Anti-inflammatory Activity of Ethanol Extract from the Leaves of *Pseuderanthemum palatiferum* (Nees) Radlk.

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**ABSTRACT**

*Pseuderanthemum palatiferum* (Nees) Radlk. is one of the most frequently used medical plants in Thailand for treating a variety of symptoms and several inflammatory diseases. This study aimed to ascertain the anti-inflammatory property of ethanol extract from the leaves of *P. palatiferum*. The anti-inflammatory activity against acute inflammation was assessed by the ethyl phenylpropiolate (EPP) induced ear edema test and cotton pellet induced granuloma model was used for chronic inflammation in albino rats. It was found that the extract showed significant anti-inflammatory activities against both acute and chronic inflammation as compared to the controls (*P* < 0.05). The extract at low dose used in acute inflammatory test (1 mg/ear) exerted comparable anti-acute inflammatory activity to phenylbutazone (PHBZ), a standard drug, whereas the extract at all doses used in chronic inflammatory test (250, 500 and 750 mg/kgBW) significantly reduced transudative weight and granuloma formation. The extract exhibited its chronic anti-inflammatory effect in a dose dependent manner and the highest dose used had higher capacity than diclofenac, a standard NSAID. Due to the decrease in malondialdehyde (MDA) and nitric oxide (NO) levels as well as the increase in superoxide dismutase (SOD) level detected in chronic inflammation-induced rats, antioxidation was suggested as the mechanism underlying the anti-inflammatory capacity of ethanol extract from the leaves of *P. palatiferum*.

**Keywords:** anti-inflammation, ethanol extract, *Pseuderanthemum palatiferum* (Nees) Radlk.

**1. INTRODUCTION**

Inflammation is the physiological responses to tissue injury caused by all kinds of aggressions. Although the inflammatory cascade could help eliminate the offending irritant and restore damaged tissues to a healthy state, untreated or inappropriately treated inflammation often causes harm to different aspects of the body and thought to be one of the leading causes of chronic disease [1,2]. The effective treatment for both acute and chronic inflammation is, thus, needed. A wide variety of anti-inflammatory
drugs are available in pharmaceutical market, but those with no or few side effects are very expensive. During the past decade, the interest in medicinal plants and herbal products has rapidly increased and the plant with anti-inflammatory property has been one of the most focused research subjects. Plant species having anti-inflammatory activity have been reported in a large body of literature. Those plants included: *Curcuma longa* Linn. [3,4], *Pluchea indica* Linn. [5,6], *Turnera ulmifolia* [7,8] and *Zingiber officinale* Roscoe [9, 10]. Nevertheless, discovery of new anti-inflammatory drugs from plants is still challenged.

*Pseuderanthemum palatiferum* (Acanthaceae), known as “Hoan Ngoc” is a medicinal plant native to Vietnam. This plant species has been introduced to Thailand about 20 years ago and it has been very popular among Thai people. It is a bush with height of 1-2 m. The leaves are in whorls, green and glossy. The flowers are pink or purple. The local Vietnamese have used it to treat a wide range of maladies, such as diarrhea, sore throat, cancer, gastric ulcer, arthritis, as well as inflamed wound [11]. Although *P. palatiferum* has been used for treating a number of inflammatory diseases, so far, its anti-inflammatory capacity has not been published based upon scientific studies. The phytochemical analysis showed that the leaves of *P. palatiferum* contain several types of antioxidants, such as flavonoid, apigenin, triterpenoids, saponin, β-sitosterol, stigmasterol, kaempferol, salicylic acid, total phenols and ascorbic acid [12,13]. Since chronic inflammation exerts its cellular side effects mainly through excessive production of free radicals and depletion of antioxidants [14,15], such antioxidant-rich plant like *P. palatiferum* may have the potential to control inflammation in the body through its potent antioxidant activity.

This study was, therefore, undertaken to evaluate the anti-inflammatory effects of the ethanol extract from *P. palatiferum* in rats using EPP-induced ear edema and cotton pellet tests. In addition, its antioxidative actions in inflammation-induced rats were also explored using lipid peroxidation and nitric oxide production, phenomena associated with oxidation stress as well as inflammation [16,17] and superoxide dismutase, a free radical scavenging enzyme, as biomarkers. Since the liver is a uniform organ with the highest antioxidant enzyme activities [18], antioxidative status in this study was conducted in liver samples of the rats.

2. MATERIALS AND METHODS

2.1 Plant Materials and Extraction

*P. palatiferum* were purchased from a market in Muang District, Chiang Mai Province on May, 2010. The plants were identified by a botanist and the herbarium specimens were deposited at the Queen Sirikit Botanical Garden, Chiang Mai (ID: WP2615). Fresh leaves of *P. palatiferum* were washed with tap water, cut and immersed in 80% ethanol for 24 hours. The extract was then filtered to remove the residue and evaporated by vacuum rotary evaporator to obtain the crude ethanol extract. The crude extract was then weighed to calculate the actual percentage yield (5.76%). Finally, the ethanol extract was dissolved in distilled water to yield concentrations of 50, 100 and 150 mg/ml, respectively and used for further experiments.

2.2 Animal

Wistar rats (*Rattus norvegicus*) were purchased from the National Laboratory Animal Center, Mahidol University, Salaya campus, Thailand. The animals were
housed in cages and had access to tap water and a standard diet (C.P. 082). The room temperature was controlled at 24-26°C in a 12 hour light/dark cycle. All procedures involving the animals were conducted with strict adherence to guidelines and procedures reviewed and approved by the Institutional Animal Care and Use Committee of Biology Department, Faculty of Science, Chiang Mai University.

2.3 Acute Oral Toxicity Study
Female albino rats weighing 100 to 120 g were used for acute toxicity study. The study was carried out as per the guidelines set by OECD [19] and no adverse effects or mortality were detected in the rat up to 2 g/kg, p.o., during the 24 h observation period. Based on the results obtained from this study, the dose for anti-inflammatory activity in chronic inflammation was fixed to be 250, 500 and 750 mg/kgBW for dose dependent study (comparable to the concentrations of 50, 100 and 150 mg/ml, respectively.)

2.4 EPP-induced Ear Edema in Rats: Acute Inflammation
The method was modified from that of Keardrit et al. (2010) [20]. The test was performed in male rats weighing 35 to 40 g. Ear edema was induced by topical application of ethyl phenylpropiolate (EPP) dissolved in acetone (50 mg/ml) in a volume of 10 μl to the inner and outer surfaces of both ears (20 μl/ear). The test sample was topically applied to the ear in a volume of 20 μl just before the irritant. The thickness of each ear was measured with a digital vernier caliper at 15, 30, 60 and 120 min after the edema induction. The edema thickness of the sample-tested group was compared to that of the vehicle group using phenylbutazone (PHBZ) at a dose of 1 mg/ear as a positive control.

2.5 Cotton Pellet-induced Granuloma in Rats: Chronic Inflammation
Rats of either sex weighing 180 to 210 g were divided into five groups (n = 6). For inflammatory induction, the rats were mildly anesthetized with thiopental and four sterile cotton pellets (50mg) were subcutaneously implanted in the dorsal region of rats, two in the axilla and two in the groin regions. After regaining their normal conditions, the inflammation-induced rats were orally administered as the following pattern: Group I served as negative control and received ddH₂O. Group II served as positive control and received diclofenac 5 mg/kgBW. Group III-V received the extracts at doses of 250, 500 and 750 mg/kgBW respectively. Additional group without inflammatory induction (normal control group) which received only ddH₂O was conducted for biochemical analysis of antioxidative status. On the 17th day, the rats were sacrificed using anesthetic ether, and the cotton pellets were dissected out without affecting the surrounding granulomatous tissues. The moist pellets were weighed and then dried at 60°C for 48 h and again weighed. The weights of the cotton pellets obtained from the extract treated groups were compared with those of control groups. The granulomatous tissues were fixed in 10% buffered formalin for histological assessment. Liver tissues were also excised and stores in 0.9% saline at -20°C for biochemical analysis.

2.6 Biochemical Analysis
The liver tissue was homogenized with tissue homogenizer in Tris-HCl buffer (0.1 M, pH 7.4). The homogenate was centrifuged at 1,000 × g, for 10 minutes in
cold centrifuge, and the supernatant was used for estimation of malondialdehyde, superoxide dismutase and nitric oxide following the methods of Wasowicz et al., 1993 [21], Marklund and Marklund, 1974 [22] and Kumar et al., 2005 [23], respectively. Protein content was determined using the method of Lowry et al., 1951 [24].

2.7 Histological Assessment

The formalin-fixed granulomatous tissues were dehydrated and processed for histology examination using standard techniques. The 6-μm-thick sections were stained with hematoxylin and eosin. A blind histological analysis was conducted by a pathologist.

2.8 Statistical Analysis

For statistical analysis, data were analyzed by one-way analysis of variance (ANOVA) using SPSS statistical software version 17.0 for windows. The results were expressed as mean ± standard deviation. A level of p value less than 0.05 was considered to be significant. To determine the relationship between cotton weights and MDA, NO and SOD levels, a bivariate correlation with a Pearson correlation coefficient (r) was used.

3. RESULTS AND DISCUSSION

The results of this study demonstrated that the ethanol extract of *P. palatiferum* possessed anti-inflammatory activity in both acute and chronic inflammation. Neither sign of toxicity nor mortality was observed when rats were treated with the extract dose up to 2,000 mg/kgBW. The extract can therefore, be considered to be relatively safe. Based on this result, the maximum dose for anti-inflammatory study was set as 750 mg/kgBW.

3.1 EPP-induced Ear Edema in Rats: Acute Inflammation

Inhibition of acute inflammation by ethanol extract from *P. palatiferum* was clearly seen in Table 1. The extract at both doses used (1 and 2 mg/ear) significantly inhibited ear edema when compared to a control group, but no significant differences among the doses was observed. During the 1st h of assessment time the percentage inhibition of ear edema in rats received both low and high doses of the extract was lower than that of rats treated with phenylbutazone (1 mg/ear). Nevertheless, the extract at a dose of 1 mg/ear displayed a comparable percent of inhibition to the anti-inflammatory drug at 2 h after topical application. Moreover, the similar time-dependent

<table>
<thead>
<tr>
<th>Groups</th>
<th>Edema thickness (μm)</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15 min</td>
<td>30 min</td>
</tr>
<tr>
<td>Control</td>
<td>583±0.07c</td>
<td>597±0.09b</td>
</tr>
<tr>
<td>Phenylbutazone</td>
<td>477±0.03a</td>
<td>486±0.05a</td>
</tr>
<tr>
<td>P. palatiferum 1</td>
<td>521±0.02b</td>
<td>515±0.05a</td>
</tr>
<tr>
<td>P. palatiferum 2</td>
<td>541±0.03b</td>
<td>511±0.06a</td>
</tr>
</tbody>
</table>

Data represent mean ± SD (n = 6); a,b,c Means in the same column and with different superscripts are different at P < 0.05; Phenylbutazone 1 mg/ear; P. palatiferum 1 = 1 mg/ear; P. palatiferum 2 = 2 mg/ear.
manner of ear edema reduction was also observed in phenylbutazone and this low dose extract treated groups. The tentative of better anti-edema effect observed in low dose group might be due to its higher degree of percutaneous penetration than the high dose group which vehicle solvent was more saturated with the extract. EPP-induced ear edema is a good model for evaluating the anti-inflammatory capacity of tested substances in acute inflammation. This assay is simple and provides a reliable result with several anti-inflammatory drugs, including phenylbutazone [25]. EPP produces local swelling by causing vasodilatation, vascular permeability and fluid accumulation and several inflammatory mediators such as histamine, kinins, serotonin (5-HT) and prostaglandins (PGs) are found responsible for those inflammation signs [26]. Inhibition of the synthesis and release of the key mediators of acute inflammation is the mechanism underlying the anti-edematous effect of phenylbutazone [27]. The results obtained from this study suggested the similar mechanism for *P. palatiferum* extract at a dose of 1 mg/ear. Due to its delay time of action as compared to anti-inflammatory drug, extensive research is required for isolating the active compound(s) with fast anti-inflammatory action.

### 3.2 Cotton Pellet-induced Granuloma in Rats: Chronic Inflammation

Granuloma formation is the repairing phase, the last phase of chronic inflammatory process, following the increase in vascular permeability and leukocytes infiltration [28]. In this study, foreign body granulomas were provoked in rats by subcutaneous implantation of cotton pellets and the diminution of granuloma formation was estimated following the administration of the extract. It was shown in Table 2 that the extract of *P. palatiferum* at all doses used could significantly reduce the wet weight of cotton pellet (*P*<0.05) and it occurred in a dose dependent manner. The wet weight of cotton reflects the transudate volume while the dry weight correlates with the amount of granulation tissue [29]. Diclofenac, a standard NSAID, could inhibit transudation by blocking the effects of serotonin, bradykinin, histamine and prostaglandin E1 on vascular membrane and by inhibiting the release of these mediators [30]. It also reduces granuloma size by inhibiting the leukocyte infiltration and collagen fiber formation. Suppression of cytokines, such as IL-1 and TNF, as well as growth factors influence fibroblast proliferation and

### Table 2. Effect of ethanol extract of *P. palatiferum* on cotton pellet-induced chronic inflammation in rats.

<table>
<thead>
<tr>
<th>Treatment (mg/kgBW)</th>
<th>Wet weight</th>
<th>Dry weight</th>
<th>% inhibition of transudative</th>
<th>% inhibition of granuloma</th>
</tr>
</thead>
<tbody>
<tr>
<td>ddH2O + cotton</td>
<td>261.88 ±19.43a</td>
<td>65.10 ±5.68a</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Diclofenac (5) + cotton</td>
<td>190.81 ±38.17b</td>
<td>49.03 ±6.26b</td>
<td>27.95</td>
<td>24.69</td>
</tr>
<tr>
<td><em>P. palatiferum</em> (250) + cotton</td>
<td>200.87 ±27.69b</td>
<td>55.17 ±5.19ab</td>
<td>25.96</td>
<td>15.25</td>
</tr>
<tr>
<td><em>P. palatiferum</em> (500) + cotton</td>
<td>197.20 ±20.00b</td>
<td>53.63 ±4.40b</td>
<td>27.04</td>
<td>17.62</td>
</tr>
<tr>
<td><em>P. palatiferum</em> (750) + cotton</td>
<td>157.08 ±29.27b</td>
<td>51.22 ±9.83b</td>
<td>40.20</td>
<td>21.32</td>
</tr>
</tbody>
</table>

Cotton: 50 mg. Values are in milligrams. Mean ± standard deviation was computed over six animals/group. Percentage inhibition was compared with negative control group. a,b Means in the same column and with different superscripts are different at *P*<0.05.
mucopolysaccharides production were suggested as the mechanisms for granuloma inhibition [31, 32]. The extract may regulate vascular permeability through the same mechanism as diclofenac, eliminating or blocking vascular mediators. Nevertheless, the capacity of *P. palatiferum* extract the highest dose used (750 mg/kgBW) gave the higher percentage of transudate inhibition than diclofenac. This potent anti-transudative property together with the non-toxic result, *P. palatiferum* in an appropriate formulation and form might be considered as an alternative to non-steroidal anti-inflammatory drugs.

An anti-granuloma effect was also achieved with the extract from *P. palatiferum*. The reduction of cotton dry weight found in rats treated with the extract (Table 2) indicated the suppression of granuloma development. The degree of granuloma inhibition was in dose-dependent manner and comparable to that of diclofenac and it was confirmed by histological results. The decrease in inflammatory cell infiltration, fibroblast proliferation, and collagen fiber density observed in the extract treated group were also in dose-dependent manner (Figure. 1 c, d and e). Aside from having some angiogenesis, *P. palatiferum* extract at the highest dose used gave the most appreciable result in histological confirmation showing relatively comparable histological features to diclofenac (Figure. 1 b and e). The possible mechanism involving anti-granulomatous effect of the extract may contribute to the reduction of some or all key mediating cytokines leading to the inhibition of

**Figure 1.** Histological section of granuloma tissue on day 17 post-surgery (a) control group (b) 5 mg/kgBW of diclofenac treated group. (c) 250 mg/kgBW of *P. palatiferum* treated group. (d) 500 mg/kgBW of *P. palatiferum* treated group. (e) 750 mg/kgBW of *P. palatiferum* treated group. Granulation tissue of the extracts contains comparatively more collagen, fibroblast and blood capillaries and few inflammatory cells (H & E stain 20x).
inflammatory cell recruitment, especially macrophage and mast cells, the key initiators for granulation [33].

3.3 Biochemical Analysis

Another suggested mechanism for anti-inflammatory effects of *P. palatiferum* probably includes the inhibition of the generation of oxygen radicals. An increase in oxygen uptake initiated by activated macrophages in inflammatory process give rise to a variety of reactive oxygen species, including O$_2$, nitric oxide (NO) and hydrogen H$_2$O$_2$ [34]. The sustained activation and phosphorylation of MAP kinases and redox-sensitive transcription factors, such as NF-KB and AP-1 were suggested as the mechanisms of enhancing inflammation [35]. The increase in gene expression of proinflammatory mediators resulted from alteration of nuclear histone acetylation and deacetylation is also caused by oxidative stress [36]. Antioxidants play a role in preventing inflammation by scavenging free radicals [37,38], lowering lipid peroxidation and maintaining the activity status of various antioxidant enzymes [39]. From our results, increased oxidative stress was reflected by the high values of MDA content, the inflammatory biomarker and indicator for lipid peroxidation in free radical interaction, in inflammatory-induced rats (Figure 2). The excessive NO radicals (Figure 3) also reflected the release of nitric oxide synthase (NOS) during inflammatory stimulation [40].

The reduction MDA and NO by *P. palatiferum* was comparable in strength to diclofenac (Figure 2 and 3) and was correlated with its capacity to inhibit granuloma development (Table 2 and Figure 1). The strength of the relationship between cotton dry weight to MDA level and to NO level was indicated by the correlation coefficient that closed to 1 (r = 0.87 and 0.85 respectively). A number of plant species containing flavonoids and other phenolic compounds such as *Scutellaria baicalensis* Georgi., *Taxillus chinensis* (DC) Danser, *Sophora japonica* (L.) Schott, and *Mahonia fortune* (Lindl.) Fedde. have been reported to exert anti-inflammatory activity by acting as the potent free radical scavengers and inhibiting cyclooxygenase and lipooxygenase pathways of arachidonate metabolism [41]. Since

**Figure 2.** Effect of ethanol extract of *P. palatiferum* on MDA level in cotton pellet-induced granuloma in rats. Values are mean ± standard deviation. * Significantly different from control (P < 0.05). (C: Cotton).
flavonoid has been reported to be one of the major phytochemicals in *P. palatiferum*, its ability to decrease MDA and NO level was, thus, not surprising.

The relationship between antioxidant and anti-inflammatory activities of *P. palatiferum* extract was further supported by the much greater increase in SOD activity that resulted when inflammatory-induced rats were treated with the extract (Figure 4). SOD level was inversely related to wet weight ($r = -0.89$). This experimental evidence strongly suggests that *P. palatiferum* is also a rich source of enzymatic antioxidant. Besides having an ability to quench the superoxide free radical, preventing of the formation of plasma-derived superoxide-dependent chemotactic factor for human neutrophils [42] was also suggested for the role of SOD in anti-inflammation process.

Since oxidative stress and inflammation are associated with the risk of several inflammatory disorders such as atherosclerosis, rheumatoid arthritis, hepatitis, glomerulonephritis and diabetes mellitus, the antioxidant and anti-inflammatory
activities of extract from the leaves of *P. palatiferum* may shed light on potential therapeutic options in those diseases.

4. CONCLUSIONS

It could be concluded from our study that ethanol extract from the leaves of *P. palatiferum* possessed potent anti-inflammatory activities in both acute and chronic inflammations. Antioxidation was proposed as the mechanism underlying its anti-inflammatory property. Our results confirm the traditional use of *P. palatiferum* for treating inflammation. Further research is needed for developing anti-inflammatory products from this plant species, either as alternatives or as adjuvants to conventional anti-inflammatory drugs.

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REFERENCES


