

IN VIVO ACUTE ORAL TOXICITY AND ANTI-INFLAMMATORY EFFECTS OF *PLUCHEA PTEROPODA* ETHANOL EXTRACT

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ABSTRACT

Pluchea pteropoda, an annual plant in the Asteraceae family, has traditionally been used in Vietnamese folk medicine as an alternative treatment for high fever, indigestion, and painful and swollen wounds. The present study investigates the acute toxicity and antiinflammatory effects of *P. pteropoda* aerial parts ethanol extract (EtPP) in animal models. EtPP exhibited no toxicity in mice with a single dose of up to 5000 mg/kg. EtPP significantly inhibited the carrageenan-induced edema paw in rats at 77.5 and 150.0 mg/kg body weight doses. The strongest inflammation inhibition effect (37.4%) was recorded at a dose level of 150.0 mg/kg after 3 h after carrageenan administration.

Keywords: *Pluchea pteropoda*; Toxicity; Antiinflammation; Carrageenan.

I. INTRODUCTION

Inflammation, recognized as a complicated biological process associated with the pathogenesis of a spectrum of diseases, including cancer,[1] age-related disorders,[2] neurological conditions,[3] and diabetes [4], holds paramount significance in contemporary biomedical research. As an indispensable facet of the innate immune

response, inflammation orchestrates the body's defense mechanism against an array of endogenous and exogenous stimuli [5]. While conventional pharmacotherapy has been instrumental in managing inflammation-associated diseases, the exploration of herbal medicine could be a source of potential therapeutic candidates primarily due to its inherent safety profile and widespread accessibility.

Pluchea pteropoda, an annual plant in the Asteraceae family, has traditionally been used in Vietnamese folk medicine as an alternative treatment for fever instead of the Chinese herb Saihubei (*Buplerum sinense*). This plant species thrives in the wild in salty coastal areas of the Northern region of Vietnam [6]. In Vietnamese folk medicine, this plant is used in remedies to treat high fever, indigestion, and painful and swollen wounds with a daily dose of 10 grams to 20 grams [6, 7]. While research on *P. pteropoda* is limited, other *Pluchea* species have been extensively studied and have shown remarkable biological effects. For instance, *P. indica* has demonstrated anti-inflammatory, antioxidant,[8] wound healing,[9] and anti-diabetic properties[10]. This species shares various genomic and morphological characteristics with *P. indica* [11, 12]. Another *Pluchea* species, *P. lanceolata*, has exhibited neuroprotective [13], immunosuppressive[14] effects in mice. Therefore, we conducted this study to explore the potential anti-inflammatory

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effects of a native species within the Southeast Asian region. In the present study, we report acute toxicity and anti-inflammatory effects of *P. pteropoda* ethanol extract on animal models.

II. MATERIALS AND METHODS

2.1. Plant materials

Aerial parts of *P. pteropoda* were collected in Cat Hai, Haiphong city, Vietnam in May 2022. The plant was identified by Ms. Nguyen Quynh Nga and Mr. Dang Minh Tu, Natural Materials Resource Center, National Institute of Medicinal Material. A voucher specimen (HPMUSP-2022-25) was deposited at the Herbarium, Centre of Marine Pharmacognosy, Haiphong University of Medicine and Pharmacy, Vietnam.

2.2. Preparation of the ethanol extract (EtPP)

The air-dried aerial parts of *P. pteropoda* (5.0 kg) were milled and subjected to reflux extraction with EtOH 96 (8:1, v:w) twice, for a total duration of 3.5 hours each time. The resulting mixture was then filtered, and the filtrate was collected and concentrated under vacuum to remove the solvent, resulting in a total of 149.7 g of ethanol extract (humidity of 3.44%). This extract was subsequently dried, sealed in a bottle, and stored in a refrigerator at 4°C until further use.

2.3. Bioassay

2.3.1. Experimental animal

Swiss albino female mice weighing between 18 and 22 grams, and Wistar rats weighing between 80 and 100 grams, were procured from the National Institute of Hygiene and Epidemiology, Vietnam. The animals were housed under standard conditions, with ad libitum access to water and a standard pellet diet. The room temperature was maintained at $25 \pm 1^\circ\text{C}$, and

the lighting schedule followed a 12-hour light-dark cycle with lighting from 7:00 to 19:00.

2.3.2. Acute toxicity study

Acute toxicity of EtPP was assessed according to acute oral toxicity – fixed dose procedure, OECD guidelines No. 420, with initial dose of 2000 mg/kg. Mice were stably raised for 5 days before the experiment. Before giving mice the test sample, let them fast overnight and drink water freely as needed. Test samples were prepared by diluting EtPP in distilled water to appropriate doses equivalently 1 mL/100 g of body weight. After the test sample has been administered using a gastric feeding tube in a single dose, food was withheld for a further 1.5 hours. Animals are observed individually after dosing every 30 mins during the first 4 h, and daily until the 15th day for changes in behaviors, skin, fur, eyes, respiration, movement, excretion.

2.3.3. Carrageenan-induced hind paw edema assay

Wistar rats weighing 80 – 100 g was stably raised for 5 days before the experiment. White rats were randomly divided into four groups (10 rats each). Before orally administered, rats were fast overnight and drink water freely as needed. The control group and the reference group received normal saline (0.9% NaCl, 2 mL/kg) and diclofenac (7 mg/kg), respectively while the test groups were treated with 77.5 and 155 mg/kg body weight of extract. After 30 mins, acute inflammation condition was induced by subcutaneous injection carrageenin solution 1% in isotonic saline to right hind paw soles with a volume of 0.1 ml each paw. Immediately, give rats 2 ml/100g of water to drink. After 1 hours, 3 hours and 5 hours of inflammation, the paw

volume was measured by a plethysmometer (Ugo Basile, model 37140, Italy) [15].

The percent of edema inhibition in treated animals versus control no treated was calculated by the following equation:

$$EI (\%) = EV_t - EV_0 (\%)$$

Whereas:

EI (%): The percent of edema inhibition

EV (%): the percent of edema volume increase at t h versus 0 h and caculated by the equation bellowing:

$$EV (\%) = \frac{V_t - V_0}{V_0} \times 100 (\%)$$

V_t: The vollum of paw edema in tested groups at t h versus 0 h

V₀: The vollum of paw edema in tested groups at t h versus 0 h,

2.4. Statistical analysis

Results were expressed as the mean ± SEM. Treated groups were compared with the controls for statistically significant differences using Mann-Whitney U-test with *p* < 0.05.

III. RESULTS AND DISCUSSION

3.1. Acute toxicity study

A mouse was given extract at a dose of 2000 mg/kg body weight. During 15 days of observation, no abnormalities were seen in the mouse's skin, fur, eyes, respiration, behavior, movement, or excretion. Therefore, the dose was increased to 5000 mg/kg in 5 rats.

Table 1. General condition and behavioral changes of the experimental mice for monitoring acute toxicity

Functions	Doses (mg/kg)					
	2000	5000				
	1	1	2	3	4	5
Mobility	+	+	+	+	+	+
Sedation	+	+	+	+	+	+
Respiration	+	+	+	+	+	+
Urination	+	+	+	+	+	+
Defecation	+	+	+	+	+	+
Diarrhea	-	-	-	-	-	-
Skin	+	+	+	+	+	+
Eyes	+	+	+	+	+	+
Fur	+	+	+	+	+	+
Convulsion	-	-	-	-	-	-
Mortality	-	-	-	-	-	-

+ normal;

- not observed

After taking the drug, all mice showed no abnormalities and had normal movement and excretion. Continue monitoring until 15th day, neither mortality, nor tremors nor convulsions were noted as shown in Table 1. Therefore, EtPP has no toxic effects or mortality with single-dose, oral ethanol

administration in rats.

3.2. Carrageenan-induced hind paw edema assay

Carrageenan has been considered as a phlogistic agent with several important advantages compared to egg albumin, kaolin, and formalin. Variability is relatively low

and the linear log of dose - response bioactivities is with a great degree of precision. The initiation of edema induced by carrageenan follows a tri-phasic progression. The initial phase transpires within 0 to 1.5 hours post carrageenan, caused by the liberation of histamine and serotonin. The

subsequent phase, occurring between 1.5 and 2.5 hours, is orchestrated by bradykinin. Finally, the third phase, spanning from 2.5 to 6 hours post-injection, has been associated with an augmented synthesis of prostaglandins [15].

Table 2. Effects of EtPP on carrageenan-induced hind paw edema in rats

Groups (n=10)	Time after 1% carrageenan injection					
	1h		3h		5h	
	EV (%)	%EI	%EV	%EI	%EV	%EI
Control	60.5±6.3		63.2±7.6		56.5±7.1	
EtPP 77.5 mg/kg	54.3±7.0	10.3	41.0±5.3*	35.2	37.2±5.6*	34.2
EtPP 155.0 mg/kg	42.5±5.5*	29.8	39.6±5.5*	37.4	37.1±4.7*	34.2
Diclofenac ^a 7 mg/kg	15.8±2.5**	73.9	19.6±2.2**	69.0	17.8±2.4**	68.5

%EV: edema volume (mL) of test substance at t h versus t=0,

% EI: percent edema inhibition of test substance compared with control group.

Significantly different from control group: *p<0.05, **p<0.01.

a: positive control.

The ethanol extract of *P. pteropoda* (EtPP) at concentration of 77.5 and 155 mg/kg was given to rats. As results showed in Table 2, EtPP suppressed a significant anti-inflammatory effect at both doses of 77.5 and 155.0 mg/kg body weight. At 1 h post carrageenan, EtPP at doses of 77.5 mg/kg reduced 10.3% paw edema, from 60.5% to 54.3% while 155.0 mg/kg of EtPP reduced 29.8%, from 63.2% to 41.0%. This demonstrated a dose-dependent anti-inflammatory effect. At 3 h and 5 h after inflammation, the effects of EtPP at these two dose levels are equivalent. The highest inflammation inhibition effect was recorded at 3 h at both dose levels with edema inhibition 35.2 and 37.4%. These results indicated that carrageenan-induced rat paw edema was inhibited by oral EtPP at both doses with a mechanism that may involve inflammatory mediators at all three stages of inflammation.

To the best of our knowledge, this is the first report on anti-inflammatory activity of *P. pteropoda*. Previous studies showed that both leaves and root extract of *P. indica* exhibit anti-inflammatory effects. Leaf extract of *P. indica* showed edema inhibition effects at doses ranging from 150 to 600 mg/kg. At dose level of 150 mg/kg body weight, leaf extract of *P. indica* reduced 37.5%, 36% and 23.5% at 1, 3 and 5 hours, respectively [16]. The root extract of this plant showed significant anti-inflammatory effect against a variety of different phlogistic agent, including histamine, serotonin, hyaluronidase, and carrageenan [8]. This result showed the similarity between these two species in anti-inflammatory activity.

IV. CONCLUSION

In conclusion, the present study demonstrated safety and anti-inflammatory effect of *P. pteropoda* aerial parts ethanol

extract. EtPP has no toxic effects or mortality with single-dose, oral ethanol administration in rats and showed significant anti-inflammatory activity in carrageenan-induced rat model. These results support usages of this herbs in folk medicine. However, further studies need to be conducted to confirm its action molecular pathway and find out single ingredients.

REFERENCES

1. **Yang, H., et al.**, Regulation of inflammation in cancer by dietary eicosanoids. *Pharmacology & Therapeutics* (2023), **248**: p. 108455.
2. **Kunz, H.E. and I.R. Lanza**, Age-associated inflammation and implications for skeletal muscle responses to exercise. *Experimental Gerontology* (2023), **177**: p. 112177.
3. **Garodia, P., et al.**, Curcumin, inflammation, and neurological disorders: How are they linked? *Integrative Medicine Research* (2023), **12**(3): p. 100968.
4. **Suren Garg, S., et al.**, Association between obesity, inflammation and insulin resistance: Insights into signaling pathways and therapeutic interventions. *Diabetes Research and Clinical Practice* (2023), **200**: p. 110691.
5. **Sugimoto, M.A., et al.**, Resolution of inflammation: what controls its onset? *Frontier Immunology* (2016), **7**: p. 160.
6. **Do Tat Loi**, *Những cây thuốc và vị thuốc Việt Nam*. 2004, Vietnam: NXB Y học.
7. **Nguyen Viet Than**, *Cây thuốc và những bài thuốc thường dùng*. Vol. 3. 2020, Vietnam: Health Medicine.
8. **Sen, T. and A.K. Nag Chaudhuri**, Antiinflammatory evaluation of a *Pluchea indica* root extract. *J Ethnopharmacol* (1991), **33**(1-2): p. 135-41.
9. **Chiangnoon, R., et al.**, Phytochemical Analysis, Antioxidant, and Wound Healing Activity of *Pluchea indica* L. (Less) Branch Extract Nanoparticles. *Molecules* (2022), **27**(3).
10. **Nopparat, J., A. Nualla-Ong, and A. Phongdara**, Ethanolic extracts of *Pluchea indica* (L.) leaf pretreatment attenuates cytokine-induced β -cell apoptosis in multiple low-dose streptozotocin-induced diabetic mice. *PLoS One* (2019), **14**(2): p. e0212133.
11. **Phùng Thị Hằng, Nguyễn Nguyễn Trân, Phan Thành Đạt, Nguyễn Phúc Đảm, Nguyễn Đức Độ and Nguyễn Trọng Hồng Phúc**, Khảo sát đặc điểm hình thái, giải phẫu và hoạt tính kháng khuẩn của Cúc tần (*Pluchea indica* (L.) Less.) và Nam sài hồ (*Pluchea pteropoda* Helms.). *Tạp chí Khoa học Trường Đại học Cần Thơ* (2022), **58**(2A): p. 132-139.
12. **Liang, Z., et al.**, The complete chloroplast genome of *Pluchea pteropoda* Hemsl, a mangrove associate plant. *Mitochondrial DNA B Resour* (2021), **6**(6): p. 1729-1731.
13. **Mundugaru, R., et al.**, Protective Effect of *Pluchea lanceolata* against Aluminum Chloride-induced Neurotoxicity in Swiss Albino Mice. *Pharmacogn Mag* (2017), **13**(Suppl 3): p. S567-s572.
14. **Bhagwat, D.P., et al.**, Immunosuppressive properties of *Pluchea lanceolata* leaves. *Indian J Pharmacol* (2010), **42**(1): p. 21-6.
15. **Winter, C.A., E.A. Risley, and G.W. Nuss**, Carrageenin-induced edema in hind paw of the rat as an assay for antiinflammatory drugs. *Proc Soc Exp Biol Med* (1962), **111**: p. 544-7.
16. **Buapool, D., et al.**, Molecular mechanism of anti-inflammatory activity of *Pluchea indica* leaves in macrophages RAW 264.7 and its action in animal models of inflammation. *J Ethnopharmacol* (2013), **146**(2): p. 495-504.