https://doi.org/10.31925/farmacia.2021.5.19 **ORIGINAL ARTICLE**

PRELIMINARY RESEARCH REGARDING CHEMICAL COMPOSITION AND ANTI-INFLAMMATORY EFFECTS OF *POLYGONUM PLEBEIUM* R. BR

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Manuscript received: December 2020

Abstract

Polygonum plebeium is a medicinal plant, traditionally used to treat diseases associated with inflammation. The purpose of this study was to assess the anti-inflammatory effect of the aqueous methanolic extract of *Polygonum plebeium* (AMEPP). The plant inhibitory potential against protein denaturation was determined by measuring the absorbance of the plant extract treated with bovine serum and egg albumin solutions. The pant *in vivo* anti-inflammatory activity was examined by using carrageenan and egg albumin induced paw oedema models. The AMEPP has inhibited the egg albumin and bovine serum albumin denaturation (72.99% and 67.51%, respectively) dose depending. The plant extract has significantly reduced inflammation (48.7% and 40.63%, respectively) against carrageenan and egg albumin-induced paw oedema models. The present study concludes that the AMEPP has a promising anti-inflammatory effect which could be related to the presence of the secondary metabolites. The current study confirmed the traditional use of *P. plebeium* extract in inflammatory conditions.

Rezumat

Polygonum plebeium este folosită în mod tradițional pentru tratamentul afecțiunilor inflamatorii. Obiectivul prezentului studiu a fost de a evalua activitatea anti-inflamatoare a extractului metanolic de *Polygonum plebeium*. Potențialul inhibitor împotriva denaturării proteinelor a fost determinat prin măsurarea absorbanței în domeniul UV-Vis a soluțiilor obținute prin tratarea extractului din plantă cu ser de bovine și de albumină. Activitatea anti-inflamatorie *in vivo* a fost evaluată folosind două modele de edem al labei induse de carrageenan (1%) și albumină (1%). Extractul metanolic a inhibat denaturarea albuminei de ou și a albuminei serice bovine (72,99%, respectiv 67,51%) într-o manieră dependentă de doză. Extractul metanolic vegetal (500 mg/kg) a redus semnificativ inflamația (48,7% și, respectiv, 40,63%). Astfel, se poate afirma că extractul metanolic de *P. plebeium* are un efect antiinflamator promițător care ar putea fi atribuit prezenței metaboliților secundari, confirmând utilizarea extractului de *P. plebeium* în patologii inflamatorii.

Keywords: albumin, carrageenan, inflammation, *Polygonum plebeium*

Introduction

The process of inflammation has a basic function in the primary defense of the body after tissue damage or infection. It helps in restricting additional damage to the affected tissue [1]. However, chronic inflammation may be responsible for non-contagious diseases including arthritis [2]. The inflammatory response is a determinant of the autoimmune diseases, developed due to the release of various players such as leukocytes, innate lymphoid cells, and macrophages. These players are activated during inflammatory conditions and release different cytokines that activate immune cells causing tissue destruction [3].

The hunt for natural phytochemicals with potential anti-inflammatory properties is still a challenge and targeting the identification and isolation of anti-inflammatory phytochemicals in plant extracts is an area of rapid growth nowadays [4, 5].

Herbal therapies are recommended for the management of various disorders for a long time before and more than 80% of the world population prefers herb-based medicines for its primary health care needs [6]. Pakistan is an agricultural land that has many plants traditionally used to treat

inflammatory diseases. From the scientific study of these medicinal plants, better candidate alternative remedies for the treatment of inflammation might be evolved [7].

Polygonum plebeium R.Br, generally referred as knotweed, belongs to the family *Polygonaceae*. It contains various phytochemicals such as essential oils, alkaloids tannins, and flavonoids. It is largely spread in different regions of the world including Pakistan, India, Bangladesh and Sri Lanka [8]. *Polygonum plebeium*, is used conventionally to treat a number of disorders such as liver disease, inflammation, dysentery, eczema and ringworms [9]. However, there is no scientific data that proves the acclaimed conventional use of this plant against inflammatory conditions.

In this study, it was prompted to investigate the anti-inflammatory potential of *P. plebeium.* For this purpose, the protective effect of *P. plebeium* against bovine serum albumin and egg albumin denaturation was determined. Carageenan and egg albumin were used to induce paw oedema models, for the *in vivo* study. Preliminary screening of the phytochemicals in the aqueous methanolic extract of *P. plebeium* (AMEPP) was also performed.

Materials and Methods

Plant material

Polygonum plebeium was bought from a shop in Sargodha, Pakistan. The plant material was identified by Dr. Ameen, Department of Botany, University of Sargodha, Pakistan. The specimen was submitted in the herbarium and assigned a voucher number SU/Voucher/115.

Extract preparation

The whole plant material was washed thoroughly, dried, and crushed to powder form. Then, the powdered plant material (1000 g) was macerated by using 5.5 L of 70% methanol for 72 hours and placed at room temperature with shaking occasionally. Then it was filtered with a muslin cloth and Whatman qualitative filter paper of grade 1. It was then re-macerated two times by using aqueous methanol for another 72 hours. Then, the filtrates were collected and evaporated through the rotary evaporator at 50ºC. The final dried product was named as the aqueous methanolic extract of *P. plebeium* and stored at -4ºC until used for the experimental study [10]. The percentage yield was 6. 68%.

Phytochemical screening

Preliminary screening of phytochemicals of AMEPP was performed for the determination of alkaloids, tannins, proteins, carbohydrates, glycoside, saponins, phenolic compounds, fixed oils and fats, gums and mucilages by using the standard procedures with slight modifications [11].

In Vitro Assays

Protein denaturation by using egg albumin

This experiment was done by preparing the reaction mixture that contains egg albumin, phosphate buffer (pH 6.4) and aqueous solution at different concentrations (6400, 3200, 1600, 800, 400, 200, 100 and 50 µg/mL) of the plant extract and ibuprofen, respectively. The control reaction mixtures were prepared in the absence of the test sample by adding only 2 mL of distilled water at each concentration level. All the test, standard and control solutions were kept in an incubator for 15 minutes at a temperature of 37°C. After that, these solutions were placed in an oven at 70°C for 5 min. Then, the solutions were removed from the oven and scanned on a UV-Visible spectrophotometer at 660 nm. The percentage inhibition property of the plant extract and ibuprofen were calculated from the absorbance of the solutions by using the formula [8]:

$$\% \text{ Inhibition} = \frac{\text{Absorbance control} - \text{Absorbance test sample}}{\text{Absorbance control}} \times 100$$

Protein denaturation by using bovine albumin

The effect of AMEPP against bovine albumin was evaluated according to the method as previously described [12]. Briefly, the reaction mixtures were prepared by adding 0.45 mL of 5% of an aqueous solution of bovine albumin to 0.05 mL of different concentrations of plant extracts in distilled water. The pH was maintained at 6.3. Those mixtures were incubated for 20 min at 37°C and heated in an oven for 30 min at 57°C. After that, the reaction mixtures were removed from the oven and cooled at room temperature. After cooling down, into the test tubes containing solution,phosphate buffer saline (pH 6.3) was added and the absorbance of the solutions was measured by using a UV-Visible spectrophotometer at 660 nm. Distilled water was used as a control. Ibuprofen was used as a reference drug (50, 100, 200, 400, 800, 1600, 3200 and 6400 µg/mL). The reference and control solutions were prepared similarly as for the test sample, in the absence of the methanolic extract. Percentage inhibition of the protein denaturation was computed according to the following equation:

$$\% \text{ Inhibition} = \frac{100 - (\text{Absorbance of test sample} - \text{Absorbance of reference control})}{\text{Absorbance of test sample}} \times 100$$

In vivo anti-inflammatory activity

Experimental animals

Sprague Dawley rats of either sex (100-200 g) have been used in this study. These animals were purchased from the University of Sargodha,

Pakistan and acclimatized to the laboratory environment. The experimental animals were feed regularly with standard diet and water ad libitum. The protocols of this study were authorized by the Institutional Ethical Committee of University of Sargodha, Sargodha. Experimental animals were used and sacrificed according to the international procedures stated in Guide for the Care and Use of Laboratory Animals, 8th edition.

Experimental design

Animals were distributed into 4 groups randomly (n = 5). Group I was served as a negative control that received distilled water orally. Group II and III were used as a test group treated with AMEPP at a dose of 250 and 500 mg/kg bw orally, respectively. Group IV received standard drug ibuprofen (20 mg/kg bw) orally and was considered as a standard group.

Carrageenan-induced paw edema in rats

The *in vivo* anti-inflammatory effect of AMEPP was evaluated by using carrageenan-induced paw edema model**.** The animals were then treated with their respective test samples. One-hour post-treatment, 0.1 mL carrageenan (1% w/v) was injected to animals in their sub-planter surface of the right hind paw to induce an edematous inflammation. The volume of right hind paw of each rat was measured with the help of a digital plethysmometer before the carrageenan injection (0 h) and at 1, 2, 3 and 4 hours after the administration of carrageenan. The edema was measured by subtracting the basal paw volume (Vo) from the inflamed paw volume in every hour by using the following formula;

$$\text{Inflammation (mL)} = Vt - Vo$$

Based on the volume of inflammation (oedema) for the control and test groups, the percentage inhibition was calculated according to the formula given below [13]:

$$\% \text{ Inhibition} = \frac{\text{Inflammation (mL) of NC} - \text{Inflammation (mL) of test group}}{\text{Inflammation (mL) of NC}} \times 100$$

Egg albumin-induced inflammatory model

Animals have fasted for 24 hours before starting the experiment. Egg albumin (0.1 mL) was injected to animals into the sub plantar surface of their right hind paw one-hour post-treatment. After that, volume of the paw of each animal was measured using a digital plethysmometer at zero (before injection of egg albumin), 1, 2, 3 and 4 hours after the injection with the inflammatory agent. Results were described as an increase in the size of paw volume (inflammation) and the percentage protection effect of AMEPP was calculated using the equation below [13]:

$$\text{Inflammation (mL) of each group} = Vt - Vo$$

$$\% \text{ Inhibition} = \frac{\text{Inflammation (mL) of NC} - \text{Inflammation (mL) of test group}}{\text{Inflammation (mL) of NC}} \times 100$$

Statistical analysis

GraphPad Prism version 8 was used to analyze the data. For the *in vivo* activity, two-way ANOVA followed by Bonferroni's *posthoc* test was used for multiple comparisons. The significance level $p < 0.05$ was considered statistically significant.

Results and Discussion

Phytochemical screening

Alkaloids, tannins, saponins, phenolic compounds, proteins, carbohydrates, gums, and mucilages were detected in the AMEPP whereas, glycoside, fixed oils and fats were not detected as presented in Table I. *Effect of P. plebeium extract on protein denaturation by using egg albumin*

In vitro anti-inflammatory effect of AMEPP was evaluated at various concentrations (6400, 3200, 1600, 800, 400, 200, 100 and 50 µg/mL). The plant extract inhibited egg albumin protein denaturation (72.99 ± 0.58) at the highest concentration. The plant extract exhibited a highly significant and dose-dependent anti-inflammatory activity in egg albumin protein denaturation model as shown in Table II. The effect of AMEPP was comparable to ibuprofen at all concentration levels.

Table I

Preliminary phytochemical screening of aqueous methanolic extract of *P. plebeium*

No.	Phytochemicals	Present/Absent
1	alkaloids	+
2	tannins	+
3	saponins	+
4	Phenols	+
5	proteins	+
6	carbohydrates	+
7	gums	+
8	mucilages	+
9	glycosides	-
10	Fixed oils	-
11	fats	-

Present +, Absent -

Table II

Inhibitory potential of aqueous methanolic extract of *Polygonium Plebium* against egg albumin protein denaturation

Concentration (µg/mL)	Percentage inhibition by *P. plebeium*	Percentage inhibition by Ibuprofen
6400	72.99 ± 0.58	76.48 ± 0.32
3200	63.35 ± 0.70	65.22 ± 0.54

Concentration (µg/mL)	Percentage inhibition by *P. plebeium*	Percentage inhibition by Ibuprofen
1600	63.13 ± 0.94	64.01 ± 0.96
800	61 ± 0.39	60.99 ± 0.79
400	55.53 ± 0.53	59.16 ± 1.1
200	54.16 ± 0.26	58.91 ± 1
100	41.91 ± 0.59	43.12 ± 0.99
50	38.04 ± 0.91	36.99 ± 0.76

Data were expressed as mean ± S.D. of n experiments (n = 3)

Effect of P. plebeium on protein denaturation by using bovine albumin

Different concentrations of the plant extract and standard drug, ibuprofen, have shown a meaningful inhibitory effect ($p < 0.001$ compared to blank control) against bovine serum albumin denaturation (Table III). The study indicated that AMEPP has an anti-inflammatory effect against bovine serum albumin denaturation with a maximum inhibition (67.51 ± 0.58) observed at a concentration of 6400 µg/mL. The standard drug ibuprofen has shown an anti-inflammatory effect with 66.44% inhibition.

Table III

The inhibitory potential of aqueous methanolic extract of *Polygonium Plebium* against bovine serum protein denaturation

Concentration (µg/mL)	Percentage inhibition by *P. plebeium*	Percentage inhibition by Ibuprofen
6400	67.51 ± 0.58	66.44 ± 0.31
3200	63.75 ± 0.7	61.48 ± 0.54
1600	61.42 ± 0.94	60.22 ± 1.1
800	60.5 ± 0.39	58.99 ± 0.21
400	54.33 ± 0.53	54.16 ± 0.2
200	51.83 ± 0.26	50.33 ± 0.45
100	50.2 ± 0.19	46.18 ± 0.32
50	42.49 ± 1.15	40.48 ± 0.79

Data were expressed as mean±S.D. of n experiments (n = 3)

Effect of P. plebeium on carrageenan-induced paw edema

The AMEPP has shown an anti-inflammatory activity in carrageenan-induced paw edema in the rat model (Table IV) which was statistically significant. Oral treatment to rats with AMEPP has decreased the level of inflammation (paw volume) in all phases as compared to the negative control. The effect of the plant extract was elevasted with the increasing dose of the plant extract against carrageenan-induced inflammation. The anti-inflammatory effect of AMEPP at a dose of 250 mg/kg bw showed a reduction in paw volume, though it was insignificant ($p < 0.05$) one-hour post carrageenan injection compared to the negative control. The anti-inflammatory property of the AMEPP (500 mg/kg bw) against carrageenan-induced oedema was relatively higher than the effect of ibuprofen, even though it was insignificant ($p > 0.05$).

Table IV

Effect of the aqueous methanolic extract of *P. plebeium* on carrageenan induced paw oedema

Treatment	0 min (mL) (% inhibition)	30min (mL) (% inhibition)	60min (mL) (% inhibition)	90 min (mL) (% inhibition)	120 min (mL) (% inhibition)
Control	0.964 ± 0.01	1.094 ± 0.3	1.214 ± 0.02	1.246 ± 0.21	1.306 ± 0.31
Aqueous methanolic extract 250 mg/kg bw	0.992 ± 0.05 ns	1.14 ± 0.32 ns (-4.2)	1.074 ± 0.2 ns (11.53)	0.996 ± 0.97** (20.06)	0.972 ± 0.3* (25.57)
Aqueous methanolic extract 500 mg/kg bw	0.894 ± 0.2 ns (7.26)	0.848 ± 0.21** (22.48)	0.758 ± 0.09** (37.56)	0.712 ± 0.93*** (42.85)	0.670 ± 0.41*** (48.7)
Ibuprofen 40 mg/kg bw	0.922 ± 0.01ns (4.3)	0.874 ± 0.02** (20.1)	0.794 ± 0.08** (34.59)	0.752 ± 0.76** (39.64)	0.7 ± 0.01*** (46.40)

Results are expressed as means ± SEM (n = 5), * = ($p < 0.05$), ** = ($p < 0.01$), *** = ($p < 0.001$) and ns = non-significant when compared to control

Effect of P. plebeium on egg albumin-induced inflammatory model

The anti-inflammatory effect of AMEPP against egg albumin-induced oedema has been presented in Table V. The AMEPP (250 and 500 mg/kg bw) ($p < 0.001$) reduced the swelling by egg albumin at one, two, three, and four hours after inflammation. The AMEPP (500 mg/kg bw) showed the maximum inhibitory effect at 4 h which was 48.69% after induction of inflammation by egg albumin.

Table V

Effect of aqueous methanolic extact of *P. plebeium* on egg albumin-induced paw oedema

Treatment	0 min (mm) (%inhibition)	30min (mm) (%inhibition)	60min (mm) (%inhibition)	90 min (mm) (%inhibition)	120 min (mm) (%inhibition)
Control	1.128 ± 0.02	1.366 ± 0.01	1.622 ± 0.31	1.582 ± 0.01	1.570 ± 0.31
Aqueous methanolic extract 250 mg/kg	1.098 ± 0.12ns (2.65)	1.340 ± 0.6 ns (1.9)	1.364 ± 0.61* (15.9)	1.252 ± 0.41* (20.85)	1.028 ± 0.5** (34.52)
Aqueous methanolic extract 500 mg/kg	1.020 ± 0.03ns (9.57)	1.182 ± 0.97 ns (13.46)	1.094 ± 0.34** (32.55)	1.026 ± 0.43** (35.14)	0.932 ± 0.39*** (40.63)
Ibuprofen 40 mg/kg	1.010 ± 0.02ns (10.46)	1.144 ± 0.93* (16.25)	1.012 ± 0.91* (37.60)	1.016 ± 0.31** (35.77)	0.91 ± 0.73*** (40.03)

Results are expressed as means ± SEM (n = 5), *** = ($p < 0.001$) and ns = non-significant when compared to control

The present study has been conducted to evaluate the anti-inflammatory effect of AMEPP by using the *in vitro* and *in vivo* techniques. According to the phytochemical screening performed, the aqueous methanolic extract of *P. plebeium* has comprised different phytochemicals such as saponins, alkaloids, phenols, proteins, gums, carbohydrates, tannins, and mucilages as shown in Table I. Phytochemical studies on the genus *Polygonum* has shown the presence of alkaloids, steroids, phenols, flavonoids, carbohydrates, tannins, sesquiterpenes, and glycosides and this class of compounds was reported to have an anti-inflammatory activity [14, 15]. Alkaloids have a variety of pharmacological activities particularly anti-proliferative as well as anti-inflammatory properties [16]. Since the major reason behind inflammation is the destruction of cells by the species of the reactive oxygen, phenolic compounds might have an outstanding radical scavenging features. Plants with the phenolic compounds have been seen to have a protective role in inflammatory conditions. *P. plebeium* contains quercetin, a strong anti-inflammatory polyphenolic compound, as reported previously [11, 17].

In the *in vitro* study, AMEPP showed inhibitory potential against egg albumin denaturation (72.99% inhibition) and bovine albumin denaturation (67.51% inhibition). This inhibition was dose-dependently and comparable to ibuprofen as shown in Table II and III. Mostly, protein denaturation is associated with inflammation to damaged tissue and eventually causes arthritis [18].

The anti-inflammatory effect of *P. plebeium* was assessed by using carrageenan and albumin-induced paw oedema models. Carrageenan-induced paw edema model is among the most reliable model which is adequate and widely used animal model to assess the anti-inflammatory activity of the phytochemicals. Carrageenan-induced swelling is an inflammatory model of an acute condition in which there is a release of inflammatory markers that cause different signs ad symptoms of inflammation [19, 20]. The carrageenan-induced inflammation presents two separate phases of inflammation. In the first phase (first hour of inflammation), serotonin and histamine are liberated whereas in the second phase there is a release of prostaglandins after the first hour [21]. The AMEPP has significantly inhibited paw oedema (25.57% and 48.70%) at both doses as shown in Table IV. Blockage of the synthesis and release of these inflammatory mediators may be responsible for the anti-inflammatory effect of AMEPP.

The anti-inflammatory activity of the AMEPP was also performed in egg albumin-induced inflammatory model. The aqueous methanolic extract has exhibited a statistically significant reduction (34.52% and 40.63%) of the a paw volume at doses 250 and 500 mg/kg bw, respectively as presented in Table V. The reduction in egg albumin-induced edema observed in the present study might explain the suppression of the synthesis, release and/or events of inflammatory mediators by the AMEPP. Histamine is a vasodilator that is responsible for the redness of a body during inflammation [22, 23].

Conclusions

It is concluded that the AMEPP has a promising anti-inflammatory effect which could be disbursed to the presence of the secondary metabolites. This study may justify and support the conventional use of *P. plebeium* to treat inflammatory conditions. It becomes mandatory to conduct further studies to identify and isolate the phytochemicals responsible for the anti-inflammatory activity and to elucidate their exact mechanism of action.

Conflict of interest

The authors declare no conflict of interest.

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