

Evaluation of *Alchemilla persica* Rothm. antidiabetic activity on mice induced by alloxan

Alloksanla indüklenen farelerde *Alchemilla persica* Rothm.'nın antidiyabetik etkisinin değerlendirilmesi

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Abstract

INTRODUCTION: *Alchemilla* species are used in Turkish folk medicine for treatment of many disease together with diabetes. *Alchemilla persica* belonging to this genus, widely distributed in East part of Anatolia as well as Caucasia, north and northeast of Iran and north Iraq.

METHODS: In present study *Alchemilla persica* aerial part and root methanolic-water extracts were evaluated for their hypoglycemic activities on alloxan induced diabetic mice in order to verify its usage in folk medicine.

RESULTS: Any of the tested extracts did not exhibit significant lowering effect on blood glucose levels. On the other hand *A. persica* aerial parts increased blood glucose level at the dose of 100 mg/kg and 200 mg/kg notably.

DISCUSSION AND CONCLUSION: *A. persica* usage for its antidiabetic activity is not confirmed in present study.

Keywords: *Alchemilla persica*, alloxan, antidiabetic activity, diabetes, hypoglycemic activity

INTRODUCTION

Alchemilla L. (Rosaceae) genus, with more than 1000 species, is distributed mainly in western Eurasia as well as South and East Africa, Madagascar, South India, Sri Lanka and Java.¹⁻² In Turkey 50 species of *Alchemilla* genus which are mainly distributed in north-east Anatolia, were recorded and this number has reached to 74 with new records.¹ *Alchemilla persica* Rothm. belonging to this genus, naturally grows in East part of Anatolia, Caucasia, north and northeast of Iran and north Iraq.³

A. vulgaris L. (Lady's mantle, Bear's foot, Lion's foot) is the best known species from *Alchemilla* genus is mainly used for women illness, wounds and skin disorders in Europe⁴⁻⁶. Its usage for non-specific diarrhea is approved by Commission E.⁷ Additionally, ESCOP Monographs described this plant usage for non-specific diarrhea, gastrointestinal disorders and dysmenorrhea based on clinical studies and long-term using.⁸ *Alchemilla* species are used for their wound healing, sedative,⁹⁻¹² antidiareic, tonic, diuretic activities,¹³⁻¹⁵ treatment for menstruation disorders,¹⁶ gynecological problems,¹⁷⁻¹⁸ liver inflammation,¹⁷ against asthma, bronchitis, cough,¹⁹ diabetes, as well as kidney, intestinal and gastric disorders²⁰⁻²¹ and skin diseases.¹⁰

Previous studies have revealed that *A. persica* aerial parts and roots 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 *A. persica* aerial parts extract exhibited a reduction in the endometrioma. However, significant reduction on the levels of cytokine; tumor necrosis factor- α (TNF- α), vascular endothelial growth factor (VEGF), interleukin-6 (IL-6) were not recorded.²³ *A. persica* displayed significant wound healing activity with the tensile strength values of 33.3%, and with the 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 the values of 26.6%.²⁴ Phenolic constituents; caffeic acid esters with sugars, flavonoids 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 *A. persica* aerial parts and its essential oil consisted of diterpenoids (19.6 %) and sesquiterpenoids (17.2%) mainly.²⁵

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

MATERIALS AND METHODS

Plant Material

Plant material was collected from Erzurum-Kop Passage, Turkey. The taxonomic identification of these plants was confirmed by H. Duman, in the Department of Biological Sciences, Faculty of Art and Sciences, Gazi University. Voucher specimens were kept in the herbarium of Ankara University, Faculty of Pharmacy (AEF 25896).

Extraction

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

Animals

Balb/C mice (22-30 g) were used for 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 purchased from Sigma (Steinheim-Germany). Alloxan and *Alchemilla persica* extracts dissolved in distilled water (w/v).

Antidiabetic activity

Diabetes was induced by injecting of 150 mg/kg of alloxan solution in ISS (Isotonic saline solution) i.p.after fasting for 18 h of mice. This procedure was repeated three times by 48 hours intervals. After 7 days of the treatment, mice blood glucose levels were measured. Mice have 200 mg/dL and over were taken into the study as diabetic mice. Diabetic animals were randomly divided into five groups of six animals each. Group I mice received 0.1 mL ISS i.p. The animals of group II and III were treated with

100 mg/kg body weight of *A. persica* aerial part extracts at 100 mg/kg and 200 mg/kg dose, group IV and V were treated by 100 mg/kg and 200 mg/kg *A. persica* root extract respectively. The animals were treated with ISS and *A. persica* extracts with single dose at the beginning of the procedure. The blood was taken from tail vein by scalpel blade and blood glucose levels were determined before treatment, 1, 2 and 4 h after treatment by applying glucose oxidase peroxidase method using Accu-Check® device (Abbott, United Kingdom).

Statistical Analyses

Statistical analysis were evaluated by using SPSS 18.0 programme. Results are reported as mean±SEM (standard error of mean) and as percentage (%). One-way analysis of variance (post-hoc Least Significant Difference-LSD test) was used for statistical analyses. Probability levels of less than 0.05 ($P < 0.05$) were considered significant.

RESULTS

In order to investigate hypoglycemic activities of *A. persica* aerial parts and roots extract on alloxan induced diabetic mice blood glucose levels were measured before and 1, 2 and 4h after treatment. Table 1 displayed the effect of the *A. persica* extracts on blood sugar levels. Current study results have revealed that none of the extracts were induced significant reduction on levels of blood sugar ($p > 0.05$). On the other hand notable increasing on blood glucose levels were observed after 1 h and 2 h treatment of *A. persica* aerial parts at 100 mg/kg dosage and 1 h treatment after 200 mg/kg dosage. Decreasing on blood glucose level was detected only by treatment of *A. persica* roots at 200 mg/kg dosage after 4 h treatment. However the results were not significant.

DISCUSSION

In Turkish folk medicine, the usage of *Alchemilla compactilis* Juz., *Alchemilla speciosa* Buser and other *Alchemilla* species for treatment of diabetes is recorded.²⁰⁻²¹ This study is not confirmed the usage of *A. persica* in folk medicine for treatment of diabetes. Previous study related to hypoglycemic effect of *A. xanthochlora* (*A. vulgaris*), have also reported that decoction of the leaves were not active on streptozotocin induced

diabetic mice.⁸ *A. mollis* aerial parts and roots were also tested for their antidiabetic activities and similar results to *A. persica* were obtained.²⁶ All study results have indicated that *Alchemilla* species; *A. mollis*, *A. vulgaris* and *A. persica* has no lowering effect on blood glucose levels. On the contrary, *A. mollis* and *A. persica* aerial parts increased blood glucose levels at 100 mg/kg and 200 mg/kg dosage after 4 hour of treatment.

CONCLUSION

According to the current study results it could be suggested *A. persica* aerial parts and roots are not useful for decreasing blood sugar levels in short time treatment. Furthermore the usage of the *A. persica* is not suitable in phytotherapy for other medicinal purposes in diabetic patients.

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Table 1. Blood sugar levels of alloxan induced diabetic mice

| Groups | Blood sugar levels (mg/dL) | | | |
|----------------------------------|---|--|--------------------------------|------------------------------------|
| | Before treatment with <i>A. persica</i> | After treatment with <i>A. persica</i> | | |
| | | 1h (0-1) | 2h (0-2) | 4h (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>A. 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>A. 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>A. 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>A. 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) |

AE: aerial parts; R: roots

Mean ± Standart Error of Mean (mean ± SEM).

Results of Post-hoc LSD test:

*: Comparision with SF group ($p < 0.05$)