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ANTI-INFLAMMATORY EFFECTS OF THE BARK OF *PHELLODENDRON AMURENSE* RUPR. IN EXPERIMENTAL MODELS

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Summary

The objective of this study was to investigate the anti-inflammatory properties of *n*-hexane, ethyl acetate (EtOAc), dichloromethane (CH₂Cl₂), and aqueous extracts of *Phellodendron amurense*. The activity was evaluated *in vitro* by nitric oxide (NO) and prostaglandin E2 (PGE2) inhibition assays, and *in vivo* using carrageenan-induced paw edema and lipopolysaccharide (LPS)-induced endotoxemia models in rats. *In vitro* anti-inflammatory activity was assessed by measuring the inhibition of NO and PGE2 production using RAW 264.7 macrophage cells. Among the tested extracts, the CH₂Cl₂ extract exhibited the most potent activity, with IC₅₀ values of 10.88 ± 2.88 µg/mL for NO inhibition and 10.82 ± 1.22 µg/mL for PGE2 inhibition, followed by the aqueous extract, which also inhibited NO and PGE2 production without causing cytotoxicity. The CH₂Cl₂ extract of *P. amurense* (CPA) was orally administered to rats in both LPS-induced endotoxemia and carrageenan-induced hind paw edema models. In the endotoxemia model, CPA significantly improved survival and markedly reduced serum IL-6 and IL-1β levels, as well as white blood cell counts. Consistently, in the carrageenan-induced paw edema model, CPA significantly suppressed edema formation, reducing paw-volume increase by 36%, 48%, and 55% at 2, 3, and 4 h, respectively, compared with the carrageenan control group (*p* < 0.05).

Keywords: *Phellodendron amurense* Rupr.; Anti-inflammatory; Carrageenan-induced paw edema; Lipopolysaccharide-induced endotoxemia; *in vitro*; *in vivo*.

1. Background

Inflammation is a hallmark of many acute and chronic debilitating disorders and is a major cause of morbidity in today's era of modern lifestyle. If unchecked, inflammation leads to the development of rheumatoid arthritis, diabetes, cancer, Alzheimer's disease, and atherosclerosis, along with pulmonary, autoimmune, and cardiovascular diseases. Inflammation involves a complex network of many mediators, a variety of cells, and the execution of multiple pathways. Current therapy for inflammatory disorders relies mainly on steroidal and non-steroidal anti-inflammatory agents (NSAIDs). Chronic use of these drugs can produce serious adverse effects such as gastrointestinal, cardiovascular, and renal complications. There is an urgent need for new anti-inflammatory agents with greater selectivity and lower toxicity. Plants and isolated phytochemicals represent promising sources of novel anti-inflammatory compounds [1],[2]. Although several studies have reported the anti-inflammatory activity of *Phellodendron amurense* and its major alkaloids, such as berberine, particularly in relation to the regulation of inflammatory mediators including nitric oxide (NO), prostaglandin E2 (PGE2), and pro-inflammatory cytokines (e.g., IL-6 and IL-1β), these findings have largely been obtained

from isolated compounds or crude extracts and in non-Vietnamese contexts. To date, limited information is available regarding the effects of solvent-partitioned bark extracts of *P. amurense* on these established inflammatory markers, especially in integrated *in vitro* and *in vivo* models. Therefore, the present study aimed to investigate whether bark extracts of *P. amurense* modulate inflammatory responses by regulating well-characterized inflammatory markers and signaling pathways *in vivo*, and to further elucidate their molecular effects on inflammatory cytokines and mediators in both *in vitro* and *in vivo* experimental systems [3],[4].

2. Materials and Methods 2.1. Plant material and extraction

The bark of *P. amurense* Rupr. was collected in Sapa, Vietnam, in 2023. The plant material was identified by MSc Nguyen Thi Quynh Nga and Dang Minh Tu (National Institute of Medicinal Materials, NIMM). A voucher specimen was deposited at the National Institute of Medicinal Materials. The contents of the characteristic alkaloids (phellodendrine, palmatine chloride, and berberine chloride) in the bark of *P. amurense* were determined using a previously developed and validated HPLC–DAD method [5]. The contents were 0.70 ± 0.01%, 0.11 ± 0.01%, and 7.93 ± 0.02%, respectively.

Extraction: The dried bark of *P. amurense* (10 kg) was ground into powder and extracted by maceration with 70% ethanol (3 times $\times$ 3 days, 1:1.5 kg/L). The combined extracts were filtered to remove plant material and concentrated under reduced pressure to remove the solvent, yielding 1082.8 g of crude extract. The crude extract (1 kg) was suspended in hot water (1:1, w/v) and then successively partitioned with solvents of increasing polarity (*n*-hexane, dichloromethane, and ethyl acetate), each performed three times at a ratio of 1:1 (v/v). The combined solvent extracts were concentrated under reduced pressure to give 68.35 g of the *n*-hexane extract, 30.94 g of the dichloromethane extract, 7.18 g of the ethyl acetate extract, and a remaining aqueous extract (121.48 g).

2.2. Chemicals

For anti-inflammatory activity, the optical density (OD) values were measured using the BioTek Elx800 spectrophotometer. Dulbecco's Modified Eagle's Medium (DMEM) and fetal bovine serum (FBS) were sourced from Life Technologies, Inc. (Gaithersburg, MD, USA). Lipopolysaccharides (LPS), sodium nitrite, sulfanilamide, *N*-1-naphthylenediamine dihydrochloride, dimethyl sulfoxide (DMSO), and dexamethasone were obtained from Sigma Chemical Co. (St. Louis, MO, USA). The RAW 264.7 cells were obtained from the University of Perugia, Italy. Aspirin (acetylsalicylic acid) was obtained from Sigma Chemical Co. (St. Louis, MO, USA).

2.3. Anti-inflammatory activity *in vitro*

2.3.1. *In vitro* anti-inflammatory activity of barks of *P. amurense* by NO inhibition assay [6]:

Griess reagent was used to measure the amount of NO production with minor adjustments. The RAW 264.7 cells were cultured for 24 hours at 37°C and 5% CO₂ at a density of 2×10^5 cells/well using DMEM. Subsequently, different doses of extracts (2.65–100 µg/mL) were applied to the cells when LPS (1 µg/mL) was added to induce inflammation. The untreated cells were used as the blank. Cells exposed to LPS with 0.1% DMSO but no extract served as the negative (vehicle) control. Nitrite accumulation was quantified after 24 h of treatment. Next, 100 µL of the supernatant was moved to a different 96-well culture plate, combined with 100 µL of Griess reagent (0.1% (w/v) naphthyl ethylenediamine dihydrochloride and 1% (w/v) sulfanilamide in 5% (v/v)

phosphoric acid). After incubation for 15 min at room temperature, OD was measured at 540 nm. The IC₅₀ value (50% of NO inhibition) was determined using the Table-Curve 2Dv4 software. The MTT assay was used to assess the cytotoxic activity. The formazan crystals were dissolved in DMSO for 60 minutes at 37°C. The quantity of formazan was measured at 590 nm. Dexamethasone (0.8 to 100 µM) was used as a positive control.

2.3.2. *In vitro* anti-inflammatory activity of barks of *P. amurense* by PGE2 inhibition assay [7]:

Step 1: The RAW 264.7 cells in DMEM were seeded into 96-well plates at a density of 1×10^5 cells/well and incubated at 37°C, 5% CO₂, for 24 hr. Subsequently, the old medium was replaced with fresh medium containing 2 µg/mL of LPS; then, the test samples were added and incubated at 37°C, 5% CO₂, for 24 h.

Step 2: PGE2 production by enzyme-linked immunosorbent assay (ELISA) was determined by transferring 50 µL of supernatant into a goat anti-mouse IgG-coated plate. After that, 50 µL each of PGE2 AChE tracer and PGE2 monoclonal antibody were added to each well and incubated at 4°C for 18 hr. The plate was washed with wash buffer; then Ellman's reagent was added and incubated at 37°C for 60–90 minutes. The OD was measured at 412 nm. The 50% inhibitory concentration (IC₅₀) was calculated. All data were expressed as mean $\pm$ standard error of the mean (SEM).

2.4. Anti-inflammatory activity *in vivo*

2.4.1. Carrageenan-Induced Paw Edema in Rats [8]:

Animals included in this study were adult male albino *Wistar* rats (200–220 g), obtained from Vietnam Military Medical University, Vietnam. They were maintained under a controlled environment and had free access to water and food. Forty rats were divided into four groups (n = 10). From day 1 to day 5 of the study, each group was orally administered aspirin (300 mg/kg), and the best active extract was selected based on *in vitro* results at different doses (150 and 300 mg/kg) as the control, reference, and test group, respectively. On the fifth day, rats were administered one hour prior to carrageenan injection. A 0.05 mL solution of 1% carrageenan in sterile normal saline solution (NSS) was injected intradermally into the plantar side of the right hind paw of the rats in all groups. The edema volumes were determined using a

plethysmometer (model 7140, Ugo Basile, Italy) before, 1, 2, 3, and 4 h after carrageenan injection. The percentage increase in paw volume throughout

4 h was determined by subtracting the paw zero-time volume from its volume at the specified time interval and then multiplying by 100.

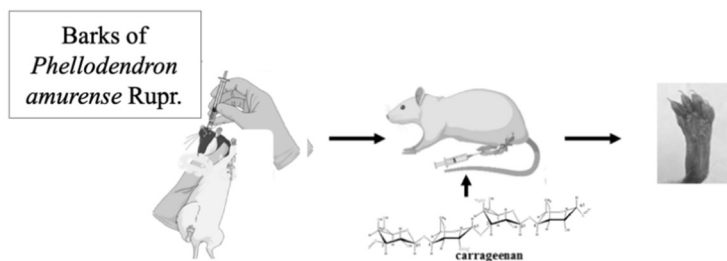


Fig. 1. Schematic representation and timeline of the carrageenan-induced paw edema assay for evaluating the acute anti-inflammatory effects of *P. amurense* extracts in rats

2.4.2. LPS-induced models [9]:

Animals included in this study were adult male albino *Wistar* rats (200–220 g) obtained from Vietnam Military Medical University, Vietnam. Animals were housed in an air-conditioned room at 22–24 °C with a 12 h dark/light cycle and were allowed food and water ad libitum. Rats were assigned to one of the following four groups, n = 10/group: (1) NOR, normal control rats treated with vehicle; (2) LPS, treated with LPS 35 mg/kg; (3) LPS + the best active extract screened *in vitro*, treated with LPS and 150 mg/kg extract; (4) LPS + the best active extract screened *in vitro*, treated with LPS and 300 mg/kg extract. After a one-week adaptation period, the LPS + 150 mg/kg and 300 mg/kg extract groups were orally administered 150

mg/kg and 300 mg/kg extract in distilled water (DW) for three consecutive days, while the NOR and LPS groups received an equivalent volume of DW as the control. On the third day, 2 h after extract administration, the LPS and LPS + 150 mg/kg and 300 mg/kg extract groups were intraperitoneally injected with LPS (*Escherichia coli* 0111:B4; BD Bioscience, Sigma, St. Louis, MO, USA) dissolved in DW to induce endotoxemia, while the NOR group received the vehicle control. Animals in each group were sacrificed under anesthesia 6 h after the LPS challenge, and their blood was collected for analysis (Table 1). Serum was separated and stored at –80°C for measurement of protein levels, cytokine expression, and white blood cell counts.

Table 1. Animal treatment and experimental group in the LPS-induced model

Groups	Day 1	Day 2	Day 3	LPS challenge	Sacrifice
NOR	Vehicle administration	Vehicle administration	Vehicle administration	0,9% NaCl (i.p) -2h after vehicle	6h after LPS challenge
LPS				LPS (i.p) -2h after vehicle	6h after LPS challenge
LPS + extract 150 mg/kg	Extract 150 mg/E g	Extract 150 mg/kg	Extract 150 mg/kg	LPS (i.p) -2h after extract 150 mg/kg	6h after LPS challenge
LPS + extract 300 mg/kg	Extract 300 mg/kg	Extract 300 mg/kg	Extract 300 mg/kg	LPS (i.p) -2h after extract 300 mg/kg	6h after LPS challenge

2.5. Statistical analysis

Data represented as Mean ± SD using Graphpad Prism 9 (USA). Statistical analysis was carried out with the one-way ANOVA test in Graphpad Prism software. $p < 0.05$ is considered statistically significant.

3. Results

3.1. Anti-inflammatory activity

A Griess assay was used to confirm the effect of the extracts on NO generation in LPS-induced RAW 264.7 cells. With IC₅₀ values of 10.88 ± 2.88 and 11.79 ± 3.20 µg/mL against

NO production, the CH₂Cl₂ and aqueous extracts showed good inhibitory efficacy among the extracts. These extracts did not exhibit any cytotoxic effects on RAW 264.7 cells, with a cell survival rate of above 80% (Table 2).

Table 2. IC₅₀ values for the inhibition of NO production of the extracts

Samples	IC ₅₀ (μg/mL)	% Cell viability at 100 μg/mL
<i>n</i> -Hexane	>100	87.52 ± 2.14
EtOAc	53.12 ± 2.05	80.96 ± 1.98
CH ₂ Cl ₂	10.88 ± 2.88	97.56 ± 2.06
Aqueous	11.79 ± 3.20	98.04 ± 4.08
Dexamethasone*	5.82 ± 0.10	95.41 ± 1.2

Data were presented as the mean ± SD (n=3); *Positive control (μM); Extracts (μg/mL).

Table 3. Percentage of inhibition and IC₅₀ values of the extracts on PGE2 production

Samples	IC ₅₀ (μg/mL)	% Cell viability at 100 μg/mL
<i>n</i> -Hexane	97.96 ± 2.44	83.35 ± 6.11
EtOAc	>100	88.25 ± 2.11
CH ₂ Cl ₂	10.82 ± 1.22	96.99 ± 5.21
Aqueous	20.11 ± 3.12	93.92 ± 0.19
Dexamethasone*	0.83 ± 0.26	81.06 ± 6.28

Data were presented as the mean ± SD (n=3); *Positive control (μM); Extracts (μg/mL).

Table 3 indicates the effect of the extracts on PGE2 production in LPS-induced RAW 264.7 cells. With IC₅₀ values of 10.82 ± 1.22 and 20.11 ± 3.12 μg/mL against PGE2 production, the CH₂Cl₂ and aqueous extracts showed good inhibitory efficacy among the extracts. These extracts did not exhibit any cytotoxic effects on RAW 264.7 cells, with a cell survival rate of above 80%. Based on the *in vitro* results, the CH₂Cl₂ extract was selected for subsequent *in vivo* studies.

3.2. *In vivo* anti-inflammatory effect

Carrageenan administration significantly increased the percentage increase in right hind paw volume at 1, 2, 3, and 4 h compared with the control group ($p \leq 0.01$). Pretreatment with the CH₂Cl₂ extract significantly attenuated carrageenan-induced paw edema at 2, 3, and 4 h compared with the carrageenan group ($p \leq 0.05$). Similarly, aspirin pretreatment significantly reduced paw edema at 2 and 3 h after carrageenan injection ($p \leq 0.001$). No significant differences were observed between the aspirin-treated and extract-treated groups (Fig. 2).

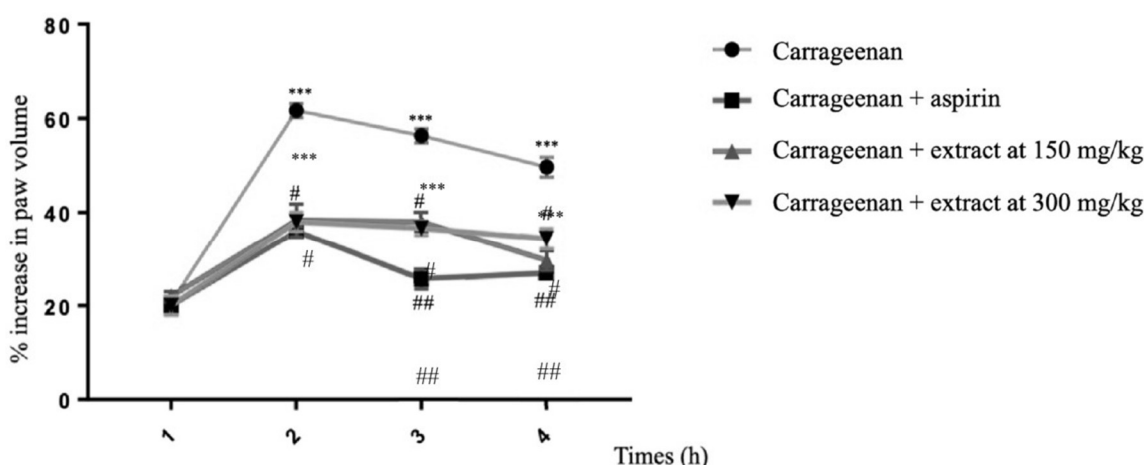


Fig. 2. Effect of the CH₂Cl₂ extract and aspirin on the % increase in paw volume measured over the period after carrageenan injection
Data are presented as Mean ± SD (n=10); *** $p \leq 0.001$ compared to control; # $p \leq 0.05$; ## $p < 0.01$ compared to carrageenan

To investigate the therapeutic effect of the CH₂Cl₂ extract on endotoxemia, we examined the survival rate of rats with LPS-induced sepsis. As shown in Fig. 3, 20% of rats

survived endotoxin shock during the 72 h after LPS injection, whereas treatment with the extract in two doses increased the survival rate to 60%.

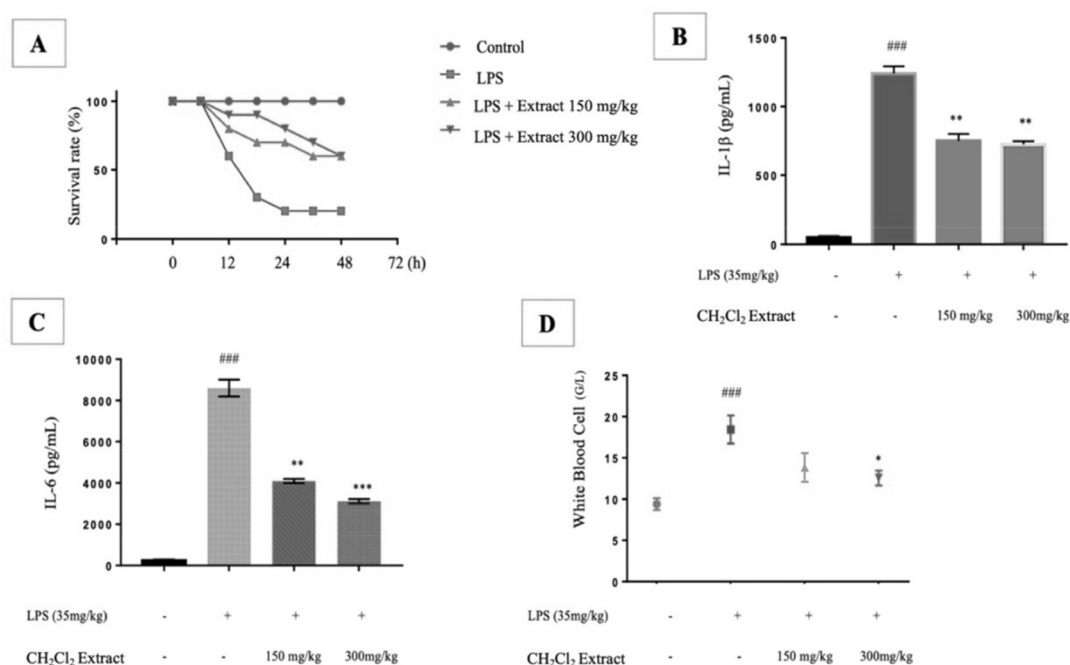


Fig. 3. Effects of the CH₂Cl₂ extract on an LPS-induced endotoxemia model in rats (A) Survival rate after LPS challenge; (B–C) Serum cytokine levels (IL-1β and IL-6); (D) Peripheral white blood cell (WBC) count. Each column represents the mean ± SD, ### *p* < 0.001 vs. NOR group; * *p* < 0.05; ** *p* < 0.01; *** *p* < 0.001 vs LPS group

To determine the effects of the CH₂Cl₂ extract on macrophage-mediated immune responses, rats were treated with or without the CH₂Cl₂ extract, and serum cytokine levels were analyzed by ELISA. The levels of IL-1β and IL-6 in serum were elevated in LPS-treated rats compared to the normal group, whereas pretreatment with the extract resulted in significant down-regulation of IL-1β and IL-6. Furthermore, white blood cell counts in the extract-treated groups decreased significantly compared to Group 2 (LPS 35 mg/kg only).

4. Discussion

Inflammation is a complex biological response involving the coordinated activation of immune cells and the release of pro-inflammatory mediators, including NO, PGE₂, and cytokines such as TNF-α, IL-1β, and IL-6. Dysregulation of these mediators plays a critical role in the pathogenesis of both acute and systemic inflammatory conditions. In the present study, we demonstrated that extracts derived from *P. amurense* bark exert significant anti-

inflammatory effects through modulation of key inflammatory mediators and signaling pathways in both *in vitro* and *in vivo* models.

LPS-stimulated RAW 264.7 macrophages were usually used to assess the anti-inflammatory effect of phytoconstituents. The stimulation of such cells with LPS produces proinflammatory cytokines and chemokines. The cells are either directly pre-treated with the supernatant of extracts (after centrifugation) or with pure phytochemicals, followed by LPS stimulation [10]. The severity of inflammation is assessed through the manifestation of pro-inflammatory mediators like TNF-α, IL-1β, NO, and cyclooxygenase (COX-1 and COX-2) in cell supernatants [11]. The enzyme immunoassay (EIA) kits can be used to quantify mediators like PGE₂, interleukins, prostaglandins, and TNF-α [11]. The cytokine levels can be measured *in vitro* using sandwich ELISA kits [12]. NO produced by activated macrophages plays a critical role in inflammatory diseases as a potent mediator of cellular damage in a wide range of

pathological conditions. Overproduction of NO is mediated primarily by iNOS, which can be up-regulated in macrophages [13]. Thus, NO may regulate most stages of the development of inflammation, particularly the early stages of inflammatory cell transmigration to sites of inflammation [14]. In our experiments, the CH₂Cl₂ extract of *P. amurensis* (CPA) exhibited an inhibitory effect on the production of NO in LPS-stimulated macrophage RAW 264.7 cells. The results showed that in *in vitro* experiments, the *n*-hexane and EtOAc extracts tested did not have PGE₂ production inhibition activity. The CH₂Cl₂ extract was the most active extract with IC₅₀ values of 10.88 ± 2.88 and 10.82 ± 1.22. µg/mL for NO and PGE₂ inhibition, respectively. These results support the use of the bark of *P. amurensis* as an anti-inflammatory drug in traditional medicine. These results were consistent with the anti-inflammatory *in vitro* effect, indicating that CPA had the strongest anti-inflammatory effect by suppressing the LPS-induced production of NO and PGE₂. Thereby, CPA has the most potential for evaluation in *in vivo* models. The *in vivo* anti-inflammatory activity of CPA was confirmed by the carrageenan-induced paw edema model. Several inflammatory mediators, for example, histamine, serotonin, kinins, PGs, complement, and pro-inflammatory cytokines, play a major role in paw edema caused by carrageenan [15],[16]. The initial phase is caused by the release of histamine and serotonin, followed by the release of bradykinin during 1–2 h after carrageenan injection [17]. The release of PGs is closely associated with leukocyte migration to the inflamed site. The presence of PGs, particularly PGE₂, in the inflammatory exudates from the injected foot can be demonstrated at 3 hours and thereafter [18]. Oral administration of CPA at two doses could reduce the swelling of carrageenan-induced paw edema with significant differences from control at 1, 2, 3, and 4 h, and was not different from the standard drug, aspirin. Oral administration of CPA at a concentration of two doses showed the anti-inflammatory activity by inhibiting the acute phase of inflammation. The main mechanism of action may be due to inhibition of PGs synthesis. Moreover, the inhibitory effect of the extract may partly involve other acute inflammatory mediators such as histamine, serotonin, bradykinin, and proinflammatory cytokines, which are released during the 1st hr after carrageenan injection.

Using an LPS-induced systemic inflammation model in rats, we demonstrated that the bark of *P. amurensis* has anti-inflammatory activity via the regulation of pro- and anti-inflammatory cytokines. Treatment with barks of *P. amurensis* rescued rats from lethal sepsis and protected them against LPS-induced endotoxemia; most LPS-treated rats died within 48 h after LPS injection and treatment with the bark extract of *P. amurensis* improved the survival rate. In response to LPS, macrophages readily respond to the release of pro-inflammatory cytokines and chemokines such as IL-6 and IL-1β [19]. IL-6 and IL-1β play critical roles in macrophage activation and are associated with acute and chronic inflammation [20],[21]. In this study, CPA inhibited IL-6 and IL-1β. These results indicate that the bark of *P. amurensis* suppresses the inflammatory response via inhibition of pro-inflammatory cytokines. *P. amurensis* and its components contain many alkaloids (e.g., berberine, palmatine, phellodendrine) known as anti-inflammatory agents [11],[22],[23],[24]. The results we obtained are similar to those reported worldwide regarding the anti-inflammatory effects of the bark of the *P. amurensis* tree and its components [11],[22],[23],[24].

5. Conclusions

This study demonstrated that the anti-inflammatory effects of *P. amurensis* bark are closely associated with the inhibition of key inflammatory mediators, including NO, PGE₂, and pro-inflammatory cytokines such as IL-6 and IL-1β. Initial *in vitro* screening in LPS-stimulated RAW 264.7 macrophages identified the CH₂Cl₂ extract as the most potent extract, showing strong inhibition of NO and PGE₂ production with low IC₅₀ values and no significant cytotoxicity.

Based on these *in vitro* findings, the CH₂Cl₂ extract was selected for subsequent *in vivo* evaluation, where it effectively attenuated carrageenan-induced paw edema and improved outcomes in an LPS-induced endotoxemia model. Collectively, these results highlight the CH₂Cl₂ extract of *P. amurensis* bark as a promising source of anti-inflammatory agents and support further investigation into its active constituents and underlying molecular mechanisms.

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References

1. Brown K. L., Cosseau C., Gardy J. L., Hancock R. E. W. (2007), Complexities of targeting innate immunity to treat infection, *TRENDS in Immunology*, 28(6), 260-266.
2. Choi Y. Y., Kim M. H., Hong J., Kim S. H., Yang W. M. (2013), Dried ginger (*Zingiber officinalis*) inhibits inflammation in a lipopolysaccharide - induced mouse model, *Evidence - Based Complementary Alternative Medicine*, 2013(1), 914563.
3. Du P., Zhu S., Lü P. (2001), Antibacterial activity of 20 kinds of Chinese medicinal materials for *Helicobacter pylori* in vitro, *Journal of Chinese Medicinal Materials*, 24(3), 188-189.
4. Mori H., Fuchigami M., Inoue N., Nagai H., Koda A., Nishioka I., Meguro K. (1995), Principle of the bark of *Phellodendron amurense* to suppress the cellular immune response: effect of phellodendrine on cellular and humoral immune responses, *Planta Medica*, 61(01), 45-49.
5. Nguyen T. H. L., Do T. H., Nguyen M. K. (2024), Quality evaluation of *Phellodendron amurense* collected in Vietnam using HPLC-DAD and QAMS methods, *Journal of Medicinal Materials*, 29(6), 347-353.
6. Park Y., Yoo S. A., Kim W. U., Cho C. S., Woo J. M., Yoon C. H. (2016), Anti-inflammatory effects of essential oils extracted from *Chamaecyparis obtusa* on murine models of inflammation and RAW 264.7 cells, *Molecular Medicine Reports*, 13(4), 3335-3341.
7. Chen J. N., De Mejia E. G., Wu J. S. B. (2011), Inhibitory effect of a glycoprotein isolated from golden oyster mushroom (*Pleurotus citrinopileatus*) on the lipopolysaccharide-induced inflammatory reaction in RAW 264.7 macrophage, *Journal of Agricultural and Food Chemistry*, 59(13), 7092-7097.
8. Winter C. A., Risley E. A., Nuss G. W. (1962), Carrageenin-induced edema in hind paw of the rat as an assay for antiinflammatory drugs, *Proceedings of the Society for Experimental Biology and Medicine*, 111(3), 544-547.
9. Butterweck V., Nahrstedt A. (2012), What is the best strategy for preclinical testing of botanicals? A critical perspective, *Planta Medica*, 78(08), 747-754.
10. Eddouks M., Chattopadhyay D., Zeggwagh N. A. (2012), Animal models as tools to investigate antidiabetic and anti-inflammatory plants, *Evidence-based Complementary Alternative Medicine*, 2012(1), 142087.
11. Kim J. H., Huh J. E., Baek Y. H., Lee J. D., Choi D. Y., Park D. S. (2011), Effect of *Phellodendron amurense* in protecting human osteoarthritic cartilage and chondrocytes, *Journal of Ethnopharmacology*, 134(2), 234-242.
12. Liao C. H., Guo S. J., Lin J. Y. (2011), Characterisation of the chemical composition and *in vitro* anti-inflammation assessment of a novel lotus (*Nelumbo nucifera* Gaertn) plumule polysaccharide, *Food Chemistry*, 125(3), 930-935.
13. Xie Q. W., Nathan C. (1994), The high-output nitric oxide pathway: role and regulation, *Journal of Leucocyte Biology*, 56(5), 576-582.
14. Zamora R., Vodovotz Y., Billiar T. (2000), Inducible nitric oxide synthase and inflammatory diseases, *Molecular Medicine*, 6(5), 347-373.
15. Di Rosa M. L., Giroud J. P., A. Willoughby D (1971), Studies of the mediators of the acute inflammatory response induced in rats in different sites by carrageenan and turpentine, *The Journal of Pathology*, 104(1), 15-29.
16. Hirschelmann R., Bekemeier H. (1981), Effects of catalase, peroxidase, superoxide dismutase and 10 scavengers of oxygen radicals in carrageenin edema and in adjuvant arthritis of rats, *Experientia*, 37(12), 1313-1314.
17. Crunkhorn P., Meacock S. C. R. (1971), Mediators of the inflammation induced in the rat paw by carrageenin, *British Journal of Pharmacology*, 42(3), 392-402.
18. Di Rosa M., Sorrentino L. (1968), The mechanism of the inflammatory effect of carrageenin, *European Journal of Pharmacology*, 4(3), 340-342.
19. Kim N. H., Son Y., Jeong S. O., Hur J. M., Bang H. S., Lee K. N., Kim E. C., Chung H. T., Pae H. O. (2010), Tetrahydroabietic acid, a reduced abietic acid, inhibits the production of inflammatory mediators in RAW 264.7 macrophages activated with lipopolysaccharide, *Journal of Clinical Biochemistry and Nutrition*, 46(2), 119-125.
20. Sampaio A. L. F., Dalli J., Brancalione V., D'Acquisto F., Perretti M., Wheatley C. (2013), Biphasic modulation of NOS expression, protein and nitrite products by hydroxocobalamin underlies its protective effect in endotoxemic shock: downstream regulation of COX-2, IL-1 β , TNF- α , IL-6, and HMGB1 expression, *Mediators of Inflammation*, 2013(1), 741804.
21. Xie C., Kang J., Li Z., Schauss A. G., Badger T. M., Nagarajan S., Wu T., Wu X. (2012), The açai flavonoid velutin is a potent anti-inflammatory agent: blockade of LPS-mediated TNF- α and IL-6 production through inhibiting NF- κ B activation and MAPK pathway, *The Journal of Nutritional Biochemistry*, 23(9), 1184-1191.
22. Choi Y. Y., Kim M. H., Han J. M., Hong J., Lee T. H., Kim S. H., Yang W. M. (2014), The anti-inflammatory potential of *Cortex Phellodendron* in vivo and in vitro: down-regulation of NO and iNOS through suppression of NF- κ B and MAPK activation, *International Immunopharmacology*, 19(2), 214-220.
23. Sun Y., Lenon G. B., Yang A. W. H. (2019), *Phellodendri cortex*: a phytochemical, pharmacological, and pharmacokinetic review, *Evidence - Based Complementary Alternative Medicine*, 2019(1), 7621929.
24. Xian Y. F., Mao Q. Q., Ip S. P., Lin Z. X., Che C. T. (2011), Comparison on the anti-inflammatory effect of *Cortex Phellodendri Chinensis* and *Cortex Phellodendri Amurensis* in 12-*O*-tetradecanoyl-phorbol-13-acetate-induced ear edema in mice, *Journal of Ethnopharmacology*, 137(3), 1425-1430.