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Myrtus communis L. Extract Ameliorates High Fat Diet Induced Kidney and Bladder Damage by Inhibiting Oxidative Stress and Inflammation

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ABSTRACT

Objective: Obesity is associated with many diseases, including urinary system disorders such as chronic kidney disease and overactive bladder syndrome. *Myrtus communis* L. (MC) extract has been reported to have antioxidant and anti-inflammatory effects. The aim of this study was to investigate the protective effects of MC extract on high-fat diet (HFD)-induced kidney and bladder damage.

Materials and Methods: Wistar albino male rats were divided into three experimental groups: control, HFD and HFD+MC. Experimental groups were fed a standard diet (control group) or HFD (HFD and HFD+MC groups) for 16 weeks. MC extract (100 mg/kg) was administered to the HFD+MC group orally during the last 4 weeks (5 days/week) of the experiment. High-density lipoprotein, total cholesterol, triglyceride and leptin levels were measured in blood serum. Tissue malondialdehyde (MDA), glutathione (GSH), 8-hydroxy-2'-deoxyguanosine (8-OHdG) and myeloperoxidase (MPO) levels were evaluated biochemically. Kidney and bladder morphology, NADPH oxidase-2 (NOX-2) and nuclear factor-kappa B (NF-κB)-positive and apoptotic cells were evaluated histologically.

Results: Lipid profiles altered and leptin levels increased in blood serum. MDA, 8-OHdG and MPO levels increased and GSH level decreased in kidney and bladder in the HFD group. Moreover, degenerated kidney and bladder morphology, increased NOX-2 and NF-κB-positive and apoptotic cells were observed in this group. All of these biochemical and histological parameters were ameliorated in the HFD+MC group.

Conclusion: HFD-induced obesity causes kidney and bladder damage by oxidative and inflammatory processes. MC extract may reduce oxidative stress and inflammation and play a protective role in obesity-related kidney and bladder damage.

Keywords: High fat diet, *Myrtus communis L. extract*, kidney, bladder, oxidative stress, inflammation

INTRODUCTION

The number of overweight and obese individuals is rising in the world. Obesity has a complex etiology, with various factors to consider, including changes in eating habits, such as high-fat diet (HFD) consumption (1,2). HFDs, mainly involving high saturated fatty acid consumption, affect weight gain and finally lead to obesity (3). Obesity is associated with the development of many diseases, including cardiovascular diseases, type

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2 diabetes, metabolic disorders, chronic kidney disease (CKD), overactive bladder syndrome and bladder cancer (2,4-7). Obesity has been shown to increase reactive oxygen species (ROS) (6,8,9). Gradual mitochondrial damage in the kidney and the bladder due to HFD feeding causes loss of efficiency of the electron transport chain, further increasing ROS production, decreasing ATP production, and causing cell dysfunction (9,10). Therefore, oxidative stress, including various reactive oxygen and nitrogen species, has an important role in the pathophysiology of HFD-induced urinary tract damage (9). An increase in NADPH oxidase (NOX), as a major source of the ROS, has been demonstrated to trigger renal inflammation (11) and transforming growth factor (TGF)-β1 mediated fibrosis (12).

Long-term hyperlipidemia due to HFD causes tissue damage in a variety of organs and systems, often resulting in significant diseases and production of ROS (13). In the course of HFD-induced obesity progression, perirenal fat accumulation directly affects renal function with the increased numbers of macrophages in adipose tissue that cause higher levels of the secretion of proinflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) (14-16). Hypertrophy of adipose tissue causes inflammation, oxidative stress and insulin resistance through numerous adipokines such as leptin, which causes inflammation in the kidney and the bladder (17,18). Studies have shown that leptin-mediated oxidative damage and mesangial cell inflammation develop in the kidney when HFD has been established (11), together with the detrusor muscle hypertrophy, collagen deposition and epithelial hypertrophy that occur in the bladder (17,19). Lower urinary tract dysfunctions associated with obesity have been shown in both experimental animals and humans (20,21). Neuronal alterations are also mentioned in addition to urothelial and detrusor smooth muscle changes for the obesity related bladder dysfunctions (22).

Herbal medicine including flavonoids and essential oils via the antioxidative and anti-inflammatory activity may have many positive effects for the prevention and treatment of the many diseases associated with obesity, including CKD (23). *Myrtus communis* L. subsp. communis (MC), a plant of the Myrtaceae family, has been utilized for antihypertensive, antitussive, antiemetic, diuretic, antidiarrheic, cardiotonic, antidiabetic, antioxidant and anti-inflammatory purposes (24-26). MC fruits and leaves contain phenolic acids, flavonoids, essential oils, alkaloids, lignans and vitamins (27,28). In several experimental studies, such as on colonic inflammation, hepatic and pulmonary fibrosis and renovascular hypertension, myrtucommulone and semimyrtucommulone extracted from MC leaves were demonstrated to exhibit antioxidant and anti-inflammatory activities (29-33).

Based on the above findings, we aimed to investigate the possible protective effects of MC on kidney and bladder damage caused by HFD-induced obesity via biochemical and histological analysis. Lipid profile and leptin level in the blood serum and oxidative damage parameters in the renal and bladder homogenates were measured using biochemical methods. Renal and bladder morphology, apoptotic, NOX-2 and nuclear factor kappa B (NF-κB)-positive cells were evaluated using histological and immunohistochemical methods.

MATERIALS AND METHODS

Animals and Experimental Groups

Wistar albino male rats (8-week-old, 250–300 g), taken from the Marmara University Animal Center (DEHAMER) were kept in a laboratory environment with a standard light/dark (12/12 hour) cycle, temperature (22±2°C) and humidity (65-70%) for the duration of this study. The animals drank tap water *ad libitum*. This study was approved by The Animal Care and Ethical Committee for Experimental Animals at Marmara University (10.2020.mar). Three experimental groups (n=8, in each) were set up in the study: control, HFD and HFD+MC groups. The rats in the control group were fed with standard lab chow. The rats in HFD and HFD+MC groups were fed with HFD (45%) for 4 months. Saline or MC extract (dissolved in saline, 100 mg/kg/day) was given orally by gavage five days a week for the last month of the experiment to the animals in HFD and HFD+MC groups. The MC dose was chosen according to the previous studies (34, 35). The rats' weight was measured weekly during the experiment. The rats were decapitated under light ether anesthesia and trunk blood and kidney and bladder samples were obtained at the end of the 4th month. Serum, kidney and bladder samples were kept at -20°C until biochemical analyses were performed.

Preparation of MC Extract

MC leaves were collected from the Turgutlu region of Manisa, Turkey and defined by botanist Dr. Gizem Bulut. Voucher specimens were kept in the herbarium in the Herbarium of the School of Pharmacy, Marmara University (MARE No: 13006). MC extract was prepared according to a previous study (35). MC leaves (100 g) were dried in the shade at room temperature. The dried powdered leaves were extracted with 96% ethanol using the Soxhlet device. After filtration, the extract was concentrated using a rotary evaporator. The MC extract powder obtained with a yield of 28.56% was kept in a dark glass bottle at 4°C until use.

Measurement of Serum High-Density Lipoprotein, Total Cholesterol, Triglyceride and Leptin Levels

High-density lipoprotein (HDL), total cholesterol, triglyceride and leptin levels were analyzed using enzyme-linked immunosorbent assay (ELISA) kits (Elabscience, Wuhan, China) in the serum. The results were given as ng/ml for HDL, triglyceride and leptin and mmol/L for total cholesterol.

Measurement of Malondialdehyde, Glutathione, 8-Hydroxy-2'-Deoxyguanosine Levels and Myeloperoxidase Activity

The malondialdehyde (MDA), glutathione (GSH) and 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels and myeloperoxidase (MPO) activity were measured in kidney and bladder homogenates using commercial ELISA kits (MyBioSource, Southern California, San Diego USA). The results were given as nmol/g for MDA and GSH, ng/mg for 8-OHdG and U/ml for MPO.

Light Microscopic Preparation

The kidney and bladder samples were fixed with 10% formalin, dehydrated in ascending alcohol series, cleared in toluene and embedded in paraffin. Paraffin sections (5 µm-thick) were stained using hematoxylin and eosin (H&E) for histopathological evaluation, picrosirius red for collagen distribution and acidified toluidine blue (pH 2.5, TB) for mast cell distribution. All stained sections were examined under a photomicroscope (Olympus BX51, Tokyo, Japan). For both samples at least 5 similar microscopical areas were evaluated semi quantitatively in H&E stained sections according to a histopathological scoring system. Scores were given as 0 – none, 1 – mild, 2 – moderate and 3 – severe for each criterion, using a semiquantitative scale as follows. In the renal tissue, (a) degeneration of glomerular structure and dilatation of Bowman's space, (b) degeneration of proximal and distal tubuli, (c) vascular congestion, interstitial edema and inflammatory cell infiltration; and in the urinary bladder (a) damage in urothelium, (b) inflammatory cell infiltration in mucosa, (c) inflammatory cell infiltration in mucosa and muscular layers (36,37). The maximum score was 9 for each tissue. Collagen distribution in picrosirius red-stained sections of both kidney and bladder and mast cell count in TB-stained sections of bladder were evaluated in five non-overlapping pictures from each section using ImageJ Software (version 1.52a, Wayne Rasband, National Institutes of Health, USA).

NOX-2 and NF-κB Immunohistochemistry

Paraffin sections were immersed in 3% hydrogen peroxide to block endogenous peroxidase activity, then microwaved in 10 mM sodium citrate (pH 6.0) for antigen retrieval. The sections were washed in phosphate buffered saline (PBS) and treated with protein blocking solution (EXPOSE Rabbit specific HRP/DAB Detection IHC Kit, Abcam, Cambridge, UK). The sections were then incubated with primary antibodies (NOX-2, 1:200, Bioss; NF-κB, 1:1000, Cell Signalling Technology, Massachusetts, USA) at 4 °C overnight. After washing in PBS, the sections were treated with a biotinylated secondary antibody (20 min, ScyTek, USA) and HRP-conjugated streptavidin (20 min, Santa Cruz, USA) at room temperature. The slides were washed and then treated with 3.30-diaminobenzidine chromogen (5 min) and then stained with hematoxylin. Five similar, randomly chosen areas were photographed in the NOX-2 and NF-κB were measured by using ImageJ Software (version 1.52a, Wayne Rasband, National Institutes of Health, USA).

Terminal Deoxynucleotidyl Transferase dUTP Nick End Labelling Analysis

A terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) kit (ApopTag Plus, In Situ Apoptosis Detection Kit, S7101, Millipore) was used for determination of apoptotic cells. TUNEL-positive cells were counted by examining five randomly chosen similar areas in the sections using ImageJ Software (version 1.52a, Wayne Rasband, National Institutes of Health, USA).

Statistical Analysis

Analysis was evaluated by an instant statistical analysis package (Prism 6.0 GraphPad Software, San Diego, CA, USA). All parameters were analyzed using a one-way analysis of variance (ANOVA). Tukey's multiple comparisons test was used for the determination of differences between groups. The data were conveyed as mean ± standard error of the mean (SEM). Significance was accepted at the $p<0.05$ level.

RESULTS

Body weight results

The body weight of rats in the experimental groups was higher in the HFD group ($p<0.001$) than the control and HFD+MC groups at the end of the study (Figure 1).

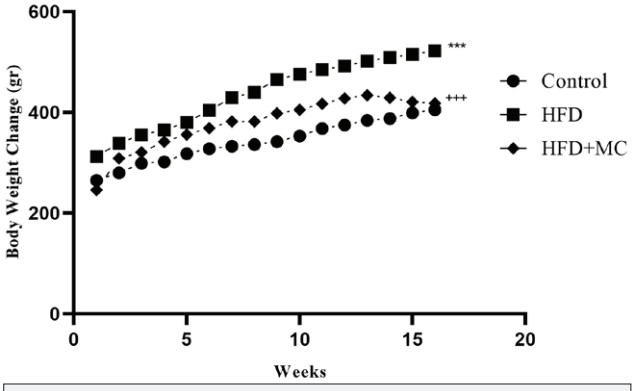

Figure 1. Weekly body weight results of the experimental groups.

***p<0.001 compared to the control group, +++p<0.001 compared to HFD group.

Serum HDL, Cholesterol, Triglyceride and Leptin Level Results

Serum HDL level decreased in the HFD group ($p<0.001$) compared to the control group and decreased in the HFD+MC group ($p<0.01$) compared to the HFD group. However, total cholesterol ($p<0.001$) and triglyceride ($p<0.001$) levels increased in the HFD group compared to the control group and total cholesterol ($p<0.001$) and triglyceride ($p<0.001$) levels reduced in the HFD+MC group compared to the HFD group. Moreover, leptin level was higher in the HFD group than the control group and lower in the HFD+MC group ($p<0.001$) compared to the HFD group (Figure 2).

MDA, GSH, 8-OHdG and MPO Level Results

Kidney MDA ($p<0.001$), 8-OHdG ($p<0.001$) and MPO ($p<0.01$) levels were higher and GSH ($p<0.01$) level was lower in the HFD group than the control group. However, MDA ($p<0.001$), 8-OHdG ($p<0.001$) and MPO ($p<0.01$) levels were lower and GSH level ($p<0.01$) was higher in the HFD+MC group than the HFD group in kidney homogenates. Additionally, bladder MDA ($p<0.001$), 8-OHdG ($p<0.001$) and MPO ($p<0.001$) levels elevated and GSH ($p<0.001$) level reduced in the HFD group compared to the control group. However, MDA ($p<0.001$), 8-OHdG ($p<0.01$) and MPO ($p<0.01$) levels were significantly re-

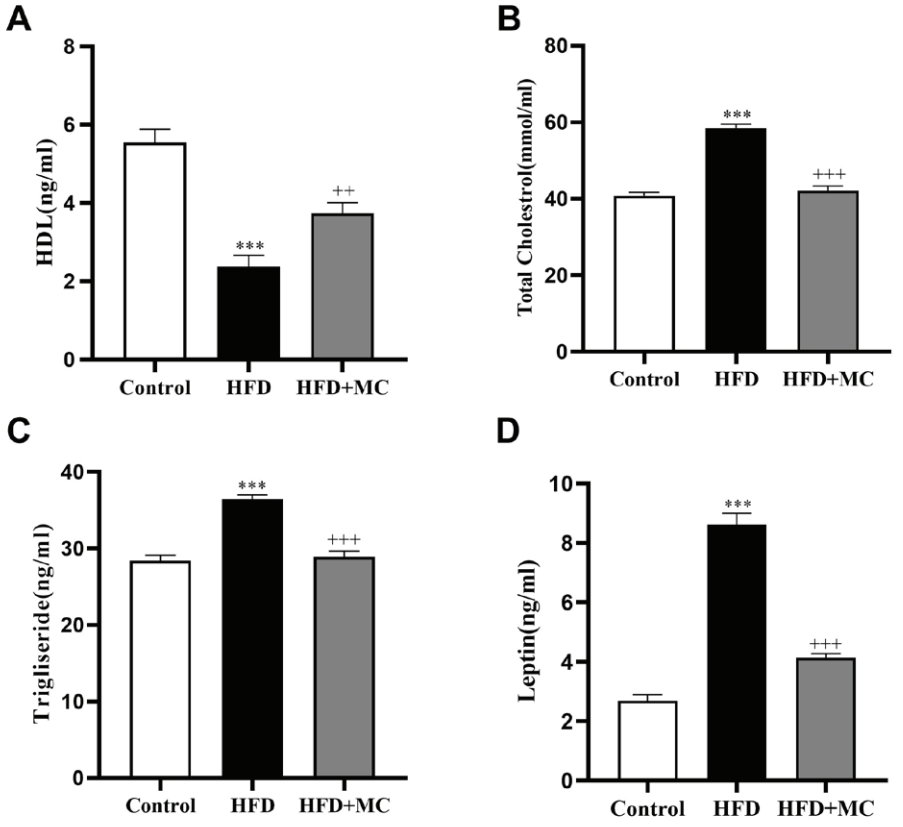

Figure 2. Serum HDL (A), total cholesterol (B), trigliseride (C) and leptin (D) levels of the experimental groups.

***p<0.001 compared to the control group, $^{++}$p<0.01 and $^{+++}$p<0.001 compared to the HFD group.

duced and GSH level (p<0.001) was elevated in the HFD+MC group compared to the HFD group in bladder homogenates (Figure 3).

Histopathological Findings

Histological evaluation of the kidney showed that regular renal corpuscles, proximal and distal tubuli and interstitial connective tissue with collagen distribution were present in the control group. However, degenerated renal corpuscles with dilatation of Bowman space, glomerular and vascular congestion, degenerated proximal and distal tubuli with luminal cellular debris and increase of collagen deposition were observed in the HFD group. All these histopathological findings decreased in HFD+MC group. Renal histopathological score and collagen deposition were significantly elevated in the HFD group (p<0.001) compared to the control group. Both histopathological score (p<0.001) and collagen deposition (p<0.01) were significantly reduced in the HFD+MC group compared to the HFD group (Figure 4).

Histological evaluation of the bladder showed that regular mucosa with urothelium and muscular layer, collagen distribution in both mucosa and muscular layers and a small number of mast cells in mucosa were present in the control group. Degenerated urothelium in the local area, vascular congestion and inflammatory cell infiltration in mucosa, increase of collagen deposition in both mucosa and the muscular layer and increased number of mast cells were observed in the HFD group. All these histological alterations were ameliorated in the MC applied HFD group. Bladder histopathological score (p<0.001), collagen deposition (p<0.001) and mast cell number (p<0.05) increased in the HFD group compared to the control group. Histopathological score (p<0.001), collagen deposition (p<0.01) and mast cell number (p<0.05) decreased in the HFD+MC group compared to the HFD group (Figure 5).

NOX-2 and NFκB Immunohistochemistry Results

In the kidney, NOX-2 and NFκB-immunopositive cells were present in glomerular and tubular cells in the control group. Although NOX-2 and NFκB-immunopositive glomerular and tubular cells increased in the HFD group, they were decreased in MC treated HFD-fed rats. The percentage of NOX-2 (p<0.05), and NFκB (p<0.001) immunostained area increased in the kidney of the HFD group compared to the control group. However, the percentage of NOX-2 (p<0.01) and NFκB (p<0.05) immunoreactive (ir) area decreased in the kidney of the HFD+MC group compared to the HFD group (Figure 6).

In bladder samples, NOX-2 and NFκB-immunopositive cells were present in urothelium, blood vessels and smooth muscle

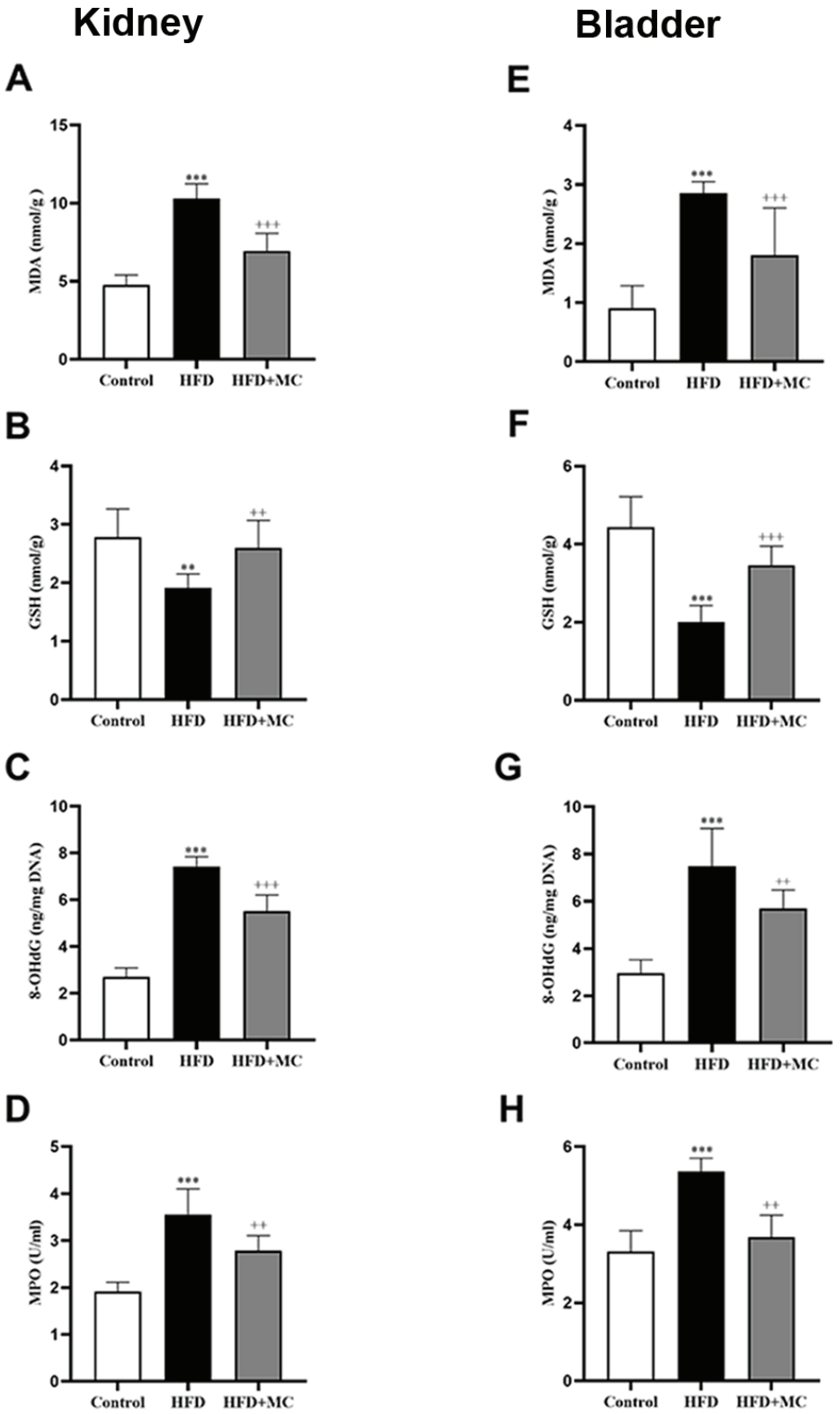

Figure 3. MDA (A, E), GSH (B, F), 8-OHdG (C, G) and MPO (D, H) levels in kidney and bladder homogenates of the experimental groups.

**p<0.01 and ***p<0.001 compared to the control group. $^{++}$p<0.01, $^{+++}$p<0.001 compared to the HFD group.

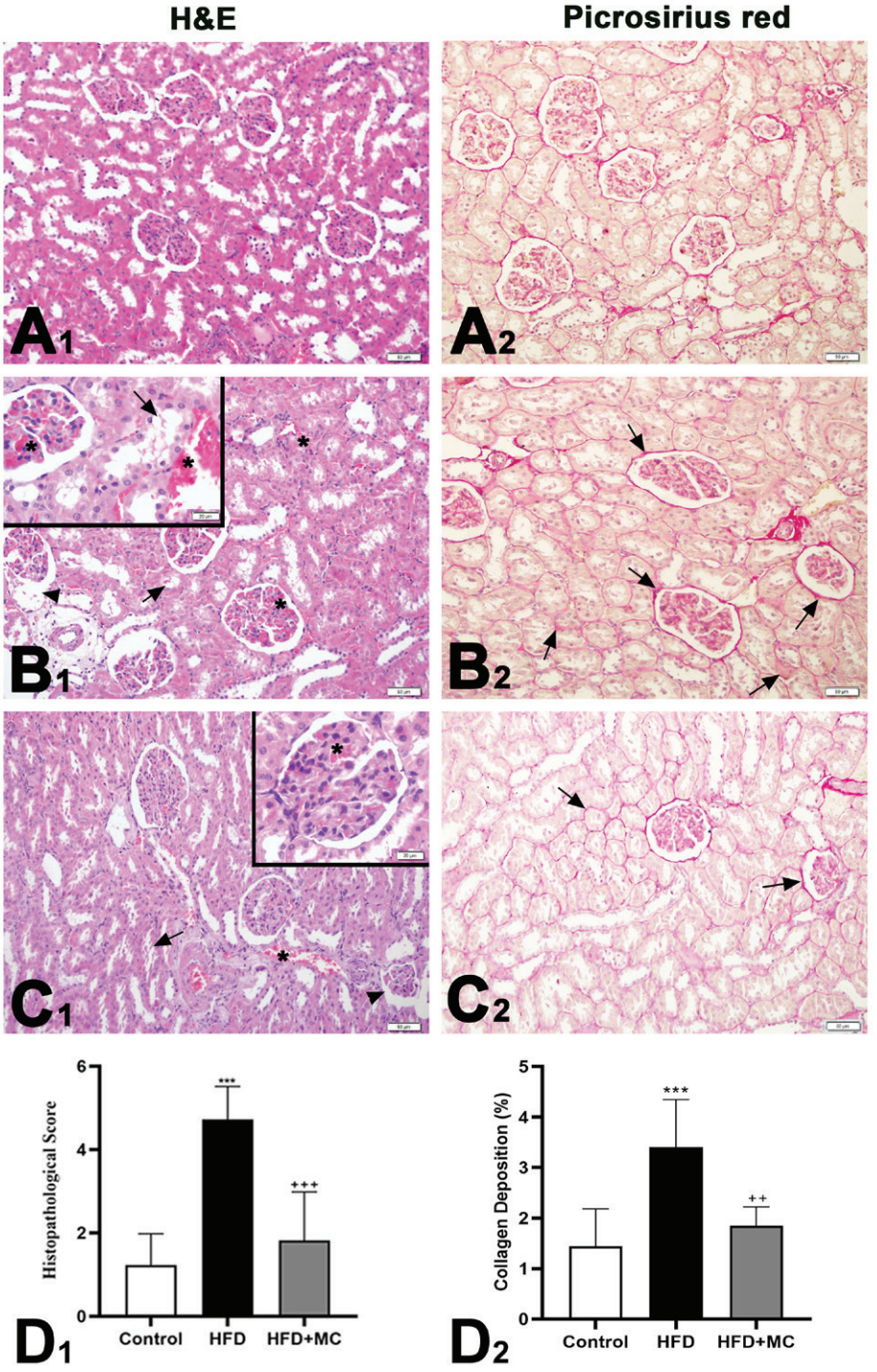

Figure 4. Representative H&E (A_1-C_1) and picrosirius red (A_2-C_2) stained light micrographs, histopathological score (D_1) and collagen deposition (D_2) of kidney in the experimental groups.

Regular renal corpuscles, and proximal and distal tubuli (A_1) and interstitial connective tissue with collagen distribution (A_2) are seen in the control group. Degenerated renal corpuscles with dilatation of Bowman space (arrowhead), glomerular and vascular congestion (*), degenerated proximal and distal tubuli (arrow) with luminal cellular debris (B_1) and increase of collagen deposition (B_2) are seen in the HFD group. Decrease of degenerated renal corpuscles with dilatation of Bowman space (arrowhead), glomerular and vascular congestion (*) and degenerated proximal and distal tubuli (arrow, C_1) and collagen deposition (C_2) are seen in the HFD+MC group. ***$p<0.001$ compared to the control group. $^{++}p<0.01$ and $^{+++}p<0.001$ compared to the HFD group. Scale bar: 50 µm, insets in B_1 and B_2: 20 µm.

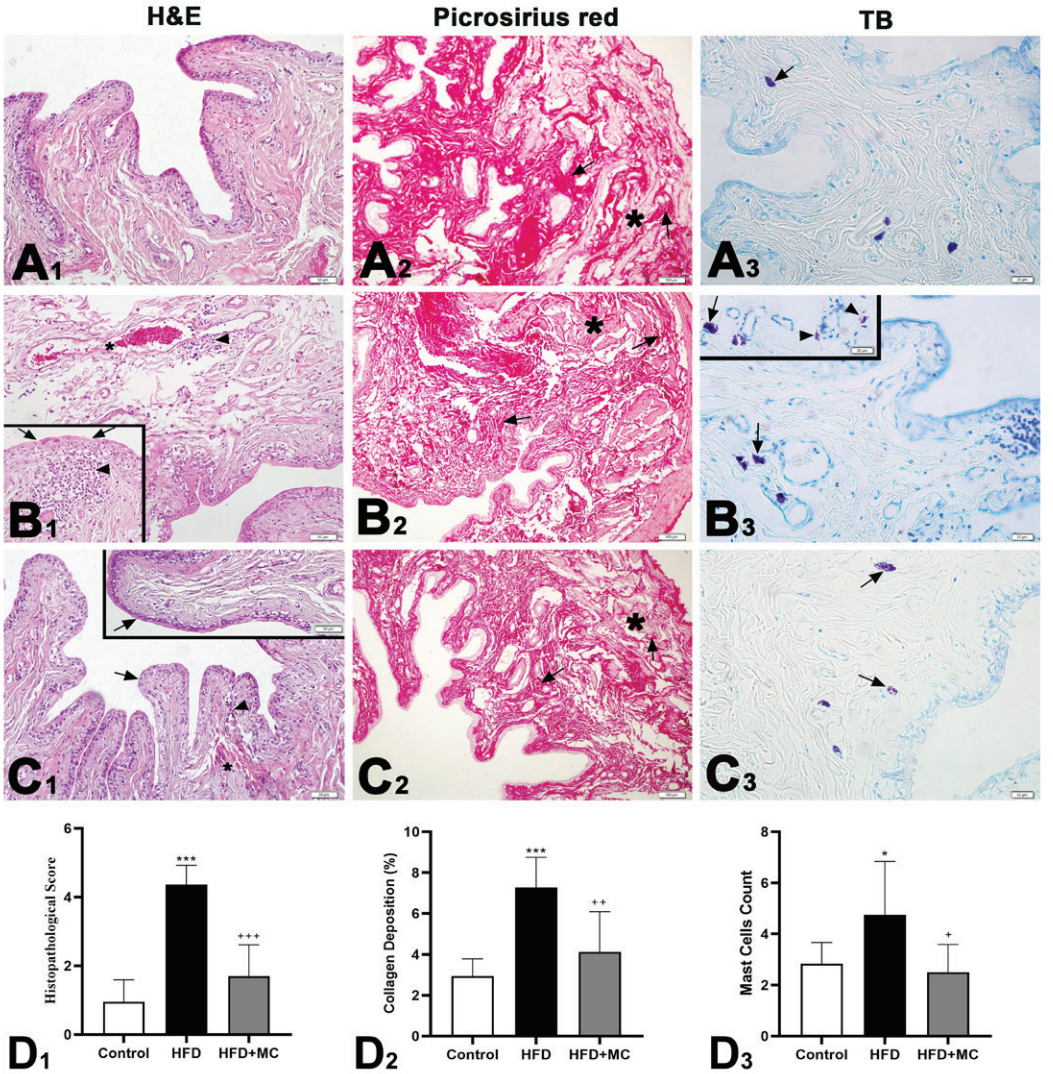

Figure 5. Representative H&E (A_1-C_1), picrosirius red (A_2-C_2) and TB (A_3-C_3) stained light micrographs, histopathological score (D_1), collagen deposition (D_2) and mast cell count (D_3) of bladder in the experimental groups.

Regular mucosal layer with urothelium and muscular layer (A_1), collagen distribution (A_2) in both mucosa and muscular layers and a few number of mast cells (A_3) in mucosa are seen in the control group. Degenerated urothelium in local area (arrow), vascular congestion (*) and inflammatory cell infiltration (arrowhead) in mucosa (B_1), increase of collagen deposition in mucosa (arrow) and around the muscle bundles (*, B_2) and increased granulated (arrow) and degranulated (arrowhead) mast cells (B_3) were observed in the HFD group. Quite regular urothelium (arrow), decreased inflammatory cells (arrowhead) and vascular congestion (*) in mucosa (C_1), decreased collagen distribution in mucosa (arrow) and around muscle bundle (*, C_2) and decreased mast cells (arrow) in mucosa (C_3) are seen in the HFD+MC group. *p<0.05 and ***p<0.001 compared to the control group. $^{+}$p<0.05, $^{++}$p<0.01 and $^{+++}$p<0.001 compared to the HFD group. Scale bar: A_1-C_2: 50 µm; A_3-C_3 and inset in B_1 and C_1: 20 µm.

cells in the control group. However, NOX-2 and NFκB-immunostained urothelial cells, inflammatory cells, blood vessels and smooth muscle cells increased in the HFD group. NOX-2 and NFκB-immunostained cells decreased in the MC-treated HFD group. The percentage of NOX-2 (p<0.05) and NFκB (p<0.05) ir area increased in the bladder of the HFD group compared to the control group. However, percentage of NOX-2 (p<0.05) and NFκB (p<0.01) ir area increased in the bladder of the HFD+MC group compared to the HFD group (Figure 7).

TUNEL Analysis Results

A small number of TUNEL-positive cells in the kidney and the bladder were present in the control groups. TUNEL-positive cells increased in the kidney (p<0.05) and the bladder (p<0.001) of the HFD group compared to the control group. MC treatment in the HFD-fed rats reduced the TUNEL-positive cell number in the kidney (p<0.05) and the bladder (p<0.001) compared to the HFD group (Figure 8).

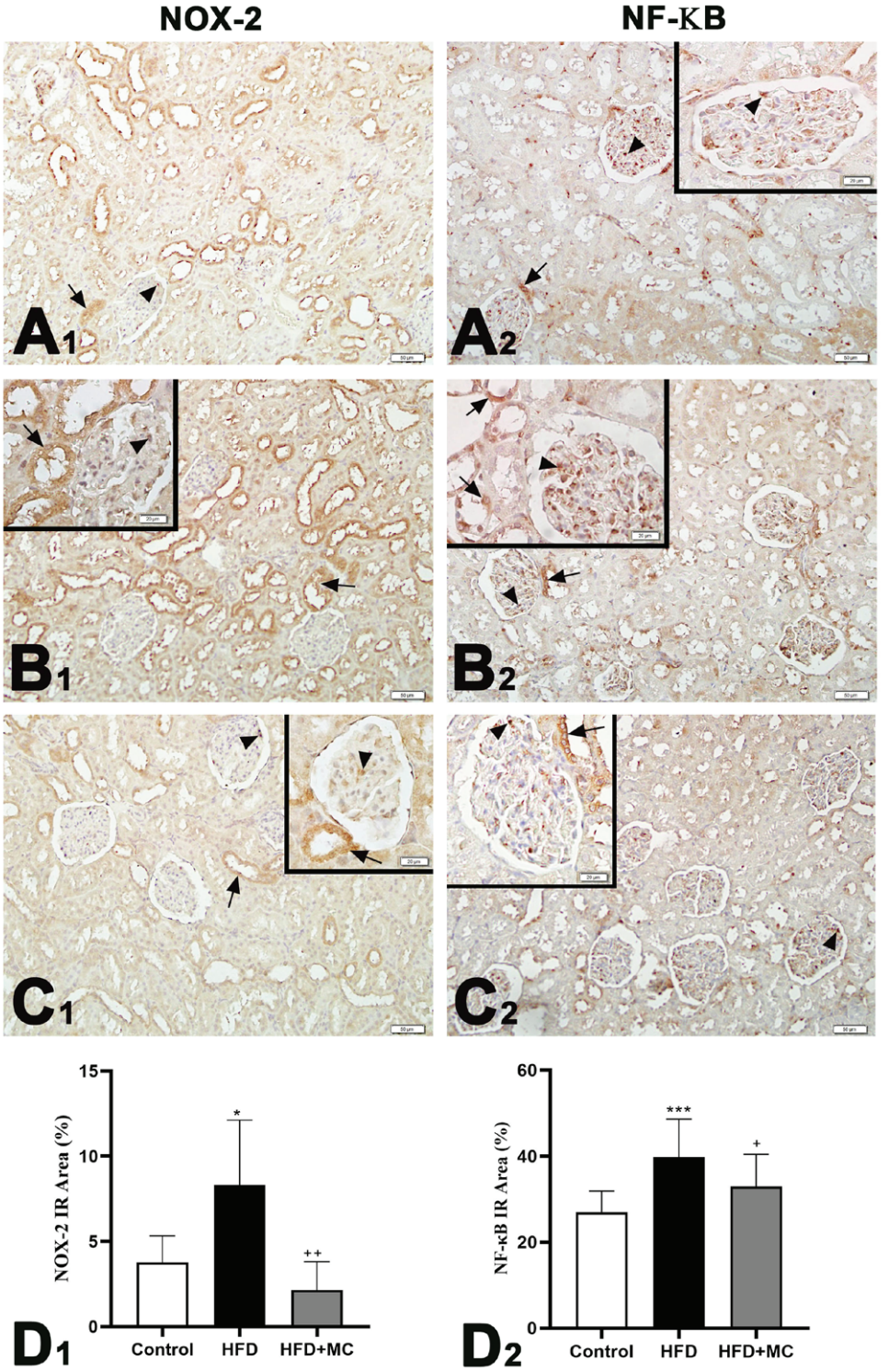

Figure 6. Representative NOX-2 (A_1-C_1) and NF-κB (A_2-C_2) immunostained light micrographs, percentage of NOX-2-ir area (D_1) and percentage of NF-κB-ir area (D_2) of kidney in the experimental groups.

NOX-2 (A_1) and NF-κB (A_2) immunopositive glomerular (arrowhead) and tubular (arrow) cells are seen in the control group. Increased NOX-2 (B_1) and NF-κB (B_2) immunopositive glomerular (arrowhead) and tubular (arrow) cells are seen in the HFD group. Decreased NOX-2 (C_1) and NF-κB (C_2) immunopositive glomerular (arrowhead) and tubular (arrow) cells are seen in HFD+MC group. *$p<0.05$ and ***$p<0.001$ compared to the control group. $^{+}p<0.05$ and $^{++}p<0.01$ compared to the HFD group. Scale bar: A_1-C_2: 50 µm; inset in B_1- C_2: 20 µm.

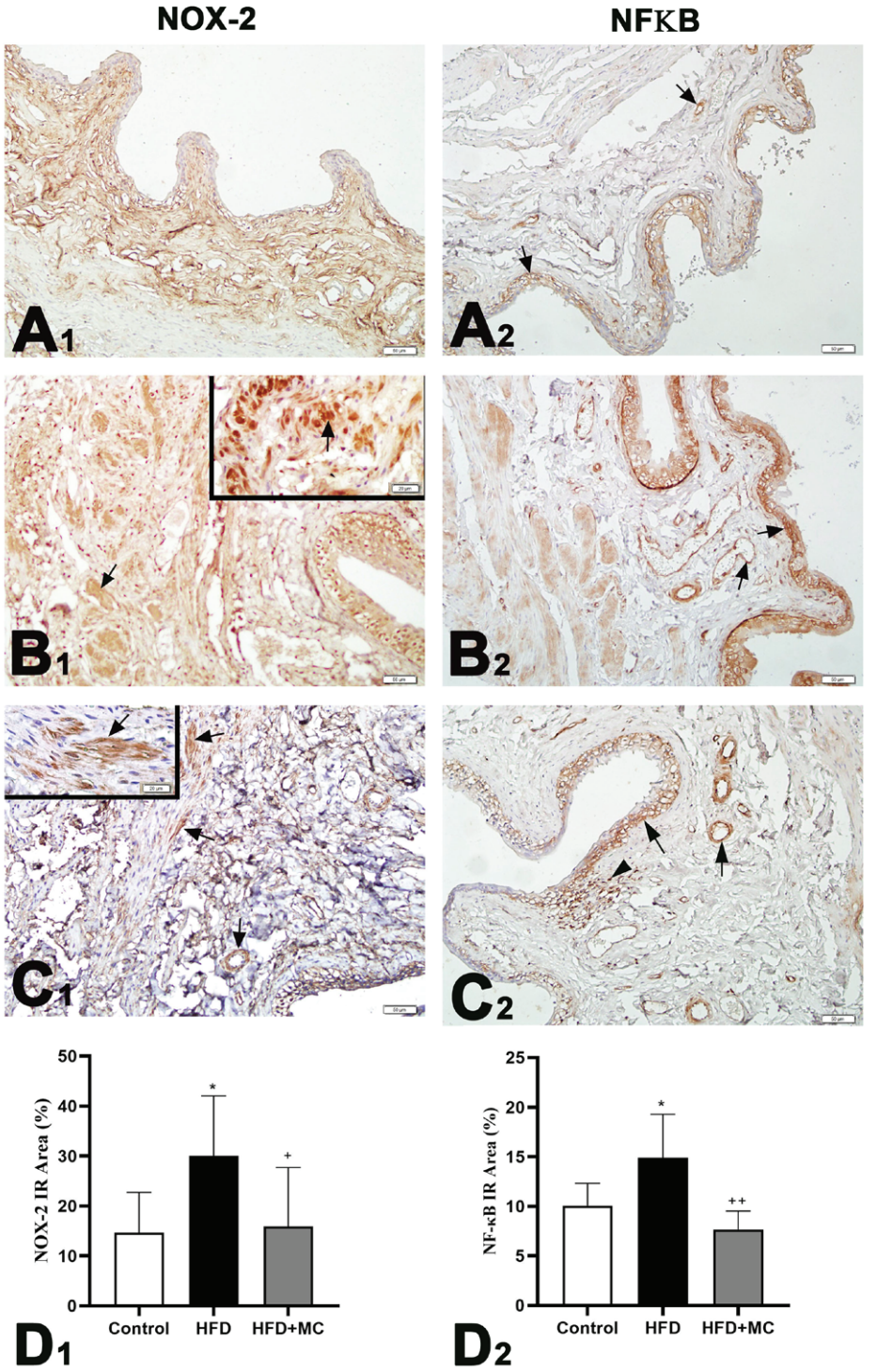

Figure 7. Representative NOX-2 (A_1-C_1) and NF-κB (A_2-C_2) immunostained light micrographs, percentage of NOX-2-ir area (D_1) and NF-κB-ir (D_2) area of bladder in the experimental groups.

NOX-2 (A_1) and NF-κB (A_2) immunopositive cells (arrow) are seen in the control group. Increased NOX-2 immunopositive muscle cells (arrow) and inflammatory cells (inset arrow, B_1) and NF-κB (B_2) immunostained (arrow) urothelial cells and blood vessels are seen in the HFD group. Decreased NOX-2 immunostained (arrow) blood vessels and smooth muscle cells (inset, C_1) and NF-κB immunostained (C_2) urothelial cells and blood vessels (arrow) and inflammatory cells (arrowhead) are seen in the HFD+MC group. *$p< 0.05$ compared to the control group. $^{+}p<0.05$ and $^{++}p<0.01$ compared to the HFD group. Scale bar: A_1-C_2: 50 µm; inset in B_1 and C_2: 20 µm.

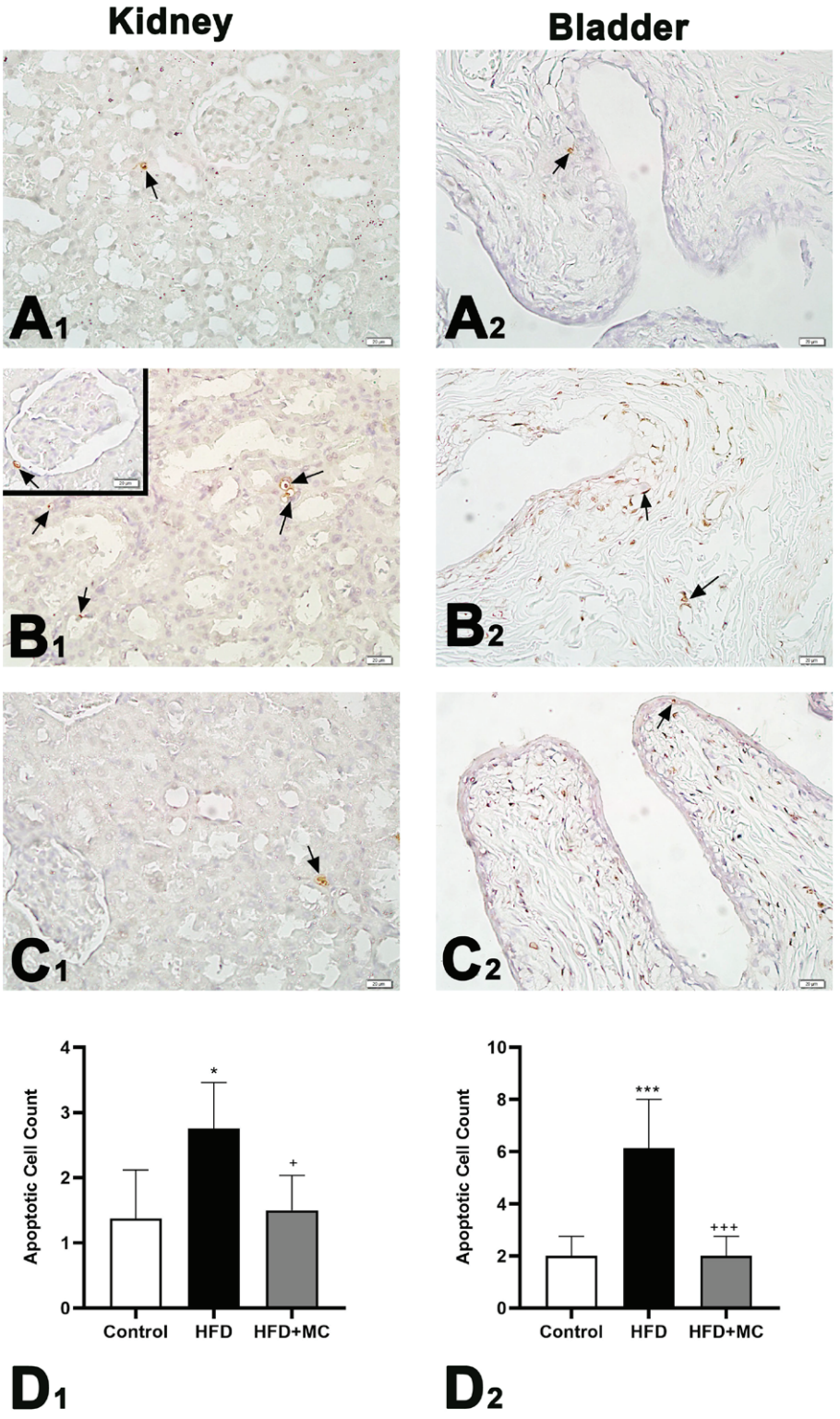

Figure 8. Representative TUNEL stained (A_1-C_2) light micrographs, apoptotic cell count of kidney (D_1) and bladder (D_2) in the experimental groups.

TUNEL positive cells (arrow) are seen in kidney (A_1) and bladder (A_2) of the control group. Increased TUNEL-positive cells (arrow) are seen in kidney (B_1) and bladder (B_2) of the HFD group. Decreased TUNEL-positive cells (arrow) are seen in kidney (C_1) and bladder (C_2) of the HFD+MC group. *$p<0.05$ and ***$p<0.001$ compared to the control group. $^{+}p<0.05$ and $^{+++}p<0.001$ compared to the HFD group. Scale bar: A_1-C_2: 20 µm

DISCUSSION

According to the findings of this study, increased body weight, hyperlipidemia and hyperleptinemia were observed in the HFD group. Additionally, increased renal and bladder MDA, 8-OHdG and MPO levels and decreased antioxidant GSH level were found in the HFD group. Parallel with the biochemical results, increased renal and bladder histopathological damage with the increase of NOX-2 and NFκB-positive cells and apoptotic cells were observed in this group. However, all these HFD-associated changes of serum lipid parameters, leptin level, renal and bladder histopathological damage and oxidative stress parameters were ameliorated by the MC extract treatment.

A body mass index over 30 kg/m^{2}in adults is defined as obese. Obesity is the underlying cause of many diseases such as metabolic syndrome, type 2 diabetes, cardiovascular diseases, non-alcoholic fatty liver disease, CKD, overactive bladder and various cancers (2,4-7). Studies in rodents have shown that body weight increases as a result of HFD feeding (19,38-42). For controlling body weight and to prevent obesity related diseases, it is recommended to change lifestyle through physical activity and eating a low calorie diet containing more vegetables and fruits (2). Parallel to the previous studies that have shown the anti-obesity activity of MC extract (40,43), our study showed that body weight increased in HFD-fed rats and MC administration reduced body weight in the HFD-fed rats.

Increased free fatty acids in the blood and accumulation of white adipose tissue are associated with obesity (6). Adipose tissue accumulates fatty acid level increased in the blood and as a result, hypertrophy and hyperplasia of adipose tissue appear. Studies have revealed that serum cholesterol and triglyceride levels increase and HDL level decrease due to HFD feeding (38-40,42). CD36 receptor was also shown to accompany lipid uptake in the kidney. It was stated that no lipid accumulation occurred in mice with CD36 mutation and that no mitochondrial ROS form due to lipid accumulation in the kidney tubule epithelium and podocyte, thus apoptosis was not observed (8,44). In another study, it was found that free fatty acids in the human detrusor muscle cell line have a negative effect on the M3 receptor in these cells, causing a decrease in the contraction force of the bladder muscle (21). These studies demonstrate that the urinary system is sensitive to lipid toxicity. It has been shown that essential oils of MC have positive effects for the reduction of the total cholesterol and antioxidant capacity in most organs including kidney (25), and polyphenolic components (myricetin-3-O-rham, myricetin-3-O-gallac, miricetin, 5-O-galloyl quinic acid and etc.) of MC aqueous extract (100mg/kg) have positive effects on rat health by increasing the Lactobacilli and Bifidobacteria colonies and antioxidative activity (27). Moreover, hypolipidemic effects of MC fruits in streptozotocin-induced diabetic rats (45) and MC L. extract in HFD-fed rats (40) have been revealed. Parallel to the previous studies, increase of serum cholesterol and triglyceride levels and decrease of HDL level as well as renal and bladder damage were observed in the HFD group, however MC administration ameliorated serum lipid profiles and tissue damage.

Obesity has been shown to be a state of hyperleptinemia (46). Leptin, one of the adipokines secreted by hypertrophic adipose tissue, binds directly to the leptin receptor (OB-R) in the central nervous system and peripheral tissue. Although the main function of leptin is appetite control, it has been shown that it has receptors in peripheral tissues such as kidney and bladder, and it activates NF-κB with a paracrine effect (47). Elevated serum leptin levels have been known to cause mesangial cell activation and tubular inflammation via the NOX-2 dependent pathway in HFD-induced non-alcoholic fatty liver disease. It was also shown that there is no renal inflammation in leptin and NOX-2 knockout mice (11). Body weight gain, hyperlipidemia and elevated serum TNF-α levels associated with increased bladder protein kinase Cζ phosphorylation, NF-κB nuclear migration and impaired muscle contractility have been demonstrated in HFD-fed mice (39). In this study, in addition to the increase in serum leptin level, increased NOX-2 and NFκ-B immunopositive cells and MPO level in kidney and bladder were also observed. MC administration reduced the leptin level in blood serum as well as inflammatory markers in both kidney and bladder.

It has been reported that as a result of an excessively lipid-rich diet, free fatty acids in the circulation cause the capacity of adipose tissue to be exceeded and accumulate in ectopic organs such as kidney (48,49). Studies have shown that free fatty acids found in high levels in the serum and the tissues are important as they may cause oxidative stress in the body. In addition, it has been established that kidney tubule epithelial cells rich in mitochondria and bladder muscle cells are sensitive to oxidative stress (49,50). HFD-induced obesity leads to kidney and bladder damage and increase of MDA, IL-6 and TNF-α levels and decrease of GSH and superoxide dismutase levels (51). Furthermore, a high-fat/high-sugar diet has been shown to induce metabolic syndrome, a decrease in glomerular filtration rate, renal tubular injury and increased oxidative stress in the renal cortex (48). In another study, MC treatment reduced the MDA level and increased GSH level and superoxide dysmutase and catalase activities in the kidney of animals with renovascular hypertension (32). In this study, an increase of MDA and decrease of endogeneous GSH levels in the kidney and the bladder in the HFD group were noted. On the other hand, MC administration decreased the oxidative stress parameters in both renal and bladder homogenates due to the strong antioxidant compounds content.

As a result of DNA damage, which becomes another target in the increase of ROS, mutation, misreplication, apoptosis or cancer formation are observed (52). The oxidative DNA damage was determined by the measurement of 8-OHdG level (53). It has been stated that the increased level of 8-OHdG in the HFD-induced obesity triggers apoptosis (8,42,51,54). Additionally, it was observed that as a result of 23-month-long high fat/high sucrose intake, GSH level and proliferative cells decreased, and TNF-α, NF-κB, caspase-3 and apoptotic cells increased in kidney together with high serum lipids in Bama minipigs (55). Moreover, tubular degeneration with degenerated mitochondria in the kidney of the HFD-fed mice (54) and glomerular basement

membrane thickening and podocyte degeneration with fusion of foot processes in HFD-fed rats (42) have been shown. In this study, we observed histopathological damage with glomerular and tubular degeneration in the kidney, urothelial degeneration and increase of inflammatory cells in the bladder, and increase of 8-OHdG level and apoptosis in renal and bladder samples of rats with four months of HFD feeding. Additionally, an increase of MDA and MPO levels and NF-κB-positive cells in both kidney and bladder samples in this group were observed. However, MC treatment reversed the oxidative stress, apoptosis and inflammation in the HFD-fed rats.

It has been shown that oxidative stress causes damage not only by stimulating the inflammatory response but also by stimulating the inflammatory response in the formation of organ damage seen in obesity (56). MPO, a heme protein produced by neutrophils, is a chlorinating enzyme that initiates the response in acute inflammation and plays a role in the spread of chronic inflammation through the production of ROS such as NOX (57). It was shown that neutrophils infiltrate into adipose tissue in the early stage of HFD, and release of oxidant and inflammatory substances such as ROS, TNF-α and MPO occur (58). Increase of neutrophils in obese people and type 2 diabetic patients is also shown. Furthermore, it was stated that there was a positive correlation between MPO activation and metabolic disorders and MPO plasma level was higher in obese patients (59). NF-κB is a family of inducible transcription factors that mediate signal-induced expression of several genes involved in immunity, inflammation, and cell growth. NF-κB is shown to be activated by pathophysiological processes in TNF-α and angiotensin II cascades in kidney and is associated with experimental and human kidney disorders. NF-κB inhibitors have been reported to prevent renal inflammation in experimental animals. Additionally, NF-κB inhibitors have been reported to prevent renal inflammation in experimental animals (60). It has been established that elevated serum leptin causes inflammation in mesangial cells in HFD-induced kidney damage (11). Increased oxidative stress, NF-κB activation and elevated inflammatory response were observed in HFD-induced nephropathy of mice (61). In another study, it was shown that NF-κB expression and fibrosis in the bladder were increased in mice of cyclophosphamide-induced chronic cystitis model as a result of inflammation (17). It has been stated that the increase in ROS in the heart and aorta of HFD-induced obese rats contributes to inflammation by causing high MPO levels. Also, increased mast cell activation in the heart was observed in the HFD-fed rats (38). It has been found that HFD feeding increases mast cell infiltration in the rat mesentery (62). In another study, increased apoptotic and mast cells in the bladder of diabetic patients with overactive bladder were shown (63). Activated mast cells release many chemokines, histamine and proteases as well as proinflammatory cytokines, which activate the ROS formation. Parallel with the previous studies, the increase of MPO level and NFκB-positive cells in both kidney and bladder and increase of mast cells in bladder of the HFD group were observed. MC treatment reduced these inflammatory markers in both kidney and bladder of the HFD-fed rats by inhibition of ROS generation.

Long-chain saturated fatty acids accumulate in various cells in the kidney, causing an increase in the production of ROS, which triggers glomerular mesangial cell inflammation through proinflammatory cytokines such as TNF-α, IL-6 and NF-κB and causes interstitial matrix fibrosis by TGF-β1 signaling (8,41). It has been revealed that free fatty acids trigger fibrosis in the detrusor muscle cell line and cause a decrease in the contraction force of the bladder muscle (21). It has been shown that there is an increase in glomerular and interstitial collagen in the kidney as a result of NOX-2-related oxidative damage (64). In another study, increase of collagen has been noted between muscle bundles of the bladder in HFD-induced obesity (19). Parallel with the previous studies, increase of collagen in interstitial connective tissue and around the glomeruli in kidney and between the muscle bundles in bladder and increase of MDA level and NOX-2 positive cells in both kidney and bladder were observed in the HFD group. MC treatment ameliorated the fibrosis in both kidney and bladder samples via the inhibition of ROS formation.

CONCLUSION

HFD-induced obesity causes hyperlipidemia and hyperleptinemia, renal and bladder fibrosis, increased oxidative, inflammatory and apoptotic activities and decreased endogeneous antioxidant activity. However, MC administration improved serum lipid and leptin levels, fibrosis, oxidative damage via the reduction of MDA and 8-OHdG levels and NOX-2-immunopositive cells, elevation of endogeneous antioxidant GSH level, inflammatory activity via the reduction of MPO activity and NF-κB-immunopositive cells and apoptotic activity in both kidney and bladder samples. The antioxidative and anti-inflammatory activity of MC treatment appear to be important for the protective effects of MC on HFD-induced renal and bladder injury, however, defining these effects of MC requires further studies with advanced molecular and biochemical techniques to show possible therapeutic use.

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