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Research Article

INHIBITION OF ARACHIDONIC ACID-INDUCED EAR EDEMA, MAST-CELL STABILIZING AND FREE RADICAL EFFECT OF DICHLOROMETHANE CRUDE EXTRACTS FROM THE LEAVES OF LABISIA PUMILA

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ABSTRACT

Objective: To evaluate the ability of dichloromethane leave extract of labisia pumila (DELP) to inhibit arachidonic acid - induced ear oedema, mast cell stabilizing effect and determination of DPPH, TPC, hydroxyl, nitric oxide and superoxide radical-scavenging activity of the extract.
Material and method: The leaf extract was prepared with dichloromethane filter and rotary evaporated and stored in desiccator. The effect of DELP on arachidonic acid - induced ear oedema, mast cell stabilizing was evaluated. DPPH, TPC, hydroxyl, nitric oxide and superoxide radical-scavenging activity of the extract was also studied.
Result: DELP was able to inhibit arachidonic acid induced ear edema in the mice. The concentration of DELP (mg/ml) needed for 50% inhibition (IC50) of superoxide radicals, DPPH and NO were 1, 1.5, and 1.5 respectively. Total Phenolic Content was 25µg GAE/mg.

Keywords: Anti-inflammation, antioxidant, ear-edema, Labisia pumila, mast-cell stabilization.

INTRODUCTION

Inflammation is a normal host defense mechanism that protects the host from infection and other insults; it initiates pathogen killing as well as tissue repair processes and helps to restore homeostasis at infected or damaged sites. It is characterized by redness, swelling, heat, pain and loss of function, and involves interactions amongst many cell types and the production of, and responses to, a number of chemical mediators. Where an inflammatory response does occur it is normally well regulated in order that it does not cause excessive damage to the host, its self-limiting and resolves rapidly. This self-regulation involves the activation of negative feedback mechanisms such as the secretion of anti-inflammatory cytokines, inhibition of pro-inflammatory signaling cascades, shedding of

receptors for inflammatory mediators, and activation of regulatory cells. Pathological inflammation involves a loss of tolerance and/or of regulatory processes. Where this becomes excessive, irreparable damage to host tissues and disease can occur. Eicosanoids are key mediators

and regulators of inflammation [1, 2] and are generated from 20 carbon polyunsaturated fatty acids (PUFAs). Because inflammatory cells typically contain a high proportion of the n-6 PUFA arachidonic acid (AA; 20:4n-6) and low proportions of other 20-carbon PUFAs, AA is usually the major substrate for eicosanoid synthesis. Eicosanoids, which include cyclooxygenase activity, in inflammatory conditions indicates the importance of this pathway in the pathophysiology of such diseases as asthma [3]. Mast cells (also known as mastocyte and labrocyte) are resident cells of several types of tissues and they contain many granules rich in histamine and heparin. Although best known for their role in allergy and anaphylaxis, mast cells play an important protective role as well, being intimately involved in wound healing and defense against pathogens. Mast cells are very similar in both appearance and function to the basophil, a type of white blood cell. However, they are not the same, as they both arise from different cell lines [4, 5, 6]

Labisia pumila popularly known as Kacip Fatimah is a sub herbaceous plant, which belongs to the Myrsinaceae family. We have previously reported the anti-inflammatory, anti-asthmatic, anti-histamine, anti-cholinergic and gastroprotective effect of dichloromethane crude extract of Labisia pumila. In this present study, the dichloromethane crude extract DELP (100, 200 and 500mg/kg) obtained from the leaf of Labisia pumila was studied for inhibition of arachidonic acid induced ear edema and mast cell stabilizing effect.

MATERIAL AND METHODS

Plant Material

The leaves of LP (Kacip Fatimah) was purchased from University Putra Malaysia, Serdang, Selangor Darul Ehsan. Mr. Shamsul Khamis, a research officer (plant taxonomy) from the Laboratory of Natural Products (NATPRO), Institute of Bioscience in University Putra Malaysia, specifically identified the plant.

Chemicals and Drugs

Analytical grade chemicals like Dichloromethane (DCM) (R&M Chemicals, England), Hexane (Chemolab, Malaysia), Ethyl Acetate (EA) (Spira, Malaysia) and Methanol (Merck, Malaysia) were used for the extraction and partial purification of the bioactive components present in the plant. Tween 40(Zulat, India), tragacanth powder (Zulat, India), glycerol (Chemolab, Malaysia), prednisolone (Sigma-Aldrich, China), Ketotifen and cimetidine (Sigma-Aldrich, China) were used in the biological assay. Some other chemicals used where sodium chloride (MERCK, Germany), potassium chloride (MERCK, Germany), Na2HPO4.2H2O (Fisher, UK) and KH2PO4 (R&M chemicals, UK). Arachidonic acid (Sigma-Aldrich, USA) was used as mediators for the biological assays.

Preparation of extracts

The leaves were air-dried for almost 3 weeks and there after grinded into fine powder using a miller. Extraction with dichloromethane was carried out by successive maceration at room temperature for a week followed by filtration. The filtration process was repeated several times to make sure all the dirt and dust were completely removed. The filtrate obtained was then concentrated using rotary evaporator at temperatures of 350C until dryness this process was repeated a few times to obtain enough DELP [7].

Animal Preparation

Forty-five (45) male Sprague Dawley rats of 6-8 month of age, weighing 150-200g and 35 ICR mice weighing 30-40g were

purchased from Institute for Medical Research (IMR). They were fed with standard pellet diet and water ad libitium. They were housed in groups of five (5) in standard cages in animal holdings units, UCSI University, Kuala Lumpur, Malaysia and maintained under standard environmental condition (temperature: 22 + 1oC; humidity 14 + 1 and light / dark schedule 12/12 hour). They were acclimatised for 2 weeks before starting the experiment. The animals were deprived of food for 24 hrs before the beginning of experiment with free access to tap water. The Project has ethical committee approval code no. ETUCSI 10013

Arachidonic acid induced ear oedema

The mice were divided into 4 groups (5 in each group). All the mice received negative control (acetone) on their left ear and on the right ear, group 1 received cimetidine (100 mg/kg) while groups 2-4 received DELP 100, 200 and 500mg/kg. 30 minutes after treatment, 20µg (2mg/kg) of arachidonic acid was topically applied to the ears of the mice and after one hour, the oedema was measured using a vernier calliper. The ear oedema was expressed as the difference in ear thickness between test animal and controls [8].

Mast cell stabilization

The rats were sensitized by a 0.5 ml subcutaneous injection of horse serum along with 0.5ml of triple antigen containing 20 000 million Bordetella pertussis organisms. The rats were then divided into 7groups (5 rats in each group). Group 1 received saline, group 2 received horse serum, group 3 received Ketotifen (50mg/kg), group 4 received prednisolone (10mg/kg), and groups 5-7 received DELP 100, 200 and 500mg/kg respectively for 14 days. On the 14th day, 3hrs after the last treatment, the rats were sacrificed and intestinal mesenteries were taken for study of mast cells. The intestinal mesenteries were kept in ringer Locke's solution at 37oC. They are then challenged with horse serum in vitro for 10mins. Pieces of the mesentery are immersed in 0.1% toluidine blue in 4% aqueous formal saline for 5-10mins. It was then transferred to xylene for 5-10mins and finally rinsed 2-3 times with acetone. The intestinal tissue pieces were cut and removed, placed on a slide and stretched with the help of needles and examined under a microscope (400 x). The number of intact and disrupted mast cells per field was counted [9]

Determination of total phenolic compounds (TPC)

The amount of total phenolic compounds present in the DELP was determined with Folin-Ciocalteu Reagent (FCR) using the method of [10]. The experiment was done by measuring 2.5ml of 10% FCR and 2ml of Na2 CO3 (2%w/v) was added to 0.5ml of each sample (3 replicates) of DELP solution (1mg/ml). The resulting mixture was incubated at 450C with shaking for 15min. The absorbance of the samples was measured at 765 nm using UV/visible light. Results were expressed as milligrams of Gallic acid (0-0.5mg/ml) dissolved in distilled water.

Determination of DPPH

Determination of DPPH (1-1-diphyenyl 2-picryl hydrazyl) radical-scavenging activity Triplicates of 1mg/ml DELP were prepared by weighing 2 mg of sample dissolved into 2 ml of ethanol (Merk, Germany) 5.9 mg of the DPPH powder was dissolved into ethanol and toped up to 100ml. A solution was made by mixing 0.2mL of the sample extract with 3.8ml of ethanol solution of DPPH radical (0.1mM). The mixture was shaken vigorously and left to stand at room temperature in the dark. After 30 minutes the absorbance was measured at 517 nm against ethanol. Mixture of DPPH 3.8 ml and ethanol solvent without DELP was used as Control sample [11]. The scavenging ability was calculated using the following equation;

DPPH Scavenging ability (%) =

$$[1-\frac{absorbance\ of\ sample}{absorbance\ of\ control}]\ x\ 100$$

Superoxide radical-scavenging activity

Mixture containing 6 µM EDTA, 3 µg NaCN , 2 µM riboflavin , 50 µM NBT, various concentration of the DELP (0.4-2.0mg/mL) and 67mM, pH 7.8 phosphate buffer in a final volume of 3ml. The tubes were uniformly illuminated with an incadescent visible light (Philips, 40W) for 15 min, and the absorbance reading was measured at 530nm. The percentage inhibition was calculated by comparing the absorbance values of control (without extract) and the experimental tubes.

Superoxide radical scavenging activity (%) =

$$\frac{absorbance\ of\ control - absorbance\ of\ sample}{absorbance\ of\ control}\ X\ 100$$

Nitric oxide scavenging activity

Nitric oxide scavenging activity was determined by the method of [12]. Sodium Nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions and the extent of nitric oxide scavenged by the test samples was determined by the Greiss illosvoy reaction [13]. Two millilitre of aqueous sodium Nitroprusside was dissolved in 0.5 ml of phosphate buffer saline (pH 7.4) and mixed with 0.5ml of DELP at various concentration (0.2, 0.4,0.6,0.8&1mg/ml).

The mixture was incubated in a water bath at 250C for 150 minutes. After incubation, the mixture absorbance was read at 540nm using methanol as blank and 0.5ml of the solution was withdrawn and mixed with 1.0 ml of Sulphanilic acid reagent (0.33% in 20% glacial acetic acid) at room temperature for 5 minutes to complete diazotization. Then 1.0 ml of 0.1% w/v Naphthylethylenediamide dichloride was added to the mixture and allowed to stand at room temperature for 30 minutes and absorbance measured at 540 nm. Nitric oxide radical scavenging activity was calculated by the following equation:

%Inhibition of NO = [A1 – A0]/A1 x 100 (Equation 3.3) where: A0 is the absorbance before reaction and A1 is the absorbance after reaction has taken place.

Statistical analysis

Data were expressed as the mean ± S.D. Experiment groups were compared using one way analysis of variance (ANOVA test) followed by the Bonferroni's test. Statistical analysis was performed using Graph-pad Prism 5.0. Values of probability p≤ 0.05 were considered statistically significant.

RESULT

The subcutaneous injection of 0.5ml of horse serum along with 0.5ml of triple antigen containing 20 000 million Bordetella pertussis organisms were able to degranulate the mast cells in the experimental rats 81.25 ± 1.35. Treatment with prednisolone 10mg/kg, ketotifen 50mg/kg, DELP 100,300,and 500mg/kg was able to stabilize the mast cells and reduced the degranulated mast cells in the experimental rats from 30.95 ± 1.32 to 69.03 ± 1.80 (prednisolone 10mg/kg) 22.87 ± 1.57 to 77.12 ± 1.70 (ketotifen 50mg/kg), 49.40 ± 1.7 to 50.60 ± 1.80 (DELP 100mg/kg), 33.60 ± 1.6 to 66.40 ± 1.60(DELP 200mg/kg), and 25.90 ± 1.7 to 70.05 ± 1.80 (500mg/kg) Fig 1.

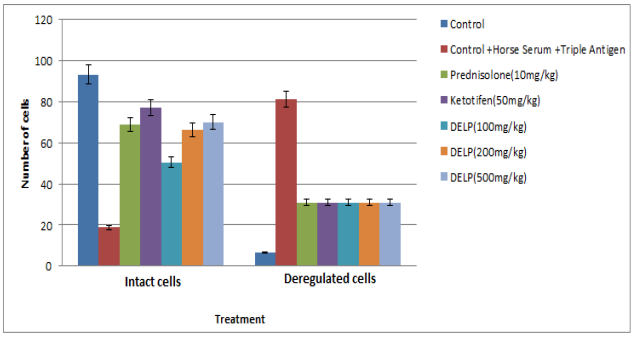

Fig. 1: Effect of prednisolone, ketotifen and DELP on horse

serum and bordetella pertussis organisms. Values were express as mean ± SD (N=5).

The topical application of 20µg (2mg/kg) of arachidonic acid on the ears of the mice were able to induce ear edema of 0.196 ± 0.004. Treatment with cimetidine 100mg/kg and DELP 100, 200 and 500mg/kg were able to inhibit the ear edema induced by arachidonic acid 0.140 ± 0.007 (29%), DELP 0.135 ± 0.005 (32%), 0.117 ± 0.005 (39%) and 0.113 ± 0.005 (42%) respectively (Fig 2). The concentration of DELP (mg/ml) needed for 50% inhibition (IC50) of superoxide radicals, DPPH and NO were 1, 1.5, and 1.5 respectively. Total Phenolic Content was 25µg GAE/mg.

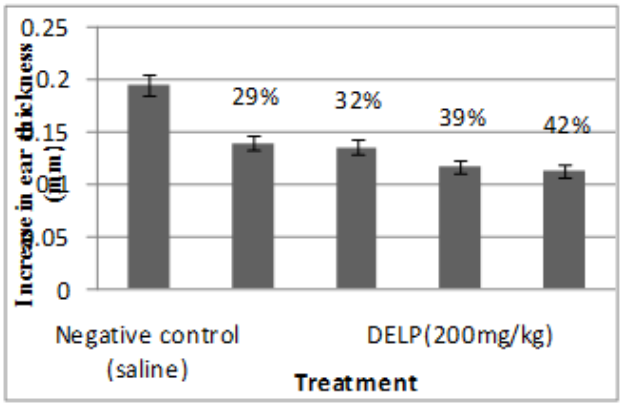

Fig. 2: Effect of Cimetidine and DELP on arachidonic-induced ear edema in experimental mice. Values were express as mean ± SD (N=5).

DISCUSSION

The oral administration of DELP at 100, 300 and 500mg/kg was able to inhibit in a concentration dependant manner the edema induced by arachidonic acid in the mice ear edema. It has been reported by [14,15], that direct application of AA to the ear of the mouse causes an immediate vasodilatation and erythema, leading to development of intense edema. The synthesis of leukotriene and prostaglandins, serotonin and histamine are responsible for the formation of ear edema [16,17]. Our earlier report has shown anti-inflammatory effect [18] (inhibitions of serotonin, prostaglandins, histamine). DELP inhibition of ear edema induced by AA suggests that it may also be interacting with 5-lipoxygenase enzyme or antagonizing the effect of leukotriene. The mast cells have a crucial role in the development of many physiological changes during anaphylactic and allergic responses. Immunoglobulin-E antibodies bind to receptors on the surface of mast cell. Allergen-IgE interaction on mast cell leads to the release of histamine, heparin, proteases and other mediators and the synthesis and secretion of leukotrienes and prostaglandins. These products result in bronchoconstriction changes in blood vessel tone, increased vascular permeability and myriad other proinflammatory effects [19]. The functions of mast cells can be manipulated for therapeutic ends by regulating their function with appropriate drugs. Plant origin constituents may influence differentiation into mast cells, chemical composition and or architecture of mast cell surface membrane. It may influence the synthesis of IgE molecules or binding of IgE on mast cell surface. It is also possible, that the plant drug may reduce the life span of mast cells. DELP markedly protected the rats against antigen-induced challenge of mast cells. The effect was almost apar with that observed with the standard drug (Prednisolone and ketotifen) used. The pathological mechanism involved in Type-I allergy has been explained as the degranulation of mast cells and basophils, followed by the release of mediators such as histamine, leukotrienes and prostaglandins from these cells [20]. The degranulation of mast cells occurs in response to the immunological stimuli in which the antigen–antibody interaction on the cell surface predominates. The present investigation indicates that the DELP is active against Type-I allergic condition because of their ability to inhibit the release of mediators from mast cells and basophils and thus influences the course of the disease. The observed biological activity of DELP may be due to its antioxidant property.

CONCLUSION

DELP inhibited arachidonic acid induced ear edema in mice, mast cell stability and antioxidant activity.

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