EFFECTS OF CRATAEGUS TANACETIFOLIA EXTRACT ON HAEMORHEOLOGICAL PARAMETERS IN RATS

CRATAEGUS TANACETIFOLIA EKSTRESİNİN SIÇANLARDA HEMOREOLOJİK PARAMETRELER ÜZERINE ETKİLERİ

A.ŞULE TAMER

Department of Physiology, İstanbul Faculty of Medicine, University of İstanbul, Çapa 34390 İstanbul TURKEY

Recently, use of natural plant extracts in the treatment of various diseases has been the subject of many studies. *Crataegus tanacetifolia* (*C. tanacetifolia*) is one of these plants studied on and it has been reported that various types of *C. tanacetifolia* species grow in Turkey. The water extract of *C. tanacetifolia* leaf which was supplied from Bolu-Şen region of Turkey, is rich in flavonoids and has antihypertensive effect. In the present study the effects of *C. tanacetifolia* leaf extract on haemorheological parameters in rats was investigated. For this purpose, 20 male Wistar-Albino rats were randomly divided into 2 groups. The rats in the experimental group (n = 10) received 50 mg.kg⁻¹ leaf extract of *C. tanacetifolia* and the rats in the control group (n = 10) received 0.9% NaCl by gastric gavage for 21 days. In the blood samples, erythrocyte count, hematocrite, mean corpuscular volume (MCV), whole blood and plasma viscosity, ED, and fibrinogen levels were examined. The results demonstrated that *C. tanacetifolia* leaf extract significantly decreased erythrocytes deformability, mean corpuscular volume, hematocrite, fibrinogen levels, and whole blood and plasma viscosity in rats after 21 days, but did not change the erythrocyte counts.

The results of the present study revealed that *C. tanacetifolia* can modify rheological parameters and can find clinical applications for hypertension and other diseases associated with abnormal rheological parameters.

Key words: *Crataegus* tanacetifolia; Erythrocyte deformability; Whole blood and plasma viscosity

Anahit kelimeler: *Crataegus* tanacetifolia; Eritrosit deformabilitesi; Tüm kan ve plazma viskozitesi

Introduction

Besides various blood proteins and lipids, whole blood viscosity, plasma viscosity, erythrocyte deformability (ED) and percentages of hematocrite (htc) in blood are the main determinants of the rheological properties of blood (5,13,14).

Deformability is the ability of a cell to alter its morphology under the influence of extrinsic forces. In microcirculation, normal erythrocytes can pass through capillaries easily due to their high deformability. ED is subject to alterations in many physiological and pathological conditions such as cell aging, intracellular calcium overload (6,8), high extracellular osmolarity, sickle cell anemia, diabetes mellitus and hypertension (1, 2, 7, 9, 11, 14).
It has been shown that cardiovascular system disorders, hypertension, diabetes mellitus and obesity are associated with haemorheological abnormalities. These findings imply that pathological changes in haemorheological parameters may play an important role in the pathogenesis of these diseases (5,15). On the other hand, recently the use of natural plant extracts in the treatment of various diseases has been the subject of many studies and *Crataegus tanacetifolia* was one of these plants. The leaves, flowers or fruits of *C. tanacetifolia* contain flavonoids, procyanidins and cardiotonic amines (12). It has been reported that various types of *C. tanacetifolia* species also grow in Turkey. The leaf extract of *C. tanacetifolia*, supplied from Bolu-Seben region of Turkey, is rich in flavonoids and has antihypertensive effect and it has been suggested that it can be used in the treatment of cardiovascular diseases (3, 6, 8, 10, 12, 19). The effects of *C. tanacetifolia* leaf extract on haemorheological parameters are yet unknown.

The aim of the present study was to investigate the effects of *C. tanacetifolia* leaf extract on haemorheological parameters in rats.

**Materials and methods**

**Preparation of the plant extract**: *Crataegus tanacetifolia* (Lam.) Pers. (Rosaceae) (identified by Prof. Dr. Kerim Alpınar and registered with number ISTE 61150 in the Herbarium of the Faculty of Pharmacy, University of Istanbul) leaves were collected in May 1989 from Seben region (Bolu, Turkey) and 50 g were macerated with 500 ml bidistilled water at room temperature for 24 h and filtered and the filtrate was concentrated in vacuum to obtain a dry extract. The extract was then dissolved in bidistilled water to give 50 mg.ml⁻¹.

**Preparation of the animals**: 20 adult male Wistar-Albino rats, weighing 200-300 g were randomly divided into 2 groups. The rats in the experimental group (*n*=10) received 50 mg.kg⁻¹ leaf extract of *C. tanacetifolia* while the control group (*n*=10) received 0.9% NaCl by gastric gavage for a period of 21 days and then all rats were anaesthetized by sodium pentothal (ip 35 mg.kg⁻¹) and blood samples were collected via heart puncture to tubes containing EDTA (1mg.ml⁻¹). In these samples, erythrocyte count, htc, mean corpuscular volume (MCV), whole blood and plasma viscosity, ED, and fibrinogen levels were determined within thirty minutes of sampling.

**Analysis in blood**: Blood and plasma viscosities were measured using Wells-Brookfield LUT cone-plate rotatory viscosimeter at a shear rate of 60 rpm (MA O20 2072 Engineering Laboratories, Stoughton, USA).

ED was determined by microfiltration technique in terms of pressure versus cell rigidity (4,16,18). Briefly, blood was filtered through cotton wool to remove leucocytes and platelets. The erythrocytes were then washed three times with Hepes buffer (137 mmol NaCl, 4 mmol KCl, 1.8 mmol CaCl₂, 0.7 mmol MgSO₄, 7H₂O, 0.8 mmol NaH₂PO₄, 7H₂O, 8.4 mmol Hepes) and finally blood samples were resuspended to htc of 5% by adding Hepes buffer. Then cell suspensions were pumped with a constant flow rate of 0.5 ml.min⁻¹ at room temperature through polycarbonate filters (Millipore, ATIP 02500) with nominal pore sizes of 5 μm (Lot no. R7KM32882). The filtration pressure was measured on the upstream side of the filter with a pressure transducer (Model TP400T) connected to an amplifier and a recorder (Nihon Kohden RM 6000). Prior to the filtration of a cell suspension, Hepes buffer solution alone was filtered to obtain the filtration pressure for suspending medium (P₀). This slope, normalized for P₀, i.e. the relative increase of filtration pressure over time was used as a parameter of RBC rigidity.

\[
k = \frac{\Delta P/t}{P_0} = \frac{(P_{20s} - P_{20s})/40s}{P_0}
\]

Erythrocytes count, MCV and htc of samples were determined by haemocounter (Sysmex SE 900, Japan) and levels of fibrinogen were determined by coagulometer (Albio Stacompart 347.4950 France).

The results were expressed as mean ± SD. Student's t-test was used for statistical analyses and statistical significance was assigned when p<0.05.

**Results**

The table shows the results of *C. tanacetifolia* received and control group rats. Whole blood viscosity and plasma viscosity, ED, levels of fibrinogen, htc, and MCV were significantly increased (p<0.01, p<0.01, p<0.01, p<0.001, p<0.05, p<0.05 respectively) in extract administered rats with comparison to control group. The erythrocytes count of the extract-received rats did not differ from the controls.

**Discussion**

Viscosity is generally defined as the resistance of a fluid to flow. Blood viscosity
is dependent on mainly three factors: contents of plasma, htc value and the rheological properties of the erythrocytes. Plasma forms the liquid phase of the blood and is the main factor affecting blood viscosity. On the other hand, it is known that diseases with an increase in htc concentrations are associated with high blood viscosity. Abnormally high htc concentrations lead to increased friction between erythrocytes and other elements of blood and this friction causes an elevation in the blood viscosity (17,20).

Table. Haemorheological parameters of *C. tanacetifolia* extract administered and control group rats (means ±SD).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sample</th>
<th>Controls (n=10)</th>
<th><em>C. tanacetifolia</em> extract (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocyte deformability (k)</td>
<td>0.053 ± 0.01</td>
<td>0.04 ± 0.01**</td>
<td></td>
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<tr>
<td>Whole blood viscosity (mmol/s)</td>
<td>4.82 ± 0.73</td>
<td>3.9 ± 0.51**</td>
<td></td>
</tr>
<tr>
<td>Plasma viscosity (mmol/s)</td>
<td>1.78 ± 0.42</td>
<td>1.28 ± 0.23***</td>
<td></td>
</tr>
<tr>
<td>Fibrinogen (mg/dl)</td>
<td>255.1 ± 21.96</td>
<td>200.8 ± 29.17***</td>
<td></td>
</tr>
<tr>
<td>Erythrocyte (10^12 μl^-1)</td>
<td>6.3 ± 1.8</td>
<td>5.7 ± 0.2</td>
<td></td>
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<tr>
<td>Haematocrit (%)</td>
<td>48.0 ± 1.18</td>
<td>43.0 ± 4.2*</td>
<td></td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>87.7 ± 6.8</td>
<td>82.2 ± 3.8*</td>
<td></td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01, ***p<0.001

The flexibility or the deformability of the erythrocytes is very important for their access to capillaries and hence for a normal microcirculation. Therefore, it is clear that the use of drugs which are capable of preventing or reducing erythrocyte membrane rigidity may be useful in conditions or diseases such as erythrocyte aging, high osmolarity, intracellular calcium overload, diabetes mellitus and hypertension (17,20,21).

In the present study we studied the effects of a plant extract prepared from *C. tanacetifolia* leaves of Bolu-Seben region of Turkey, on haemorheological parameters in rats. The results demonstrated that *C. tanacetifolia* leaf extract significantly decreased ED, MCV, htc, fibrinogen levels, and whole blood and plasma viscosities in rats after 21 days, but did not change the erythrocyte counts. On the other hand, in our previous study (19) we showed that the leaf extract of *C. tanacetifolia* has anti-hypertensive effect and the results of the present study reveal that *C. tanacetifolia* can modify rheological parameters.

Our findings imply that *C. tanacetifolia* can find clinical applications against hypertension and other diseases associated with abnormal rheological parameters. Further studies are needed to clarify the effects of *C. tanacetifolia* leaf extract on rheological parameters.

Acknowledgements are to Prof. Dr. A.H. Meričli for his kind gift of *C. tanacetifolia* sample.

References


Accepted: 13.12.2000