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Effect of *Crataegus tanacetifolia* Extract on Total Body Ion Concentration in Normal Rats

Crataegus tanacetifolia Ekstresinin Normal Sıçanlarda Vücut İyon Konsantrasyonuna Etkisi

Hüsniye Birman*,[1] Nihal Salmayenli[2], Gülay Melikoğlu[3] and Ali H. Meriçli[3]

[1]Istanbul University, Faculty of Medicine, Department of Physiology, Çapa Istanbul, Turkey
[2] Istanbul University, Faculty of Medicine, Department of Clinical Biochemistry, Çapa Istanbul, Turkey
[3] Istanbul University, Faculty of Pharmacy, Department of Pharmacognosy, Beyazıt Istanbul,Turkey

Abstract

Since ancient times *Crataegus* species have been used in traditional medicine all around the world, especially flavonoids, procyanidins, aromatic acids as well as amine type constituents were found to be responsible for its cardioactivity. In the present study we have investigated body ion concentration of the rats giving aqueous leaf extract of *C. tanacetifolia* (50mg/kg), for thirty days via gavage.

We observed that the potassium, phosphat and calcium concentrations have increased significantly.This data suggest that ion changes may play a role in hypotensive activity.

Key words: *Crataegus tanacetifolia*; chronic use; body ion concentration; rat ; potassium

Introduction

Crataegus species (*Hawtorn)* has been used traditionally against various ancient times. They have been used against gout in France, hepatitis in Hungry, urinary system diseases in Chzeshoslovakia, pulmoner diseases in China and angina pectoris in USA (Ullsperger,1953). Most of the studies with *Crataegus* species focus on heart and cardiovascular system (Loew,1999).Such as *Crataegus* extract has positive inotropic effect (Chen *et al.*,1998), and coronary blood flow by coronodilatator effect (Taskov,1977), it also exhibits antiarrhytmic effect (Thompson *et al.*, 1974). In Germany its extracts were used in the treatment of mild heart failures, it was shown that the extract blocks potassium current in guinea pig ventricular cardiac myocytes (Müller *et al.*,1998). The *Crataegus monogyna* extract has also antioxidant activity (Bahorun *et al.*,1994). It has been concluded that the *Crataegus* extract containing procyanidins, flavonoids, aromatic acids and cardiotonic amins may play a role in these activities (Wagner *et al.*,1982). Some investigators have been considered that the activities on the cardiovascular system are caused by flavonoids while the others blame for procyanidins.

It has been reported that flavonoids may play a role in vasorelaxing activity and the regulation of blood pressure, while cardiotonic amins may play a role in positive inotropic effect.

* *Corresponding author. E-mail:birmanh@istanbul.edu.tr*

The experimental observation of *C.tanacetifolia* plant leaf extract which is grown in Turkey, an endemic type is very important. In our previous studies, we studied whether it has toxic effect on kidney and liver. In conclusion, it has no toxic effect. We had already reported that *C. tanacetifolia* leaf extract has hypotensive effect. In our another study, the effect of this plant when it is used for a long time was positive on the cardiovascular system. It has been seen that the total cholesterol and triglyceride levels significantly reduced.
In this study, we investigated the effects on the body ion concentration of *C. tanacetifolia* plant leaf extract, an endemic type of *Crataegus* plant when it is used for a long time.

Materials and Methods

Plant materials: The leaves of *Crataegus tanacetifolia* (Lam.)Pers. (*Rosaceae*) were collected in May 1989 from Seben region, Bolu (Turkey). The plant was identified by Prof .Dr. Kerim Alpınar (Istanbul), and deposited in the Herbarium of the Faculty of Pharmacy, University of Istanbul (ISTE 61150).
Preparation of Crataegus tanacetifolia extract: The dried leaves of *C.tanacetifolia* were powdered and 50 grams of the material were macerated with 500 ml of water at room temperature for 24 hours, the mixture was filtered and the filtrate was concentrated in vacuo to obtain a dry residue (yield, 9.5%).The residue was then diluted with water so as to obtain a concentration of 50 mg/ml.
Animals: The experiments were carried out on groups of 15 Wistar Albino adult male rats weighting 275±52 gr. The control group consisted of 10 male rats. All of the rats in their separate cages were fed with standart food and tap water.
Physiological methods: Extract of *C. tanacetifolia* (50 mg/kg) was given by gavage to the rats in the morning during the same hours for 30 days. The control group received only saline. On the 30^{th} day of the application the rats were anaesthetised intra- peritoneally by pentothal sodium (35 mg/kg). In addition to these groups, also a positive control group was used by giving the potassium (K^+) at the same concentration with those of the *Crataegus* group in order to determine hypotensive activity.
The cannulated femoral artery and vein were used to determine blood pressure and for drug application, respectively. The parameters were determined by means of a Hugo Basil Blood Pressure Transducer. After determining the blood pressure, the chests were opened and 4-4.5 ml of blood were taken from the left ventricule into the anticoagulant-free vacuum tubes and the serum was separated by centrifugation for the biochemical tests.
Blood was also taken into the purple vacotainers containing EDTA for hematological cell counting. Calcium(Ca^{++}), inorganic phosphat (PO_4^-) levels were determined colorimetrically on Technicon DAX-72 autoanalyzer. Sodium (Na^+), potassium (K^+), chloride (Cl^-) and magnesium (Mg^{++}) levels were measured by the ion selective method on the same system. The Blood Cell counting was performed on The Advia 120 Hematological system (Bayer).
All results were expressed as means ± SD. Statistical analysis was done with Student t-test. The values in Table 2 were calculated using one way ANOVA test.

Results and Discussions

Table 1 shows the levels of Na^+, K^+ Ca^{++} , Cl^- Mg^{++} and PO^-_4 in the experimental and control groups. Table 2 shows the values of the arterial blood pressure in the group using *C. tanacetifolia* leaf extract for a long-term (n=15) and the group given K^+ (n=15) and the control group (n=10). Table 3 shows the hematological values in the experimental and control groups.
In this study, we investigated chronic usage of *C. tanacetifolia* leaf extract experimentally. We found that the mean arterial blood pressure was reduced in the experimental group compared to

the control group (Table 2). We also determined the hematological values and the variations of body ion concentrations (Table 1 and 3)

Table 1. Total body ion concentration of the experimental group.

Parameters	Control group (n=10)	Experimental group (n=15)
Sodium (Na^+) mmol/L	141.30 ± 4.08	141.13 ± 3.90
Potassium (K^+) mmol/L	5.08 ± 0.32	5.76 ± 0.29**
Clor (Cl^-) mmol/L	101.80 ± 2.09	100.66 ± 1.17
Magnesium (Mg^{++}) mmol/L	0.99 ± 0.02	1.01 ± 0.06
Phosphat (PO_4^-) mg/dL	4.62 ± 0.44	8.20 ± 0.45**
Calcium (Ca^{++})mg/dL	9.19 ± 0.42	9.54 ± 0.40*

** P<0.001 , *P< 0.05

Tablo 2. Values of arterial blood pressure control, K^+ Administrated and groups of long term using *C.tanacetifolia* leaves extract.

Parameters	Control group (n=10)	*Crataegus* (n=15)	Normal + K^+ (n=15)
Mean arterial blood pressure (mmHg)	135 ± 5.7	105 ± 3.1**	120 ± 6.8*
Beat per minute (bpm)	312 ± 15	325 ± 12.4	329 ± 5.3

* P<0.05, ** P<0.01

Tablo 3. Hemotological values of the experimental and control groups

Parameters	Control group (n=10)	Experimental group (n=15)
WBC10^3/μl	4.16 ± 0.62	4.38 ± 0.48
RBC 10^6//μL	7.55 ± 0.50	7.80 ± 0.57
HCT (%)	38.79 ± 2.68	39.2 ± 1.66
HGB (g/dL)	13.38 ± 0.62	13.54 ± 0.68
NEUT (%)	67.2 ± 12.4	69.9 ± 15.3
LYMPH (%)	28.7 ± 13.9	25 ± 12.7

In this study, we investigated chronic usage of *C. tanacetifolia* leaf extract experimentally. We found that the mean arterial blood pressure was reduced in the experimental group compared to the control group. We also determined the hematological values and the variations of body ion concentrations.

In the experimental group, hemoglobin values, leukocyte and red blood cell count increased slightly, but these levels were not significantly high as given in (Table 3). In this group when compared to those of the control group, there were no significant difference in body ion concentrations of Na^+, Cl^-, Mg^{++} however, K^+, Ca^{++} and PO_4^- concentrations increased in the experimental group using *C.tanacetifolia* extracts. This increase was significantly meaningful for K^+ and PO^-_4 ($p< 0.001$) but for Ca^{++} ($p<0.05$).

The changes of Na^+ and K^+ concentrations in extracellular fluids can trigger potentials of myocardial fibers, because the electrical activity of heart depends on diffusion of these ions at muscle cell membrane. The rest potential of membrane muscle fiber decreases when the extracellular K^+ concentration increases and thus stimulation of fibers becomes harder. We established that the blood pressure decreases while K^+ ion concentration increases in the experimental group (*Crataegus* group).

The effect of *Crataegus* species on cardiovascular system has been investigated in many studies. Some investigators attributed the activity to nitric oxide, others to procyanidins which are present in plant extracts. In our previous investigations with *C. tanacetifolia* leaf extract we established that the flavonoids should be responsible for the activity (Tamer *et al.*, 1999).

In this study, we established increased extracellular K^+ ion concentrations in the experimental group. This increase may cause arteriolar dilatation by increasing Na^+ ion excretion and suppressing renin secretion. Thus, it may reduce blood pressure. This can occur by stimulating activity of Na^+-K^+ ATPase and decreasing intracellular Ca^{++} concentration. In addition to this effect, it can reduce blood pressure by decreasing response to endogen vasoconstrictors with the help of flavonoids in the *Crataegus* leaf extract.

In a study, it is suggested that accumulation of K^+ in hypertensive rats reduces blood pressure and also incidence of stroke (Tobian, 1986). There are some studies suggesting treatment with Ca^{++} and K^+ in essential hypertension (Kaplan, 1988 ;Hardman, 1996).

The accumulation of 48 mmol K^+ per day account for decrease in systolic and diastolic blood pressure in the medium level hypertensive patients (Siena *et al.*, 1987). This result is in accordance with our study. In our study, although the animals were normotensive, increased extracellular K^+ level is probably caused by decreased blood pressure. There are also some studies suggesting the accumulation of K^+ ion in the body may have protective effect against ventricular ectopy and stroke (Khaw *et al.*,1987). Also, another study shows that *Crataegus* extracts blocks the flow of K^+ ions in the ventricular cardiac myocytes, but it has no effect in the calcium flow (Müller *et al.*,1999).

In our study, we have also seen that the application of *Crataegus,* chronically increases the inorganic phosphat concentration. The phosphorus is found in ATP, cAMP compounds and proteins. It is connected with phosphorylation and dephosphorylation of proteins. We found no study dealing with the change of ion concentration of the body among studies made on *Crataegus* species. However, this extract may regulate blood pressure by increasing extracellular K^+ ion levels.

Özet

Crataegus türleri geleneksel tedavide çok eski zamanlardan beri kullanılmıştır.Bitkinin bileşiminde bulunan prosiyanidinler,aromatik asitler, amin tipi bileşikler ve özellikle de flavonoidler kardiyoaktif etkilerden sorumlu tutulmaktadır.

Bu çalışmada *Crataegus tanacetifolia* sulu yaprak ekstresi normal sıçanlara uzun süreli olarak uygulanarak, total vücut iyon konsantrasyonlarına olan etki incelenmiştir.

Hayvanlara 50mg/kg. *C.tanacetifolia* ektresinden mide gavajı yoluyla 1 ay süre ile uygulandığında, potasyum,inorganik fosfat ve kalsiyum iyonlarının deney grubunda kontrol grubuna göre anlamlı olarak arttığı tespit edilmiştir.

Bu sonuç bitkinin gösterdiği hipotansif etkide, muhtemelen iyon değişikliklerinin de rolü olabileceğini düşündürmektedir.

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