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Anti-Inflammatory Activity of Polysaccharide from *Pholiota nameko*

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Abstract—*Pholiota nameko* polysaccharide (PNPS-1) has been isolated and purified by enzymatic hydrolysis, hot water extraction, ethanol precipitation, and ion-exchange and gel-filtration chromatography. The anti-inflammatory activity of PNPS-1 was evaluated in rodents using xylene-induced ear edema, egg albumin-, carrageenin-, and formaldehyde-induced paw edema, cotton pellet granuloma test, adhesion of peritoneal leukocytes *in vitro*, and ulcerogenic activity. The results showed that PNPS-1 (5 mg/ear) inhibited topical edema in the mouse ear and at 100, 200, and 400 mg/kg (intraperitoneally) it significantly suppressed the development of egg albumin-, carrageenin-, and formaldehyde-induced paw edema in the animals. PNPS-1 (100, 200, and 400 mg/kg, per oral) significantly inhibited the growth of granuloma tissues induced by subcutaneously implanted cotton pellets in rats by 10.96, 18.07, and 43.75%, respectively. PNPS-1 also inhibited spontaneous and phorbol-12-myristate-13-acetate-activated adhesion of peritoneal leukocytes *in vitro*. Further, both acute as well as chronic administration of PNPS-1 (100, 200, and 400 mg/kg, per oral) did not produce any gastric lesion in rats. In conclusion, these data indicated that PNPS-1 possesses significant anti-inflammatory activity suggesting its potential as an anti-inflammatory agent for use in the treatment of various inflammatory-related diseases.

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The interest of researchers in mushrooms as natural sources of active principles has noticeably increased during the decades and particular attention has to be paid to the polysaccharide components of various mushrooms. Polysaccharides are well known to be the major structural components of mushrooms and to possess immunomodulatory and anti-inflammatory activities [1, 2]. Polysaccharides isolated from different mushrooms have shown good anti-tumor activity [3-5]. Inflammation, especially chronic inflammation, contributes to the development of cancer [6-8]. From our experience, anti-tumor polysaccharides from mushrooms can exert their effects through anti-inflammatory activity in cancer tissues.

Pholiota nameko is a widely cultivated mushroom in China and Japan. It was reported to be very effective in anti-tumor activity by the polysaccharides extracted from it [9, 10]. However, there was no scientific evidence as regards to the anti-inflammatory activity of the polysaccharide from *P. nameko*, and its pharmacological basis for the treatment of inflammation disorders had not been understood.

With a growing number of polysaccharides belonging to the anti-inflammatory family exhibiting unusual biological properties, we were prompted to study the efficacy of the polysaccharides of *P. nameko* in anti-inflammatory activities. The study of anti-inflammatory activity is pertinent in this aspect since the anti-inflammatory activity of *P. nameko* is essential for its anti-tumor biological function. Moreover, *P. nameko* also has the potential to serve as effective therapeutic agent for inflammatory diseases, especially the inhibitors of inflammation-induced tumor. The aim of the present study was to isolate and examine the anti-inflammatory activity of the polysaccharide isolated from *P. nameko* using various models of inflammation.

Abbreviations: ASA) acetylsalicylic acid; C-PNPS) crude *P. nameko* polysaccharide; PMA) phorbol-12-myristate-13-acetate; PNPS-1) *Pholiota nameko* polysaccharide.

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MATERIALS AND METHODS

Isolation and purification of *P. nameko* polysaccharide. *Pholiota nameko* polysaccharides were isolated and

purified in our laboratory as reported by Li and Wang [11] with some modifications.

Isolation of crude polysaccharide. One hundred grams of lyophilized *P. nameko* dissolved in distilled water (1 : 8 w/v) was digested with crystallized trypsinase (200 U/ml; Sigma, USA) at 37°C, pH 8.0, for 50 min. The digest was extracted twice with hot water (90°C), each for 2.5 h, and then centrifuged at 3000*g* for 15 min. The supernatant was concentrated to 300 ml under reduced pressure using a rotary evaporator. Then two volumes of 95% ethanol solution (v/v) was added and maintained for 12 h. The precipitate, dissolved with 250 ml distilled water, was deproteinated by the Sevage method eight times. The resulting solution was precipitated with two volumes of 95% ethanol solution (v/v) and the precipitate was washed with ethanol, acetone, and ether, each for two times. This yielded 10 g crude *P. nameko* polysaccharide named C-PNPS.

DEAE-cellulose chromatography. The C-PNPS dissolved in 50 ml of distilled water was applied to a DEAE-cellulose column (Whatman DE52) equilibrated with 0.1 M $NaHCO_3/Na_2CO_3$ buffer, pH 7.5. The column was eluted with two volumes 0.1 M $NaHCO_3$, two volumes 0.3 M $NaHCO_3$, two volumes 0.5 M $NaHCO_3$, and two volumes 0.1 M NaOH; elution was monitored using the phenol–sulfuric acid method [12]. The C-PNPS was combined into four fractions named PNPS-A (30.6 mg), PNPS-B (1.4 g), PNPS-C (20.6 mg), and PNPS-D (8.5 mg). The pooled PNPS-B, the major polysaccharide-containing population, was dialyzed against distilled water at 4°C overnight and condensed by filtration through filter paper. Thus, 1.4 g PNPS-B was obtained.

Sephadex G-100 chromatography. PNPS-B (1.4 g) dissolved in 50 ml distilled water was applied to a Sephadex G-100 (Pharmacia, Sweden) column equilibrated with 0.1 M NaCl. The column was eluted with 0.1 M NaCl. There were two fractions pooled: PNPS-1 (1.2 g) and PNPS-2 (8.1 mg). The pooled PNPS-1 was dialyzed against distilled water at 4°C for 36 h and lyophilized for the further study of its anti-inflammatory activity.

Animals. Adult Swiss mice (weighing 18-25 g) and adult Sprague–Dawley rats (weighing 140-160 g) of both sexes were purchased from the animal house of the Tianjin Pharmaceutical University. They were housed under standardized conditions (at constant room temperature 25°C with alternating 12-h periods of light and darkness, humidity 50-60%), and they were fed on a standard rodent pellet diet with water *ad libitum* before use. This study was approved by the Committee for Ethics in Animal Research of our university and experiments were carried out in accordance with the norms of Animal Experimentation established by Tianjin University of Commerce.

Xylene-induced topical edema in the mouse ear. The effect of PNPS-1 on acute topical inflammation was evaluated according to the method of Atta and Alkohafi [13] with some modifications. Groups of five Swiss mice of either sex were allotted to each experimental group. The treatment group received PNPS-1 samples (5, 10, and 20 mg/ear, dissolved in saline) applied on the anterior surface of the right ear. Topical inflammation was instantly induced on the posterior surface of the same ear by application of xylene (0.05 ml). The control group (negative control) only received the vehicle (distilled water), while the positive control group received indomethacin as anti-inflammatory agent (5 mg/ear; Sigma). Two hours after induction of inflammation, mice were killed by overdose of ether anesthesia and both ears removed. Circular sections (7 mm diameter) of both the right (treated) and left (untreated) ears were punched out using a cork borer, and weighed. Edema was quantified as the weight difference between the two earplugs. The anti-inflammatory activity was evaluated as percent edema reduction/inhibition in the treated animals relative to control animals.

Egg albumin-induced paw edema in rats. Egg albumin-induced acute inflammation was measured in terms of change in volume of the rat hind paw induced by subplantar injection of egg albumin, conducted following the methodology used by Okoli and Akah [14]. Animals of five per group received 100, 200, and 400 mg/kg of PNPS-1 samples (dissolved in saline) administered intraperitoneally, respectively. Thirty minutes later, edema was induced with 0.1 ml of fresh undiluted egg albumin injected into the subplantar region of the right hind paw of the rats. The volume of distilled water displaced by the treated paw was measured before and 1, 2, 3, and 4 h after induction of edema. The control group (negative control) only received equivalent volume of vehicle (3% (v/v) Tween 85), while the positive control group received acetylsalicylic acid as anti-inflammatory agent (100 mg/kg; Sigma). Inflammation was assessed as the difference between the zero time volume of the treated paw and the volume at the various times after the administration of the phlogistic agent.

Carrageenin-induced paw edema in mice. The carrageenin-induced mouse hind paw edema model was used with modifications in measuring periods for determination of anti-inflammatory activity [15]. Animals in groups of five received 5, 10, and 20 mg/kg of PNPS-1 samples (dissolved in saline) administered intraperitoneally, respectively. Sixty minutes later, edema was induced with 25 μl (0.5 mg) of freshly prepared colloidal suspension of carrageenin (Sigma) in physiological saline (154 nM NaCl) injected into the subplantar region of the right hind paw of the rats. As the control group, equivalent volume (25 μl) of saline solution was injected into that of the left hind paw. Paw edema was measured every 1 h during 4 h after induction of inflammation. Piroxicam (5 mg/kg) (Sigma) was used as the reference drug. The difference in footpad thickness between the right and left

foot was measured with a pair of dial thickness gauge calipers (Fanke Tools Co., China). Mean values of treated groups were compared with mean values of a control group and analyzed using statistical methods.

Formaldehyde-induced hind paw edema in rats. The experiment was performed according to the technique developed by Brownlee [16]. Pedal inflammation was induced by injecting 0.1 ml of 4% formaldehyde solution below the plantar aponeurosis of the right hind paw of the rats. The paw volume was recorded immediately prior to formaldehyde administration (0 h) and then at 1.5, 24, and 48 h after formaldehyde injection. Vehicle (1 ml/kg, per oral), PNPS-1 (100, 200, and 400 mg/kg, per oral) dissolved in saline and standard drug, acetylsalicylic acid (ASA) (Sigma; 300 mg/kg, per oral), were administered 1 h prior to formaldehyde injection.

Cotton-pellet granuloma test in rats. The cotton-pellet granuloma model test in rats, performed according to Winter and Porter [17] with slight modification, was used to evaluate the effect of PNPS-1 samples on chronic inflammation. The animals were anaesthetized with pentobarbitone (30 mg/kg, subcutaneously; Sigma). The back skin was shaved and disinfected with 70% ethanol. An incision was made in the lumbar region. Subcutaneous tunnels were formed with a blunted forceps. Two pre-weighed autoclaved cotton pellets (30 ± 1 mg) were aseptically placed on both sides in the scapular region of the previously depilated back of the rats. On day 1, the rats of either sex received orally 100, 200, and 400 mg/kg of PNPS-1 samples (dissolved in distilled water), respectively. The control group (negative control) only received equivalent volume of vehicle (3% (v/v) Tween 85), while the positive control group received dexamethasone (5 mg/kg; Sigma) as anti-inflammatory agent. PNPS-1 samples were administered once daily for the next seven days. On day 8, the animals were killed by overdose of pentobarbitone. Then, the pellets were dissected out, freed of tissue attachments, and dried in the oven overnight at 60°C until the weight remained constant. The dry pellets were weighed and the mean weight of the granuloma tissue formed around each pellet determined. The level of inhibition of granuloma tissue development was calculated using the relation:

$$\text{inhibition of granuloma tissue (\%)} = 100\left(\frac{T_C - T_T}{T_C}\right),$$

where T_C is weight of granuloma tissue of control group and T_T is weight of granuloma tissue of treated group.

Adhesion of peritoneal leukocytes *in vitro*. The experiment was performed according to the method described by Popov et al. [18]. Peritoneal cells were obtained from mice by peritoneal lavage with phosphate buffered saline (5 ml). The cells were washed by centrifugation (10*g*, 10 min) and then re-suspended in Hanks' medium supplemented with 10% fetal calf serum. PNPS-1 samples (100, 200, and 400 μg/ml) were incubated with the cells (100 μl) in 96-well flat-bottom tissue culture plate (Corning Corp., USA; No. 3896) at 37°C for 10 min, with the suspension of peritoneal cells containing about 10^6 cells/ml of macrophages. Phorbol-12-myristate-13-acetate (PMA, 2.5 μg/ml; Sigma) as a stimulating agent and saline were added to the wells for activation and spontaneous adhesion. The non-adherent cells were washed out of the wells and the adherent cells were fixed in ethanol and stained with azure-eosin. The colored material was solubilized in methanol and absorbance (OD) of the solution was measured at 590 nm using a Cary 50 microplate reader (Varian Inc.) [19].

Ulcerogenic activity. The ulcerogenic activity of PNPS-1 was assessed using the procedures described by Amresh et al. [20].

Acute ulcerogenic activity. The ulcerogenic potential of PNPS-1 at three different doses (100, 200, and 400 mg/kg, per oral) was tested in male rats fasted for 24 h. After the fasting period, the control group was administered vehicle (1 ml/kg, per oral), while the other group received standard drug ASA (300 mg/kg, per oral), respectively. Five hours after drug administration, animals were killed with anesthetic ether. The stomachs were removed, cut along the lesser curvature, and opened up to expose the mucosal surface. The mucosal surface was washed with diluted formaldehyde solution (2.5%). A few minutes later, mucosa of the stomach was observed for petechial hemorrhages and ulcers, if any. The degree of ulceration was graded according to the arbitrary scale as described by Sairam et al. [21].

Chronic ulcerogenic activity. Male rats were used with free access to food and drinking water throughout the period of experiment. The rats were administered vehicle (1 ml/kg, per oral), PNPS-1 (100, 200, and 400 mg/kg, per oral), and ASA (300 mg/kg, per oral), once daily for 14 consecutive days. Twenty-four hours after the administration of the last dose of the drug, all the animals were killed with anesthetic ether and the stomachs were removed and examined as in the acute experiment.

Statistical analysis. Results were analyzed using One Way ANOVA (Fisher LSD post hoc test) and expressed as mean ± S.E.M. Difference between means of treated and control groups was considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Due to the increasing frequency of intake of non-steroidal anti-inflammatory drugs and their common side effects reported, there is need to focus on the scientific exploration of natural products having fewer or no side effects. So, there is a continuous search for highly effective natural ingredients from food, such as mushroom, which can provide relief from inflammation reported in

Table 1. Effect of PNPS-1 on xylene-induced acute topical edema of the mouse ear

Group	Dose, mg/ear	Edema, mg	Inhibition, %
Control	–	0.52 ± 0.11	–
Indomethacin	5	0.25 ± 0.02*	51.92
PNPS-1	5	0.33 ± 0.10*	36.54
	10	0.26 ± 0.05*	50.00
	20	0.18 ± 0.06*	65.38

* $P < 0.05$ (ANOVA; LSD post hoc test). Values of edema shown are mean ± S.E.M. ($n = 5$). Percent inhibition was calculated relative to control.

many references [22]. To give scientific validation to PNPS-1, an attempt was made to study its anti-inflammatory activity.

The PNPS-1 was proved chemically homogeneous by gel filtration. The molecular weight of the PNPS-1 estimated by gel filtration was 114 kD [11]. The sugar composition of PNPS-1 was determined by PC and GLC as alditol acetate derivatives. PNPS-1 is composed mainly of Man, Glc, Gal, Ara, and Xyl in a molar ration of 1 : 8.4 : 13.6 : 29.6 : 6.2.

Table 1 shows that PNPS-1 samples, which were topically applied onto the mouse ear, could potentially suppress the acute edema induced by xylene. Xylene caused instant irritation of the mouse ear, which led to fluid accumulation and edema characteristic of the acute inflammatory response. Suppression of this response was a likely indication of antiphlogistic effect [13]. The topical anti-inflammatory effect suggested that PNPS-1 might relieve rheumatism and offer the additional advantage of suppressing inflammatory response initiated by tissue injury.

In the egg albumin-induced anti-inflammatory test, PNPS-1 (100 mg/kg, per oral) exhibited no inhibition of the development of paw edema induced by egg albumin, but caused a significant ($P < 0.05$) dose-related inhibition when administered PNPS-1 amounted to 200 and 400 mg/kg, per oral, with activity higher than that of ASA (100 mg/kg) (Table 2).

PNPS-1 (20 mg/kg, per oral) exhibited a dose-related inhibition higher than that of piroxicam (5 mg/kg) in the carrageenin-induced edema experiment (Table 3). The result suggested that PNPS-1 had significant anti-inflammatory activity in mice, which reduced the edema induced by carrageenin by 61% on oral administration of 5 mg/kg, as compared to the untreated control group. Carrageenin-induced inflammation is a biphasic phenomenon [23]. The first phase of edema is attributed to release of histamine and 5-hydroxytryptamine. A plateau phase is maintained by kinin-like substances and second accelerating phase of swelling is attributed to prostaglandin like substances. The knowledge of these mediators involved in different phases is important for interpreting the mode of PNPS-1 action. PNPS-1 might have inhibited the release or actions of the various chemical mediators such as histamine, 5-HT, kinins, and prostanoids known to mediate acute inflammation induced by phlogistic agents likely also involved in both egg albumin- and carrageenin-induced acute edema. The carrageenin-induced paw edema increased progressively with time while egg albumin-induced paw edema began to increase at 1 h and progressively decreased with time, which suggested that PNPS-1 may suppress both the early and later phases of the acute inflammatory response.

The result of anti-inflammatory activity of PNPS-1, which was tested in a model of paw edema formation in rats induced by formaldehyde, is documented in Table 4. It is evident that PNPS-1 demonstrated a significant anti-inflammatory effect against formaldehyde-induced inflammation in doses of 100-400 mg/kg. The anti-inflammatory effect was positively dose-related and the maximum inhibition of edema volume produced by PNPS-1 (400 mg/kg, per oral) was higher than that of ASA (300 mg/kg, per oral) (54 versus 49% at 1.5 h). PNPS-1 (100, 200, and 400 mg/kg, per oral) significantly diminished the mean paw edema volume at 1 h (23, 35, and 54%) ($P < 0.05$) and 24 h (3, 27, and 30%) ($P < 0.05$). Further, the effect of PNPS-1 lasted for a period of 24 h,

Table 2. Effect of PNPS-1 on egg albumin-induced acute inflammation in rats

Group	Dose, mg/kg	Edema, ml			
		1 h	2 h	3 h	4 h
Control	–	0.33 ± 0.06	0.32 ± 0.02	0.29 ± 0.03	0.27 ± 0.02
ASA	100	0.26 ± 0.12 (21)	0.22 ± 0.07 (27)	0.17 ± 0.11 (32)	0.12 ± 0.04 (56)
PNPS-1	100	0.30 ± 0.10 (9)	0.28 ± 0.02 (7)	0.28 ± 0.04 (3)	0.24 ± 0.02 (11)
	200	0.20 ± 0.05 (39)*	0.18 ± 0.04 (40)*	0.15 ± 0.01 (48)*	0.08 ± 0.02 (70)*
	400	0.17 ± 0.08 (48)*	0.14 ± 0.06 (53)*	0.11 ± 0.04 (62)*	0.05 ± 0.04 (81)*

* $P < 0.05$ (ANOVA; LSD post hoc test). Values of edema are mean ± S.E.M. ($n = 5$) while those in parentheses represent percent inhibition of edema. ASA, acetylsalicylic acid.

Table 3. Effect of PNPS-1 on carrageenin-induced acute inflammation of mouse paw edema

Group	Dose, mg/kg	Swelling thickness $\times 10^{-2}$, mm			
		1 h	2 h	3 h	4 h
Control	–	42.2 ± 2.11	46.4 ± 2.05	50.3 ± 2.33	49.3 ± 2.14
Piroxicam	5	42.4 ± 2.39 (NI)	39.2 ± 2.76 (37)	36.7 ± 2.82 (68)	35.9 ± 2.21 (88)
PNPS-1	5	42.4 ± 3.45 (NI)	41.7 ± 3.44 (6)*	38.2 ± 2.48 (50)*	37.8 ± 2.12 (61)*
	10	42.5 ± 3.61 (NI)	39.0 ± 2.72 (40)*	36.8 ± 2.49 (66)*	35.3 ± 2.36 (85)*
	20	42.7 ± 2.33 (NI)	38.6 ± 2.02 (44)*	35.1 ± 2.24 (87)*	34.5 ± 2.29 (95)*

* $P < 0.05$ (ANOVA; LSD post hoc test). Values of edema are mean ± S.E.M. ($n = 5$), while those in parentheses represent percent inhibition of edema. NI, no inhibition.

Table 4. Effect of PNPS-1 on formaldehyde-induced paw edema in mice

Group	Dose, mg/kg	Formaldehyde-induced hind paw edema volume, ml		
		1.5 h	24 h	48 h
Control	–	0.74 ± 0.05	0.97 ± 0.02	0.55 ± 0.02
ASA	300	0.38 ± 0.01 (49)*	0.92 ± 0.01 (5)	0.53 ± 0.01 (4)
PNPS-1	100	0.57 ± 0.02 (23)*	0.82 ± 0.03 (15)*	0.53 ± 0.02 (4)
	200	0.48 ± 0.01 (35)*	0.71 ± 0.02 (27)*	0.54 ± 0.03 (2)
	400	0.34 ± 0.02 (54)*	0.68 ± 0.02 (30)*	0.51 ± 0.01 (7)

* $P < 0.05$ (ANOVA; LSD post hoc). Values of formaldehyde-induced hind paw edema volume (ml) shown are mean ± S.E.M. ($n = 5$), while those in parentheses represent percent inhibition of edema.

while the effect of ASA was significant only at 1.5 h, which suggested the long duration of action of PNPS-1. Inflammation induced by formaldehyde is biphasic, the edema being produced by formaldehyde and mediated by substance P and bradykinin in the early phase, followed by tissue-mediated response induced by histamine, 5-HT, prostaglandins, and bradykinins [24]. Thus, the anti-inflammatory activity of PNPS-1 may be attributed to inhibition of one or some of these inflammatory mediators.

The values of the inhibitory action of PNPS-1 against granuloma formation in rats induced by cotton implantation are shown in Table 5. PNPS-1 (100, 200, and 400 mg/kg, per oral) was found to significantly ($P < 0.05$) reduced the granuloma formation with inhibition of 10.96, 18.07, and 43.75% as compared with dexamethasone (5 mg/kg, per oral), which showed significant ($P < 0.05$) inhibition on granuloma formation (35.71%). In the cotton pellet granuloma model, which is widely used to assess its efficacy against proliferative phase of inflammation in which tissue degeneration and fibrosis occur, inflammation and granuloma develops during the period of several days. This model is an indication for the proliferative phase of inflammation [25]. Inflammation involves proliferation of macrophages, neutrophils, and fibroblasts, which are basic sources of granuloma formation. The results show that PNPS-1 was inhibitory in its action and is proportional to the doses employed, thus proving its activity in the proliferative phase of inflammation. As expected, PNPS-1 has the potential to be used as curative in different inflammatory conditions due to its significant anti-inflammatory activity exhibited in chronic models of cotton pellet granuloma inflammation.

Table 5. Effect of PNPS-1 on granuloma tissue formation in rats

Group	Dose, mg/kg	Granuloma tissue weight, mg	Inhibition, %
Control	–	68.77 ± 1.05	–
Dexamethasone	5	44.21 ± 3.29*	35.71
PNPS-1	100	61.23 ± 1.56*	10.96
	200	56.34 ± 2.67*	18.07
	400	38.68 ± 6.57*	43.75

* $P < 0.05$ (ANOVA; LSD post hoc). Values of granuloma tissue weight shown are mean ± S.E.M. ($n = 5$).

Table 6. Effect of PNPS-1 on adhesion of peritoneal leukocytes *in vitro* (OD_{590})

Group	Dose, μg/ml	Spontaneous	PMA (2.5 μg/ml)
Control	–	0.453 ± 0.076	0.532 ± 0.086
PNPS-1	50	0.449 ± 0.033 (0.8)	0.524 ± 0.011 (1.5)
	100	0.406 ± 0.045 (10)*	0.456 ± 0.066 (14)*
	200	0.127 ± 0.021 (72)*	0.185 ± 0.032 (65)*
	400	0.043 ± 0.010 (91)*	0.084 ± 0.014 (84)*

* $P < 0.05$ (ANOVA; LSD post hoc) compared with Hanks' medium. Values of adhesion shown are mean ± S.E.M. ($n = 5$) while those in parentheses represent percent inhibition of adhesion. Spontaneous, spontaneous adhesion of peritoneal leukocytes; PMA, phorbol-myristate-acetate-stimulated adhesion of peritoneal leukocytes.

The influence of PNPS-1 on adhesion of peritoneal macrophages *in vitro* is presented in Table 6. Microscopic evaluation indicated that ca. 90% of peritoneal macrophages were adherent in the control wells after 15 min of incubation. With the addition of PNPS-1, its anti-inflammatory effect changes in a dose-dependent inhibition of macrophage adhesion *in vitro*. Inhibition of adhesion in the absence of activation by PMA was shown to be equal to 10, 72, and 91% at 100, 200, and 400 μg/ml of PNPS-1, respectively, while PMA-activated adhesion failed to be affected by low concentrations (<100 μg/ml) of PNPS-1, with the inhibition 14, 65, and 84% at 100, 200, and 400 μg/ml of PNPS-1 (Table 6).

Leukocytes play an integral role in the pathogenesis of different inflammatory disorders [26]. Inflammation is characterized histologically by the accumulation of leukocytes at the inflamed site due to the directional migration of circulating leukocytes. And leukocyte adhesion represents one of the first steps in inflammatory response initiation, and it is essential for accumulation of active immune cells at sites of inflammation [27]. Hence, inhibition of peritoneal leukocytes adhesion *in vitro* of PNPS-1 was evaluated in order to elucidate involvement of leukocytes in the mechanism of the anti-inflammatory effect.

The ulcerogenic effect of PNPS-1 was tested on fasted rats. Rats administered PNPS-1 as a single dose or chronically for 14 days were found to be devoid of gastric lesions in contrast to the standard anti-inflammatory agent (ASA) that significantly ($P < 0.05$) induced the gastric lesions with the mean score of severity of 1.96 ± 0.12 in acute and 2.56 ± 0.14 in chronic studies, as compared with the vehicle treated rats (data not presented). The gastric lesions were associated with accumulation of fluid in the stomach, erosion or congestion of gastric mucosa, pinpoint hemorrhagic spots, and a few ulcers. The main side effect of non-steroidal anti-inflammatory drugs is thought to impair the mucosal defense of the stomach and the intestine to produce gastrointestinal lesions [28]. They act by inhibition of cyclooxygenase and, therefore, inhibit the production of gastric prostaglandins. This leads to a reduction in the production of gastric mucus and an increase in mucosal permeability [29]. During the acute and chronic ulcerogenic studies, PNPS-1 did not induce any adverse effect on gastric mucosa, indicating lack of ulcerogenic activity.

The present study demonstrated potent anti-inflammatory activity of PNPS-1 in different models of inflammation, i.e. acute exudative (xylene-induced mouse topical ear edema, egg albumin-induced rat edema, carrageenin-induced mouse paw edema), subacute (formaldehyde-induced rat paw edema) and chronic proliferative inflammation (cotton pellet granuloma), adhesion of peritoneal leukocytes *in vitro*, especially without any ulcerogenic activity, which may derive from inhibition of prostaglandins synthesis, inhibition of increased vascular permeability, inhibition of neutrophil migration into inflamed tissues, and stimulation of lymphocyte accumulation, and may enhance tissue repair and healing. The advantages of PNPS-1, viz., better and safer anti-inflammatory profile without ulcerogenic activity deserves further studies to identify the possible mechanism of action as well as establishing the therapeutic value in the treatment of inflammatory-related diseases.

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