Original Article

Anti-inflammatory effects of *Sanguisorba minor* Scop. subsp. *muricata* (Spach) Briq. and *Cirsium libanoticum* DC. subsp. *lycaonicum* (Boiss. & Heldr.) Davis & Parris in rat

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Abstract. An ethnobotanical research conducted in Pınarbaşı, Kayseri, revealed that *Sanguisorba minor* Scop. subsp. *muricata* (Spach) Briq. (Smm) is commonly used for wounds and burns and *Cirsium libanoticum* DC. subsp. *lycaonicum* (Boiss. & Heldr.) Davis & Parris (Cll) is used especially to treat pressure wounds suffered by bedridden patients. This study was performed to assess any possible anti-inflammatory effect of these two folk medicinal plants.

Aqueous extracts of Smm and Cll were prepared at room temperature with maceration. Extracts were given at doses of 25, 50 and 100mg/kg to rats. Anti-inflammatory test was performed to animals after 30 minutes of intraperitoneal injection. Smm and Cll extracts exerted anti-inflammatory activity in a dose dependent fashion.

Control group shows 1.1% (mL) paw edema following lambda-carrageenan injection. Aqueous extracts of Smm showed 41.9, 76.4 and 83.4% anti-inflammatory activity at 25, 50 and 100 mg/kg doses respectively. Similarly aqueous extracts of Cll showed 20.9, 34.2 and 53.5% anti-inflammatory activity at 25, 50 and 100mg/kg doses respectively.

In addition, Smm was more potent anti-inflammatory plant than Cll. These findings suggest that traditional use of these subspecies of Smm and Cll for anti-inflammatory activity have an ethnopharmacological relevance.

Key words: *Sanguisorba, Cirsium,* anti-inflammatory, paw edema, carrageenan

1. Introduction

Genus *Sanguisorba* (*Rosaceae* L.) includes perennial herbs/small shrubs and consists of approximately 27 species distributed in temperate to sub-Arctic region of the Northern Hemisphere (1,2). The genus comprises of three species in Turkey and one of them is endemic (3). Especially, *S. officinalis* is well known plant of its high variety of biological activities and folk medicinal implementations. There have been records for *S. officinalis* as analgesic, astringent (4), anti-wrinkle (5), anti-viral (6,7), anti-oxidative, anti-atherogenic, vasorelaxant (8), anti-inflammatory, anti-cancer and neuroprotecive (9). In Anatolia this plant is used to treat goiter (10), as diuretic, appetizing, stomachic and constipating (11). Recent studies revealed that its ethanolic extract exerts inhibitory effect on PGE production and suggests a potent anti-inflammatory activity mediated by NF-κB and AP-1 inhibitory properties (8). Extract of *S. minor* subsp. *muricata* was found to have anti-ulcerogenic activity (12) but there has been no record about the anti-inflammatory activity of this subspecies. Anti-inflammatory activity findings of *S. officinalis* and the folk medicinal data about the usage of *S. minor* subsp. *muricata* for wounds and burns (13) require the clarification of this effect for this subspecies.

Genus *Cirsium* (Asteraceae L.) is among the several genera commonly grouped as thistles and

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Received: 06.05.2014
Accepted: 05.02.2015

most *Cirsium* species are considered as noxious weeds. Turkey has more than 60 *Cirsium* taxa and nearly one third are endemic to Turkey (14-16). Many *Cirsium* plants are commonly used in traditional medicine and some of them are scientifically investigated (17). Even the name "Cirsium" comes from the Greek word "*kirsos*" meaning "swollen vein" and thistles were among the plants used as cure for swollen veins. This usage of the plant suggests an activity of the plant on smooth muscles such as smooth muscles of vessels. Bioactivity studies reveal that molecules from aqueous extract of *Cirsium japonicum* affects rat thoracic aorta (18). In addition to that, *Cirsium* species were found to have activities such as anti-cancer (19), anxiolytic (20), immunomodulatory (21), anti-diabetic (22) anti-microbial (23,24) and hepatoprotective (25). Anti-inflammatory effect of this genus was tested in *C. chanroenicum* (26) but there have been no pharmacological records on the anti-inflammatory activity of endemic plant, *C. libanoticum* subsp. *lycaonicum* which is used to treat pressure wounds suffered by bedridden patients in Pınarbaşı-Kayseri (13).

Inflammation is an active procedure that occurs in response to the chemical, mechanical injuries, microbial infections, burns and other stimuli that may threaten the welfare of the organism. This process increases vascular permeability, causes blood flow changes and destruction of tissues via the activation and migration of leucocytes with oxidative burst. It leads to the synthesis of local inflammatory mediators such as lipooxygenases, cyclooxygenases, phospholipase A_2 induced platelet-activating factors and prostaglandins (27). The acute inflammation terminates infections and supports tissue recover by stimulating immune cells (leukocytes, mast cells) while chronic inflammation is a crucial factor in the pathogeny of many malady conditions including cancer, diabetes, cardiovascular, neurodegenerative and degenerative joint diseases. Additionally, macrophage activation causes toxic byproducts including reactive oxygen species and some proteases which are responsible for tissue damage (28).

Although steroidal and non-steroidal anti-inflammatory drugs are currently used for acute inflammatory states, they have not been perfectly successful in healing chronic inflammatory diseases. Additionally, these drugs are accompanied by unexpected side effects. For that reason, there is an urgent need to find safer compounds with anti-inflammatory effect. Plants with anti-inflammatory effect have long been used as folk medicine for inflammatory conditions like pain, fever and arthritis. As the inflammatory causes of some diseases become clear, anti-inflammatory plants and natural products become of greater interest (29). The present study was undertaken to investigate any possible antiinflammatory activity of folk medicinal plants *Sanguisorba minor* subsp. *muricata* (*Smm*) and *Cirsium libanoticum* subsp. *lycaonicum* (*Cll*) using carrageenan-induced hind-paw edema in rats.

2. Materials and methods

2.1. Plant material

Flowering aerial parts of *Smm* were collected from Central Anatolia: Kayseri, Pınarbaşı, southern slopes of Şirvan Mount, at 1780m., in May 2006. The endemic plant *Cll* was also collected at flowering period from Kayseri, Pınarbaşı, Kılıçmehmet Village meadows, at 1480m., in May 2006. Voucher specimens retained for verification purposes in Ankara University, Faculty of Pharmacy Herbarium (Herbarium numbers: AEF 21219 and AEF 22786, respectively).

2.2. Preparation of plant extracts

Air-dried and powdered plant material was subjected to active maceration in distilled water by using a Heidolph mechanic shaker at 300rpm at room temperature for 8h. The extracts obtained were filtered from filter paper and dried in a freeze dryer (Christ Gamma 2-16 LSC, Osterode am Harz, Germany).

2.3. Animals

Sprague-Dawley rats were maintained in the Animal House of Yuzuncu Yil University, Faculty of Medicine. The animals were bred in this institutional animal house but the lineage originally obtained from Ankara Health Protection Institute, a governmental organization. The animals were housed in standard cages with food and water *ad libitum*, provided with pelleted food (Van Animal Feed Factory, Van-TURKEY). The approval of Animal Ethics Committee was obtained. All procedures involving animals were done in accordance with the guidelines and international rules considering the animal experiments and rights about biodiversity.

3.4. Chemicals

Lambda-carrageenan Type IV and indomethacin was obtained from Sigma (Steinheim, Germany). Lambda-carrageenan was dissolved in distilled water (w/v) and indomethacin was dissolved in ethyl alcohol (w/v).

2.5. Anti-inflammatory activity

The method of Winter et al. (30) was used with slight modification. Forty-eight rats were divided into eight groups of six animals each. The rats were fasted for 12h and deprived of water only during the experiment. Deprivation of water was to ensure uniform hydration and to minimize variability in edematous response. Inflammation of the hind paw was induced by injecting 0.05 mL fresh lambda carrageenan (phlogistic agent) into the subplantar surface of the right hind paw. The control group and the reference group were given ISS (0.1mL) and indomethacin (3mg/kg, ip), respectively (31) while the remaining nine groups received *Smm* water extract at doses of 25, 50, and 100mg/kg, *Cll* water extract at doses of 25,50, and 100mg/kg, ip.

The measurement of foot volume was accomplished by displacement technique using the plethysmometer (Ugo Basile 7140 plethysmometer, Italy), immediately before and three hours after the injection. The percentage inhibition of the inflammatory reaction was determined for each animal by comparison with controls and calculated by the formula (32):

I%=[(1-(dt/dc)] x 100 where *dt* is the difference in paw volume in the drug-treated group and *dc* the difference in paw volume in the control group.

2.6. Statistical analysis

Results were reported as mean ± standard error of mean (SEM). The total variation was analyzed by performing one-way analysis of variance (ANOVA). LSD (least significant difference) test and Dunnett's test were used for determining significance. Probability levels of less than 0.05 were considered significant. Medium effective dose (ED_{50}) value was calculated by non-linear regression analysis (SigmaPlot 2004 for Windows Version 9.01).

3. Results

There were no deaths or serious complications due to injection of the extracts. Control group shows 1.1% (mL) paw edema following lambda-carrageenan injection. *Smm* showed 41.9, 76.4 and 83.4% anti-inflammatory activity at 25, 50 and 100mg/kg doses, respectively.

Aqueous extracts of *Cll* showed 20.9, 34.2, 53.5% anti-inflammatory activity at 25, 50 and 100mg/kg doses respectively.

Indomethacin (3mg/kg) was given as a positive control and exerted a potent anti-inflammatory activity (96%) as expected. Results concerning anti-inflammatory activity of drugs and plant extracts are presented in Table 1.

4. Discussion

In this study possible anti-inflammatory activity of aqueous extracts of *Smm* and *Cll* - which is endemic to inner Anatolia- were tested.

In all of the doses applied for *Smm* and *Cll* with the increase in doses of the extracts, anti-inflammatory activity increased. According to our

Table 1. Effects of S. minor and C. libanoticum aqueous extracts on rat paw edema

Groups	Dose	Paw edema (mL %)	Inhibition (%)
*Control (ISS)	0.1 mL	1.106 ± 0.073	-
Indomethacin	3 mg/kg	[a] 0.037 ± 0.027	96.16
S. minor	25 mg/kg	[b] 0.643 ± 0.201	41.90
S. minor	50 mg/kg	[a] 0.261 ± 0.061	76.43
S. minor	100 mg/kg	[ac] 0.181 ± 0.012	83.64
C. libanoticum	25 mg/kg	[bde] 0.643 ± 0.201	20.93
C. libanoticum	50 mg/kg	[bde] 0.261 ± 0.061	34.24
C. libanoticum	100 mg/kg	[ab] 0.181 ± 0.012	53.49
F/p values	*5.972 / 0.000*		

Data were presented as mean ± standard error of the mean (n=6).

*ISS: Isotonic saline solution (0.9% NaCl).

ED50 value of *S. minor*: 33.913mg/kg.

ED50 value of *C. libanoticum*: 57.833mg/kg.

Post-hoc LSD (least significant difference) and Dunnett's test:

a : $p<0.05$ compared to control (ISS) group,

b : $p<0.05$ compared to indomethacin group,

c : $p<0.05$ compared to *S. minor* 25 mg/kg.

d : $p<0.05$ compared to *S. minor* 50 mg/kg.

e : $p<0.05$ compared to *S. minor* 100 mg/kg.

findings it is possible to say that *Smm* and *Cll* extracts showed anti-inflammatory activity in a dose dependent fashion. Studies in literature also revealed anti-inflammatory activity. Aqueous extracts of *C. subcoriaceum* has anti-inflammatory activity in rats. In addition to whole extract, anti-inflammatory activity pointed also to a flavonoid glycoside-pectolinarin- (33). In addition to aqueous extracts, methanolic extracts of *Cirsium* species such as *C. chanroenicum* (26) and *C. maackii* (34) showed activity in different anti-inflammatory and antinociceptive models. A study by Lee et al. (35) conducted on roots of *C. setidens* demonstrate a grading in anti-inflammatory activity in accordance with the solvent used for extraction. Potency of the activity was ordered as n-butanol > ethanol > water >ethyl acetate>dichloromethane. Syringin isolated by chromatographic methods revealed the most active constituent for this activity.

Smm was found to be a more potent plant in anti-inflammatory activity than *Cll*. In all doses of *Sanguisorba*, percent inhibition rate which indicates the anti-inflammatory activity of the extract was significantly higher than its dose counterparts of *Cirsium* extracts. Literature records indicate involvement of phenolic phytochemicals in anti-inflammatory activity. Interestingly trace metals seems to contribute to this activity (36). Another study by Yu et al. (8) showed that ethanol extract of *S. officinalis* possesses anti-inflammatory activity by NF-κB, and AP-1 and via suppressing Src and MAPK.

Indomethacin (3 mg/kg) was observed as a much more potent anti-inflammatory agent in our experiment (96% inhibition) as expected, however, side effects of this drug should also be considered in this context. In this study no activity was tested for its ulcerogenic potency or for other side effects. Further studies are needed for such investigations which will give us a more comprehensive understanding for its value as an anti-inflammatory plant source.

Further bioactivity guided fractionation on their extracts can reveal the responsible molecule/s related with this activity. Continuing work on these and other species of *Sanguisorba* genus and *Cirsium* genus in Turkey can yield valuable molecules concerning their pharmacological activities.

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