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Effect of Crataegus and Hyperoside on renal dysfunction and renin release in L-NAME-induced hypertensive rats

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Abstract
Crataegus has long been used as a folk medicine all around the world. The pharmacological effects of *Crataegus* have mainly been attributed to the flavonoids. Epidemiological studies have been suggested that flavonoids decrease death from coronary heart disease and stroke incidence. In this study, we investigated the effect of *Crataegus tanacetifolia (CT)* and *hyperoside* on renal dysfunction and renin release in L-NAME-induced hypertensive rats. Twenty-eight male Wistar albino rats were used in this study. Rats were divided randomyl info four groups. Group 1: Saline control group (n=8); Group 2: N^{ω} nitro-L-arginine methyl ester (L-NAME) (50 mg/kg)-induced hypertensive group (n=8); Group 3: L-NAME+*Crataegus tanacetifolia* (*CT*) (100 mg/kg/day by gavage**)** group (n=6); Group 4: L-NAME+*Hyperoside* (6 mg/kg/day by gavage) group (n=6). To produce long-term hypertension, L-NAME was dissolved in the drinking water at a concentration of 0.5 gr/L and then given to three groups for four weeks. Mean arterial blood pressure decreased significantly in *CT* and *hyperoside* groups, compared to non-treated L-NAME hypertensive rats. Glomerular filtration rate (GFR)**,** urine osmolality (U_{osm}), water, Na^+ and Cl^- excretion, number of renin-positive areas in kidney cortex were increased in L-NAME+*CT* group compared to hypertensive group. Urinary excretion of water (UV) decreased significantly while GFR and urine osmolality showed a significant increase in *hyperoside* treatment group compared to hypertensive group. However, renin-positive areas were significantly increased in kidney cortex in this group. In conclusion, in this study showed that *CT* especially the *hyperoside* can be partially prevented L-NAME-induced renal injury and increased renin granule in rats.

Keywords: *Crataegus tanacetifolia*, *Hyperoside*, Kidney Structure, Renin, L-NAME

1. Introduction

Crataegus (*hawthorn*) species has been used for many years because of its protective effects on the cardiovascular system (Chen et al. 1998; Garjani et al. 2000; Kim et al. 2000; Lacaille-Dubois et al. 2001). It was concluded that the *Crataegus* extract containing procyanidins, flavonoids, aromatic acids and cardiotonic amins may play a role in these activities (Wagner and Grevel 1982; Tost et al. 2000). *Hyperoside* is a native compound containing the total mixture of flavonoids isolated from the leaves of *Crataegus tanacetifolia* (*CT*).

Flavonoids are present in the human diet and isolated from various plant extracts. Several epidemiological studies have shown an inverse association between dietary flavonoids and coronary heart disease mortality and incidence of stroke (Hirvonen et al. 2001). Recent studies report that quercetin reduced elevated blood pressure, endothelial dysfunction and cardiac hypertrophy in spontaneously hypertensive rats (Duarte et al. 2001). In addition, quercetin also has antihypertensive and end-organ

protective effect in chronic nitric oxide deficient rats (Duarte et al. 2002). Some of the herbal drugs have been used as alternative treatment for hypertension (Kagathara et al. 2009; Patel et al. 2010).
It has been recently suggested that quercetin and its metabolites showed endothelium independent vasodilator effect (Pérez-Vizcaino et al. 2002) and antiaggregant effect (Gryglewski et al. 1987) which may explain their cardiovascular protective effects (Tamer et al. 1999). Vasodilatory effect of monoacetyl-vitexinrhamnoside (a flavonoid found in *Crataegus* species) may be mediated in part by endothelium-derived relaxing factor (EDRF) in addition to phosphodiesterase inhibition (Schüssler et al. 1995).
We have observed that extract of the *CT* leaf exhibits marked acute hypotensive effect at an i.v. dose of 100 mg/kg (Birman et al. 2001). Our previous study showed that the leaf extract of *C. tanacetifolia* and especially the *hyperoside* prevented the rise in arterial blood pressure induced by L-NAME (Kocyildiz et al. 2006). In the same study, histopathological data of coronary arteries demonstrated reduced in artery wall thickness and dilating of the lumen in both the plant extract and *hyperoside*-treated groups. It also causes significantly lower plasma lipid levels in the *Crataegus* extract group compared to the L-NAME group (Kocyildiz et al. 2006).
Considering a number of reports on antihypertensive effect of flavonoids, it is interesting that few literatures are available regarding on kidney function and renin secretion in L-NAME-induced hypertensive rats. For this reason in the present study we investigated the protective effects of *C. tanacetifolia* and its natural flavonoid *hyperoside* on kidney function and renin secretion in normal and L-NAME-treated rats. Thus, the present study is important to identify new knowledge's for the treatment of hypertension and for future benefits.

2. Materials and Methods

2.1 Plant material

Identified by Kerim Alpınar (Istanbul), and deposited in the Herbarium of the Faculty of Pharmacy, University of Istanbul (ISTE 61150), the leaves of *Crataegus tanacetifolia* (Lam.) Pers. (*Rosaceae*) were collected in May 1999 from Seben region, Bolu, Turkey.

2.2 Leaf extract

The dried leaves of the *C. tanacetifolia* were powdered and 50 g 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 vacuum to obtain a dry residue (yield 9.5%), which was then diluted with water in order to obtain a concentration of 50 mg/ml (Melikoglu et al. 2004).

2.3 Isolation of hyperoside

The material (1 kg), which was dried at room temperature, was first extracted with petroleum benzine to separate lipophilic compounds. After removing the lipophilic compounds the remaining material was extracted with 96% ethanol. The alcoholic extract was evaporated and then diluted with water and extracted with benzene, chloroform and ethylacetate subsequently 25.7 g (yield 2.5%) ethylacetate extract was gained from 1 kg leaf material, 6 g of this extract was chromatographied over Kieselgel 60 (0.2-0.5 mm). Elution was made with the changing percentages of toluen-ethanol mixture. Chromatography was ended by eluating with pure ethanol 100 ml of each fraction was taken. The similar fractions were gathered by controlling with paper chromatography (PC) and thin layer chromatography (TLC). 69-98 fractions were purified with water-ethanol in poliamid column and then only with methanol in Sephadex LH-20 colum. A total of 1840 mg (yield 0.184%) *hyperoside* was obtained.

2.4 Experimental design

Animal experiments were reviewed and approved by the Animal Care and Use Committee of Istanbul University (No: 140/2008). Twenty-eight Wistar albino male rats were housed in groups of two to four per cage in a controlled temperature room (22±3˚C). They were fed with standard pellet diet and tap water ad *libitum*. In the experiments, a total of 28 rats (260±35 g) divided into four groups, and received the following treatments for 4 weeks.

1. Group Control (n=8) Received a daily saline 2 ml/kg for 4 weeks by gavage.
2. Group L-NAME (n=8) Received 50 mg/kg L-NAME (Sigma Chemical Co. St Louis, USA) and was named L-NAME-induced hypertensive group (Poyraz, 2000). To produce long-term hypertension, L-NAME was dissolved in the drinking water at a concentration of 0.5 g/L and then given to three groups for four weeks.

3. Group L-NAME+*CT* (n=6) Received 50 mg/kg L-NAME followed by daily administration 100 mg/kg/day extract of *CT* leaves, by gavage.
4. Group L-NAME+*Hyperoside* (n=6) Received 50 mg/kg L-NAME followed by daily administration 6 mg/kg/day *hyperoside*, by gavage.

Systemic arterial blood pressure was measured by the tail-cuff method (Blood presssure monitor-Rhema-Labortechnic) at the beginning of the experiments (before any treatments) and at the end of the first, second, third and fourth week. For measuring systolic blood pressure, conscious rats were placed individually into the plexiglass resteiner from a warm chamber maintained at 37°C for 1 hour. The tail-cuff device was placed around the rat's tail and at least six pressure measurements were recorded for each rat and then the avarage systolic blood pressure was calculated (Normal blood pressure 60-90/75-120) (Poyraz, 2000). At the end of experiment, animals were placed individually into metabolism cages in order to collect urine in graduated cylinders. Urine volume and electrolyte concentrations were measured. Urine sodium, potassium, chloride and creatinin concentrations were determined by Cobas Integra 400 Plus Autoanalyser (Roche). Serum sodium, potassium, chloride and creatinine concentrations were determined by Roche Analytic Moduler System (DPP). Glomeruler filtration rate (GFR) was evaluated by creatinine clearance. Urine and plasma osmolality were determined using an osmometer (Gonotec osmometer). After these procedures, rats were anaesthetised with Pentothal sodium (35 mg/kg, i.p). Their chests were opened, approximately 2-2.5 ml of blood were taken from the left ventricule into the anticoagulant-free vacuum tubes, and the serum was separated by centrifugation for the the plasma osmolality tests.

2.5 Histological examination

The kidney tissue was fixed in 10% neutral formalin for 24 hours and then a routine paraffin embedding method was used to obtain histological sections of 5-µm thickness that were subsequently stained with Hematoxylin and eosin (HE) for evaluation of morphological changes under light microscope and photographed. Renin granules stained with Bowie's method (Pitcock and Hartroft 1958).

2.5.1 Preparation of Bowie's stock solution

One gram of Biebrich scarlet was dissolved in 250 ml of distilled water and filtered through a filter paper into a beaker. Two gram of ethyl violet was dissolved in 500 ml of distilled water and filtered into the same beaker. The mixture was then be filtered and the precipitate dried. The stock solution was made by dissolving 0.2 g of the dried precipitate in 20 ml of 95% alcohol.

2.5.2 Staining procedure for renin granules

The paraffin sections were rapidly taken through xylols and alcohols to alcoholic iodine (in 50% alcohol 1%) for 3 minutes. They were immersed in sodium thiosulfate (5%) for 3 minutes, and then washed in running tap water for 5 minutes. Slides were mordanted in 2.5% potassium bicromate at approximately 40°C over night, then rinsed with distilled water. Subsequently, they were immersed in sterilizier for 3 hours, and then in 20% ethanol to which 10 to 15 drops of Bowie's stock solution per 100 ml were added over night at room temperature. Slides were dried with blotting paper, and dipped quickly 2 to 3 times in two changes of acetone to remove excess stain. Sections were differentiated in a 1:1 mixture of xylol and clove oil for 15-20 minutes until red or reddish purple appears. Sections were rinsed with changes of xylol followed by changes of benzene and mounted with permount ((Pitcock and Hartroft 1958). Kidney renin activity is quantitatively determined by renin-positive areas in kidney cortex. Counts of renin-positive areas were done in 20 areas (each area is 0.26 mm^2) from five slides prepared from all experimental groups, using an 40x objective with an 10x ocular at light microscope.

2.6 Statistical analysis

The results were expressed as mean ± SD. One-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests were used for statistical analysis. **P*<0.05, ***P*<0.01 values were considered statistically significant.

3. Results

As shown in Table 1, rats receiving L-NAME showed a progressive increased systolic blood pressure as compared to control animals (Birman et al. 2011).

Table 1. Systolic arterial blood pressure, mean arterial blood pressure and standard deviations in experimental groups (*Nobel Med*. 7: 17-22, 2011)

Blood pressure (mmHg)	Initial SABP (mmHg)	1. week SABP (mmHg)	2. week SABP (mmHg)	3. week SABP (mmHg)	4. week MABP (mmHg)
Control (n=8)	103 ± 5.8	105 ± 3.8	108 ± 2.4	107 ± 3.3	107 ± 4.6
L-NAME (n=8)	108 ± 5.7	124 ± 9.2	139 ± 12.7	161 ± 9.6	178 ± 10.3***
L-NAME+*CT* (n=6)	116 ± 8.4	138 ± 18.5	150 ± 25.0	152 ± 20.4	166 ± 11.6
L-NAME+*Hyperoside* (n=6)	122 ± 9.6	131 ± 15.7	141 ± 8.4	150 ± 18.5	136 ± 15.0 ***

****P*<0.001 compared to L-NAME group
SABP: Systolic arterial blood pressure, MABP: Mean arterial blood pressure, L-NAME + *CT*: L-NAME plus *Crataegus tanacetifolia*

3.1 Kidney function tests

Glomerular filtration rate, excretion of water, sodium, and urinary osmolality were significantly increased in L-NAME+*Crataegus* group compared to the control. While Cl^- was decreased in hypertensive group compared to the control, Cl^- was increased in L-NAME+*CT* group ($P<0.05$) compared to L-NAME group. Glomerular filtration rate and urinary osmolality (Table 2) showed an increase of significance while urinary excretion of water decreased significantly in L-NAME+*Hyperoside* group compared to hypertensive group (Table 3). Whereas urinary osmolality decreased in L-NAME group compared to the control, a significant increase was seen in L-NAME+*CT* and L-NAME+*Hyperoside* groups compared to L-NAME group.

Table 2. Effect of oral administration of *Crataegus tanacetifolia* and its flavonoid *hyperoside* on glomerular filtration rate (GFR), urine (U_{osm}) and plasma (P_{osm}) osmolality in normal and experimentally hypertensive rats

Groups	GFR (ml/min/kg)	U_{osm} (Mosm/Kg d'H_2O)	P_{osm} (Mosm/Kg d'H_2O)
Control (n=8)	4.81 ± 0.6	1264 ± 26	309 ± 2.9
L-NAME (n=8)	5.37 ± 0.4	998 ± 35*	367 ± 3.1
L-NAME+*CT* (n=6)	7.01 ± 0.5**	1410 ± 47*	335 ± 7.4
L-NAME+*Hyperoside* (n=6)	6.16 ± 0.3*	1211 ± 52*	330 ± 1.2

Values are mean±S.D. **P*<0.05, ***P*<0.01 compared to L-NAME group

Table 3. Effect of oral administration of *CT* and *hyperoside* on urinary excretion of water (U_V, ml/kg/24h) and urinary electrolytes elemination in the experimental groups

Groups	UV (ml/kg/24 h)	$U_{Na}V$ (mEq/kg/24 h)	U_KV (mEq/kg/24 h)	$U_{Cl}V$ (mEq/kg/24 h)
Control (n=8)	21 ± 2.8	15 ± 2.3	150 ± 5.6	135 ± 6.4
L-NAME (n=8)	30 ± 5.2*	39 ± 3.6	142 ± 4.3	106 ± 7.2
L-NAME+*CT* (n=6)	36 ± 3.1*	65 ± 2.4**	150 ± 2.5	127 ± 5.3*
L-NAME+*Hyperoside* (n=6)	16 ± 4.2*	34 ± 5.4	150 ± 3.9	108 ± 8.2

Values are mean±S.D. **P*<0.05, ***P*<0.01 compared to L-NAME group

3.2 Kidney renin activity

Figure 1 shows that renin activity in L-NAME+*Hyperoside* group was increased compared to only L-NAME and L-NAME+*Crataegus*-administered groups. The results of renin activity are quantitatively expressed as renin-positive areas in kidney cortex in Figure 2.

3.3 Histopathologic results in kidney

The histopathologic results are summarized in Figure 3. The renal histological injury was detected in rats treated with L-NAME compared to controls. Fig. 3 B shows blood cells in the interstitial areas in rats' kidney cortex. L-NAME group showed thickening of the vascular wall, tubular atrophy, dilatation of Bowman capsule, rupturing of the vessels and extraverted interstitial erythrocytes. In this study, kidney cortex of *hyperoside* flavonoid-administered rats were normal and similar to those of the control rats (Fig. 3 D and A) and *Crataegus*-administered rats (Fig. 3 C). It was observed that dysfunctional effect of L-NAME on kidney was converted to normal structure when using *hyperoside* and *Crataegus* extract.

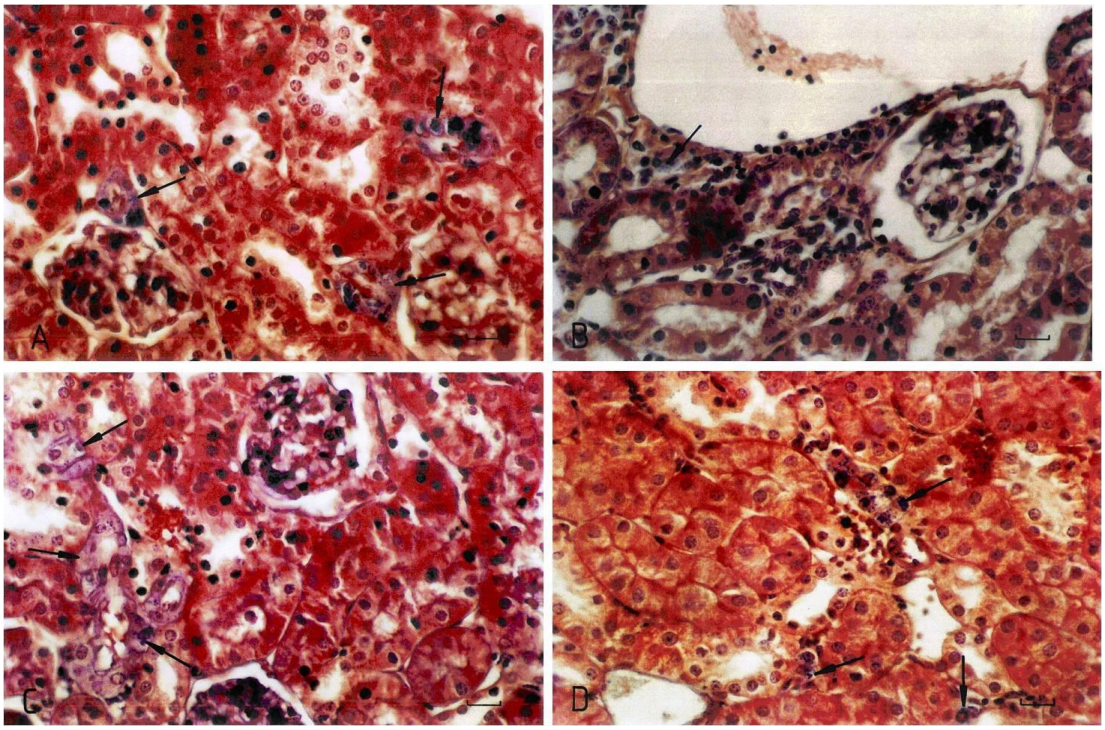

Figure 1 A-D. Renin granular areas in the kidney sections, A) Control, B) L-NAME, C) L-NAME+*Crataegus tanacetifolia* extract and D) L-NAME+*Hyperoside* treated animals in the kidney cortex. Arrows show Bowie's Method-stained areas. Scale bar: 5 µm.

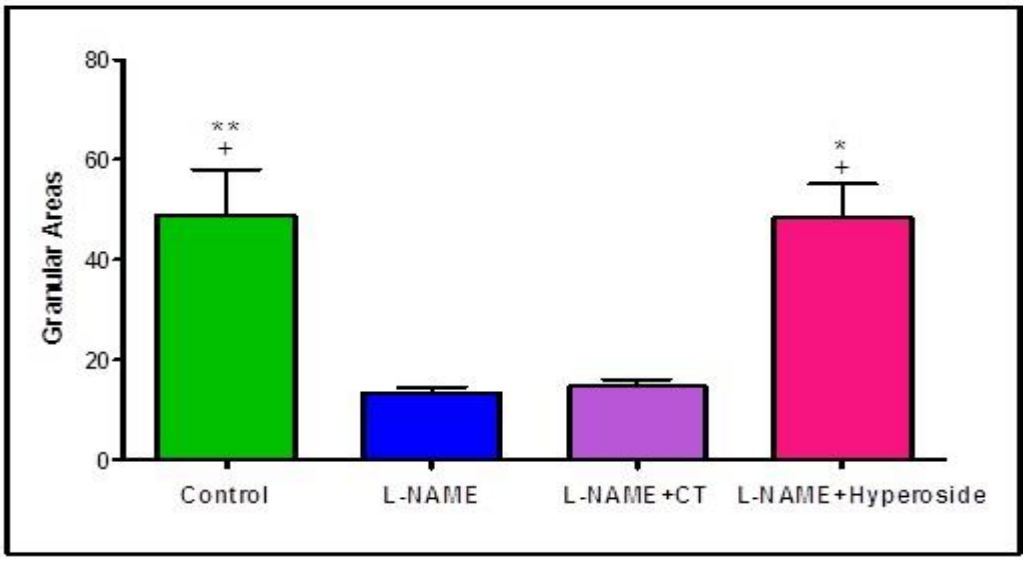

Figure 2. Renin granular areas in the kidney cortex
*: $P<0.05$, **: $P<0.01$ compared to L-NAME group, +: $P<0.05$ compared to L-NAME+*Crataegus* group

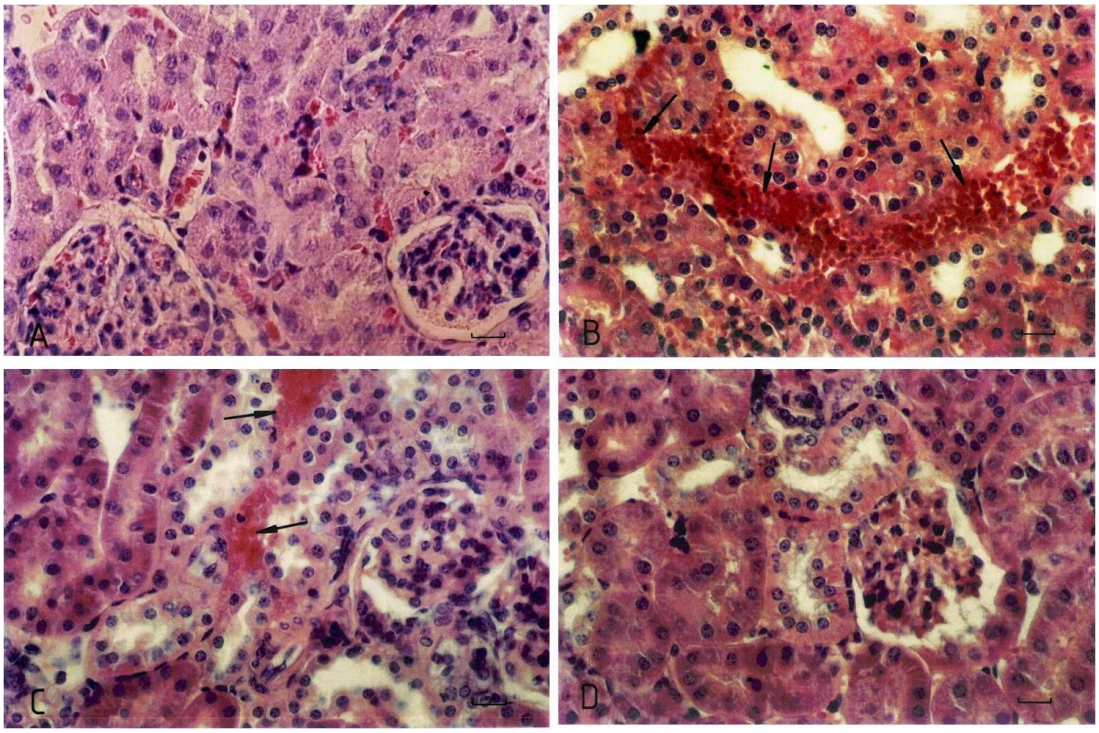

Figure 3 A-D. Histological staining of kidney sections of experimental groups. A) Control, B) L-NAME, C) L-NAME+*Crataegus tanacetifolia* extract, D) L-NAME+H*yperoside* treated animals. Arrows show blood cells in the interstitiel areas in the rat kidney cortex. Hematoxylin-eosin (HE), Scale bar: 5 µm.

4. Discussion and Conclusion

In the present study we have investigated the effects of *CT* and *hyperoside* on blood pressure, kidney function and structure in L-NAME-induced hypertensive rats. *Crataegus* extract improved GFR, excretion of water, sodium, chloride and urinary osmolality. We found that L-NAME had no effect on GFR. Tost et al. (2000) reported that NOS inhibition results in a decrease in GFR. However, GFR increased in L-NAME+*Crataegus* and L-NAME+*Hyperoside* groups in our study. An interesting finding for *hyperoside* which is known to have an antihypertensive activity, this situation may be related to nitric oxide synthesis in the kidney. In our previous study we have determined an increase in NOS activity in kidney tissue by means of *hyperoside* flavonoid of *Crataegus* plants (Kocyildiz et al. 2006). Although L-NAME was reported to inhibit all NOS isoforms (Mitchell et al. 1993) this was neglected for kidney by application of *hyperoside* flavonoid in our study. In spite of blokade of NOS, increased NOS activity in kidney of *Crataegus* and *hyperoside*-administered rats enabled blood pressure to lower in those animals, thus resulting in increament of GFR and sodium excretion. Alteration in NO synthesis may be an important factor in the control of sodium and water balance. Production of NO prevents excess vessel contraction and results in normalized excretion of sodium and water. It is known that inhibition of NO production by blocking drugs increases kidney vessel resistance and causes an increase in blood pressure and a decrease in GFR and sodium (He et al. 1995). In another study which has been performed with quercetin flavonoid, there was no change in aortic NOS activity (Duarte et al. 2002) while plasma NOS activity increased in other tissues. Similarly, procyanidin compounds of *Crataegus* caused a relaxation in rat aorta (Kim et al. 2000).

In our study, we determined that increased blood pressure was resulted from L-NAME and prevented by administration of *Crataegus* and *hyperoside*. In addition, excretion of sodium and water was higher in L-NAME+*CT* group. This finding probably points out that diuretic activity is much more effective on blood pressure reducing effect of *CT*. In L-NAME+*Hyperoside* group, excretion of water and sodium was not high, but dispersion of renin granule areas was increased compared to L-NAME group. That is why increased NOS activity in the kidney may cause renin production to elevate. It is reported that NO has a stimulating effect on renin excretion (Mitchell et al. 1993). Distribution of renin granules in kidney was inhibited in L-NAME receiving animals, whereas renin granules significant increment was observed in *hyperoside* group. This finding supports the assumption that *hyperoside* reduces blood pressure by increasing kidney NOS activity (Kocyildiz et al. 2006). Reduced blood pressure and the increasing

amount of renin granules may be related to uptake of *hyperoside*. When blood pressure increases with L-NAME, renin secretion decreases absolutely. It is mentioned by several studies that kidney's renin activity decreased before NOS was inhibited (He et al. 1995; Mundel et al. 1995). Thus, NOS inhibition by L-NAME significantly decreased renin-positive areas in the kidney, and this effect, at least in part, was due to the appropriate inhibition of the renin system. It was reported that flavonoids could be effective by ACE inhibition on blood pressure (Lacaille-Dubois et al. 2001). A recent study showed antihypertensive and negative chronotropic effects of plant extract including flavonoid, and suggested that this activity might be due to blockade of renin angiotensin system or renal antioxidant effect (Patel et al. 2010). On the other hand, one study put forward that L-NAME increased blood pressure and renal renin mRNA in spontaneously hypertensive rats (Ishiguro et al. 2007). In our study, amount of kidney renin granules decreased by L-NAME, and were returned to normal by application of *hyperoside*. Also in the study by Kocyildiz et al. (2006), NOS activity in kidney has shown a significant increase by *hyperoside*. These findings might suggest that flavonoids are antioxidants. Therefore, it is thought that increase in NO production causes action which decreases blood pressure. Studies on the mechanisms reported that the effects of flavonoids on blood pressure are mediated by antioxidant system. Flavonoids are known to decrease oxidative stress by inhibiting some enzymes (NADPH oxidase, xanthine oxidase) which produce oxygen radicals, or by preventing free radicals and by performing chelation by metal ions (Duarte et al. 2002). By using *hyperoside*, flavonoids both diminish partly the kidney damage caused by L-NAME and decrease the blood pressure much more efficiently. Similar findings are available in literature (Zatz and Baylis 1998; Duarte et al. 2002). Proposed mechanisms for antihypertensive effect of some flavonoids include decreased oxidative stress, improved endothelial function, direct action on the vascular smooth muscles, inhibition of angiotensin converting enzyme activity and modulation in cell signaling system (Larson et al. 2010). Some studies suggest that flavonoids are vasodilator through a variety of mechanisms, one of which is likely interaction with ion channels. Therefore, they recover ischemia-reperfusion injury (Akhlaghi and Bandi 2009).

In conclusion, these results suggest that *CT* and its flavonoid *hyperoside* prevents development of hypertension by means of partial recovery of renal dysfunction occuring with chronic NOS inhibition and return of kidney renin secretion to normal.

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Conflict of interest

The authors declare that there are no conflicts of interest.

References

Akhlaghi, M., Bandi, B. (2009). Mechanisms of flavonoid protection against myocardial ischemia-reperfusion injury. Journal of Molecular and Cellular Cardiology, 46, 309-317.

Birman, H., Akgün-Dar, K., Kapucu, A., Olgac, V., Gürel, E. (2011). Deneysel hipertansif sıcanlarda Crataegus tanacetifolia (Alıc) ve Hiperozit'in tükürük bezi ile böbrek dokusu üzerine etkileri ve nitrik oksit (NO) ile iliSkisi. Nobel Medicus, 7, 17-22.

Birman, H., Tamer, S., Melikoglu, G., Mericli, A. H. (2001). Hypotensive activity of Crataegus tanacetifolia. Journal of Faculty Pharmacy of Istanbul University, 34, 23-26.

Chen, Z. Y., Zhang, Z. S., Kwan, K. Y., Zhu, M., Ho, W. K., Huang, Y. (1998). Endothelium-dependent relaxation induced by hawthorn extract in rat mesenteric artery. Life Sciences, 63, 1983-1991.

Duarte, J., Jiménez, R., O'Valle, F., Galisteo, M., Pérez-Palencia, R., Vargas, F., Pérez-Vizcaíno, F., Zarzuelo, A., Tamargo, J. (2002). Protective effects of the flavonoid quercetin in chronic nitric oxide deficient rats. Journal of Hypertension, 20, 1843-1854.

Duarte, J., Pérez-Valencia, R., Vargas, F., Ocete, M. A., Pérez-Vizcaino, F., Zarzuelo, A., Tamargo, J. (2001). Antihypertensive effects of the flavonoid quercetin in spontaneously hypertensive rats. British Journal of Pharmacology, 133, 117-124.

Garjani, A., Nazemiyeh, H., Maleki, N., Valizadeh, H. (2000). Effects of extracts from flowering tops of Crataegus meyeri A. Pojark. on ischaemic arrhythmias in anaesthetized rats. Phytotherapy Research, 14, 428-431.

Gryglewski, R. J., Korbut, R., Robak, J., Swies, J. (1987). On the mechanism of antithrombotic action of flavonoids, Biochemical Pharmacology, 36, 317-322.

Guyton A. C., Hall J. E. (1996). Textbook of Medical Physiology. Nobel Tıp, Istanbul, 327.

He, X. R., Greenberg, S. G., Briggs, J. P., Schnermann, J. B. (1995). Effect of nitric oxide on renin secretion. II. studies in the perfused juxtaglomerular apparatus, American Journal of Physiology, 268, F953-959.

Hirvonen, T., Pietinen, P., Virtanen, M., Ovaskainen, M. L., Häkkinen, S., Albanes, D., Virtamo, J. (2001). Intake of flavonols and flavones and risk of coronary heart disease in male smokers. Epidemiology, 12, 62-67.

Ishiguro, K., Sasamura, H., Sakamaki, Y., Itoh, H., Saruta, T. (2007). Developmental activity of the renin-angiotensin system during the "critical period" modulates later L-NAME-induced hypertension and renal injury. Hypertension Research, 30, 63-75.

Kagathara, V. G., Ambikar, D. B., Vyawahare, N. S. (2009). Hypertension-animal models and phytomedicines: A review, Pharmacologyonline, 2, 436-461.

Kim, S. H., Kang, K. W., Kim, K. W., Kim, N. D. (2000). Procyanidins in Crataegus extract evoke endothelium-dependent vasorelaxation in rat aorta. Life Sciences, 67, 121-131.

Kocyildiz, Z. C., Birman, H., Olgac, V., Akgün-Dar, K., Melikoglu, G., Mericli, A. H. (2006). Crataegus tanacetifolia leaf extract prevents L-NAME-induced hypertension in rats: a morphological study. Phytotherapy Research, 20, 66-70.

Lacaille-Dubois, M. A., Franck, U., Wagner, H. (2001). Search for potential angiotensin converting enzyme (ACE)-inhibitors from plants. Phytomedicine, 8, 47-52.

Larson, A. J., Symons, J. D., Jalili, T. (2010). Quercetin: A treatment for hypertension? A review of efficacy and mechanisms. Pharmaceuticals, 3, 237-250.

Melikoglu, G., BitiS, L., Mericli, A. H. (2004). Flavonoids of Crataegus microphylla, Natural Product Research, 18, 211-213.

Mitchell, J. A., Kohlhaas, K. L., Sorrentino, R., Warner, T. D., Murad, F., Vane, J, R, (1993). Induction by endotoxin of nitric oxide synthase in the rat mesentery: lack of effect on action of vasoconstrictors, British Journal of Pharmacology, 109, 265-270.

Mundel, P., Gambaryan, S., Bachmann, S., Koesling, D., Kriz, W. (1995). Immunolocalization of soluble guanyl cyclase subunits in rat kidney. Histochemistry and Cell Biology, 103, 75-79.

Patel, S. S., Verma, N. K., Ravi, V., Gauthaman, K., Soni, N. (2010). Antihypertensive effect of an aqueous extract of Passiflora nepalensis wall. International Journal of Applied Research in Natural Products, 3, 22-27.

Pérez-Vizcaino, F., Ibarra, M., Cogolludo, A. L., Duarte, J., Zaragozá-Arnáez, F., Moreno, L., López-López, G., Tamargo, J. (2002). Endothelium-independent vasodilator effects of the flavonoid quercetin and its methylated metabolites in rat conductance and resistance arteries. The Journal of Pharrmacology and Experimental Therapeutics, 302, 66-72.

Pitcock, J. A., Hartroft, P. M. (1958). The juxtaglomerular cells in man and their relationship to the level of plasma sodium and to the zona glomerulosa of the adrenal cortex. American Journal of Pathology, 34, 863-883.

Poyraz Ö. (2000). Laboratuvar Hayvanları Bilimi. Kardelen Ofset, Ankara, 212.

Schüssler, M., Hölzl, J., Rump, A. F., Fricke, U. (1995). Functional and antiischaemic effects of Monoacetyl-vitexinrhamnoside in different in vitro models. General Pharmacology, 26, 1565-1570.

Tamer, S., Birman, H., Melikoglu, G., Mericli, A. H. (1999). The comparative investigation of the leaf, flower and fruit extracts of Crataegus tanacetifolia and the medicinal species C. monogyna on their effects on the cardiovascular system. Acta Pharmaceutica Turcica, 41, 117-119.

Tost, H., Hably, C., Lengyel, M., Gógl, A., Lendvai, A., Bartha, J. (2000). Effect of nitric oxide synthase inhibition on renal circulation and excretory function in anaesthetized rats. Experimental Physiology, 85, 791-800.

Wagner, H., Grevel, J. (1982). [Cardioactive drugs IV. Cardiotonic amines from Crataegus oxyacantha (author's transl)], Planta Medica, 45, 98-101.

Zatz, R., Baylis, C. (1998). Chronic nitric oxide inhibition model six years on, Hypertension, 32, 958-964.