

EVALUATION OF ANTI-INFLAMMATORY AND ANALGESIC ACTIVITY OF THE AQUEOUS METHANOLIC EXTRACT OF *ASPRARAGUS RACEMOSUS* IN EXPERIMENTAL MODELS

HASEEB AHSAN^{1*}, IHTISHAM HAIDER², MUHAMMAD NAVEED MUSHTAQ³, IRFAN ANJUM³

¹College of pharmacy, University of Sargodha, Sargodha, Pakistan

²Nawaz Sharif medical college, University of Gujrat, Pakistan

³Faculty of Pharmacy, Department of Pharmacology, The University of Lahore, Lahore, Pakistan

*corresponding author: haseeb.ahsan@uos.edu.pk

Manuscript received: March 2018

Abstract

The aim of the present study was to evaluate the anti-inflammatory and analgesic activity of the aqueous methanolic extract of the root of *Asparagus racemosus* in Albino mice model. Oedema models were induced by injection of carrageenan and fresh egg albumin into right hind paw. Acetic acid and formalin were used to induce pain models. The aqueous methanolic extract showed significant reduction in both paw oedema models. In pain models, the extract also significantly inhibited the acetic acid-induced writhing and formalin-induced paw lickings. Our findings showed that aqueous methanolic extract of *Asparagus racemosus*'s root reduced inflammation and pain in experimental models. The results proposed a potential use of *Asparagus racemosus*'s roots in treating conditions associated with inflammation and pain.

Rezumat

Scopul studiului a fost de a evalua activitatea antiinflamatorie și analgezică a extractului hidroalcoolic din rădăcina de *Asparagus racemosus* într-un model experimental murin. Edemul a fost indus prin injectarea de carragenan și albumină proaspătă din ou în laba dreaptă din spate. S-au folosit acid acetic și formalină pentru a induce modele de durere. Extractul hidroalcoolic a demonstrat o reducere semnificativă a ambelor modele de edem de labă. În modelele de durere, extractul inhibă, de asemenea, în mod semnificativ simptomatologia specifică fiecărui test. Constatările noastre au arătat că extractul hidroalcoolic din rădăcina de *Asparagus racemosus* a redus inflamația și durerea în modelele experimentale testate. Rezultatele au sugerat o posibilă utilizare a rădăcinilor *Asparagus racemosus* în tratarea afecțiunilor asociate cu inflamația și durerea.

Keywords: *Asparagus racemosus*, anti-inflammatory, carrageenan, pain, formalin

Introduction

Inflammation is a primary defence mechanism and protects body against toxic substances, allergens, infections and a number of harmful agents. Under various pathophysiological conditions, the inflammation process becomes uncontrolled and leads to chronic diseases [1]. Generally, inflammation is regarded as a protective response intended to eliminate causes of injury such as noxious chemicals or microbial agents [2]. It is a complicated process that is mediated by variety of signals produced by leukocytes, macrophages and mast cells. Cyclooxygenases (COX) play a key role in the production of potent pro-inflammatory prostaglandins (PGs) [3]. Different synthetic drugs are used as anti-inflammatory agents. These include narcotics e.g., opioids or non-narcotics e.g., salicylates and corticosteroids e.g., hydrocortisone [4]. But the side effects of the currently available anti-inflammatory drugs present a major problem. Hence there is need to develop safe drugs. Asian have written evidence for

the use of natural products for various disease [5]. Traditionally, natural products are approved as safe drugs in most of the societies as compare to allopathic medicines [6]. A large number of phytochemicals isolated from natural sources have been used to treat inflammation and other pain-associated conditions. These phytochemicals showed low toxicity and higher therapeutic effect [7].

Asparagus racemosus is a commonly occurring plant in Punjab, Pakistan from *Liliaceae* family. The common name of this plant is satmuli and the part used as therapeutic agent is the root. It normally grows at the height of 1 - 2 m, presenting needles like leaves and white flowers [8].

Asparagus racemosus is used traditionally as carminative, antispasmodic, antidiarrheal and in dyspepsia and rheumatism [9]. There is a need to prove its biological activities pharmacologically. The aim of the present study was to evaluate the anti-inflammatory and analgesic activity of the aqueous methanolic extract of the root

of *Asparagus racemosus* (AMEAC) in carrageenan and albumin- induced paw oedema and formalin-induced paw licking and acetic acid-induced abdominal writhing in albino mice respectively.

Materials and Methods

Plant Material

The plant *Asparagus racemosus* was collected from Gujrat, Pakistan. The plant was identified by Dr. Amin Ullah Shah, Department of Botany, University of Sargodha, Pakistan. The extraction of the root of *Asparagus racemosus* (4 kg) was performed by cold maceration method by adding 9 L (70:30 ratio of methanol to distilled water) solvent. The powder was soaked for 72 hours at room temperature with infrequent shaking and filtered through muslin cloth and Whatman qualitative Grade 1 filter paper. The final filtrate was evaporated with the help of rotary evaporator and then lyophilized to give a yield of 4.8% of extract. The extract was dissolved in distilled water for administration [10, 11].

Animals

Adult healthy Swiss albino mice of both sexes weighing 20 - 30 g were used for this study. The polypropylene cages were used for housing and animals were allowed free access to water and were given palatable clean food. Humidity and temperature of the animal house were maintained constant. Dark and light cycle of 12/12 hours was preserved. The National Institute of Health (NIH) guidelines were followed for the treatment of these animals. The followed experimental protocol was according to internationally approved guidelines (Guide for the care and use of laboratory animals, NIH, United States) for animal use and care. The animals were acclimatized for a period of 7 days. The study protocols were approved by the local ethical committee of the University of Sargodha, Pakistan.

Drug administration

The extracts were administered to the animals by the following routes.

Oral administration

Oral gavage was used for administration of plant extract and standard drug. The needle of the 1 cc BD syringe was removed and Ryle tube #4 attached to needle no. 17 was used.

Anti-inflammatory activity

Carrageenan-induced paw oedema in mice

Anti-inflammatory activity of aqueous methanolic extract in 250 and 500 mg/Kg bw doses was performed by using carrageenan-induced paw oedema model in mice [12]. Twenty animals were divided into four groups having five animals each. Group I received distilled water (2 mL/kg bw p.o.) and served as control group. Group II and III received aqueous methanolic extract of *Asparagus racemosus* in 250 and 500 mg/kg bw p.o. doses respectively through

oral route. Group IV received the drug ibuprofen in 40 mg/kg bw dose p.o. and was considered as standard group. One-hour post treatment, oedema was developed by injection of carrageenan (0.1 mL, 1%, w/v in saline) into the sub plantar tissue of the right hind paw. The linear paw circumference was then measured at 0, 30, 60, 90 and 120 min. of the administration of phlogistic agent, using the vernier calliper. The % inhibition was calculated by using the following formula:

$$\text{Inhibition (\%)} = [(V_c - V_t)/V_c] \times 100,$$

where, V_c and V_t represent the average paw volume of the control and the treated animals respectively [13, 14].

Egg albumin-induced paw oedema in mice

The anti-inflammatory activity of the extract was also evaluated on egg albumin-induced inflammation model in mice by following the method described in literature with some modification [12]. Briefly, mice weighing 20 - 30 g were grouped into four groups consisting of five mice each. The animals were kept fasting for 12 hours before the beginning of the experiment. Group I (control) was treated with distilled water 2 mL/kg bw orally. Animals of Groups II and III, were given aqueous methanolic extract of *Asparagus racemosus* in 250 and 500 mg/Kg bw doses orally, whereas Group IV (standard group) received ibuprofen as a standard drug (40 mg/kg bw p.o.). 0.1 mL of fresh egg albumin was injected into the paw to induce inflammation, after one hour of treatment. The linear diameter of paw was measured at 0, 1, 2, and 3 hours after the administration of inflammatory agent using a vernier calliper. The above-mentioned formula was used to calculate % inhibition [15].

Analgesic activity

The analgesic activity of the extract was assessed by using two models i.e., acetic acid-induced writhing model and formalin-induced paw licking model in mice.

Acetic acid-induced writhing in mice

The aqueous methanolic extract of *Asparagus racemosus* was evaluated for its analgesic activity using acetic acid induced writhing method in mice. The different groups of the animals were given the extract (250 and 500 mg/kg bw), ibuprofen (40 mg/kg bw) and distilled water orally. After 30 minutes post treatment, acetic acid (0.6%, v/v in saline, 10 mL/kg, i.p.) was injected to every mouse in order to induce pain. The number of writhings developed in each mouse after administration of acetic acid (characterized by contraction of the abdominal musculature and extension of the hind limbs) were observed after 5 min and continued for 10 min.

$$\% \text{ inhibition} = \{[\text{No. of writhings (Control)} - \text{No. of writhings (Treated)}] / \text{No. of writhings (Control)}\} \times 100$$

Formalin-induced paw licking in mice

Each dose of the aqueous methanolic extract, normal saline and standard drug (ibuprofen) were administered to 12 hour fasting mice divided into 4 groups of five mice each. Group I serving as control was treated with 2 mL/kg bw saline, orally. Groups III and IV were treated with 250 and 500 mg/kg bw doses of the extract respectively, while Group IV was treated with 40 mg/kg bw p.o. of ibuprofen. Pain was induced by injection of 0.05 mL of 2.5% formalin into sub-planter of right hind paw. The time that the mice spent licking the injected paw, was recorded and considered as indicative of pain. This response was calculated for 30 minutes. The percentage inhibition was calculated by the below given formula:

$$\% \text{ Inhibition} = \{[\text{Reaction Time (Control)} - \text{Reaction Time (Treated)}] / \text{Reaction Time (Control)}\} \times 100$$

Statistical analysis

Data were analysed as mean ± SEM. One-way ANOVA analysis followed by Dunnett's test was used to compare data with control group by using Graph pad prism 5. Student t test was applied in the case of paw licking and writhing data.

Results and Discussion

In the carrageenan-induced paw oedema model, the aqueous methanolic extract of *Asparagus racemosus* caused significant reduction of the swelling induced in the paw. The plant extract in 250 and 500 mg/kg bw doses showed negligible reduction at 0 time. After 30 minutes of induction of oedema, both doses of extract caused significant anti-inflammatory effect comparable to control group at the respective time. As time proceeded, the extract produced a maximum inhibitory effect. Ibuprofen, used as a standard drug also produced a significant reduction of paw oedema compared to control group as shown in Table I.

Table I

Effect of aqueous methanolic extract of *Asparagus racemosus* at 250 and 500 mg/kg bw doses on carrageenan - induced paw oedema in mice

Treatment (Dose, mg/kg bw)	0 min (mm) (% inhibition)	30 min (mm) (% inhibition)	60 min (mm) (% inhibition)	90 min (mm) (% inhibition)	120 min (mm) (% inhibition)
Control (2 mL/kg bw)	3.11 ± 0.049	3.36 ± 0.056	3.58 ± 0.071	3.80 ± 0.051	4.0 ± 0.045
Aqueous methanolic extract (250 mg/kg bw)	3.06 ± 0.084 ^{n.s} (1.61%)	2.94 ± 0.07 ^{***} (12.5%)	2.80 ± 0.17 ^{***} (21.78%)	2.72 ± 0.13 ^{***} (28.42%)	2.76 ± 0.11 ^{***} (31%)
Aqueous methanolic extract (500 mg/kg bw)	3.01 ± 0.12 ^{n.s} (3.21%)	2.74 ± 0.04 ^{***} (18.45%)	2.58 ± 0.02 ^{***} (27.93%)	2.42 ± 0.05 ^{***} (36.31%)	2.3 ± 0.05 ^{***} (42.5%)
Ibuprofen (40 mg/kg bw)	3.08 ± 0.05 ^{n.s} (0.96%)	2.7 ± 0.32 ^{***} (19.64%)	2.59 ± 0.12 ^{***} (27.65%)	2.44 ± 0.21 ^{***} (35.18%)	2.34 ± 0.14 ^{***} (41.5%)

Results are expressed as means ± SEM (n = 5) Key: where, *** = (p < 0.001) and n.s = non-significant when compared to control.

The aqueous methanolic extract of *Asparagus racemosus* at both doses (250 and 500 mg/kg bw) showed significant reduction in the swollen paw induced by albumin from 30 min post phlogistic administration to 120 min. But this extract caused a maximum inhibition

of the swollen paw after 2 hours of induction of oedema. Ibuprofen at a dose of 40 mg/kg bw also significantly reduced oedema, but this effect is time dependent. These results were comparable with control group as shown in Table II.

Table II

Effect of aqueous methanolic extract of *Asparagus racemosus* at 250 and 500 mg/kg bw on egg albumin-induced paw oedema in mice

Treatment (Dose, mg/kg bw)	0 min (mm) (% inhibition)	30 min (mm) (% inhibition)	60 min (mm) (% inhibition)	90 min (mm) (% inhibition)	120 min (mm) (% inhibition)
Control (2 mL/kg bw)	3.28 ± 0.15	3.7 ± 0.071 ^{***}	4.02 ± 0.06 ^{***}	4.4 ± 0.1 ^{***}	4.53 ± 0.09 ^{***}
Aqueous methanolic extract (250 mg/kg bw)	3.16 ± 0.15 ^{n.s} (3.6%)	3.14 ± 0.14 ^{n.s} (15.1%)	3.05 ± 0.08 ^{**} (24.1%)	2.95 ± 0.06 [*] (32.9%)	2.74 ± 0.08 [*] (39.5%)
Aqueous methanolic extract (500 mg/kg bw)	3.24 ± 0.09 ^{n.s} (1.2%)	3.04 ± 0.08 ^{**} (17.8%)	2.64 ± 0.1 ^{***} (34.3%)	2.34 ± 0.051 [*] (46.8%)	2.22 ± 0.08 ^{**} (50.9%)
Ibuprofen (40 mg/kg bw)	3.20 ± 0.12 ^{n.s} (1.3%)	2.8 ± 0.38 ^{**} (24.3%)	2.6 ± 0.14 ^{***} (35.3%)	2.39 ± 0.32 ^{***} (45.7%)	2.20 ± 0.02 ^{***} (51.4%)

Results are expressed as means ± SEM (n = 5); *** = p < 0.001; ** = p < 0.01; * = p < 0.5; and n.s = non-significant vs. control

In the case of analgesic models, the aqueous methanolic extract significantly reduced the number of writhings induced by acetic acid with 54.78 ± 1.93 and 45.32 ± 1.73 at the dose of 250 and 500 mg/kg bw respectively

as compared to control group (75.50 ± 0.93), whereas in the case of standard group, ibuprofen in 40 mg/kg bw dose caused highly inhibition (69.05%) in number of writhings as shown in Table III.

Table IIIEffect of aqueous methanolic extract of *Asparagus racemosus* on acetic acid-induced writhing in mice

Treatment (Dose, mg/kg bw)	No. of writhings	% inhibition
Control (10 mL/kg bw)	75.50 ± 0.93	-
Aqueous methanolic extract (250 mg/kg bw)	54.78 ± 1.93*	27.24
Aqueous methanolic extract (500 mg/kg bw)	45.32 ± 1.73*	39.97
Ibuprofen (40 mg/kg bw)	23.36 ± 1.47*	69.05

Results are expressed as means ± SEM (n = 5); * = p < 0.05 when compared to control (Student t test)

The aqueous methanolic extract of *Asparagus racemosus* at 250 and 500 mg/kg caused significant reduction of pain response produced by formalin with maximum inhibition 76.08% and 81.62% in the first phase (first 60 min.) and 67.94% and 75.88% in the second phase

respectively. This inhibitory effect observed in the case of standard drug, ibuprofen, was 87.7% and 91.7% in first and second phase respectively as shown in Table IV.

Table IVEffect of aqueous methanolic extract of *Asparagus racemosus* on 2.5% formalin induced paw licking in mice

Treatment (Dose, mg/kg bw)	First phase		Second phase	
	Licking time (s)	% inhibition	Licking time (s)	% inhibition
Control (10 mL/kg bw)	168.1 ± 0.85	-	208.1 ± 0.92	-
Aqueous methanolic extract (250 mg/kg bw)	40.20 ± 5.46*	76.08	66.70 ± 5.22*	67.94
Aqueous methanolic extract (500 mg/kg bw)	30.89 ± 7.95*	81.62	50.19 ± 1.61*	75.88
Ibuprofen (40 mg/kg bw)	20.52 ± 2.55*	87.79	17.23 ± 0.58*	91.72

Results are expressed as means ± SEM (n = 5); * = p < 0.05 when compared to control (Student t test).

The anti-inflammatory study of the aqueous methanolic extract of *Asparagus racemosus* was studied in two inflammatory models, carrageenan and albumin-induced oedema model. The first method mimics acute inflammation in which carrageenan causes release of inflammatory mediators at the site of injury. This type of inflammation is indicated by swelling, pain and fever. This extract showed significant anti-inflammatory effect that might be due to the blockade of the release of mediators. Our experimental work showed resemblance with previous studies [16].

The results of our experimental work proposed that the extract showed comparable anti-inflammatory activity to standard drug ibuprofen. In first inflammatory model, carrageenan caused induction of oedema in two phases. Previous studies have shown that during the first phase (first 60 min) histamine and serotonin are released while inflammatory mediators such as prostaglandins (PGs), bradykinin and lysozyme are released in the second phase (after 60 min) [12]. Steroidal and non-steroidal anti-inflammatory drugs produce their response in the second phase of inflammation. Our extract produced anti-inflammatory effects by reducing paw oedema (2.94 ± 0.07 and 2.74 ± 0.04) at both doses 250 mg/kg bw and 500 mg/kg bw dose that was continued at all observable times as shown in Table I. This activity might be due to an inhibitory effect on mediators of inflammation released in both phases.

The anti-inflammatory activity of the aqueous methanolic extract of *Asparagus racemosus* was also studied in egg albumin-induced oedema model. The extract showed significant reduction (2.74 ± 0.08 and 2.22 ± 0.08) in paw oedema at both doses 250 and 500 mg/kg bw as shown in Table II. It is well established that

histamine released from mast cells is a potent vasodilator and induces inflammation [15]. The present experimental work proposed that the extract exhibited anti-inflammatory effect that might be due to inhibition of release of inflammatory mediators such as histamine, serotonin, cytokines and prostaglandins as it was found in previous studies [17].

Acetic acid-induced writhing model has been used to evaluate the peripherally acting analgesic drugs [18]. These mediators are responsible for pain perception. In the current study, acetic acid produced 75.50 ± 0.93 writhing in the control group as shown in Table III. The extract, in a dose dependent manner, reduced acetic acid induced writhing showing peripherally mediated analgesic activity of the extract. This peripherally mediated analgesic activity might be due to activation of peritoneal receptors present on the surface of cell lining of the peritoneal cavity [19, 20]. The extract at both doses (250 and 500 mg/kg bw) caused significantly reduction (54.78 ± 1.93 and 45.32 ± 1.73) respectively in abdominal constrictions and jerkings. This analgesic activity might be peripherally mediated due to the inhibitory effect on the release of prostaglandins and other endogenous pain mediators in agreement with previous studies [21]. Formalin-induced paw licking model is another method used for analgesic activity evaluation. It has two phases. During the first phase, there is a direct chemical stimulation of the nociceptors, whereas the second phase depends on inflammatory mediators [22]. The extract was evaluated for both central and peripheral actions. Formalin causes increases in activation of C-afferent fibre, indicated as paw licking in animals [23]. In the present study, paw licking time for formalin was 168.1 ± 0.85 seconds and 208.1 ± 0.92 seconds

respectively as shown in Table IV. The aqueous extract in 250 and 500 mg/kg bw doses exhibited a significant reduction (40.20 ± 5.46 and 30.89 ± 7.95) in the first phase as well as in the second phase (66.70 ± 5.22 and 50.19 ± 1.61) respectively (Figure 1). These results suggest that the analgesic effect might be due to its central as well as peripheral actions as reported by previous studies [24, 25].

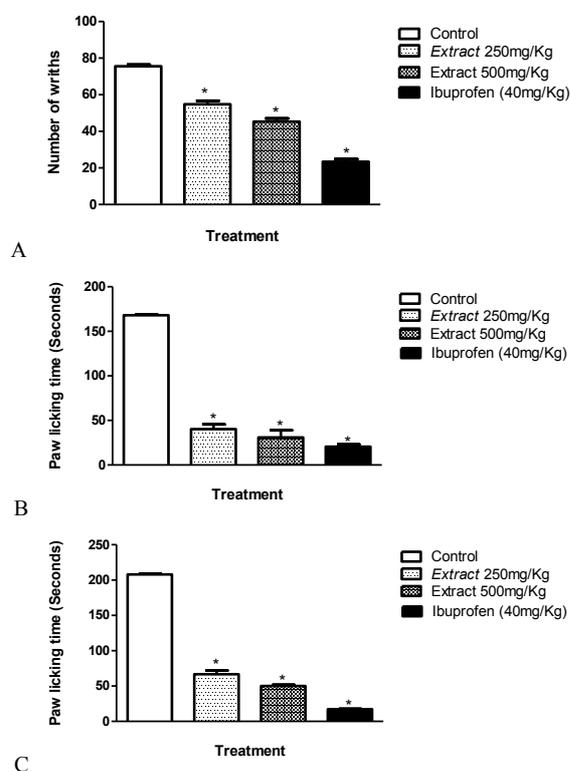


Figure 1.

Effect of *Asparagus racemosus*'s extract on acetic acid-induced number of writhes (A) and on formalin-induced paw licking time, Phase-I (B) and Phase-II (C) in albino mice.

(* p < 0.05 compared to control group; n = 5)

Conclusions

Our results had shown that the aqueous methanolic extract of *Asparagus racemosus* exhibited analgesic and anti-inflammatory effects. It might inhibit the release of a number of mediators involved in inflammation induced by carrageenan and egg albumin in mice inflammatory models. Moreover, it significantly reduced writhing induced by acetic acid and paw lickings by formalin in mice models. Thus, these findings might be helpful for discovering new biological activities of *Asparagus racemosus*.

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