove it as an effective therapeutic agent for inflammation.

CONCLUSION

P. zeylanica extract showed active against carrageenan-induced rat paw edema in a dose-dependent manner. At 500 mg/kg P. zeylanica was comparable to diclofenac (25 mg/kg) in the inhibition of paw edema. The effect of DMEPZ may be attributed to its free radical scavenger activity and protection of apoptosis. In the experimental models, DMEPZ was found to exhibit significant (p<0.001) anti-inflammatory activity.

Fig. 2: High-performance liquid chromatography profiles of the dichloromethane extract of Plumbago zeylanica

Fig. 1: (a) Effect of dichloromethane extract of Plumbago zeylanica (DMEPZ) on carrageenan-induced rat (b) effect of DMEPZ on carrageenan-induced paw edema (basal time) rat paw edema (after 1 hr) (c) effect of DMEPZ on carrageenan-induced rat paw edema (after 3 hrs)
and the results were comparable to standard drug of diclofenac. Thus, the present study revealed DMEPZ phytoconstituents exerts the desired effects against hypersensitivity and inflammation.

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