



Evaluation of the Antidiabetic Activity of *Alchemilla persica* Rothm. in Mice with Diabetes Induced by Alloxan

Alloksanla İndüklenen Farelerde *Alchemilla persica* Rothm.'nın Antidiyabetik Etkisinin Değerlendirilmesi

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ABSTRACT

Objectives: *Alchemilla* species are used in Turkish folk medicine for the treatment of many diseases together with diabetes. *Alchemilla persica*, belonging to this genus, is widely distributed in Eastern Anatolia as well as in Caucasia, northern and northeastern Iran, and northern Iraq.

Materials and Methods: Methanolic-water extracts of the aerial parts and roots of *A. persica* were evaluated for their hypoglycemic activities in mice with alloxan-induced diabetes in order to verify its usage in folk medicine.

Results: None of the tested extracts exhibited a significant lowering effect on blood glucose levels. However, the aerial parts notably increased blood glucose levels at doses of 100 mg/kg and 200 mg/kg.

Conclusion: *A. persica* usage as an antidiabetic is not confirmed in the present study.

Key words: *Alchemilla persica*, alloxan, antidiabetic activity, diabetes, hypoglycemic activity

ÖZ

Amaç: *Alchemilla* türleri Türk halk tıbbında diyabetin yanında birçok hastalığın da tedavisinde kullanılmaktadır. Bu cinse ait olan *Alchemilla persica*, Doğu Anadolu, Kafkasya, Kuzey ve Kuzeydoğu İran ile Kuzey Irak'ta yetişmektedir.

Gereç ve Yöntemler: Bu çalışmada *Alchemilla persica*'nın halk arasındaki kullanımını doğrulamak amacıyla; toprak üstü kısım ve köklerinin sulu-metanollü ekstraktlarının alloksan indüklü diyabetik fareler üzerindeki hipoglisemik etkileri test edilmiştir.

Bulgular: Test edilen ekstraktlardan hiçbiri kan glukoz düzeyini kayda değer biçimde düşürmezken, *A. persica* toprak üstü kısımları kan glukoz düzeyini 100 mg/kg ve 200 mg/kg dozlarda önemli ölçüde artırmıştır.

Sonuç: Bu çalışma *A. persica*'nın antidiyabetik olarak kullanımını doğrulamamıştır.

Anahtar kelimeler: *Alchemilla persica*, alloksan, antidiyabetik aktivite, diyabet, hipoglisemik aktivite

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INTRODUCTION

The genus *Alchemilla* L. (Rosaceae), with more than 1000 species, is distributed mainly in western Eurasia as well as in southern and eastern Africa, Madagascar, southern India, Sri Lanka, and Java.^{1,2} In Turkey 50 species of the genus *Alchemilla*, which are mainly distributed in Northeast Anatolia, were recorded and this number has reached 74 with new records.¹ *Alchemilla persica* Rothm., belonging to this genus, grows naturally in Eastern Anatolia, Caucasia, northern and northeastern Iran, and northern Iraq.³

Alchemilla vulgaris L. (lady's mantle, bear's foot, lion's foot) is the best known species from the genus *Alchemilla* and is mainly used for treating women's illnesses, wounds, and skin disorders in Europe.⁴⁻⁶ Its usage for nonspecific diarrhea is approved by Commission E.⁷ Additionally, ESCOP Monographs described this plant's usage for nonspecific diarrhea, gastrointestinal disorders, and dysmenorrhea based on clinical studies and long-term use.⁸ *Alchemilla* species are used for their wound healing, sedative,⁹⁻¹² antidiuretic, tonic, and diuretic activities,¹³⁻¹⁵ and in treatment for menstruation disorders,¹⁶ gynecological problems,^{17,18} liver inflammation,¹⁷ asthma, bronchitis, cough,¹⁹ and diabetes, as well as kidney, intestinal, and gastric disorders²⁰⁻²¹ and skin diseases.¹⁰

Previous studies have revealed that the aerial parts and roots of *A. persica* showed antioxidant activity by DPPH radical scavenging (IC₅₀ 0.055 M and 0.151 M, respectively) and reducing MDA levels (5.9 nmol/mL and 19.08 nmol/mL respectively).²² Extract of the aerial parts of *A. persica* exhibited a reduction in the endometrioma. However, no significant reduction in the levels of cytokine, tumor necrosis factor- α , vascular endothelial growth factor, or interleukin-6 were recorded.²³ *A. persica* displayed significant wound healing activity with tensile strength values of 33.3% and contraction values of 43.5% in linear incision and circular excision wound models, respectively. Hydroxyproline estimation and histopathological analysis also confirmed the results. *A. persica* showed significant anti-inflammatory activity with a value of 26.6%.²⁴ Phenolic constituents, namely caffeic acid esters with sugars, flavonoid glycosides, catechin and epicatechin, condensed tannins related to gallic acid, such as pedunculatin/pedunculagin, agrimoniin, casuarictin, castalagin/vescalagin, and sanguin H-10 isomers, were identified by HR-MS Q-TOF in the aerial parts of *A. persica* and its essential oil consisted of diterpenoids (19.6%) and sesquiterpenoids (17.2%) mainly.²⁵

The current study was designed to evaluate the hypoglycemic activities of the roots and aerial parts of *A. persica* using an alloxan-induced diabetic mice test model to test its traditional usage for treatment of diabetes in Turkish folk medicine.

MATERIALS AND METHODS

Plant material

Plant material was collected from the Kop Pass, Erzurum, Turkey. The taxonomic identification of these plants was confirmed by H. Duman, at the Department of Biological Sciences, Faculty of Arts and Sciences, Gazi University. Voucher specimens

were deposited in the herbarium of the Faculty of Pharmacy at Ankara University (AEF 25896).

Extraction

Aerial parts and roots of *A. persica* were extracted with a methanol:water (80:20) solvent system for 8 h at room temperature by stirring and then filtered. The methanol was evaporated under vacuum at 35-40°C and then the remaining water was lyophilized to obtain crude extracts.

Animals

Balb/C mice (22-30 g) were used for testing antidiabetic activity. The study protocol (30/09/2015-69) was approved by the Ethical Committee of İstanbul Medipol University. The animals were housed in standard cages (48 cm×35 cm×22 cm) at room temperature (22±2°C), with artificial light from 7.00 am to 7.00 pm, and provided with pelleted food and water *ad libitum*. The procedures followed were in accordance with animal rights as per the Guide for the Care and Use of Laboratory Animals.

Chemicals

Alloxan was purchased from Sigma (Steinheim, Germany). The alloxan and *A. persica* extracts were dissolved in distilled water (w/v).

Antidiabetic activity

Diabetes was induced by injecting 150 mg/kg of alloxan solution in isotonic saline solution (ISS) i.p. into the mice after fasting for 18 h. This procedure was repeated three times at 48 h intervals. After 7 days of treatment, the mice's blood glucose levels were measured. The mice with 200 mg/dL or over were included in the study as diabetic mice. The diabetic animals were randomly divided into five groups of six animals each. Group I mice received 0.1 mL of ISS i.p. The animals in groups II and III were treated with 100 mg/kg body weight of extracts of the aerial parts of *A. persica* at 100 mg/kg and 200 mg/kg doses, while those in groups IV and V were treated with 100 mg/kg and 200 mg/kg root extract of *A. persica*, respectively. The animals were treated with ISS and *A. persica* extracts in a single dose at the beginning of the procedure. Blood was taken from the tail vein by scalpel blade and blood glucose levels were determined before treatment and 1, 2, and 4 h after treatment by applying the glucose oxidase peroxidase method using an Accu-Check® device (Abbott, United Kingdom).

Statistical analysis

The statistics were analyzed using SPSS 18.0. The results are reported as mean ± standard error of mean and as percentages (%). One-way analysis of variance (*post-hoc* least significant difference test) was used for the statistical analysis. Probability levels of less than 0.05 ($p < 0.05$) were considered significant.

RESULTS

In order to investigate the hypoglycemic activities of extracts of the aerial parts and roots of *A. persica* on alloxan-induced diabetes in mice blood glucose levels were measured before and 1, 2, and 4 h after treatment. Table displays the effect of the *A. persica* extracts on blood sugar levels. The current study's

Table. Blood sugar levels of mice with alloxan-induced diabetes

Groups	Blood sugar levels (mg/dL)			
	Before treatment with <i>Alchemilla persica</i>	After treatment with <i>Alchemilla persica</i>		
		1 h (0-1)	2 h (0-2)	4 h (0-4)
Control (ISS)	470.00±23.62	471.00±13.78 (0.74±3.99)	493.80±15.55 (5.54±3.73)	494.20±27.32 (4.97±2.18)
<i>Alchemilla persica</i> (AE) 100 mg/kg	306.67±36.13	501.67±27.97 (70.97±15.01)*	484.67±22.12 (67.15±17.39)*	387.66±20.77 (36.35±17.91)
<i>Alchemilla persica</i> (AE) 200 mg/kg	297.40±38.09	410.00±25.37 (42.81±10.85)*	354.20±33.90 (22.50±12.27)	290.60±59.97 (1.36±26.06)
<i>Alchemilla persica</i> (R) 100 mg/kg	337.00±27.28	379.83±52.56 (14.45±15.87)	425.33±52.26 (29.80±17.04)	355.83±61.67 (8.87±18.78)
<i>Alchemilla persica</i> (R) 200 mg/kg	405.17±24.40	445.00±44.83 (9.01±7.30)	427.83±50.04 (4.64±9.13)	305.80±63.67 (-23.73±13.49)

Mean ± standard error of mean; Results of *post-hoc* LSD test; *: Comparison with SF group (p<0.05)
AE: Aerial parts, R: Roots, LSD: Least significant difference, ISS: Isotonic saline solution

results revealed that none of the extracts induced significant reductions in the levels of blood sugar (p>0.05). On the other hand, notable increases in blood glucose levels were observed 1 h and 2 h after treatment with the aerial parts of *A. persica* at 100 mg/kg dosage and 1 h after treatment with 200 mg/kg dosage. A decrease in blood glucose level was detected only with treatment of *A. persica* roots at 200 mg/kg dosage 4 h after treatment. However, the results were not significant.

DISCUSSION

In Turkish folk medicine, the use of *Alchemilla compactilis* Juz., *Alchemilla speciosa* Buser, and other *Alchemilla* species for the treatment of diabetes is recorded.^{20,21} The present study did not confirm the usage of *A. persica* in folk medicine for the treatment of diabetes. A previous study related to the hypoglycemic effect of *Alchemilla xanthochlora* (*A. vulgaris*) also reported that decoction of the leaves was not active on streptozotocin-induced diabetic mice.⁸ The aerial parts and roots of *Acanthus mollis* were also tested for their antidiabetic activities and results similar to those for *A. persica* were obtained.²⁶ All study results indicated that the *Alchemilla* species *A. mollis*, *A. vulgaris*, and *A. persica* had no lowering effect on blood glucose levels. In contrast, the aerial parts of *A. mollis* and *A. persica* increased blood glucose levels at 100 mg/kg and 200 mg/kg dosages 4 h after treatment.

CONCLUSIONS

Based on the current study results, the aerial parts and roots of *A. persica* are not useful for decreasing blood sugar levels in short-term treatment. Furthermore, *A. persica* is not suitable in phytotherapy for other medicinal purposes in diabetic patients.

Conflict of Interest: No conflict of interest was declared by the authors.

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