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Original Article

Effects of Myrtus Communis L' Extract and Apocynin on Lens Oxidative Damage and Boron Levels in Rats Fed with High Fat-Diet

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Abstract

Objectives: Nutritional obesity causes oxidant damage in the body and cataract formation in the lenses by increasing the formation of free radicals. Myrtus Communis leaf extracts (Myr) have antioxidant properties, and Apocynin (Apo) is an effective NADPH-oxidase inhibitor. The data on tissue boron levels are quite lacking. The aim of this study, which was carried out for the first time in the literature, was to investigate the effects of Myr and Apo treatment on boron levels and oxidative lens damage in rats fed with high-fat diet (HFD).

Materials and Methods: Wistar albino male rats were randomly divided into four groups: Control Group; HFD Group; HFD + Myr Group; and HFD + Apo Group. Prior of the experiment body weight and blood lipids were determined. After decapitation, the lenses of rats were enucleated and homogenated. Catalase (CAT) and superoxide dismutase (SOD) activities and boron, malondialdehyde (MDA), and reduced glutathione (GSH) levels were determined in the lens homogenates.

Results: The HFD increased serum triglyceride ($p < 0.05$), total cholesterol level ($p < 0.001$), body weights ($p < 0.001$), and lens MDA levels ($p < 0.01$), and decreased lens GSH ($p < 0.05$) and boron level ($p < 0.01$), SOD ($p < 0.001$), and CAT activity ($p < 0.001$). However, Myr and Apo treatment reduced the rats' body weight ($p < 0.001$), serum triglyceride ($p < 0.05$), and total cholesterol level ($p < 0.001$); increased lens boron ($p < 0.01$; $p < 0.001$), GSH levels ($p < 0.05$; $p < 0.01$), and CAT activity ($p < 0.001$).

Conclusion: Both Myr and Apo may be able to reduce oxidative stress in the lenses of obese rats caused by HFD by increasing boron levels.

Keywords: obezite, lens, bor, antioksidanlar, Myrtus, apocynin

Introduction

Obesity is described as excessive or abnormal fat accumulation and it is known that it causes diabetes, hypertension, dyslipidemia, sleep apnea, respiratory problems, osteoarthritis, cardiovascular disease, and cancer. One of the mechanisms related to obesity and related comorbidities are the formation of excess oxidant and reactive oxygen species (ROS).[1] In various studies, it has been stated that increased ROS formation in high-fat diet (HFD), causes oxidant damage in the lens and, cataract development.[2,3]

Reactive oxygen species are produced during normal cellular oxygen metabolism. They are essential for numerous enzymatic reactions and biological functions. However, in some pathological conditions, they appear in excessive amounts and cause harmful effects at cellular level.[4] Peroxidation of polyunsaturated fatty acids in biomembranes often occurs by exposure to ROS. Malondialdehyde (MDA) occurs in the peroxidation of fatty acids containing three or more double bonds. MDA, which is one of the major end products of lipid peroxidation, is frequently used in evaluating oxidant damage.[5] Cells try to protect themselves from the harmful effects of ROS by developing various antioxidant systems. Endogenous antioxidants are catalase (CAT), superoxide dismutase (SOD),

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and glutathione (GSH), etc. Dietary antioxidants contribute significantly to the endogenous antioxidant system in relieving oxidative stress.[6]

It has been shown that plant phytochemicals have preventive actions against oxidative stress in various animal models.[7,8] *Myrtus communis* is commonly known as Myrtle, which is one of the edible foods and medicinal plants in the Mediterranean and the Black Sea regions, including Turkey and grows mainly in swamps and forests.[9] *Myrtus communis* leaf extracts (Myr) have been reported to have anti-inflammatory, antibacterial, and antioxidant properties.[10,11] Nicotinamide adenosine dinucleotide phosphate oxidase (NADPH oxidase) is a multi-enzyme complex that catalyzes the one-electron reduction of molecular oxygen to the superoxide anion. Therefore, this reaction is the major source of ROS.[12] Apocynin (Apo) which can be obtained from the *Apocynum cannabinum* plants' root, is a potent NADPH-oxidase inhibitor.[13]

The biological importance of boron is increasing day by day.[14,15] Although boron is not yet considered an essential element for humans, it is placed in a possible essential element class.[16] The data on tissue boron levels, boron metabolism, and boron mechanism of action are quite lacking. There is no previous study in the literature that determines lens boron levels.

The aim of this study was to investigate the effects of Myr and Apo treatment on boron levels and oxidative lens damage in rats fed with high-fat diet (HFD). To our knowledge, this study is the first study to show that the lens boron level is evaluated and that the HFD, Myr, and Apo can affect the lens boron levels.

Materials and Methods

Animals and Conditions

Wistar albino male rats (n = 20) aged 2 months were used and supplied by the XXX University Application and Research Center for Experimental Animals. Rats were placed in an air-conditioned room with light-dark cycles of 12h:12h, where the relative humidity (65% to 70%) and the temperature (22 ± 2°C) were kept constant. The ethical approval was obtained XXX University Animal Care and Use Committee (30.2019.mar).

Plant samples and preparation of extract of *Myrtus Communis*

Plant samples used in this study were collected from the Manisa city (Turgutlu region) in 2010. These samples were identified by a botanist in the XXX University, Faculty of Pharmacy. Voucher specimens were deposited in the Herbarium of XXX University, Faculty of Pharmacy (MARE no: 13006). Leaves of *Myrtus Communis* (100 g) were dried in the shade at room temperature. The dried pulverized leaves were extracted with 96% EtOH using a Soxhlet apparatus. Then, they were evaporated at 40°C until dry in a vacuum. This extract was stored in a dark container in the refrigerator (4°C) until use.

Study Groups

After seven days of the acclimation period, the rats were weighed and randomly divided into four groups as follows:

Control Group (n=5): Rats were fed with standard rat diet for 16 weeks.

HFD Group (n=5): Rats were fed with HFD including 45% fat for 16 weeks.

HFD + *Myrtus communis* L. Group (n=5): Rats were fed with an HFD for 16 weeks and Myr (100 mg/kg) was administered with orogastric gavage during the last 4 weeks of the study.

HFD + Apocynin Group (n=5): Rats were fed with an HFD for 16 weeks and Apo (Merck, Darmstadt, Germany) (25 mg/kg, in Dimethyl sulfoxide (%15), i.p.) was given during the last 4 weeks of the study.

Biochemical Analysis

At the end of 16 weeks, the rats were weighted again and decapitated, blood samples were collected for the total cholesterol, HDL-cholesterol, and triglyceride determinations and then the lenses were enucleated and homogenated to prepare 5% lens homogenates with 0.9% of NaCl solution. Lens homogenates were kept in deep-freeze at -80°C until assay time. Boron, reduced GSH and MDA levels, SOD, CAT activities were determined in the lens homogenates by the methods of modified carminic acid[17], Ellman[18], Ledwozwy[19], Mylorie[20], and Aebi[21], respectively.

Statistics Analysis

Statistical analysis was done using GraphPad Prism 5.0 (GraphPad Software, San Diego, USA). All data were expressed as mean ± standard error. Analysis of variance (ANOVA) was used for multiple comparisons followed by Tukey as posthoc test. The p-value of less than 0.05 was considered as significant.

Results

In this study, an obesity model was induced by an HFD. The weight values at the beginning and at the end of the experiment are shown in Figure 1. At the end of the 16th week, HFD group rats were significantly heavier than those of the control group

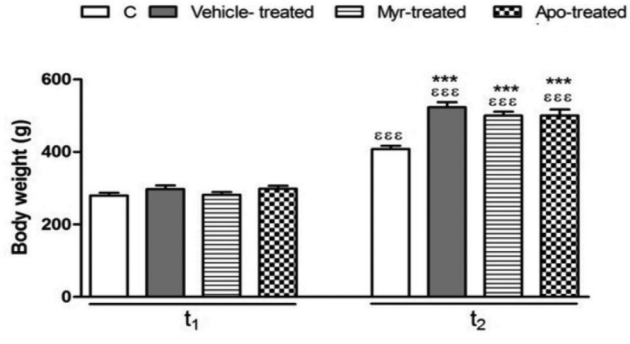

Figure 1. Bodyweight of groups recorded at the beginning (t1) and end of the study (t2)
The values were given as mean ± standard error. εεε: p<0.001 significantly from t1. ***: p<0.001 significantly different from control values

C: Control, HFD: High fat diet, Myr-treated: High fat diet + Myrtus communis L. Apo-treated: High fat diet + apocynin

(p<0.001); moreover, Myr and Apo-treatment significantly reduced this increase in bodyweights.

The total cholesterol, triglyceride, and HDL-cholesterol of rats at the end of 16 weeks are shown in Figure 2. The triglyceride (p<0.05) (Figure 2a) and total cholesterol (p<0.001) (Figure 2b) levels of the rats in the HFD group were higher, and HDL-cholesterol (p<0.001) (Figure 2c) levels were significantly lower than those of the control group. However, the total cholesterol and triglyceride levels of the rats receiving Myr and Apo-treatment were significantly lower and HDL-cholesterol levels were significantly higher than those of the HFD group.

At the end of 16 weeks, HFD groups' lens MDA levels were significantly higher than those of the control group (p<0.01) (Figure 3a). Lens MDA levels of Apo-treated rats were significantly lower than those of the control group (p<0.05) and the HFD group (p<0.001). Moreover, the lens MDA levels of the Apo-treated group were significantly lower than those of the Myr-treated group (p<0.001). Lens GSH levels in the HFD group were significantly lower than those of the control group (p<0.05) (Figure 3b). The lens GSH level of the Apo-treated (p<0.01) and Myr (p<0.05) groups was significantly higher than those of the HFD group. Lens CAT (Figure 3c) and SOD (Figure 3d) activities in the HFD group were significantly lower than those of the control group (p<0.001). There was no significant difference in SOD activity between the Apo-treatment and HFD groups. However, Myr-treatment groups' SOD activity was higher than those of the HFD group (p<0.05). Lens CAT activity in Myr and Apo-treatment groups was significantly higher than those of the control and HFD groups (p<0.001).

Lens boron levels in the HFD, Myr, and Apo-treatment groups were significantly lower than those of the control group (p<0.001). Moreover, Myr (p<0.05) and Apo-treatment (p<0.001) groups' lens boron levels were higher than those of the HFD group (Figure 4).

Discussion

It is known that HFD is strongly related to obesity. HFDs have been used for decades to induce dyslipidemia and obesity model in rodents.[22] In the present study, the body weights of HFD-fed rats (45% fat) were significantly higher than the control group (standard rat diet). However, this increase in body weight decreased in Myr and Apo-treatment groups. Similar to our study, it has been shown that the Myr treatment (200 and 400 mg/kg bw) in rats[23] and Apo treatment (5 mM, dissolved in drinking water) in mice, fed with HFD diets, causes decreased the bodyweight.[24] It has been shown that the polyphenols and flavonoids regulate the activity of PPAR-γ (peroxisome proliferator-activated receptor), inhibition of angiogenesis in adipose tissue, and SREBP (Sterol regulatory-element binding proteins) pathway.[25-26] Myr has a rich content of polyphenols and flavonoids. Therefore, it is thought that it can reduce body weight. It has been suggested that Apo can achieve this by preventing insulin resistance.[27]

In recent years, the use of plant extracts and plant-derived compounds has been increasing in research studies for the prevention and treatment of many cardiovascular diseases.[28] Rosa et al.[29] have reported that the semi myrtucommulone and myrtucommulone-A compounds contained in the Myr has antiatherogenic effects. Meng et al.[27] have shown that Apo significantly improves dyslipidemia in obese mice induced by HFD. In the present study, the total cholesterol and triglycerides levels of rats fed with HFD were significantly higher than those of the control group, while the levels of HDL cholesterol were lower. However, triglyceride and total cholesterol levels were significantly lower in Myr and Apo treatment groups than those of the HFD group, while HDL cholesterol was higher.

Oxidative damage is an important factor that causes cataracts, which make up almost half of human blindness cases worldwide. Generally, oxidation is considered to be a key feature of cataract formation.[30] HFD can cause cataract formation by the effect of

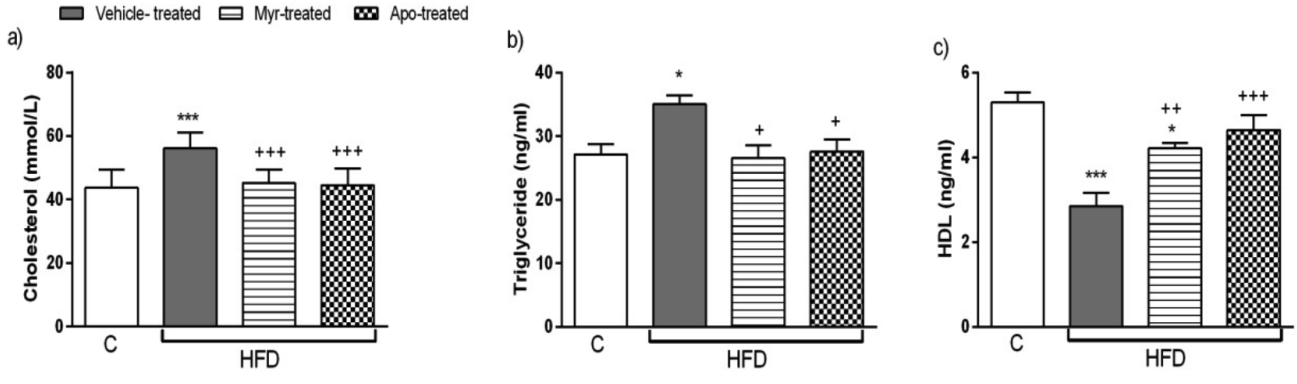

Figure 2. Total cholesterol, triglyceride, and HDL-cholesterol levels
The values were given as mean ± standard error
a: Total cholesterol, b: Triglyceride, c: HDL-cholesterol, *p<0.05, ***p<0.001: significantly different from control group. +p<0.05, ++p<0.01, +++p<0.001: significantly different from vehicle-treated group,

C: Control, HFD: High fat diet, Myr-treated: High fat diet + Myrtus communis L. Apo-treated: High fat diet + apocynin

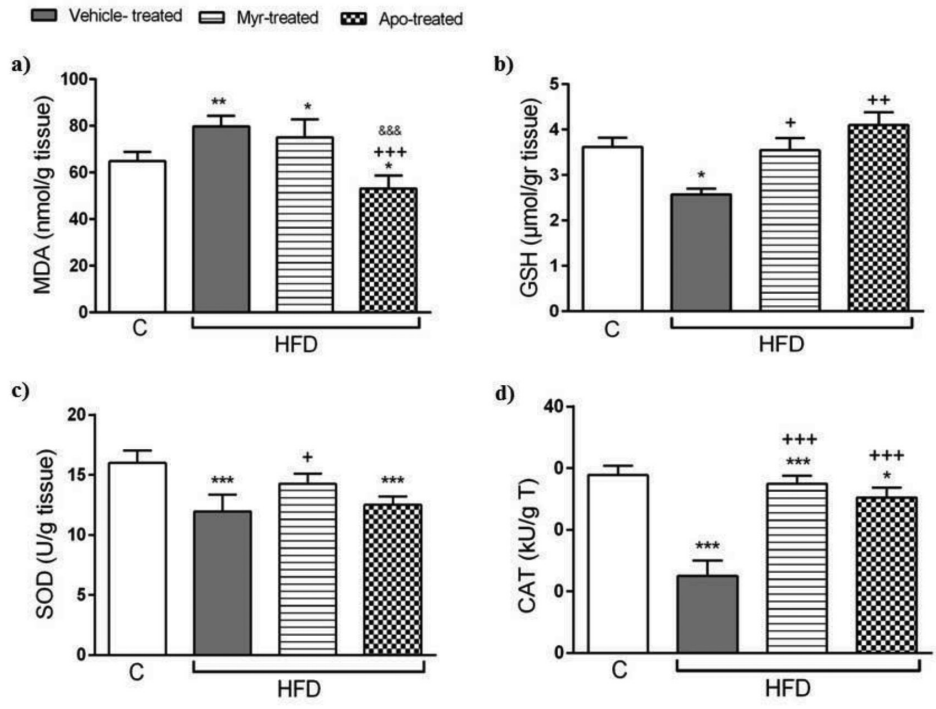

Figure 3. Lens malondialdehyde and glutathione levels, superoxide dismutase and catalase activities
The values were given as mean ± standard error. a: malondialdehyde (MDA); b: glutathione (GSH); c: superoxide dismutase (SOD); d: catalase (CAT). *p<0.05, **p<0.01, ***p<0.001: significantly different from control group. +p<0.05, ++p<0.01, +++p<0.001: significantly different from vehicle-treated group. &&&: p<0.001: significantly different from Myr-treated group.

C: Control, HFD: High fat diet, Myr-treated: High fat diet + Myrtus communis L. Apo-treated: High fat diet + apocynin

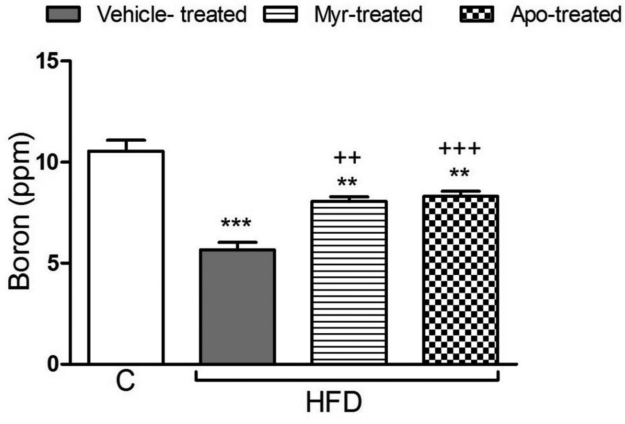

Figure 4. Lens boron levels
The values were given as mean ± standard error. **p<0.01, ***p<0.001: significantly different from control group. ++p<0.01, +++p<0.001: significantly different from vehicle-treated group.

C: Control, HFD: High fat diet, Myr-treated: High fat diet + Myrtus communis L. Apo-treated: High fat diet + apocynin

increased ROS in the lens.[2,3] In a case-control study conducted to evaluate the relation between diet and cataract risk, has been stated that the risk of cataracts increases as the total amount of fat intake with the diet increases (p <0.001).[31]

Ocular tissues include many antioxidants such as enzymes, proteins, ascorbic acid, glutathione, cysteine, and tyrosine to protect against excess ROS. The lens is a tissue most affected by oxidative damage.[32] It is also known that in cataract patients, the level of H_2O_2 in the lens may triple compared to a healthy lens.[33] It has been shown that SOD prevents the lens from the oxidative damage of H_2O_2 in rats.[34] It is known that GSH in the lens contributes to the protection of lens transparency.[35] GSH protects lens proteins' thiol groups against ROS. This is very important for the normal function of the epithelium of the lens, Na-K-ATPase enzyme, which affects cell permeability[35]. It is known that NADPH-oxidase is the main source of ROS and Apo is an effective NADPH-oxidase inhibitor.[36] As the major end product of lipid peroxidation, MDA is considered a toxic compound in the eye due to its high cross-linking ability with the lipid membrane.[37] In the present study, tissue oxidative damage was monitored with lens MDA levels. In rats fed with HFD, lens MDA levels were significantly higher than those of the control group. This result shows that HFD increases oxidative damage. Moreover, the lens levels of MDA in the Apo treatment group were significantly lower than those of the control and HFD groups. Furthermore, the lens GSH and CAT activity in the Apo-treatment group were significantly higher than those of the HFD group. These results show that Apo can protect the lens from oxidative damage. In a study, 2.4 g/L (in drinking

water) apocynin treatment for 5 weeks in mice fed HFD has been reduced systemic and hepatic oxidative stress.[27] It has been also found that cataract progression was reduced in rabbits given 20 mg/kg/day Apo (i.p.).[38]

Various studies have shown that *Myrtus Communis* has antimicrobial, anti-inflammatory, and anti-oxidant effects.[39-41] In the literature, studies showing the effect of *Myrtus Communis* on lens antioxidant status are limited. In STZ-induced diabetic rats, Myrtus extract has been shown to increase lens GSH ($p < 0.05$) and MDA levels ($p < 0.05$).[42] In the present study, the lens MDA levels were not a significant difference between HFD and Myr-treatment groups. However, Myr-treatment groups' GSH levels, CAT and SOD activities were significantly higher than those of the HFD group.

Boron is present in human tissues and body fluids as a natural result of boron intake of foods and drinking water.[14] Studies on the distribution of boron in tissues are limited in the literature.[15] Data on the mechanism of action of boron is insufficient. It is reported that boron may react with the cis-hydroxyl group containing biomolecules such as polysaccharides, adenosine-5-phosphate, pyridoxine, flavin (e.g., FAD) dehydroascorbic acid, and pyridine (e.g., NAD+ or NADP).[14] Having low atomic weight and being able to make compounds with organic molecules is thought to be important for the biological function of boron. It is also thought that boron may be effective in hormone receptors and trans-membrane signals, cell membrane functions, and stability.[43]

When boron compounds are taken orally, it is stated that they are rapidly converted into boric acid in the gastrointestinal tract and almost all of them are absorbed and distributed to the tissues through the blood.[14] Studies have shown that 84-85% of boron taken by diet is excreted from the body with urine. Although it is known that passive diffusion and/or sodium-dependent borate carrier-1 (NaBC1) is effective in the distribution of boron into tissues, it has not been fully elucidated yet.[44] Studies should be done about how boron is transported to the lens. In rats, 'no observed adverse effect level' (NOAEL) for developmental effects of boron is 9.6 mg boron/kg body weight/day. The oral lethal dose (LD50) for boron in rats is 400-700 mg boron/kg body weight.[45-46] The available human exposure studies are very limited due to geographical conditions and dietary differences, and the toxic oral reference dose and recommended dietary allowance (RDA) of boron for humans have not been clearly established. However, it is known that it is not possible to exceed the safe intake level (20mg/day) and toxic dose (500 mg/day).[47]

There is no previous study in the literature that examines lens boron levels. Therefore, we could not compare these results. However, it has been shown that the boron level decreases in plasma, kidney, brain, and liver tissue of rats receiving malathion which induces oxidative stress. Boron levels in these tissues were found to be quite lower than our lens boron levels.[48] Similar to the above study the lens boron level also decreased with an HFD in the present study. Various studies have shown that boron plays a role in energy and lipid metabolism. It causes to increases thermogenesis by causing the expression of uncoupling proteins in adipose tissue[49], and inhibits transcription activity of sterol regulatory element-binding protein (SREBP).[50] In rats fed HFD, it has been shown that increased boron intake reduces body weight by altering serum L-carnitine and insulin-like growth factor 1 (IGF-1) levels.[51] In humans, it has been reported that high dietary boron intake increases serum and saliva boron levels, and reduces body weight, serum LDL, VLDL, total cholesterol, and triglyceride levels.[44]

Study Limitations

The limitation of the present study was the lack of the determination of boron intake by food and water. Drinking water was given from the same source to all groups. So it can be considered that boron intake by water would be similar for all groups. However, the boron intake of the HFD group may be lower than those of treated HFD groups. The reason for the increased boron levels may arise from both the antioxidant properties of Myr and Apo, and also from the boron intake by Myr. Increased boron levels may support the effects of Myr and Apo. The increase in lens boron level in HFD + APO group rats suggests that boron may be important in preventing lens oxidative damage. Boron can be a mediator in the prevention of lens oxidative damage. Further studies are necessary to be conducted with boron supplements on HFDs and lens boron levels.

Conclusion

Both Apo and Myr may be able to reduce oxidative stress in the lenses of obese rat caused by HFD by increasing boron levels. More detailed studies are required to be done about the boron mechanism in the lens. It should be further investigated whether boron has any effects on cataract formation. The boron levels may be a novel indicator of reduced oxidative stress. Furthermore, the distribution and mechanism of action of boron should be clarified.

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