**Effect of Yucca schidigera extract on blood pressure, antioxidant activity and some blood parameters in the L-name-induced hypertensive rats**

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**Summary:** The present study was aimed at investigating the effects of dietary supplementation of Yucca schidigera extract (YSE) on blood pressure, antioxidant activity and some biochemical parameters in the L-NAME-induced hypertensive rats. The study was performed on eighteen male Sprague-Dawley rats divided into 3 groups with 6 animals per group: 1) Control, fed standard chow ad libitum, 2) N-nitro-L-arginine methyl ester (L-NAME)-treated group, 75 mg/L drinking water, 3) YSE+L-NAME group, YSE (125 mg/kg diet)+L-NAME (75 mg/L drinking water), through study period lasted at 4 weeks. L-NAME-treated rats had significantly (P<0.05) higher the mean arterial blood pressure (MABP) as compared with the rats in the control and YSE+L-NAME groups. L-NAME+YSE-treated rats had lower heart rate (HR) as compared to the L-NAME-treated and control rats and, higher vascular NO. The results may suggest that YSE ameliorates the increase of MABP in the L-NAME-induced hypertension by enhanced generation of vascular NO.

Key words: Cholesterol, glucose, nitric oxide, vitamin A, yucca schidigera extract

**L-NAME ile hipertansiyon oluşturulan çiçelerde diyet Yucca schidigera ekstrakti (YSE) ilavesinin kan basıncı, antioksidan aktivite ve bazı biyokimyasal parametreler üzerine etkisi**

**Özet:** Bu çalışmada, L-NAME ile hipertansiyon oluşturulan çiçelerde diyet Yucca schidigera ekstrakti (YSE) ilavesinin kan basıncı, antioksidan aktivite (AA) ve bazı biyokimyasal parametreler üzerine etkileri araştırıldı. Araştırdamda, 18 adet erkek Sprague-Dawley çiçeğin beslenmesi ve siêuklar; 1 standart diyetle ad libitum beslenen kontrol grubu, 2) içme suları günde 75 mg/L N-nitro-L-arginine methyl ester (L-NAME) uygulanan grup ve 3) içme sularında L-NAME alan grubun diyetine 125 ppm YSE uygulanan grup olmak üzere her biri 6 siêuanden oluşan üç grubu ayrıldı. Araştırma 4 hafta sonra sonlandırıldı. L-NAME uygulanan siêuklararda ortalama arteriyel kan basıncı (OAKB) kontrol ve YSE+L-NAME gruba göre yüksek çıktı. Kalp atım sayısı ise YSE+L-NAME uygulanan siêuklarda kontrol ve L-NAME grubuna göre düşük belirlendi. L-NAME uygulaması NO metabolitleri (NOx) ile vitamin A düzeyini azaltırken, β-karoten ve methemoglobin değerlerini artırdı. Sonuç olarak, L-NAME ile hipertansiyon oluşturulan çiçelerin diyetine YSE ilavesinin NO oluşumunu artıracak bazı ortalama arteriyel kan basıncı düzenlenebileceği düşünülmektedir.

Anahtar sözcükler: Glikoz, Kolesterol, Nitrik oksit, vitamin A, yucca schidigera ekstrakti

**Introduction**

Nitric oxide (NO) released by endothelial cells is an important regulator of vascular function. The ability of NO to maintain vascular tone is deficient in hypertensive condition (16). Therefore, long-term blockade of NO synthesis causes systemic arterial hypertension (18). Saponins are widely distributed in many plant species and have complex chemical structures consisting of a variety of triterpenoidal or steroidal aglycons and various carbohydrate moieties. Although saponins have always been considered as deleterious by scientists, lately, they are accepted as functional components in food because of their physiological properties (28). Experiments demonstrating the physiological, immunological and pharmacological properties of saponins have provoked considerable clinical interest in these substances (6,13). *Yucca schidigera* (YS) originates from the lily family of plants and grows wild in the high deserts of the...
American southwest and the mountains of northern Chili, known and used for food and medicine by native people for centuries (6). YS has the highest saponin content of any Yucca species and, is the most common commercial source of steroidal saponins (26). Therefore, the condensed juice of this plant finds wide commercial application as food, cosmetic and pharmaceutical additive (6). YS extracts have been used for centuries in folk medicine to treat a wide variety of inflammatory disorders, especially headaches, gonorrhea, arthritis and rheumatism (13,20) but in literature there are no data about its physiological characteristics such as antihypertensive and relating blood lipid peroxidation with hypertension in rats fed a diet containing YSE. Therefore, the aim of this study was to investigate whether NO-deficient hypertensive rats could be ameliorated by the administration of YSE. To evaluate the blood pressure change and oxidative stress by YSE in the L-NAME-induced hypertensive rats, we have also studied the effect of dietary supplementation of YSE on blood pressure, antioxidant activity and some biochemical parameters in the L-NAME-induced hypertensive rats.

**Materials and Methods**

Eighteen rats (Sprague-Dawley strain with a body weight of 220-250 g) fed with a standard laboratory chow and water were used. The study protocol was approved by the Ethical Committee of the Afyon Kocatepe University (B.30.2.AKÜ.0.8Z.00/092). Rats were randomly divided into 3 groups containing six animals per group and placed in separate cages during the study. The groups occurred as 1) Control, fed standard chow ad libitum, 2) N-nitro-L-arginine methyl ester (L-NAME)-treated group, 75 mg/L drinking water (25), 3) L-NAME+YSE treatment group, L-NAME, 75 mg/L drinking water and 125 mg/kg supplementation of a commercial source of YSE (De-Odorase® Alltech Inc., USA) powder into diet of the rats (4).

At the end of this study lasted at 4 weeks, the mean arterial blood pressure (MABP) was measured by the tail-cuff method, via the tail cuff method using a model ML 125 rat tail NIBP system / Blood Pressure Amplifier/Pump from U.K. The heart rate (HR) was measured by MLS310 heart rate variability from Power Lab /4st. UK. The blood samples were taken from all rats after the animals were fasted overnight and sacrificed by anesthetizing with a combination of ketamine (80 mg/kg i.p.) and xylasine HCl (10mg/kg i.p.). The plasma was prepared by centrifugation (3000 x g, 10 min, +4 °C) to measure the biochemical parameters.

The malondialdehyde (MDA) as a biomarker for oxidative stress was estimated according to Draper and Hardley (10). Plasma antioxidant activity (AA) was measured according to Koracevic et al. (21). Nitric oxide was assayed by the colorimetric method of Griess (24) in the plasma. Methemoglobin level was measured spectrophotometrically according to Fairbanks and Klee (12). The plasma vitamin A and β-carotene concentrations were estimated by the method of Suzuki and Katoh (32) using a spectrophotometer. Total protein, total cholesterol and triglycerides were measured using commercially available assay kits (TECO Diagnostics, California, USA). Statistical analyses of data from the experiment were analyzed with SPSS (13.0) statistical software. Comparisons between different groups were performed by one-way ANOVA; if ANOVA revealed significant differences, the post-hoc comparisons were performed by Duncan’s multiple range test. Differences between means of p<0.05 were considered significant. The results were expressed as mean ± SE.

**Results**

The effects of L-NAME and L-NAME + YSE treatments on the MABP and HR during the 4-week periods of blockade of NO synthesis with L-NAME are shown in Table 1. Oral administration of L-NAME increased the MABP but unchanged the HR (p<0.05) versus control. Oral administration of L-NAME + YSE dropped significantly (p<0.05) the MABP and HR as compared with the control and L-NAME treated groups. Plasma concentration of NO metabolites (NOx) was decreased (p<0.05) by the treatment of L-NAME compared to the control group. The supplementation with YSE for 4 weeks reversed the decreased release of NOx by L-NAME-treatment. However, plasma concentration of NOx in the L-NAME + YSE treated rats was also higher (p<0.05) than in the control rats (Table 1). L-NAME+YSE treatment increased (p<0.05) the plasma MDA, but decreased plasma AA, cholesterol and total protein levels significantly (p<0.05) as compared with the control and L-NAME-treated rats. The treatment of L-NAME increased plasma cholesterol concentration versus the control, but decreased plasma vitamin A concentration as compared with the control and L-NAME + YSE treated rats and they were both statistically significant (p<0.05). The methemoglobin level in blood of the L-NAME + YSE treated rats was lower (p<0.05) than that of rats in the L-NAME treated rats, but similar to that of rats in the control group (Table 1).

**Discussion and Conclusion**

Pharmacological long-term blockade of NO synthesis by the chronic administration of L-NAME, an inhibitor of nitric oxide synthase (NOS), produces systemic arterial hypertension, vascular structural change, and renal dysfunction (7,18). Because NO
induces vasodilatation and inhibits renal tubular sodium reabsorption, reduced bioavailability of NO leads to peripheral vasoconstriction and sodium retention (34). We also observed in the present study that chronic oral administration of L-NAME into rats increased the blood pressure in association with decreased plasma NOx levels.

The use of plants and plant parts for therapeutic purposes has a long history. Recently, many studies have been performed to find more suitable antihypertensives from natural sources. Among them, extracts of saponin-rich plants have the hypotensive effects due to diuresis (29,35), endothelium-dependent vasorelaxation or direct stimulation of NO release (2,16,17) and inhibition of angiotensin-converting enzyme (9). In this study, the administration of YSE into diet attenuated the increase of MABP in L-NAME+YSE-treated rats. The plasma NOx were also reversed by co-administration of YSE through this experiment as in accordance with previous studies (16,17). In addition, the decrease of HR in L-NAME+YSE-treated rats observed in this study may be due to the inhibition effect of saponins on catecholamine secretion (22). The saponins from Henaria glabra (29) or Korea red ginseng (16,17) have an antihypertensive effect on conscious spontaneously hypertensive rats or the L-NAME-induced hypertension via enhancement of NO in the vascular tissues. Accordingly, YSE may also have an antihypertensive effect via stimulation of NO system in the vascular tissues.

Detection of lipid hydroperoxides and conjugated dienes and triobarbituric acid-reactive substances (TBARS) such as MDA, often applies to the study of lipid peroxidation reactions (4,11,19). Thus, the presence of MDA is taken as an indicator of free-radical damage through membrane lipid peroxidation (3). Hypertension may result in yielding reactive oxygen species (ROS). However, in this study, MDA and AA in the blood of L-NAME-treated rats were not different as compared to control rats. Cracowski et al. (8) found that lipid peroxidation and oxidative stress were not increased in untreated mild- to-moderate hypertension, and suggested that ROS may not be critical in the early stages of hypertension, but could be more important in severe hypertension. On the other hands, the increase of MDA and the decrease of AA in L-NAME+YSE-treated rats is contradictory to the reports of Piacente et al. (27) suggested that the significant activities exhibited by the phenolic fraction and its constituents showed the potential use of YS as a source of antioxidant principles. In the present study, an increased NO production in L-NAME+YSE-treated rats may be responsible for the elevated MDA and decreased AA indicating lipid peroxidation because of being a free radical with vasodilatory properties of NO (31).

The molecular studies are useful to understand biochemical pathways that have been altered during the development of hypertension. The L-NAME-treated rats had higher cholesterol levels in plasma than the other groups in this study. However, plasma cholesterol level in the L-NAME+YSE-treated rats was lower than in the L-NAME-treated and control rats. This result is in consistent with report that saponins are known to be hypcholesterolemic because of forming an insoluble complex with cholesterol, which prevents its absorption from the small intestine (15). Similarly, the L-NAME+YSE treated rats had significantly lower plasma glucose and total protein concentrations than the rats of control and L-NAME treated groups, as in agreement.

### Table 1. Effect of the supplementation of YSE to diet on blood pressure, antioxidant activity and some blood parameters in hypertensive rats (n=6, ±SE).

<table>
<thead>
<tr>
<th>Groups*</th>
<th>Control</th>
<th>L-NAME</th>
<th>L-NAME+YSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>MABP (mmHg)</td>
<td>103.50±2.41a</td>
<td>148.10±2.52b</td>
<td>123.20±3.88c</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>405.80±7.41a</td>
<td>399.50±7.82a</td>
<td>298.60±6.40b</td>
</tr>
<tr>
<td>NOx (µmol/L)</td>
<td>8.40±0.38a</td>
<td>8.20±1.24a</td>
<td>10.20±0.72b</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>67.10±2.74a</td>
<td>88.50±7.32b</td>
<td>56.10±4.58c</td>
</tr>
<tr>
<td>Total protein (g/dl)</td>
<td>6.20±0.24ab</td>
<td>6.70±0.29a</td>
<td>5.40±0.31b</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>130.80±14.38a</td>
<td>98.60±27.25ab</td>
<td>81.80±14.52b</td>
</tr>
<tr>
<td>Vitamin A (µg/dl)</td>
<td>62.80±4.80a</td>
<td>45.80±4.24b</td>
<td>56.30±2.30a</td>
</tr>
<tr>
<td>β-carotene (µg/dl)</td>
<td>3.30±0.30ab</td>
<td>3.80±0.23a</td>
<td>3.30±0.15b</td>
</tr>
<tr>
<td>Methemoglobin (%)</td>
<td>6.70±0.68a</td>
<td>12.50±1.68b</td>
<td>7.0±1.29a</td>
</tr>
</tbody>
</table>

Control, fed standard chow ad libitum; L-NAME, treated group, 75 mg/L drinking water; L-NAME+YSE, L-NAME treated 75 mg/L drinking water and 125 YSE mg/kg supplementation of a commercial source of YSE. Differences among treatments in same line are significant (p<0.05).

A,b,c: Differences among treatments in same line are significant (p<0.05).
with previous results in chicks (15). In addition, this result may be due to decreased absorption of amino acids across the small intestine, because saponins reduce protein digestibility probably by the formation of scarcely digestible saponin-protein complexes (29).

Vitamin A plays an important role in many physiological activities through its metabolites, particularly 9-cis and all-trans retinoic acids (atRA) (5). Previous studies have shown that a high intake of vitamin A and β-carotene is associated with a decreased risk for coronary artery disease (1,14,30). In the present study, the treatment of L-NAME decreased the vitamin A level and, increased the β-carotene level in the plasma of the rats. Lansink et al. (23) suggested that endothelial cells are exposed to the highest concentration of circulating atRA, express retinoid receptors, and play a significant role in atRA metabolism compared with other cell types. Achan et al. (1) demonstrated that atRA increases NO synthesis in endothelial cells without increasing eNOS expression and the expression of dimethylarginine dimethylaminohydrolase II (DDAH II), which is the predominant DDAH isoform in endothelial cells. The asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of NOS and metabolized by the enzyme DDAH (34). Therefore, the increase of the enzyme DDAH II in endothelial cells contributes to the increase of NO production (33). Our results may indicate that endothelial cells in the L-NAME treated rats received more vitamin A from circulating to product NO because of the decrease of NO production in the endothelial cells by the treatment of L-NAME known as an inhibitor of eNOS. In the present study, the increase of β-carotene in the L-NAME treated rats may also suggest that the biosynthesized vitamin A from the β-carotene in the liver and peripheral tissues is diminished by the L-NAME treatment because a significant fraction of β-carotene is absorbed from the intestine and is then converted to metabolites (retinoids) in the liver and peripheral tissues (15).

Triterpenoid saponins from Gypsophila and Quillaja included in the diet appear to interfere with the absorption of vitamins A and E in chicks. However, feeding a steroid saponin at the same level had no effect on any of these parameters (15). Our results confirmed the results of Jenkins and Atwal (15) and, may also indicate no effect of YSE containing steroidal saponins on the absorption of fat-soluble vitamins from the small intestine.

Alteration of hemoglobin-oxygen supply may be involved in the pathogenesis of hypertension. Zinchuk et al. (36) indicated that the endothelial dysfunction in patients with arterial hypertension leads to significant impairments in blood oxygen transport indices. In the present study, the treatment of L-NAME increased methemoglobin level, but the treatment of L-NAME +YSE did not effect it in blood of rats as compared with the blood of the control rats.

In conclusion, the inactivation of NO system was reversed by co-administration with YSE in the L-NAME-induced hypertension. YSE attenuated the increase of MABP in the L-NAME-induced hypertension via enhancing NO concentrations.

References


