Original Article

CENTRAL ASIAN JOURNAL of MEDICAL SCIENCES
CAJMS

Cent Asian J Med Sci. 2023 Sept;9(3): 140-146 https://doi.org/10.24079/CAJMS.2023.09.005

The Effect of *Panzeria Alaschanica* Kupr. on Carrageenan-Induced Acute Inflammation in Rats

Hu Bi Si Ha La Tu[1,2], Khaliunaa Tumurbaatar[3,4], Chimedragchaa Chimedtseren[3], Tsend-Ayush Damba[1], Tsogt Bukhbayar[1,5]

[1]International School of Mongolian Traditional Medicine, Mongolian National University of Medical Sciences, Ulaanbaatar, Mongolia;
[2]Ordos Mongolian and Chinese Medicine Hospital, Ordos, China;
[3]Research Center, Institute of Traditional Medicine and Technology, Ulaanbaatar, Mongolia;
[4]Jiangxi University of Traditional Chinese Medicine, Nanchang, China;
[5]Inner Mongolia National University Medical Committee, Inner Mongolia, China.

Submitted: May 14, 2023
Revised: June 11, 2023
Accepted: September 09, 2023

Corresponding Author
Hu Bi Si Ha-La Tu (MD)
Ordos Mongolian and Chinese Medicine Hospital, Ordos 017020, China.

Tel: +86-18686131926
E-mail: 13604777196@163.com
ORCID: https://orcid.org/0009-0003-4958-7623

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Objectives: In the present study, we aimed to investigate the effect of *Panzeria alaschanica* Kupr. (*P. alaschanica*) on carrageenan-induced acute inflammation in rats. **Method:** Carrageenan-induced rat paw edema was used to evaluate the anti-inflammatory activity. *P. alaschanica* extract (32, 64, and 160 mg/kg/ BW) or vehicle was given orally 60 minutes before the subplantar injection of carrageenan. Ibuprofen (100 mg/kg) was used as a standard drug. The carrageenan-injected paw was measured 30, 60, 120, 180, and 240 minutes after the carrageenan injection. The levels of serum tumor necrosis factor (TNF)-α, interleukin (IL)-ELISA measured 1β, and IL-6. **Results:** The *P. alaschanica* at all given doses significantly ($p<0.01$) inhibited carrageenan-induced rat paw edema. Moreover, it significantly reduced TNF-α, IL-1β, and IL-6 serum levels at different doses ($p<0.01$). The anti-inflammatory effect of *P. alaschanica* was comparable to ibuprofen. **Conclusion:** *P. alaschanica* has an anti-inflammatory impact on carrageenan-induced paw edema in rats. The mechanism of action may partly be via reducing the levels of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6.

Keywords: *Panzeria Alaschanica* Kupr., Carrageenan, Inflammation, Cytokine

Introduction

World Health Organization (WHO) reported that about 70-80% of the world's population relies on nonconventional medicine, mainly from herbal sources, in their primary healthcare, and its demand is increasing daily in developing countries [1, 2]. In 2022, 170 of the 194 WHO Member States have reported using traditional medicine [3].

The subcutaneous injection of either carrageenan or complete Freund's adjuvant produces local inflammation, designated by the 5 cardinal signs: hypersensitivity, redness, swelling, heat, and loss of function [4, 5].

Inflammation is the immune system's response to harmful stimuli, such as pathogens, damaged cells, toxic compounds, or irradiation [6], and acts by removing harmful stimuli and

initiating the healing process. Inflammation is, therefore, a defense mechanism that is vital to health [7, 8].

During inflammation, inflammatory cells, including neutrophils and macrophages, are activated. Activated macrophages stimulate the expression of a series of genes involved in host defense, which results in the release of different inflammatory mediators, pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6 and the pro-inflammatory enzymes including inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX)-2 [9].

Panzeria alaschanica Kupr. (*P. alaschanica*) has been used in traditional medicine as a remedy for postpartum abdominal pain, irregular menstruation, and dysmenorrhea due to its effects on menstruation and blood circulation. *P. alaschanica* is a perennial herbaceous plant of the family Labiate and is distributed primarily in the Ordos and Alasha of Inner Mongolia, China [10, 11]. *P. alaschanica* is one of the best-known traditional herbal medicines frequently used to treat pelvic inflammation [12, 13].

It is widely used in Mongolia as a substitute for *Leonurus artemisia*, which is used in the treatment of irregular menstruation, dysmenorrhea, amenorrhea, endless lochia, and acute nephritis edema. However, there are few reported pharmacological studies [14] to support the therapeutic and medicinal effects of *P. Alaschanica*, and the mechanism of its beneficial effects has yet to be demonstrated. Previous phytochemical studies have revealed that *P. alaschanica* contains two new flavonoids, glycosides and phenylethanoids [15-16].

In this study, we investigated the effects of *P. alaschanica* as an anti-inflammatory candidate to inhibit paw edema formation in carrageenan-induced rat models of acute inflammation. In addition, this study seeks to determine the possible effects on the levels of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6.

Materials and Methods

Chemicals

Carrageenan was purchased from Sigma Aldrich Co. (USA). Enzyme-linked immune sorbent assay (ELISA) kits were purchased from MLBio Co. (China).

Collection of *P. alaschanica*

P. alaschanica was collected from Ordos and Alasha of Inner Mongolia. The herb was identified in May 2018 by the plant taxonomist of the pharmaceutical industry of the Mongolian Pharmaceutical Hospital Committee of Ordos City, Inner Mongolia, China. The herb was dried, pulverized, and stored in the dark at room temperature.

Preparation of *P. alaschanica*

An extract of *P. alaschanica* was prepared by mixing 10 g of the root powder in 100 ml 40% ethanol, creating a 1:10 liquid extract. The liquid was evaporated using a vacuum evaporator.

Animals

Male adult Wistar rats (12-14 weeks, 150-200 g) were provided from the Animal House of the Research Center, Institute of Traditional Medicine and Technology of Mongolia. Rats were housed in a standard cage with a 12-hour light/dark cycle, and temperature was maintained at $20^0C \pm 2$. Rats were given free access to food and water.

The National Institute of Health Guide conducted the animal study for the Care and Use of Laboratory Animals (NIH Publications No. 80-23), revised in 1996. Formal approval to conduct the experiments was obtained from the Ethical Committee of the Mongolian National University of Medical Sciences (Protocol No.2018/3-18). All efforts were made to minimize the number of animals used and their suffering.

Experimental Design

The experiment was conducted at the Research Center of the Institute of Traditional Medicine and Technology of Mongolia.

60 rats were divided into the following groups: 1. Normal (oral administration of saline+subplantar injection of saline), 2. Control (oral administration of saline+subplantar injection of carrageenan), 3. *P. alaschanica* 32 mg/kg (oral administration of *P. alaschanica* 32 mg/kg+subplantar injection of carrageenan), 4. *P. alaschanica* 64 mg/kg (oral administration of *P. alaschanica* 64 mg/kg+subplantar injection of carrageenan), and 5. *P. alaschanica* 160 mg/kg (oral administration of *P. alaschanica* 160 mg/kg+subplantar injection of carrageenan), 6. Ibuprofen group (oral administration ibuprofen+subplantar injection of carrageenan).

The paw swelling was measured before and 30, 60, 120, 180, and 240 minutes after the carrageenan injection using a pletismometer (Ugo Basile Co., Italy, 2002).

Carrageenan-induced acute inflammation

Paw edema was induced with carrageenan, as described

previously [17]. Briefly, rats were inflamed by subplantar injection of 0.1 ml of 1% carrageenan into the right hind paw. Saline (0.9%, 10 ml/kg), *P. alaschanica* extract (32, 64, and 160 mg/kg BW), and ibuprofen (100 mg/kg BW) were given orally one hour before the carrageenan injection.

Blood sampling

Four hours after the carrageenan administration, the animals were anesthetized with ketamine hydrochloride (80-90 mg/kg), and the blood samples were collected by cardiocentesis. Plasma was separated by centrifugation (10 min at 3000 rpm) and kept at -20^{0}C until further analysis.

Enzyme-linked immune sorbent assay (ELISA)

Levels of cytokines in serum samples were determined by enzyme-linked immunosorbent assay (ELISA) following the manufacturer's instructions for using kits selective for rat TNF-α, IL-1β, and IL-6 (ChroMate-4300, Awareness Technology Co., USA).

Statistical Analysis

We expressed continuous variables, including carrageenan-induced rat paw edema and the levels of pro-inflammatory cytokines as the mean and standard deviation. The dependent variable was edema size (ml). The edema was measured repeatedly from 0 to 240 minutes. There was a control group and 4 treatment groups; the study groups were unrelated. We evaluated the change in edema size for each group by one-way repeated measures ANOVA. The treatment effect (study groups) was calculated using the "between-subject factor" in the analysis model. The treatment group's differences in changes in edema size were calculated using post hoc tests. Analysis were performed using STATA 17.0 software; $p<0.05$ was considered significant. A one-way ANOVA test in study groups evaluated pro-inflammatory cytokine changes after treatment.

Results

Effect of *P. alaschanica* on carrageenan-induced paw edema

The subplantar administration of carrageenan generated an increase in the paw size of rats due to edema. This paw edema peaked after 120 min of the experiment, thus indicating an acute paw inflammation. The data of the *P. alaschanica*–treated groups (32, 64, and 160 mg/kg/ BW) showed a significant decrease in paw edema size (p=0.018, 0.031, 0.037). Similarly, the data of the ibuprofen (100 mg/kg BW) treated groupshowed a significant decrease in paw edema (p=0.029) (Figure 1).

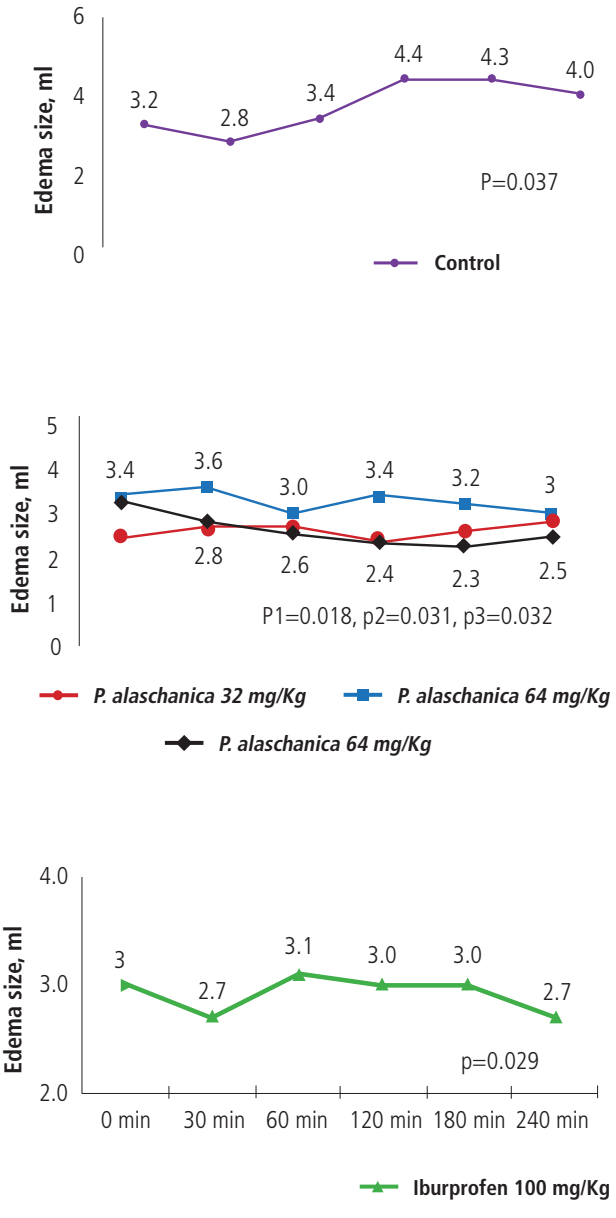

Figure 1. Effects of P. alaschanica oncarrageenan-induced paw edema (ml) by study groups, Repeated measures ANOVA in each group

Edema increased in the control group and decreased with statistically significant differences in the treated groups. We assessed differences in edema across treatment groups using post hoc repeated measures ANOVA (Table 1).

Table 1. The effect of *P. alaschanica* on carrageenan-induced paw edema was evaluated post hoc analysis p- value for least significant difference

Variable (i)	Variable (j)	Mean difference	P-value	Change difference
Control	*P. alaschanica (32 mg/kg)*	1.07	0.0001	Yes
	P. alaschanica (64 mg/kg)	0.42	0.014	Yes
	P. alaschanica (160 mg/kg)	1.03	0.0001	Yes
	Ibuprofen (100 mg/kg)	0.77	0.001	Yes
P. alaschanica (32 mg/kg)	*P. alaschanica (64 mg/kg)*	-0.65	0.012	Yes
	P. alaschanica (160 mg/kg)	-0.03	0.689	No
	Ibuprofen (100 mg/kg)	-0.30	0.068	No
P. alaschanica (64 mg/kg)	*P. alaschanica (160 mg/kg)*	0.62	0.017	Yes
	Ibuprofen (100 mg/kg)	0.35	0.062	No

P-value for least significant difference

Effects of *P. alaschanica* on carrageenan-induced increases in serum levels of TNF-α, IL-1β, and IL-6

We assessed the effects of *P. alaschanica* on carrageenan-induced increases in serum levels of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6.

Serum levels of TNF-α significantly elevated after the carrageenan injection.TNF-α level was significantly different in post-treatment (p=0.0001). Administration of *P. alaschanica* at doses of 64 and 160 mg/kg prevented paw edema with respective percentage inhibition of21.43% and 22.48%, while ibuprofen inhibited the paw edema by 28.% (Figure 2).

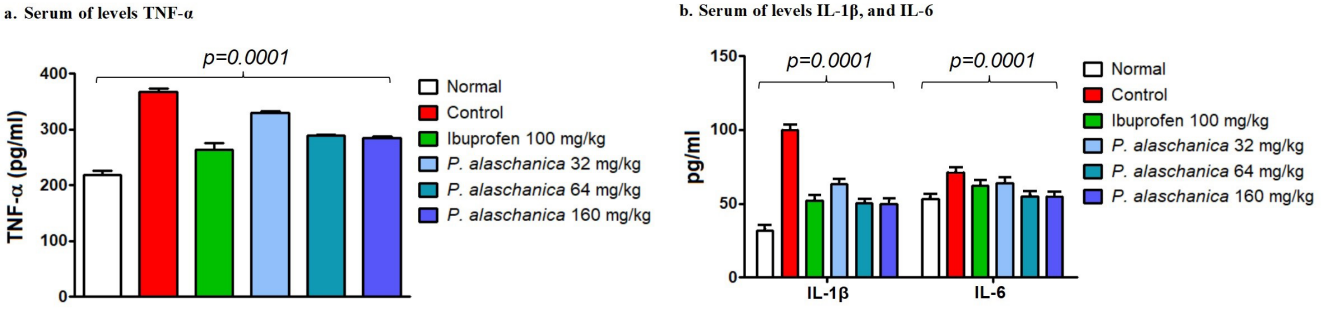

Figure 2. Effects of *P. alaschanica* on carrageenan-induced increases in serum of levels TNF-α, IL-1β, and IL-6

Injection of carrageenan significantly increased the levels of IL-1β and IL-6 (p=0.0001, 0.0001). These TNF-α, IL-1β, and IL-6 levels were similar in the treatment with different doses of *P. alaschanica* compared to the ibuprofen group. However, there were no significant differences in TNF-α, IL-1β, and IL-6 levels between the higher doses of *P. Alaschanica* groups (Table 2).

Table 2. The effect of *P. alaschanica* on carrageenan-induced pro-inflammatory cytokines was evaluated post hoc analysis

Comparison groups	Significance level		
	TNF-α	IL-1β	IL-6
Normal*Control	**	**	**
Normal*Ibuprofen (100 mg/kg)	**	**	**
Normal*P. alaschanica (32 mg/kg)	**	**	**
Normal*P. alaschanica (64 mg/kg)	**	**	**
Normal*P. alaschanica (160 mg/kg)	**	**	**
Control*Ibuprofen (100 mg/kg)	**	**	**
Control*P. alaschanica (32 mg/kg)	**	**	**
Control*P. alaschanica (64 mg/kg)	**	**	**
Control*P. alaschanica (160 mg/kg)	**	**	**
Ibuprofen (100 mg/kg)*P. alaschanica (32 mg/kg)	*	*	*
Ibuprofen (100 mg/kg)*P. alaschanica (64 mg/kg)	*	*	*
Ibuprofen (100 mg/kg)*P. alaschanica (160 mg/kg)	*	*	*
*P. alaschanica (32 mg/kg)*P. alaschanica (64 mg/kg)*	*	*	ns
*P. alaschanica (32 mg/kg)*P. alaschanica (160 mg/kg)*	*	*	ns
*P. alaschanica (64 mg/kg)*P. alaschanica (160 mg/kg)*	ns	ns	ns

* - $p \leq 0.05$ for posthoc test: LSD-least significant difference,

** - $p \leq 0.01$ for posthoc test: LSD-least significant difference,ns - $p > 0.05$

Discussion

The present study evaluated the anti-inflammatory activities of *P. alaschanica* in experimental rodent models.

A common animal model for investigating anti-inflammatory drugs is λ-carrageenan injection into a mouse's foot [18]. These trigger increased permeability of local blood vessels, resulting in foot swelling. This method is often employed to evaluate the efficacy of anti-inflammatory agents and the anti-edema effects of natural products.

The response to the injection of λ-carrageenan-induced paw edema is biphasic, and the injected λ-carrageenan releases various substances with time to induce inflammation. In phase I, within 1 to 2 h of λ-carrageenan injection, histamine, serotonin, and other mediators are released. During phase II, within 3 to 5 h of injection, prostaglandin is released. Between the two phases, substances like kinin are released [19].

At the site of inflammation, cytokines have been studied to have an important role in the formation of edema by inducing vasodilatation and increase of vascular permeability [20]. In particular, recent studies have shown that carrageenan induces peripheral release ofNO as well as PGE2 [21]. In addition, it has been shown that carrageenan also induces the release of TNF-α, which subsequently promotes IL-1 and IL-6 production in the tissues [22].

In this study, we successfully induced paw swelling by subplantar injection of carrageenan into the right hind paw of rats. The significant inhibitory activity shown by *P. alaschanica* over a period of 120-240 min in carrageenan-induced paw edema was comparable to that elicited by ibuprofen.

The ethyl acetate extract (EtOAc) from *P. alaschanica* was found to contain flavonoid compounds. Flavonoids are known to target prostaglandins, which are involved in the late phase of acute inflammation and pain perception [23]. Therefore, the presence of flavonoids and phenylpropanoids in the EtOAc extract of *P. alaschanica* has anti-inflammatory activity, which also supports our study.

The anti-inflammatory effect and high-performance liquid chromatography (HPLC) analysis from the EtOAc extract of the aerial parts of *P. alaschanica* was investigated against inflammation induced by carrageenan, and egg-albumin in rats [24].

The pro-inflammatory cytokines such as TNF-α, IL-1, and IL-6 are small secreted proteins that mediate and regulate immunity

and inflammation. TNF-α is a major mediator in inflammatory responses, inducing innate immune responses by activating T cells and macrophages and by stimulating the secretion of other inflammatory cytokines [25]. IL-1β is another inflammatory cytokine, which is found in the circulation following Gram-negative sepsis, and IL-6 is also an inflammatory cytokine mainly synthesized by macrophages and plays a role in the acute phase response [26, 27].

In the present study, *P. alaschanica* at doses of 32, 64, and 160 mg/kg BW significantly decreased carrageenan-induced increases in serum levels of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6.

In conclusion, our study has shown that *P. alaschanica* has an anti-inflammatory effect in carrageenan-induced paw edema of rats, which is quite comparable to that of the standard drug, ibuprofen. The mechanism of action may partly be via reducing the levels of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6. Therefore, to overcome the model's limitations, further investigations are needed to clarify various models of inflammatory disorders and levels of pro-inflammatory cytokines studies as well.

Conflict of Interest

The authors state no conflict of interest.

Acknowledgements

I would like to express my sincere gratitude to the Research Center, Institute of Traditional Medicine and Technology, Ulaanbaatar, Mongolia for providing me with the laboratory to conduct experimental research.

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