Global Journal of Pharmacology 6 (2): 126-130, 2012
ISSN 1992-0075

DOI: 10.5829/idosi.gjp.2012.6.2.6590

In vivo and *In vitro* Anti-Asthmatic Effects of Dichloromethane Crude Extract from the Leaves of *Labisia pumila*

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Abstract: This present study was aimed at evaluating the anti-asthmatic effects of crude dichloromethane leaf extract (DELP) (100, 300 and 500 mg/kg), from *Labisia pumila (*LP*)*. *In vivo* experiment was done by subcutaneous injection of 0.1ml of 1% histamine, serotonin and bradykinin, dissolved in saline, into the sub-plantar of the experimental rat model to induce edema and 0.2% of histamine and acetylcholine aerosol was used in a transparent glass box using nebulizer. The *in vitro* anti-histaminergic, anti-cholinergic anti- serotonin and anti- bradykinin studies were examined by using guinea pig ileum (GPI) in an organ bath containing Krebs-Henseleit solution (pH 7.4) gassed with 95% O_2 and 5% CO_2 and maintained at 37°C. Phytochemical screening was done using simple biochemical colorimetric test. The result showed a concentration-dependent inhibition of paw edema induced by histamine, serotonin and bradykinin and bronchoconstriction induced by histamine and acetylcholine. The extracts also inhibited the contraction induced by histamine, acetylcholine, serotonin and bradykinin in GPI. Preliminary phytochemical screening revealed the presence of flavonoids, steroids, saponins, alkaloids and tannins. The present study confirmed that the DELP exhibited bronchodilator activity and inhibited actions of the inflammatory mediators involved in asthma.

Key words: Labisia Pumila · Asthma · Anti-Inflammatory · Bronchodilators

INTRODUCTION

One of the common disorders encountered in clinical medicine in both adults and children is asthma and it is characterized by inflammation of the airways which causes airway dysfunction [1]. Asthma is currently a worldwide problem with around 300 million people around the globe suffering from it and world deaths of about 250 thousand annually [2]. Inhaled bronchodilators and anti-inflammatory drugs are available and effective and they require long-term use and are associated with side effects [3, 4]. This is why alternative and complementary medicine is being sort after to prevent these side effects [5]. Several medicinal plants have anti-inflammatory effect and have proved effective in the treatment of asthma. *L. pumila* is a sub-herbaceous plant with Creeping Stems. It is widely spread in the rainforest lowland and hill of Malaysia, Thailand, Indochina, Philippines and New Guinea [6-8]. Previously in our study we have reported that *L. Pumila* leaf extract possess gastro-protective, anti-inflammatory, anti-pyretic and anti-nociceptive activities [9-11]. Therefore in this study the anti-asthmatic activities of DELP were investigated and simple preliminary phytochemical analysis of the extract was carried out.

MATERIALS AND METHODS

Plant Material: The leaves of LP (Kacip Fatimah) were purchased from University Putra Malaysia, Serdang, Selangor Darul Ehsan. The plant was specifically identified by Mr. Shamsul Khamis, a research officer (plant taxonomy) from the Laboratory of Natural Products (NATPRO), Institute of Bioscience in University Putra Malaysia voucher specimen number LP50 was given to it.

Preparation of Extracts: The leaves were air-dried for almost 3 weeks and were then grounded into fine powder using a miller. Extraction with dichloromethane (DCM) was carried out by successive maceration at room

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temperature for a week followed by filtration. The filtration process was repeated several times to make sure all the dirt and dust are completely removed. The filtrate obtained was then concentrated using rotary evaporator at temperatures of 35¢J until dryness this process was repeated a few times to obtain enough crude extract of LP (DELP) [11].

Animal Preparation: Seventy-five (75) male Sprague Dawley rats of 6-8 month of age, weighing 150-200g, twenty (20) ICR male mice weighing 30-40g and fifty (50) Guinea pigs weighing 250-300g 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 $\pm$ 1°C; humidity 14 $\pm$ 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 experiments with free access to tap water. The animal ethical committee approval was obtained before the commencement of the experiment.

Paw Oedema (Histamine, Serotonin and Bradykinin): The rats were divided into 5 groups as follows: Group I received saline, Group 2 received the reference drug indomethacin 10mg/kg and Groups 3-5 received DELP at 100, 300 and 500mg/kg respectively. The drugs were orally administered to the respective groups of 5 rats each (N=5). After one hour, paw oedema was induced in the rats by subcutaneous injection of 0.1ml of 1% histamine and serotonin into the sub-plantar of the experiment rats and the paw volume was measured with a plethysmometor at 0hr, 1hr, 2hrs, 3hrs, 4hrs and 5hrs after the injection of the mediators. The procedure was repeated for bradykinin, but bradykinin was injected along side with perindopril arginine and paw volume was measured at 0, 10, 20, 40 and 60 minutes. [12].The inhibitory activity was calculated according equation 3.2 [13].

Equation 3.2

$$\text{Percentage inhibition} = \frac{\text{100 - Average Inflammation of Treated Group at Time (T)}}{\text{Average inflammation of control at time (t)}} \times 100$$

Histamine and Acetylcholine Induced Bronchoconstriction: The guinea pigs were divided into 5 groups: Group I received saline, Group 2 received pyrelamine maleate and Groups 3-5 received DELP at concentration of 100, 300 and 500mg/kg. The drugs were orally administered to the respective groups of 5 rats each (N=5). After overnight fasting of the rats, they were exposed to 0.2% histamine and acetylcholine aerosol in an air-tight chamber and the pre-convulsion time (PCT) (time of aerosol exposure to the onset of dyspnoea leading to appearance of convulsion) was recorded and they were immediately removed from the chamber and placed in fresh air to recover. The rats were left for 24 hours to recover and were given their various treatments according to the grouping above and were again exposed to histamine and acetylcholine aerosol at 1hr, 4hrs and 24hr after treatment and their PCT was again recorded this was PCT after the administration of the drugs[14].

Equation 3.3

Percentage protection = $[1 - (T_1 / T_2)] \times 100$

Histamine, Acetylcholine, Serotonin and Bradykinin Induced Contraction in GPI: The abdomen of the guinea pig was opened and the part of the ileum nearest to the caecum was removed and placed in a beaker filled with aerated Krebs-Henseleit solution(in mM): NaCl, 118.41; KCl, 4.69; $NaHCO_3$, 25.0; $CaCl_2.2H_2O$, 2.52; $MgSO_4.7H_2O$, 1.22; KH_2PO_4, 1.18; glucose anhydrous, 9.991) [15]. 1cm Ileum (1cm) was cut off and placed in a petri dish containing aerated Krebs-Henseleit solution and the residues left in the luminal area were carefully washed off with Krebs- Henseleit solution with the aid of an oral feeding gauge. After washing, the tissue was suspended in a 50 ml organ bath filled with aerated Krebs-Henseleit solution with one end of the tissue attached to a tissue holder and the other end to an isometric force transducer using a waved cotton thread. This preparation was incubated for 30 - 60 minutes during which the bathing solution was changed every 15 minutes. The tissue was maintained at 1 g tension [16]. The contraction induced by histamine, acetylcholine, serotonin and bradykinin was challenged with 100ug and 500ug of DELP in the organ bath [17]. The concentration –response curves were recorded.

Phytochemical Screening: Conventional standard protocols [18, 19] for detecting the presence of different chemical constituents in the plant extract were employed. The chemical constituents tested include flavonoids, alkaloids, tannins, steroids and saponins.

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 $p \leq 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Pre-treatment with standard drug indomethacin (19mg/kg), DELP at 100, 200 and 500mg/kg significantly (p<0001) decreased the rat paw oedema induced by histamine, serotonin and bradykininas compared to negative control (Fig 1). Histamine, serotonin and bradykinin are an important inflammatory mediator, potent vasodilator substance that causes increases vascular permeability. This result is in agreement with our earlier report on the inhibition of first phase of carrageen induced paw oedema which involves the release of histamine and serotonin [10]. DELP 100, 200 and 500mg/kg showed a significant (p<0001) increase in preconvulsion time in the experimental guinea pig at the fourth hour (4hrs) when exposed to either histamine or acetylcholine aerosol, the effect similar to the control drug pyrelamine maleate (Fig 2). Histamine and acetylcholine are known bronchocontrictors which increases airway resistance and cause difficulty in breathing, DELP have been shown to inhibit this effect. DELP was able to inhibit in a concentration –dependent manner the contraction induced by histamine, acetylcholine, serotonin and bradykinin on the GPI (Fig 3). These mediators caused depolarisation and tonic contractions of intestinal smooth muscles. It's generally accepted that an increase in concentration of cytoplasmic-free calcium ions is indispensable f or smooth muscle contraction. The activation of the necessary receptors of longitudinal smooth muscle of guinea – pig small intestine produces an increased frequency of action potential discharge and depolarisation which results in a contraction [20]. According to [21] smooth muscle contractile tone can be relaxed by increased levels of adenosine 3`, 5` – cyclic monophosphate (cAMP). Therefore the relaxatory effect observed by DELP maybe as a result of its ability to increase cAMP [independent of any specific receptor activity, then reduction in Ca^{2+} levels. On the other hand, DELP may have inferred with access of calcium ions to the cytoplasm, by blocking the voltage-activated or receptor-operated calcium channels [22].

The effects of the extract may be because of presence of phytochemicals such as flavonoids, saponins, steroids and tannins, known to possess similar effects. Flavonoids have been reported to have antioxidant and anti-inflammatory activity [23,24]. Saponins are well known for anti-inflammatory and cell stabilizing activity [25, 26]. Saponins inhibit the formation of cyclooxygenase metabolites viz. prostaglandins and thromboxanes and the putative antipholgistic activity of saponins was ascribed to the inhibition of arachidonic acid metabolism [27]. Another mechanism of action behind the anti-inflammatory effects of saponins is due to inhibition of histamine, bradykinin and serotonin along with its antioxidant effects which in turn inhibits the formation of reactive oxygen species having important role in inflammation [28]. Tanins have been reported to inhibit COX enzyme, decrease vascular permeability and antioxidant activity [29, 30]. Steroids possess a very strong anti-inflammatory activity by inhibiting cytokine release IL-1, 2 and 6; migration of leucocytes and induction of lipocortin 31, 32, 33.

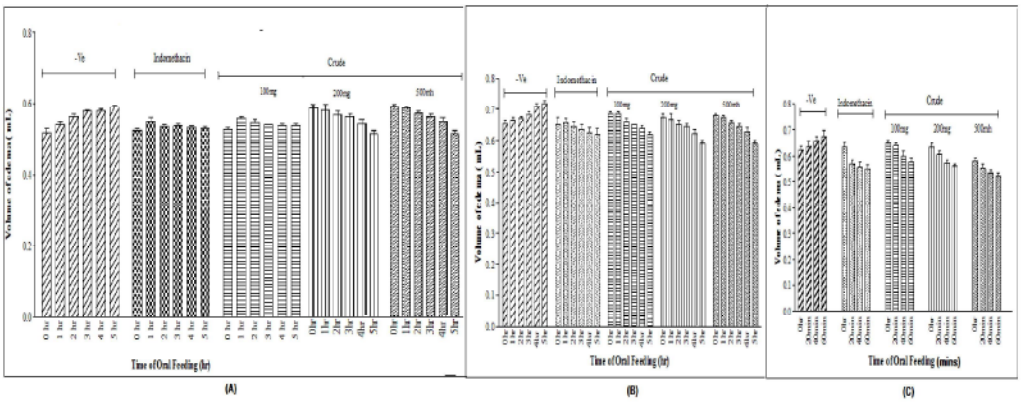

Fig. 1: The effect of DELP on (a) histamine, (b) serotonin and (c) bradykinin induced paw edema in rats.Values were expressed as mean ± SD (N = 5). Statistical significance (p< 0.001) was calculated by ANOVA followed by Bonferroni's test.

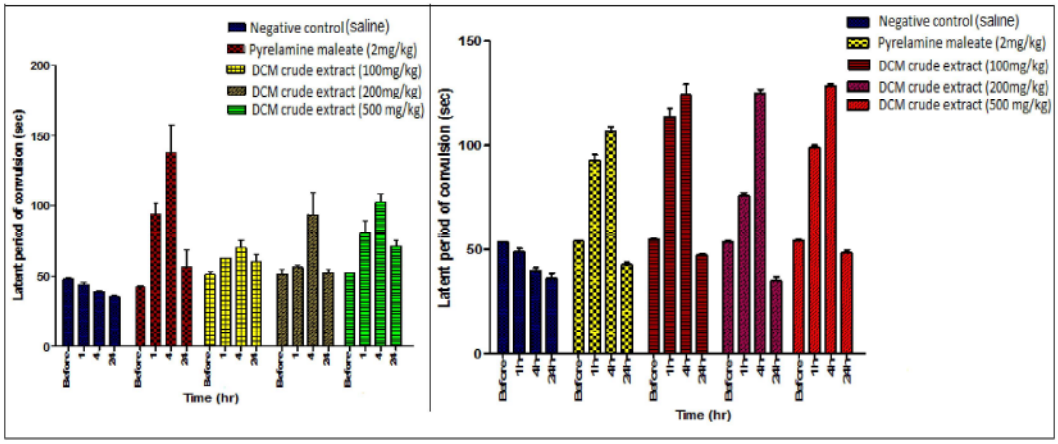

Fig. 2: Effect of DELP on (A) histamine and (B) acetylcholine induced bronchoconstriction in guinea pigs.Values were expressed as mean ± SD (N = 5). Statistical significance (p< 0.001) was calculated by ANOVA followed by Bonferroni's test

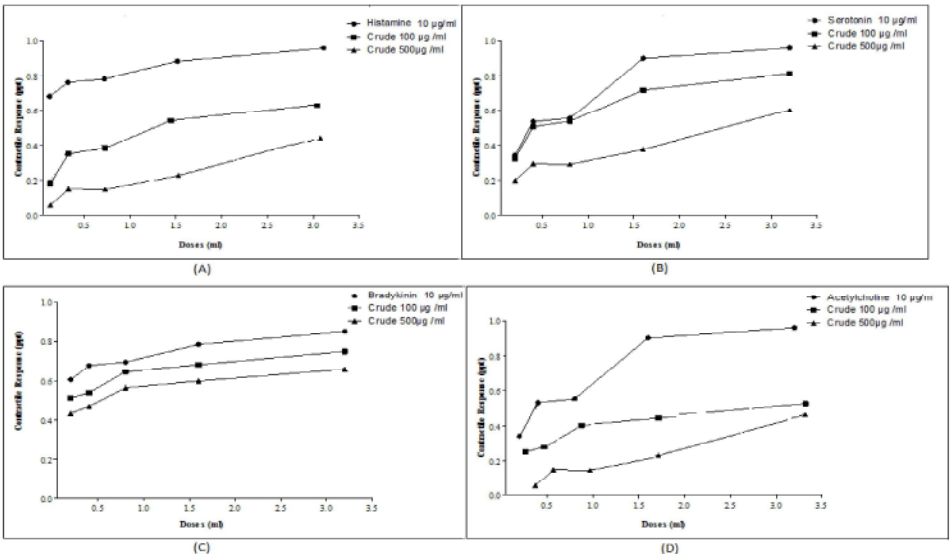

Fig. 3: Effect of DELP on (A) histamine, (B) serotonin, (C) bradykinin and (D) acetylcholine- induced contraction.Values were expressed as mean ± SD (N = 5). Statistical significance (p< 0.001) was calculated by ANOVA followed by Bonferroni's test.

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