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Original Article

Effects of *Myrtus communis* L. Extract and Apocynin on Lens Oxidative Damage and Boron Levels in Rats with a 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 novel study was to investigate the effects of Myr and Apo treatment on boron levels and oxidative lens damage in rats fed a high-fat diet (HFD).

Materials and Methods: Wistar albino male rats were randomly divided into four groups: the control group, HFD group, HFD + Myr group, and HFD + Apo group. Body weight and blood lipids were determined before and after the experiment. After decapitating the rats, the lenses were removed and homogenized. Catalase (CAT) and superoxide dismutase (SOD) activities and boron, malondialdehyde (MDA), and reduced glutathione (GSH) levels in the lens homogenates were determined.

Results: The HFD increased serum triglyceride ($p<0.05$), total cholesterol level ($p<0.001$), body weight ($p<0.001$), 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 ($p<0.001$) and 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: Obesity, lens, boron, antioxidants, *Myrtus communis*, apocynin

Introduction

Obesity is described as excessive or abnormal fat accumulation and is known to cause diabetes, hypertension, dyslipidemia, sleep apnea, respiratory problems, osteoarthritis, cardiovascular disease, and cancer. One of the mechanisms related to obesity and its associated comorbidities is the formation of excess oxidants and reactive oxygen species (ROS).[1] Various studies have indicated that increased ROS formation in a high-fat diet (HFD) causes oxidant damage in the lens and cataract development.[2,3]

ROS are produced during normal cellular oxygen metabolism and 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 through exposure to ROS. Malondialdehyde (MDA)

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is generated by 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 include catalase (CAT), superoxide dismutase (SOD), and glutathione (GSH). Dietary antioxidants contribute significantly to the endogenous antioxidant system in relieving oxidative stress.[6]

Plant phytochemicals have been shown to exhibit preventive activity against oxidative stress in various animal models.[7,8] *Myrtus communis*, commonly known as myrtle, is among the edible foods and medicinal plants found in the Mediterranean and the Black Sea regions (including Turkey) and grows mainly in swamps and forests.[9] *M. communis* leaf extracts (Myr) have been reported to have anti-inflammatory, antibacterial, and antioxidant properties.[10,11] Nicotinamide adenosine dinucleotide phosphate (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 root of the *Apocynum cannabinum* plant, is a potent NADPH oxidase inhibitor.[13]

The biological importance of boron is increasingly coming to light.[14,15] Although boron is not yet considered an essential element for humans, it is classified as a possible essential element.[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 an HFD. To our knowledge, this study is the first to evaluate boron levels in the lens, and our results show that an HFD, Myr, and Apo can affect lens boron levels.

Materials and Methods

Animals and Conditions

The study was conducted in 2-month-old male Wistar albino rats (n=20) supplied by the Marmara University Application and Research Center for Experimental Animals. The rats were housed in an air-conditioned room with light-dark cycles of 12h:12h and constant relative humidity (65-70%) and temperature (22±2 ºC). Ethical approval was obtained from the Marmara University Animal Care and Use Committee (30.03.2019).

Plant samples and preparation of *Myrtus communis* extract

The plant samples used in this study were collected from the city of Manisa (Turgutlu region) in 2010. The samples were identified by a botanist in the Marmara University, Faculty of Pharmacy. Voucher specimens were deposited in the Herbarium of Marmara University, Faculty of Pharmacy (MARE no: 13006). *M. communis* leaves (100 g) were dried in the shade at room temperature. The dried pulverized leaves were extracted with 96% EtOH using a Soxhlet apparatus. They were then evaporated in a vacuum at 40 ºC until dry. This extract was stored in a dark container in the refrigerator (4 ºC) until use.

Study Groups

After a 7-day acclimation period, the rats were weighed and randomly divided into four groups as follows:

- Control group (n=5): Rats were fed a standard rat diet for 16 weeks.
- HFD group (n=5): Rats were fed an HFD including 45% fat for 16 weeks.
- HFD + *M. communis* L. group (n=5): Rats were fed an HFD for 16 weeks and received Myr (100 mg/kg) via orogastric gavage during the last 4 weeks.
- HFD + Apo group (n=5): Rats were fed an HFD for 16 weeks and received Apo (Merck, Darmstadt, Germany) (25 mg/kg, in 15% dimethyl sulfoxide) via intraperitoneal injection during the last 4 weeks.

Biochemical Analysis

At the end of 16 weeks, the rats were weighed again and decapitated. Blood samples were collected for measurement of total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride levels and the lenses were removed and homogenized in 0.9% of NaCl solution to prepare 5% lens homogenates. The lens homogenates were stored at -80 ºC until assaying. Boron, reduced GSH and MDA levels, SOD, and CAT activities in the lens homogenates were determined using the modified carminic acid[17], Beutler [18], Ledwozwy et al.[19], Mylorie et al.[20], and Aebi[21] methods, 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's post-hoc test. A p-value less than 0.05 was considered significant.

Results

This study used an HFD-induced obesity model. Weight values at the beginning and end of the experiment are shown in Figure 1. At the end of week 16, rats in the HFD group were significantly heavier than those in the control group ($p<0.001$), whereas treatment with Myr and Apo significantly reduced this increase in weight.

The total cholesterol, triglyceride, and HDL-cholesterol of rats at week 16 are shown in Figure 2. Rats in the HFD group had higher triglyceride ($p<0.05$) (Figure 2a) and total cholesterol levels ($p<0.001$) (Figure 2b) and lower HDL-cholesterol levels ($p<0.001$) (Figure 2c) than the control group. Rats that received Myr and Apo also had significantly lower total cholesterol and triglyceride levels and significantly higher HDL-cholesterol levels than those in the HFD group.

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

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

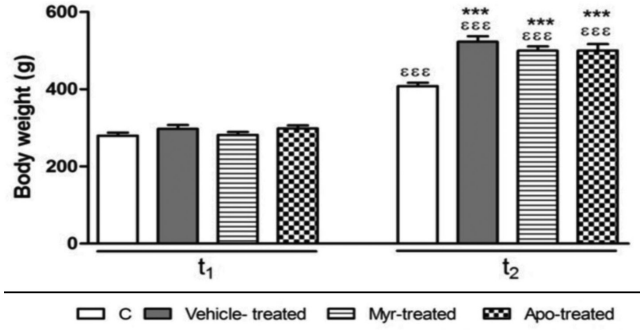

Figure 1. Body weight of the groups recorded at the beginning (t1) and end (t2) of the study
C: Control group, HFD: High-fat diet group, Myr-treated: HFD + *Myrtus communis* L. extract, Apo-treated: HFD + apocynin group. Values are given as mean ± standard error. ***: p<0.001: significantly different compared to t1, ***: p<0.001: significantly different compared to the control group.

Discussion

It is known that HFD is strongly associated with obesity. HFDs have been used for decades to induce dyslipidemia and obesity in rodents.[22] In the present study, body weight was significantly higher in HFD-fed rats (45% fat) compared to the control group (standard rat diet). However, this increase in body weight was less pronounced in the Myr- and Apo-treated groups. Similar to our study, it has been shown that Myr treatment (200 and 400 mg/kg) in rats[23] and Apo treatment (5 mM, dissolved in drinking water) in mice reduced weight gain in animals receiving an HFD.[24] It has been shown that polyphenols and flavonoids regulate the activity of PPAR-γ (peroxisome proliferator-activated receptor), the inhibition of angiogenesis in adipose tissue, and the SREBP (sterol regulatory-element binding proteins) pathway.[25,26] Myr is rich in 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] reported that the semi-myrtucommulone and myrtucommulone-A compounds in Myr has antiatherogenic effects. Meng et al.[27] showed that Apo significantly improved dyslipidemia in mice with HFD-induced obesity. In the present study, the total cholesterol and triglycerides levels of rats fed an HFD were significantly higher than those of the control group, while their levels of HDL cholesterol were lower. However, triglyceride and total cholesterol levels were significantly lower in the Myr and Apo treatment groups than in the HFD group, while HDL cholesterol was higher.

Oxidative damage is an important factor that causes cataracts, which are responsible for almost half of all cases of human blindness worldwide. Generally, oxidation is considered to be a key feature of cataract formation.[30] HFD may contribute to cataract formation by increasing ROS in the lens.[2,3] A case-

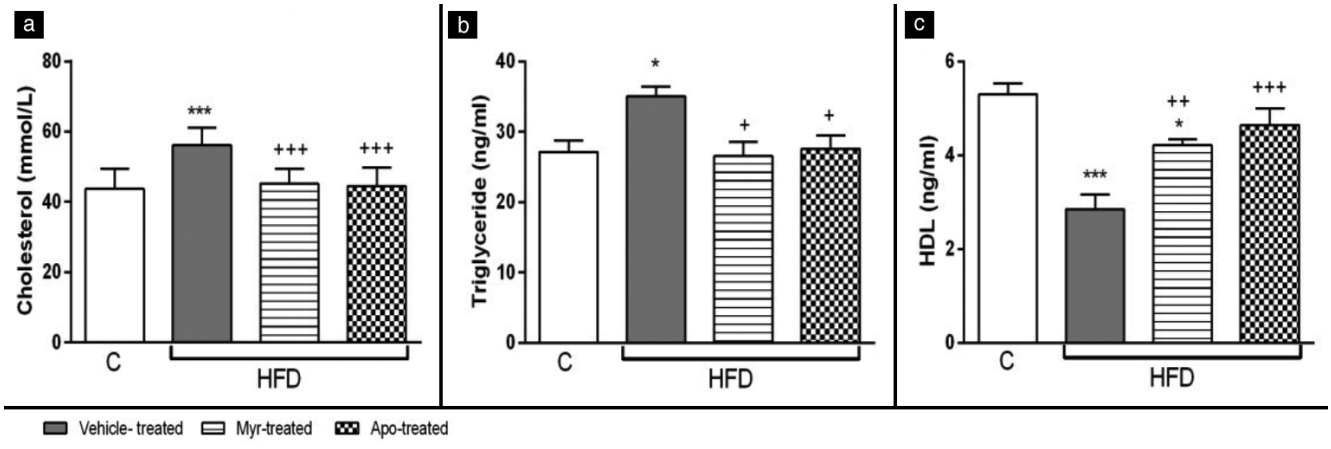

Figure 2. Total cholesterol (a), triglyceride (b), and HDL-cholesterol (c) levels
C: Control group, HFD: High-fat diet group, Myr-treated: HFD + *Myrtus communis* L. extract, Apo-treated: HFD + apocynin group. Values are given as mean ± standard error. *p<0.05, ***p<0.001: significantly different from the control group. +p<0.05, ++p<0.01, +++p<0.001: significantly different from the HFD group

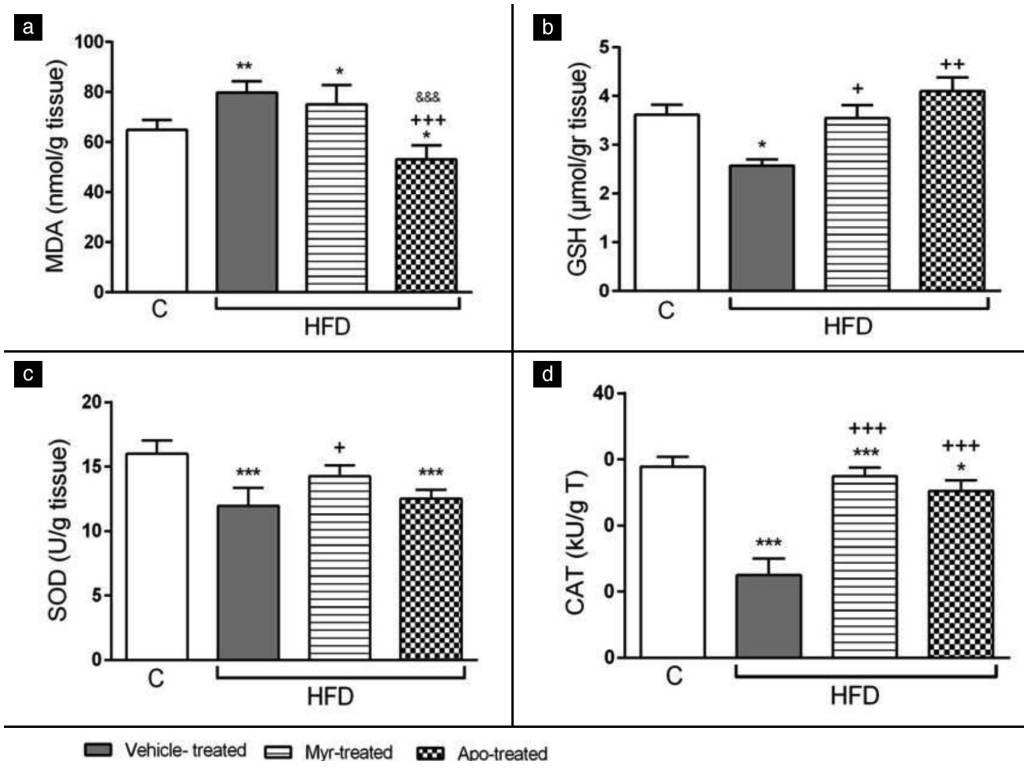

Figure 3. Lens malondialdehyde (MDA; a) and glutathione (GSH; b) levels and superoxide dismutase (SOD; c) and catalase (CAT; d) activities
C: Control group, HFD: High-fat diet group, Myr-treated: HFD + *Myrtus communis* L. extract, Apo-treated: HFD + apocynin group. Values are given as mean ± standard error. *p<0.05, **p<0.01, ***p<0.001: significantly different from the control group. +p<0.05, ++p<0.01, +++p<0.001: significantly different from the HFD group. &&&: p<0.001: significantly different from the HFD+Myr group

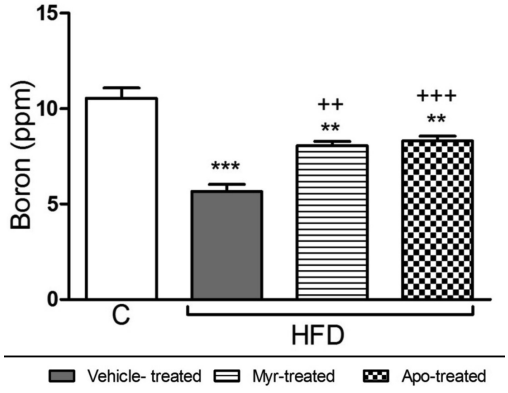

Figure 4. Lens boron levels
C: Control group, HFD: High-fat diet group, Myr-treated: HFD + *Myrtus communis* L. extract group, Apo-treated: HFD + Apocynin group. Values are given as mean ± standard error. **p<0.01, ***p<0.001: significantly different from the control group. ++p<0.01, +++p<0.001: significantly different from the HFD group

control study evaluating the relationship between diet and cataract risk showed that the risk of cataract increased with total dietary fat intake (p<0.001).[31]

Ocular tissues contain many antioxidants such as enzymes, proteins, ascorbic acid, glutathione, cysteine, and tyrosine to protect against excess ROS. The lens is a tissue that is particularly vulnerable to oxidative damage.[32] It is also known that in cataract patients, the level of hydrogen peroxide (H_2O_2) in the lens may triple compared to a healthy lens.[33] It has been shown that SOD protects the lens from oxidative damage from H_2O_2 in rats.[34] It is known that GSH in the lens contributes to the preservation of lens transparency.[35] GSH protects thiol groups in lens proteins against ROS. This is very important for the normal function of the lens epithelium 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 an HFD, lens MDA levels were significantly higher than those of the control group. This result shows that HFD increases oxidative damage. Moreover, lens MDA levels in the Apo treatment group were significantly lower than in the control and HFD groups. Furthermore, lens GSH and CAT activity in the Apo treatment group were significantly higher than in the HFD group. These results show that Apo can protect the lens from oxidative damage. In another study, treatment with 2.4 g/L Apo (in drinking water) for 5 weeks in mice fed an HFD reduced systemic and hepatic oxidative stress.[27] It was also reported that cataract progression was reduced in rabbits given 20 mg/kg/day Apo intraperitoneally.[38]

Various studies have shown that *M. communis* has antimicrobial, anti-inflammatory, and antioxidant effects.[39,40,41] In the literature, studies showing the effect of *M. communis* on lens antioxidant status are limited. In streptozotocin-induced diabetic rats, *M. communis* extract was shown to increase lens GSH ($p<0.05$) and MDA levels ($p<0.05$).[42] In the present study, lens MDA levels did not differ significantly between the HFD and Myr-treated groups. However, GSH levels, CAT and SOD activities were significantly higher in the Myr-treated group than in the HFD group.

Boron is present in human tissues and body fluids as a natural result of boron intake from food 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 cis-hydroxyl group-containing biomolecules such as polysaccharides, adenosine-5-phosphate, pyridoxine, flavins (e.g., flavin adenine dinucleotide), dehydroascorbic acid, and pyridine (e.g., NAD+ or NADP).[14] Having low atomic weight and being able to make compounds with organic molecules are 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]

Boron compounds ingested orally are rapidly converted to boric acid in the gastrointestinal tract and are nearly completely absorbed and distributed to the tissues through the blood.[14] Studies have shown that 84-85% of dietary boron is excreted in urine. Although it is known that the distribution of boron into tissues involves passive diffusion and/or sodium-dependent borate carrier-1 (NaBC1), it has not been fully elucidated yet.[44] Studies are needed to determine how boron is transported to the lens. In rats, the "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/kg.[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 (20 mg/day) and toxic dose (500 mg/day) through dietary and water intake.[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 considerably lower than our lens boron levels.[48] Similar to the above study, lens boron levels also decreased with an HFD in the present study. Various studies have shown that boron plays a role in energy and lipid metabolism. It increases thermogenesis by causing the expression of uncoupling proteins in adipose tissue[49] and inhibits transcription activity of SREBP.[50] In rats fed an HFD, it has been shown that increased boron intake reduces body weight by altering serum L-carnitine and insulin-like growth factor 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 low-density lipoprotein cholesterol, very-low-density lipoprotein cholesterol, total cholesterol, and triglyceride levels.[44]

Study Limitations

A limitation of the present study was that we did not determine boron intake by food and water. Drinking water from the same source was given to all groups. Therefore, boron intake by water can be assumed to be similar for all groups. However, the boron intake of the HFD group may have been lower than that of the treated HFD groups. The reason for the increased boron levels may arise from both the antioxidant properties of Myr and Apo, as well as from boron intake with Myr. Increased boron levels may enhance 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 evaluating the effects of boron supplements on HFDs and lens boron levels are needed.

Conclusion

Both Apo and Myr may be able to reduce oxidative stress in the lenses of HFD-induced obese rats by increasing boron levels. More detailed studies are needed to elucidate boron's distribution and mechanism of action in the lens and whether it has any effect on cataract formation. Boron levels may be a novel indicator of reduced oxidative stress.

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Ethics

Ethics Committee Approval: Ethical approval was obtained from the Marmara University Animal Care and Use Committee (30.03.2019).

Informed Consent: Informed consent is not necessary because our study is an experimental study.

Peer-review: Externally peer reviewed.

Authorship Contributions

Surgical and Medical Practices: G.Ş., F.E., Concept: G.Ş., F.E., A.Y., Design: G.Ş., F.E., A.Y., Data Collection or Processing: A.Ş., G.Ş., F.E., A.Y., Analysis or Interpretation: R.K.Y., D.K., A.Ş., G.Ş., F.E., A.Y., Literature Search: R.K.Y., D.K., A.Y., Writing: R.K.Y., D.K., A.Y.

Conflict of Interest: No conflict of interest was declared by the authors.

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