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Polygala anatolica Boiss. et Heldr.: Is A Potential Remedy for Inflammation and Pain?

[*Polygala anatolica Boiss. et Heldr*.: ¿Es un remedio potencial para la inflamación y el dolor?]

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Abstract: Species of *Polygala* genus have been used for the treatment of inflamation and pain in Turkish traditional medicine. The aim of the present study is to assess the anti-inflammatory and analgesic activities of *P. anatolica*. n-Hexane, ethyl acetate and methanol extracts of the aerial parts and roots of *P. anatolica* were investigated for their anti-inflammatory and analgesic effects. The methanol extracts prepared from the aerial parts and roots of *P. anatolica* were found to be active in carrageenan- and PGE_2-induced paw edema models and in Whittle method. Methanolic extract of the aerial part inhibited serotonin-induced hind paw edema, while the root extract did not exert inhibitory effect in the same model. In addition, Fr. B and C obtained from the methanol extract of *P. anatolica* aerial parts showed significant anti-inflammatory activity. Morover, the analgesic effect of the methanol extracts prepared from the roots and aerial parts and Fr.B and Fr.C were found to be statistically significant without inducing ulceration. The methanol extract obtained from the aerial parts of the plant and its saponoside and flavonoid fractions showed anti-inflammatory and analgesic activities in the trials.

Keywords: *Polygala anatolica*; Polygalaceae; Anti-inflammatory; Analgesic

Resumen: Las especies del género *Polygala* se han utilizado para el tratamiento de la inflamación y el dolor en la medicina tradicional turca. El objetivo del presente estudio es evaluar las actividades antiinflamatorias y analgésicas de *P. anatolica*. Se investigaron los extractos de n-hexano, acetato de etilo y metanol de las partes aéreas y raíces de *P. anatolica* por sus efectos antiinflamatorios y analgésicos. Los extractos de metanol preparados a partir de las partes aéreas y raíces de *P. anatolica* se encontraron activos en modelos de edema de pata inducidos por carragenina y PGE_2 por el método de Whittle. El extracto metanólico de la parte aérea inhibió el edema de la pata trasera inducido por serotonina, mientras que el extracto de raíz no ejerció un efecto inhibidor en el mismo modelo. En suma, la fracción B y C obtenidos a partir del extracto metanólico de partes aéreas de *P. anatolica* mostraron actividad antiinflamatoria significativa. Además, el efecto analgésico de los extractos de metanol preparados a partir de las raíces y las partes aéreas y la fracción B y C resultaron ser estadísticamente significativas sin inducir la ulceración. El extracto de metanol obtenido de las partes aéreas de la planta y sus fracciones de saponósidos y flavonoides mostraron actividades antiinflamatorias y analgésicas en los ensayos.

Palabras clave: *Polygala anatolica*; Polygalaceae; Antiinflamatorio; Analgésico

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INTRODUCTION

Polygala is a large genus and has a widespread distribution. Its species have been commonly used in traditional medicine, thereof. Twelve Polygala species namely, *Polygala anatolica* Boiss. et Heldr., *P. alpestris* (Rchb.), *P. comosa* Schkuhr., *P. major* Jacq., *P. monspeliaca* L., *P. papilionaceae* Boiss., *P. pruinosa* Boiss., *P. stocksiana* Boiss., *P. supina* Schreb., *P. venulosa* Sibth. & Sm., *P. vulgaris* L. and *P. transcaucasia* Tamamsch. are growing wild in Turkey (Cullen, 1965). In the ethnobotany studies, various *Polygala* species were determined to be utilized for their therapeutic features among people living in Anatolia. The roots of *P. pruinosa* subsp. *pruinosa* have been used as expectorant, galactagogue, diaphoretic and tonic in Elazığ province (Çakılcıoğlu *et al.,* 2007). The decoction prepared from the aerial parts of *P. anatolica* has been used to releive edema, as expectorant, galactagogue and diuretic in the west Anatolia (Honda *et al.,* 1996; Deniz *et al.,* 2010).

Due to the widespread distribution, *Polygala* species have also been frequently used in traditional medicines worldwide. *P. japonica* Houtt. has been used for the treatment of acute tonsillitis, pharyngitis, pulmoner tuberculosis, esophageal cancer and pertussis; as sedative, expectorant and tonic in China (Harvey, 2008; Schmidt *et al*., 2008; Li *et al*., 2012). The roots of *P. tenuifolia* Willd. are utilized as tranquilizer and sedative and for the treatment of dementia and neurasthenia (Li *et al*., 2008; Kim *et al*., 2013). In Japan, the roots of the same plant are used as sedative, diuretic and expectorant as well as for the treatment of amnesia and inferitility (Chopra, 1956). *P. sibirica* L. is used as tonic for sedative and expectorant purposes, (Song *et al*., 2013); the roots of *P. chinensis* L. are used as antipyretic in China (Chopra, 1956). The decoction prepared from the root and branch barks of *P. arillata* Buch.-Ham. is used to treat diarrhea in Thailand (Suksri *et al*., 2005); root decoction of *P. glomerata* Lour. in the treatment of various inflammatory diseases (Chopra, 1956); *P. paenea* L. as diuretic and expectorant in India (Polonsky *et al*., 1960); the roots of *P. elongata* Klein ex Willd. and *P. crotalarioides* Buch.-Ham. ex DC. for the treatment of gall bladder diseases and constipation and in the treatment of snake bites (Chopra, 1956). The roots of *P. senega* L. are used against cough, chronic bronchitis, pharyngitis and rattlesnake bites by the north Americans (Lin *et al*., 2005; Li *et al*., 2006; Wu *et al*., 2008). In central America *P. rosmarinifoli* Wight & Arnin is used against snake bites and as expectorant (Alagammal *et al*., 2013); *P. cyparissias* St. Hillaire & Moquin, growing wild in Brazil's Atlantic coast, is utilized for antiaging (Zhang *et al.,* 1994), aginst rheumatic pain, intestinal and kidney disorders and cancer (Stevenson & Weber, 1991; de Campos *et al*., 1997); *P. spectabilis* roots in the treatment of hemorrhoids and amoebic infection (Andrade *et al*., 1977); decoction prepared from the aerial parts of *P. amara* L. and *P. vulgaris* L. for the treatment of urinary tract disorders and as galactagogue in central Europe.

Previous phytochemical studies on *Polygala* species revealed that these species are rich in antrhacenes, oligosaccharides, flavonoids, xanthones, coumarins and saponins (Andrade *et al*., 1977; Lin *et al.,* 2005; Song *et al*., 2013). In this study, it was intended to explain the availability of *P. anatolica* in phytotherapy in terms of its potential anti-inflammatory and analgesic effects by using *in vivo* analgesic and anti-inflammatory activity models.

MATERIALS AND METHODS

Plant material

Aerial parts and roots of *Polygala anatolica* Boiss. et Heldr. were collected from Tulumtaş village, Gölbaşı, Ankara, Turkey in June 2013. A voucher specimen authenticated by Prof.Dr. Hayri Duman (Department of Biology, Faculty of Science, Gazi University) is deposited at the Herbarium of the Faculty of Pharmacy, Gazi University (GUE 3234).

Extraction and Fractionation

Preparation of the extracts

An amount of 500 g of shade dried and powdered aerial parts and roots of the plant was subjected to successive solvent extractions with *n*-hexane, ethyl acetate (EtOAc) and methanol (MeOH) at room temperature for 48 h (6 x 5 L). After filtration, the extracts were evaporated by using a rotary evaporator (Buchi, Switzerland) at 40° C to dryness *in vacuo*. Yields of the aerial part and root extracts were 2.65% for *n*-hexane, 20.08% for EtOAc and 46.85% for MeOH; 5.02% for *n*-hexane 17.23% for EtOAc and 43.18% for MeOH, repectively.

Fractionation of the methanol extract

Two grams of the fraction was subjected to chromatographic separation in Silica gel column (Silica gel 70-230 mesh, 60 A°, Merck Art. 7734) using $CHCl_3$/MeOH (99:1); (98:2); (97:3); (96:4); (95:5); (90:10); (85:15); (80:20); (75:25); (70:30); (60:40) eluent systems and eluents were combined as follows after TLC control using $CHCl_3$/Gl. acetic acid/MeOH/H_2O (60:32:12:8) as mobile phase: [Fr. A] (72.3 mg), [Fr. B] (315.8 mg) and [Fr. C] (254.7

mg).

Biological activity tests

Animals

Male Swiss albino mice weighing 20–25 g were purchased from Laboratory of Experimental Animals, Kobay, Turkey. The animals were left for 3 days at room conditions for acclimatization and maintained on standard pellet diet and water *ad libitum* throughout the experiment. A minimum of six animals were used in each group. Throughout the experiments, animals were processed according to the suggested European ethical guidelines for the care of laboratory animals. The present study was performed according to the international rules considering the animal experiments and biodiversity rights (Gazi University Ethical Council Project Number: G.U.ET-14.79).

Preparation of test samples for bioassay

Test samples were given orally to test animals after suspending in a mixture of distilled water and 0.5% sodium carboxymethyl cellulose (CMC). The control group animals received the same experimental handling as those of the test groups except that the drug treatment was replaced with appropriate volumes of the dosing vehicle. Either indomethacin (10 mg/kg) or acetyl salicylic acid (ASA) (100 mg/kg) in 0.5% CMC was used as reference drugs.

Anti-inflammatory activity

The anti-inflammatory activity was assessed using carrageenan-, prostaglandin E_2 (PGE_2)-, and serotonin-induced hind paw edema and acetic acid–induced capillary permeability in the mice.

Carrageenan-induced hind paw edema

For the determination of the effects on acute inflammation, carrageenan-induced paw edema model was employed with some modifications (Kasahara *et al*., 1985). Sixty minutes after the oral administration of either test sample or dosing vehicle, each mice was injected with freshly prepared (0.5 mg/25µl) suspension of carrageenan (Sigma, St. Louis, Missouri, USA) in physiological saline (154 mM NaCl) into subplantar tissue of the right hind paw. As the control, 25 µl saline solution was injected into that of the left hind paw. Paw edema was measured in every 90 min during 6 h after induction of inflammation. The difference in footpad thickness between the right and left foot was measured with a pair of dial thickness gauge callipers (Ozaki Co., Tokyo, Japan). Mean values of treated groups were compared with mean values of a control group and analyzed using statistical methods.

Serotonin-induced hind paw edema

The method of Kasahara *et al*. (1985) was used. Sixty minutes after the oral administration of test sample or dosing vehicle each mouse was injected with serotonin (serotonin creatinin sulfate, Merck, Art. 7768) in Tyrode's solution (0.5 µg/5µl) into subplantar tissue of the right hind paw and 5 µl of Tyrode's solution into that of the left as secondary control. Measurements were done and evaluated as described above in every 6 min during 30 min.

Acetic acid-induced increase in capillary permeability

Effect of the test samples on the increased vascular permeability induced by acetic acid in mice was determined according to Whittle method with some modifications (Yeşilada, 1991). Each test sample was administered orally to a group of 10 mice in 0.2 ml/20 g body weight. Thirty minutes after the administration each mice was injected with 0.1 ml of 4% Evans blue (Sigma, St. Louis, Missouri, USA) in saline solution (iv.) at the tail. Then, 10 min after the iv. injection of the dye solution, 0.4 ml of 0.5% (v/v) AcOH was injected ip. After 20 min, the mice were killed by dislocation of the neck, and the viscera were exposed and irrigated with distilled water, which was then poured into 10 ml volumetric flasks through glass wool. Each flask was made up to 10 ml with distilled water, 0.1 of 0.1 N NaOH solution was added to the flask, and the absorption of the final solution was measured at 590 nm (Beckmann Dual Spectrometer). In control animals, a mixture of distilled water and 0.5% CMC was given orally, and they were treated in the same manner as described above.

PGE_2-induced hind paw edema model

PGE_2-induced hind paw edema model was used for determination of anti-inflammatory activity (Kasahara *et al*., 1985). The difference in footpad thickness between the right and left foot was measured with a pair of dial thickness gauge calipers (Ozaki Co., Tokyo, Japan). Mean values of treated groups were compared with mean values of a control group and analyzed using statistical methods. 60 min after the oral administration of test sample or dosing vehicle, each mouse was injected with freshly prepared (5 µg/5µl) suspension of PGE_2 (Fluka Chemie AG, Art. 82475) in Tyrode's solution into subplantar tissue of the right hind paw. As the control, 5 µl Tyrode's solution was injected into that of the left hind paw. Paw edema was

measured in every 15 min during 75 min after induction of inflammation. The difference in footpad thickness was measured by a gauge calipers (Ozaki Co., Tokyo, Japan). Mean values of treated groups were compared with mean values of a control group and analyzed using statistical methods. Indomethacin (10 mg/kg) was used as the reference drug.

Analgesic activity

P-benzoquinone-induced abdominal constriction test was used to evaluate the analgesic activity in mice.

p-Benzoquinone-induced abdominal constriction test

An hour after orally administering *D. vulgaris* fruit extracts or the reference agent ASA (100 mg/kg) to different groups of mice, a 2.5% *p*-benzoquinone solution dissolved in distilled water was injected intraperitoneally to produce pain at a dose of 0.1 ml/10 g. After 5 min, the writhing reflex response of each animal was counted for 15 min. The inhibition in reflex response was statistically evaluated (Okun *et al.*, 1963; Yesilada & Küpeli, 2007).

Statistical analysis of data

Data obtained from the animal experiments were expressed as the mean ± standard error of mean. Statistical differences between the treatment and control groups were calculated using analysis of variance and Students–Newman–Keuls post hoc tests. A probability of $p<0.05$ was considered to be significant (* $p<0.05$; ** $p<0.01$; *** $p<0.001$).

RESULTS

Carrageenin-induced paw edema model was selected as *in vivo* experimental model for broad spectrum of anti-inflammatory activity determination (Winter *et al*., 1968). As shown in Table No. 1, extracts of the aerial parts and roots of the plant demonstrated comparable anti-inflammatory effect with the reference drug indomethacin. The methanol extract obtained from the aerial parts was selected as the material for further biological activity analyses due to its higher efficacy over the methanol extract obtained from the roots of the plant. Two extracts were found to have similar groups of compounds in TLC analysis.

As a result of fractionation by column chromatography three subfractions were obtained from the methanol extract. When the obtained subfractions were applied to the experimental animals, Fr.A was found to be ineffective in the carrageenin-induced paw edema model; Fr.B (24.1% inhibition) and Fr.C (26.9% inhibition) were found to have siginifcant activity close to methanol extract (Table No. 1). TLC analysis on the fractions that were employed in the BAGF assays revealed that Fr.B and Fr.C were determined to have high saponin and flavonoid contents, respectively.

In order to verify the results of carrageenin edema model, plant extracts and fractions were applied on the other experimental models of inflammation. In "Whittle Method", based on acetic acid-induced inhibition of increased capillary permeability, methanol extracts of the aerial parts and roots of the plant were found to have significant anti-inflammatory activity, however, the other extracts devoid of such effect. Fr.B and Fr.C obtained from the aerial part-methanol extract, were found to provide high rates of inhibition (Figure No. 1).

To verify the findings in carrageenin-induced hind paw edema model, other anti-inflammatory activity determination method, the "serotonin-induced hind paw edema" model was used. As shown in Table No. 2, serotonin edema model outcome supported the results obtained in the carrageenan edema model. Therefore, the findings showed that both aerial part and root extracts in various polarity were not effective in this model.

In the carrageenan edema model, edema formation is considered as a 2-stage phase. In the first hour, edema occurs depending on the trauma injury and is characterized by serotonin and histamine release. Second stage of three hours or longer, prostaglandin is primarily responsible for the development of edema. This study showed that methanol extracts prepared from the aerial parts and roots of the plant significantly inhibited edema formation at periods when the prostaglandin release occured. This effect was close to the reference drug, indomethacin, a prostaglandin biosynthesis inhibitor. Our study revealed that ehanol extract and subfractions, Fr.B and Fr.C have compounds directly responsible from the anti-inflammatory activity.

As it is known, anti-inflammatory effects can arise in many different ways. Especially in the elucidation of the mechanism of action of the compounds, inhibitory effects on the various enzymes and mediators give valuable insight (Singh *et al.*, 1987). Prostaglandins occurred via cyclooxygenase pathway are the most important mediators of inflammation in the body (Yeşilada, 1991). Nonsteroidal anti-inflammatory drugs such

as aspirin shows its effect by inhibiting the synthesis of prostaglandins. In order to determine the role of prostaglandins, anti-inflammatoy effect of the extracts and farctions were invesitgated by using "PGE_2- induced hind paw edema" model. In this model, the methanol extracts and its fractions, Fr.B and Fr.C., were found to have equivalent activity to that of indomethacin, which supported the results of carrageenan-induced hind paw edema model (Table No. 3).

Table No. 1

Preliminary anti-inflammatory activity assessment of extracts of *P. anatolica* aerial parts (AE) and roots (R) and subfractions of the MeOH extract of prepared from *P. anatolica* aerial parts using carrageenan-induced paw edema model in mice

Material	Parts used	Dose mg/kg	Swelling thickness (x 10-2mm) ± SEM (Inhibition %)			
			90 min	180 min	270 min	360 min
Control			47.5±2.8	50.9±3.1	58.6±4.2	61.7±3.9
***n*-Hexane extract**	AE	100	51.4±3.7	54.8±4.0	59.2±3.6	64.8±4.3
	R	100	46.4±3.0 (2.3)	49.6±3.3 (2.6)	59.1±3.8	62.2±4.1
EtOAc extract	AE	100	46.1±2.8 (2.9)	44.2±3.3 (13.2)	59.1±4.3	64.6±3.7
	R	100	43.5±3.8 (8.4)	45.9±4.2 (9.8)	53.6±5.1 (8.5)	62.8±5.2
MeOH extract	AE	100	39.6±2.5 (16.6)	41.5±2.1 (18.5)	44.3±2.7 **(24.4)*,**†	42.8±2.4 **(30.6)**,**†
	R	100	40.3±3.0 (15.2)	43.7±2.8 (14.1)	46.2±3.2 (21.1)	45.3±2.9 **(26.6)*,**†
Indomethacin		10	37.4±2.3 (21.3)	37.8±2.0 **(25.7)***	39.5±2.4 **(32.6)****	36.2±2.5 **(41.3)*****
Control			39.7±2.3	43.5±2.8	45.9±2.1	49.7±2.4
Fr. A		100	42.5±3.2	44.8±3.5	49.6±4.2	51.9±4.8
Fr. B		100	35.7±2.1 (10.1)	38.3±1.8 (11.9)	35.4±2.2 (22.9)	37.7±1.9 **(24.1)*,** †
Fr. C		100	32.3±1.7 (18.6)	36.1±2.0 (17.0)	34.2±2.1 **(25.4)*,** †	36.3±1.7 **(26.9)**,** †
Indomethacin		10	30.1±2.0 **(24.2)***	33.5±1.6 **(22.9)***	31.3±1.9 **(31.8)****	31.4±2.1 **(36.8)*****

Compared with respect to control, values are mean ± SEM (n=6), * p<0.05. **p<0.01. *** p<0.001. ns p<0.05; Compared with respect to control, values are mean ± SEM (n=6), † p<0.05. ††p<0.01. ††† p<0.001. ns p<0.05. Data were analyzed by ANOVA followed Dunnett's multiple comparison t-test; AE: Aerial part; R: Root

Increase in body temperature and pain are among the most obvious symptoms observed in case of inflammation (Yeşilada, 1991). Pain is usually interpreted as a marker of inflammation. The occurrence of chronic, non-inflammatory pain syndromes is higher amongst patients with inflammatory diseases than in the population (Lee, 2013). Goldring reported that increase of inflammatory markers would rise pain (Goldring & Otero, 2011). Similarly, Omoigui based pain theory offered that the origin of all pains are inflammation and tissue injury stimulates inflammatory markers that leads to changes in markers (Omoigui, 2007). It is therefore ideal for an active compound to have the effects of both anti-inflammatory and analgesic (Kasahara *et al.*, 1985). In our study, the analgesic effect of the extracts and fractions were investigated by using "*p*-benzoquinone-induced pain test" and the methanol extracts prepared from the roots and aerial parts of the plant and Fr.B and Fr.C were found to be statistically significant without inducing ulceration (Figure No. 2).

Table No. 2
Effects of the extracts of P. anatolica aerial parts (AE) and roots (R) on serotonin-induced paw edema in mice

			Swelling thickness (x 10-2mm) ± SEM (Inhibition %)					
Material	**Parts used**	**Dose mg/kg**	**0 min**	**6 min**	**12 min**	**18 min**	**24 min**	**30 min**
Control			3.9±1.3	11.6±3.7	24.8±3.4	32.9±2.5	34.1±2.9	39.9±4.2
***n*-Hexane extract**	AE	100	4.1±1.7	14.2±2.3	27.9±3.0	34.1±3.3	40.2±3.4	43.3±3.8
	R	100	4.0±1.9	16.2±3.9	29.6±5.7	39.1±4.3	43.0±4.8	49.6±5.7
EtOAc extract	AE	100	3.9±1.6	17.2±3.8	28.5±3.1	37.4±4.2	40.8±5.1	45.7±5.4
	R	100	3.8±1.1	13.4±3.2	24.9±4.2	35.0±3.7	39.6±3.2	41.1±5.0
MeOH extract	AE	100	4.0±1.2	11.9±2.3	21.7±2.9 (12.5)	29.9±3.4 (9.1)	35.4±3.8	41.0±4.7
	R	100	3.9±1.3	12.6±3.9	20.2±3.1 (18.5)	34.8±3.7	35.6±4.1	42.4±4.5
Indomethacin		10	3.9±1.1	10.2±2.4 (12.1)	20.3±2.0 (18.1)	25.1±2.2 **(23.7)***	23.7±2.4 **(30.5)****	28.1±2.5 **(29.6)***

Compared with respect to control, values are mean ± SEM (n=6), * p<0.05. **p<0.01. *** p<0.001. ns p<0.05; Compared with respect to control, values are mean ± SEM (n=6), † p<0.05. ††p<0.01. ††† p<0.001. ns p<0.05. Data were analyzed by ANOVA followed Dunnett's multiple comparison t-test; AE: Aerial part; R: Root

Table No. 3
Effects of the extracts of P. anatolica aerial parts (AE) and roots (R) and subfractions of the MeOH extract of prepared from P. anatolica aerial parts on PGE2-induced paw edema in mice

			Swelling thickness (x 10-2mm) ± SEM (Inhibition %					
Material	**Parts used**	**Dose mg/kg**	**0 min**	**15 min**	**30 min**	**45 min**	**60 min**	**75 min**
Control			1.9±0.4	11.6±1.1	14.0±1.5	18.4±1.3	15.2±1.4	10.8±1.7
***n*-Hexane extract**	AE	100	2.1±0.9	12.7±1.4	14.6±1.9	19.7±2.2	17.8±2.1	13.5±2.5
	R	100	2.2±1.3	14.5±2.0	16.8±3.1	19.7±3.4	20.8±2.7	19.6±2.3
EtOAc extract	**EtOAc extract**	AE	100	2.4±1.1	14.6±1.2	11.5±1.5 (17.9)	15.6±1.7 (15.2)	18.1±1.8
		R	100	2.0±0.8	10.4±1.1 (10.3)	13.2±1.3 (5.7)	19.1±1.1	21.2±1.9
MeOH extract	AE	100	1.8±0.7 (5.2)	10.2±1.1 (12.1)	9.5±0.4 **(32.1)**, ns**	12.2±0.7 **(33.7)**, ns**	9.8±0.6 **(35.5)**, ns**	9.4±0.9 (12.9)
	R	100	1.9±0.7	9.4±1.2 (18.9)	10.1±1.3 **(27.9)*,** †	13.2±1.1 **(28.2)*,** †	10.3±0.9 **(32.2)**,** †	9.1±1.0 (15.7)
Indomethacin		10	1.8±0.9 (5.2)	8.3±1.1 **(28.4)***	9.2±1.0 **(34.3)****	11.3±0.8 **(38.6)*****	9.4±0.8 **(38.2)*****	9.7±1.3 (10.2)
Fr. A		100	1.6±0.5	11.8±1.1	15.7±1.5 (6.5)	19.9±1.8	15.4±0.9 (9.9)	14.2±1.3
Fr. B		100	1.4±0.6 (12.5)	10.7±0.9	11.2±1.1 **(33.3)**,** †	15.3±1.4 (20.7)	12.8±0.7 **(25.1)**,** †	12.2±1.1 (10.3)
Fr. C		100	1.9±0.8	9.7±1.0 (4.9)	11.6±0.9 **(30.9)**,** †	14.8±0.7 **(23.3)*,** ††	12.1±0.8 **(29.2)**,** ††	11.9±1.2 (12.5)
Indomethacin		10	1.7±1.1	8.2±1.3 (19.6)	10.4±0.7 **(38.1)*****	11.3±1.0 **(41.5)*****	10.4±0.8 **(39.2)*****	9.7±0.5 **(28.7)****

Compared with respect to control, values are mean ± SEM (n=6), * p<0.05. **p<0.01. *** p<0.001. ns p<0.05; Compared with respect to control, values are mean ± SEM (n=6), † p<0.05. ††p<0.01. ††† p<0.001. ns p<0.05. Data were analyzed by ANOVA followed Dunnettís multiple comparison t-test; AE: Aerial part; R: Root

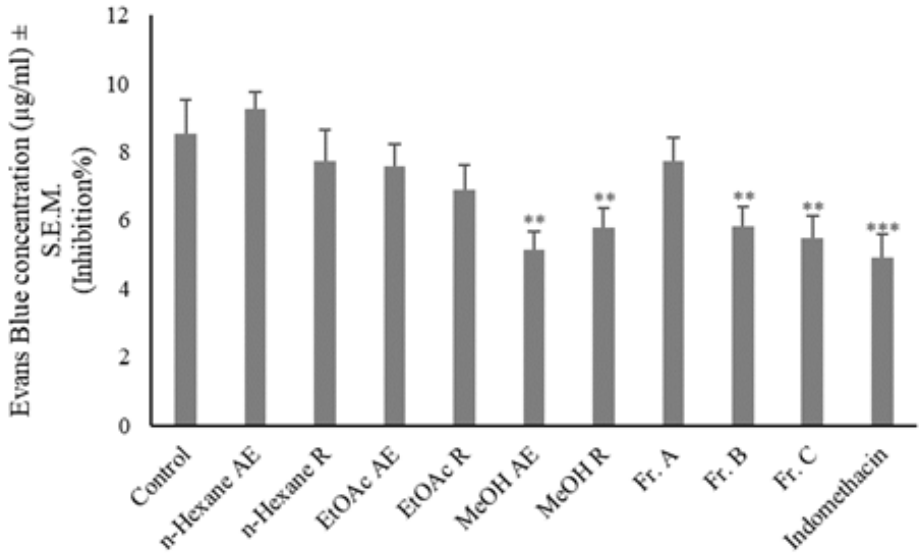

Figure No. 1
Effects of the extracts of *P. anatolica* aerial parts and roots and subfractions of the MeOH extract of prepared from *P. anatolica* aerial parts on increased vascular permeability induced by acetic acid in mice

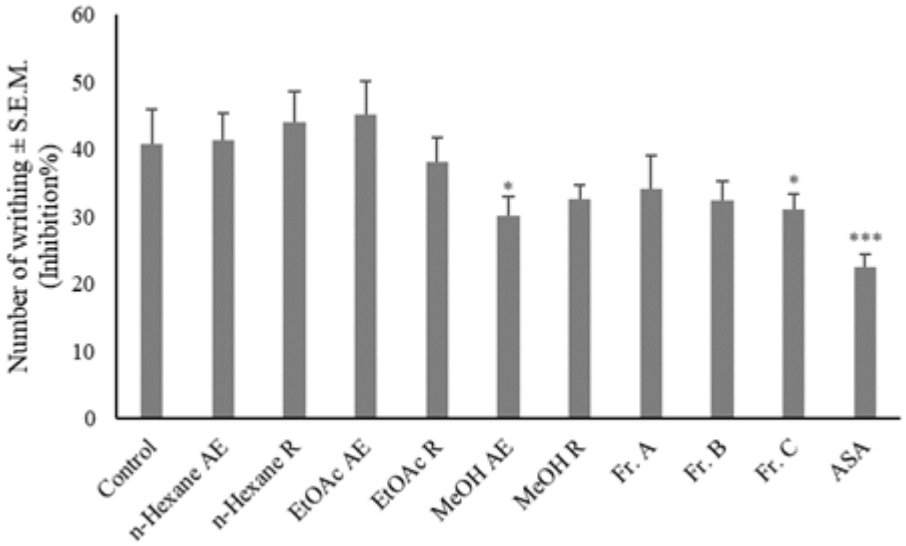

Figure No. 2
Analgesic effects of the extracts of *P. anatolica* aerial parts and roots and subfractions of the MeOH extract of prepared from *P. anatolica* aerial parts

DISCUSSION

Polygala species are important and popular plants used in folk medicine as well as in the modern medical system. *P. senega* and *P. tenuifolia* roots, registered in various pharmacopoeia monographs as officinal species, are used as expectorants due to their secretion enhancing effects; to treat inflammation of the mucous membrane, chronic bronchitis and as tranquilizer and sedative in case of insomnia (Samuelsson, 2004; Sun *et al.*, 2007).

In ethnobotanical field studies conducted in our country, it has been reported that different species of *Polygala* have been used for the treatment of various diseases in Anatolia. *P.*

anatolica, the plant material used in the present study, was reported to be used as expectorant, galactagogue, diuretic and diaphoretic as well as to treat inflammation when prepared as decoction from the aerial parts in Afyon, Kütahya, Denizli, Muğla and Aydın and Uşak Provinces (Honda *et al*., 1996; Deniz *et al*., 2010). In the present study, anti-inflammatory and analgesic effects of the aerial parts and roots of *P. anatolica*, one of the *Polygala* species widespread in Turkey, were investigated in order to explain the usage of the traditional medicine and to emphasize its importance in phytotherapy.

COX-1 inhibitors such as nonsteroidal anti-inflammatory drug, aspirin, causes gastric ulceration, while COX-2 inhibitors, do not inhibit the gastric mucosa protective effect of the prostaglandins (PGE 1) (Christopher, 1995). In our study, the observed lesions and ulcers in the stomachs of mice treated with the extracts and fractions also supports the idea of inhibition of prostaglandins.

Several studies were conducted on *Polygala* species that were used for the treatment of various inflammatory diseases. In a previous study conducted by El Sayah *et al*. (1999), it was reported that aqueous ethanol extracts of *P. cyparissias* non-competitively inhibited the inhibitory mediators, histamine, bradykinin, PGE-2 and thromboxane A2 in guinea pig trachea (El Sayah *et al*., 1999). Zhang *et al*. (2008) isolated the compound, reinioside C from *P. fallax* and reported that the mentioned compound inhibited the asymmetric dimethylarginine-induced TNF-α production in monocytes and weakened the the formation of reactive oxygen species and NF-kB activity (Zhang *et al*., 2008). Bai *et al*. (2009) also demonstrated that reinioside C, reduced the adhesion of monocytes to the endothelial cells, attenuated the mRNA expression of NADPH oxidase, ROS formation and NF-kB activation in endothelial cells. Kou *et al*. (2006) reported that the aqueous extract prepared from the leaves of *P. japonica*, used for the treatment of inflammatory diseases in folk medicine, have significant inhibitory effect on histamine-induced peritoneal and cutaneous vascular permeability, and picryl chloride-induced ear swelling. Extract was also shown to decrease histamine-induced paw edema and prostaglandin E_2 formation in carrageenan-induced air pouch.

Triterpenic saponins, 3-O-β-D-glucopyranosylbayogenin, 28-O-β-D-xsylopyranosyl (1→4) -α-L-rhamnopyranosyl (1→2)-β-D-glucopyranosyl ester, poligalasaponin V and bayogenin-3-O-β-D-glucopyranoside isolated by Wang *et al*. (2008), were reported to have anti-inflammatory activity in carrageenin-induced hind paw edema model in mice. Bayogenin-3-O-β-D-glucopyranoside inhibited the inflammatory mediators without affecting macrohage viability in LPS-induced RAW264.7 macrophages (Wang *et al*., 2008). In a study conducted by Van *et al*. (2009) LPS-induced murine macrophage production effects of the polysaccharide fraction, polyphenolic fraction and EtOAc/H_2O fraction of *P. senega* was evaluated on RAW164.7 cells. Only polyphenolic fraction showed increases in IL-6 level; other fractions decreased the production of IL-1β, TNF-α and IL-6 levels (Van *et al*., 2009).

In a study conducted by Hong et al., it was determined that intrarectal injection of 2,4,6-trinitrobenzene sulfonic acid (TNBS) in murine model of experimental colitis, *P. tenuifolia* roots reduced the inflammation degree and IFN-γ levels, improved the infiltration of polymorphonuclear cells and histological changes such as multiple erosive lesions and increased IL-4 production (Hong *et al*., 2002).

The aqueous extract prepared from the roots of *P. tenuifolia* inhibited LPS induced TNF-α secretion showing its anti-inflammatory activity (Kim *et al*., 1998). In a similar study, tenuifolioside A, isolated from the roots of *P. tenuifolia*, inhibited the NO, iNOS, PGE_2 and COX-2 production and supressed TNF-α ve IL-1β secretion and inhibited NF-κB ve MAPK pathways in LPS-stimulated RAW264.7 and murine peritoneal macrophages (Kim *et al*., 2013).

There are several biological activity studies contucted on saponins due to their wide distribution and their physical, chemical and physiological differences. In the previous activity studies, haemolytic, expectorant, analgesic, anti-inflammatory, antipyretic, antioxidant, hypoglycemic antiphototoxic, hypocholesterolemic, antiobesity, diuretic, neuroprotective, antispasmodic, antiulcer, estrogenic, antiviral, antifungal, anti-allergic, chemopreventive, cytotoxic, atigenotoxic, antimutagenic activities have been reported for saponins (Fleming *et al*., 2012; Liu *et al*., 2012; Weng *et al*., 2012; Podolak *et al*., 2013). Due to the wide range of biological activity they show saponin containing drugs are used for various purposes in traditional folk medicine and cosmetic industries. Saponins are considered to be the main components of the many herbal drugs used in folk medicine. The pharmacological action of these drugs are also related to the saponins. Indeed, saponins and polyphenols play a key role in traditional Chinese medicine. These two secondary metabolite groups

were determined to be responsible for the majority of the observed biological activities (Guclu-Ustundag & Mazza, 2007). On the other hand, flavonoids isolated from various plant also have antioxidant (Sun *et al*., 2007; Hu *et al*., 2009), antibacterial (Capra *et al*., 2010), antiviral (Lee *et al*., 2004), antifungal (Koo *et al*., 2000; Kawashima *et al*., 2004), antiplasmodial and anticancer (Katselis *et al*., 2007) effects. In structure-activity studies, these compounds were determined to have anti-inflammatory effect by inhibiting NF-kB, COX-2, iNOS and LOX (Winter *et al*., 1968; Kupeli Akkol *et al*., 2010;). The findings obtained in the present study supported the literature information given on saponins and flavonoids.

CONCLUSION

The findings obtained in the present study experimentally verified the utilization of *P. anatolica* for the treatment of inflammatory diseases and revealed its efficacy on the treatment of pain. Saponin and flavonoid fractions obtained from the plant as the active ingredients were found to have prostaglandin inhibitory effect which results in ulceration. Therefore, it is recommended that *Polygala anatolica* should be carefully utilized for the treatment of inflammatory disorders.

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