Clinical and Experimental Health Sciences

Apocynin Ameliorates Testicular Toxicity in High-Fat Diet-Fed Rats by Regulating Oxidative Stress

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Received: 10.12.2021 **Accepted:** 15.09.2022

ABSTRACT

Objective: The purpose of this study was to examine the effects of apocynin (APC), an inhibitor of NADPH oxidase (NOX), on high-fat diet (HF)-induced testis cytotoxicity.

Methods: Wistar albino rats were divided into three groups as control, HF and HF+APC groups. Rats in HF and HF+APC groups were fed using HF for 16 weeks and in the last four weeks of this period vehicle solution or APC (25 mg/kg) was administered orally five days a week, respectively. Control group was fed with standart lab chow for 16 weeks. Cholesterol, triglyceride, high-density lipoproteins, leptin, estrogen, testosterone, LH and FSH were estimated in blood serum. Sperm parameters were analysed from the epididymis. Testicular malondialdehyde, 8-hydroxy-2-deoxyguanosine, glutathione, superoxide dismutase and myeloperoxidase levels were estimated biochemically. Testicular morphology, proliferative, apoptotic and NOX2-positive cells were analysed histologically.

Results: HF-induced obesity caused significant alterations in serum lipid and hormone profiles. Testicular malondialdehyde, 8-hydroxy-2-deoxyguanosine, and myeloperoxidase levels increased, glutathione and superoxide dismutase levels decreased in this group. Moreover, altered sperm parameters, increased degenerated seminiferous tubules, apoptotic and NOX2 – positive cells and decreased proliferative cells were observed in the HF group. All these biochemical and histological alterations improved in the HF+APC group.

Conclusion: HF-induced obesity causes altreations in lipid values, sperm parameters and testicular morphology by increasing oxidative stress through NOX2 activity. Apocynin might prevent testis damage via regulating oxidant/antioxidant balance.

Keywords: High fat diet, apocynin, testis, apoptosis, oxidative stress

1. INTRODUCTION

Obesity, one of the main worldwide health problems of the last five decades, is a chronic disease that effects the physiological, economic and psychological quality of individual life, regardless of cultural, financial or ethnic origin (1). Obesity is defined by body mass index (BMI) more than 30 kg/m^2 and it is related to the development of numerous health disturbances, including cardiovascular disorders, type 2 diabetes, insulin resistance, hepatic and renal failure, respiratory disorders, different types of cancer, and infertility (2). High BMI is related to male infertility determined by altered sperm parameters as low sperm concentration, decreased number of motile sperm and spermatozoa with normal morphology (3).

Human and experimental studies have shown the effects of oxidative stress in the pathogenesis of obesity. High fat high carbohydrate food consumption induces intracellular pathways causing oxidative stress by biochemical mechanisms such as superoxide generation from NADPH oxidase (NOX) and oxidative phosphorylation (4). Oxidative stress caused by reactive oxygen species (ROS) have a crucial role in male infertility with DNA damage and testicular germ cell apoptosis in testis, and reduced sperm motility (5) and changes of sperm concentration, motility and morphology (6). In different studies, it was shown that obesity leads to excessive reactive oxygen species (ROS) production which is related to the alterations in sperm parameters, testicular damage with an increase of oxidative stress, inflammation and cell death (7,8).

Activation of NOX initiates the ROS production from oxygen (9). Apocynin (APC, 4´-hydroxy-3´-methoxyacetophenone) is a naturally originating methoxy-substituted catechol taken out from the *Picrorhiza kurroa* (Scrophulariaceae) and *Apocynum cannabinum* (Canadian hemp), known as NOX inhibitor and used in traditional Indian medicine (10). It was shown that

DOI: 10.33808/clinexphealthsci.1035133

APC decreases apoptosis by increasing antioxidant protection in experimental cerebral artery occlusion (11). Moreover, APC protected testicular tissue from cisplatin-induced (12), metotrexate-induced (13), streptozotocin-induced (14) and ischemia-reperfusion-induced (15) testis damage by inhibiting ROS formation and apoptosis.

The protective effects of APC through its antioxidant and antiapoptotic properties have been demonstrated in many testicular injury models (12-16). However, the effects of APC, on testis damage in HF-induced obesity were not examined before. The purpose of this study was to examine the anti-oxidant and anti-apoptotic impacts of APC on testicular cytotoxicity in high-fat diet-fed rats.

2. METHODS

2.1. Animals and Experimental Groups

Wistar albino male rats (250–300 g, eight-week-old) were kept in a laboratory environment with a regular light/dark (12/12 hour) cycle at 22 ±2°C for the duration of the experiment. The animals accessed to water *ad libitum*. This study was appoved by The Animal Care and Ethical Committee for Experimental Animals at Marmara University (68.2017.mar).

The rats were divided into 3 experimental groups as control, HF and HF+APC groups. Rats in the control group (n=8) were fed with standart diet (6% fat). Rats in the HF (n=10) and HF+APC (n=10) groups were fed with high-fat diet (45% fat) for 16 weeks and treated with either vehicle solution (15% dimethyl sulfoxide) or 25 mg/kg APC orally 5 days a week for the last 4-weeks of the experiment, respectively. In a previous study, APC was applied to the control group to observe if it had any adverse effects; but no adverse effect was observed (12). Based on this finding, and also to avoid sacrificing any more animals, an APC treated control group was not created in this study. The solvent agent, dosage and administration of APC were based on previous studies (12, 15). The rats weight was measured every week during the experiments. Rats were decapitated under light ether anesthesia and blood, epididymis and testis samples were obtained at the end of the 16th week. The trunk blood samples were kept at room temperature (30 min), centrifuged at 4000 rpm (15 min) and then separated serum samples were kept at – 20°C for lipid profile and hormone analysis. Testis samples were weighed. Epididymis was dissected for evaluation of sperm parameters. The right testis was processed for histological examinations. The left testis was homogenized in phosphate buffered saline (PBS) and kept at – 80 °C until biochemical evaluation.

2.2. Measurement of Serum Cholesterol, Triglyceride and High-Density Lipoprotein Levels

Total cholesterol, triglyceride and high-density lipoprotein (HDL) levels were estimated with ELISA kits (Elabscience, Wuhan, China). The results were given as mmol/L for cholesterol and ng/ml for triglyceride and HDL.

2.3. Measurement of Serum Leptin, Estrogen, Testosterone, LH and FSH levels

Serum leptin, estrogen, testosterone, LH and FSH levels were estimated with the commercial ELISA kits (Elabscience, Wuhan, China). The results were given as ng/ml for leptin, estrogen and testosterone, and IU/L for LH and FSH.

2.4. Measurement of Malondialdehyde, 8-Hydroxy-Deoxyguanosine, Glutathione, Superoxide Dismutase and Myeloperoxidase Levels in Testis

The malondialdehyde (MDA), 8-hydroxy-deoxyguanosine (8-OHdG), glutathione (GSH), superoxide dismutase (SOD) and myeloperoxidase (MPO) levels were surveyed in testis homogenates by ELISA kits (MyBioSource, Southern California, San Diego USA). The results were given as nmol/g for MDA and GSH, ng/mg for 8-OHdG, % inhibition for SOD, and U/g for MPO.

2.5. Evaluation of Epididymal Sperm Parameters

Caudal epididymis samples were cut into small pieces in Earle's balanced salts solution (5 ml, Sigma, USA) for sedimentation, and supernatants were removed. Using the routine density gradient method, pellets were centrifuged at 1800 rpm (18 min). After removing supernatants, they were diluted with a sperm washing medium (0.3 ml, SAGE, UK) to centrifuge at 2000 rpm (10 min) and supernatants were discharged. Pellets diluted with fertilization medium (0.3 ml, SAGE, UK) were evaluated with Macler Counting Chamber (Sefi Medical Instruments, Haifa, Israel) for the motility rate and sperm counting under a photomicroscope. All of the sperms in 100 squares were counted and multiplied by a million. Smears were stained using the Diff-Quick kit (Medion Diagnostics, Grafelfing, Germany). In each smear slide, 100 sperms were evaluated morphologically at 1000x magnification under the photomicroscope (17).

2.6. Light Microscopic Preparation

The right testes were fixed with 10% formaldehyde solution. Following fixation, the tissues were processed routinely for paraffin embedding. Paraffin sections were stained using hematoxylin and eosin (H&E) for histological analysis. The diameter and epithelial thickness of 20 seminiferous tubules were measured using the Image J (NIH-USA) program. Twenty seminiferous tubules were evaluated in accordance with the modified Johnsen scoring method (17,18). Each tubule was scored from 1 (absence of both germinal and Sertoli cells) to 10 (full spermatogenesis).

2.7. Proliferating Cell Nuclear Antigen Immunohistochemistry

Paraffin sections were treated with 3% hydrogen peroxide (H_2O_2; 30 min) for endogenous enzyme blockade. Sections were incubated with 300 W microwave in citrate buffer for antigen retrival (20 min). The cooled sections were washed with PBS (3x5 min), kept in blocking solution (10 min, Invitrogen) and then kept in rabbit anti-proliferating cell nuclear antigen (PCNA, Novus) primary antibody (1:2000) overnight at 4°C. The sections were washed in PBS (5 min), then kept in biotinylated secondary antibody (30 min, Thermo), washed in PBS, incubated in streptavidin peroxidase (10 min, Invitrogen), washed in PBS, waited in 3,3'-diaminobenzidine (DAB) chromogen (5 min), rinsed in distilled water and then were counterstained with hematoxylin. The proliferation index was estimated by dividing the PCNA – positive cells by the total number of cells into 20 seminiferous tubules in each section.

2.8. Terminal Deoxynucleotidyl Transferase Dutp Nick End Labelling Method

The terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) technique was performed in accordance with the manufacturer's intsruction (ApopTag Plus, In Situ Apoptosis Detection Kit, S7101, Millipore). TUNEL-positive cells were estimated by examining 20 seminiferous tubules in each section. The apoptotic index was calculated by dividing the number of seminiferous tubules with three or more TUNEL-positive cells by the total number of seminiferous tubules.

2.9. NOX2 Immunohistochemistry

Paraffin sections were treated with 3% H_2O_2 solution for endogenous peroxidase blockage and then incubated in microwave in citrate buffer solution (pH 6.0) for antigen retrival. Then the slides were washed in PBS, treated with protein blocking solution (EXPOSE Rabbit specific HRP/DAB Detection IHC Kit, Abcam, Cambridge, UK). The slides were incubated in anti-NOX2/gp91phox primary antibody (1:100 dilution, bs-3889 R, Bioss Inc, Massachusetts, USA) overnight at 4ºC. After washing in PBS, sections were treated with biotinylated secondary antibody (10 min, Ultra Tek Hrp Anti-Polyvalent, ScyTek). Then the slides were washed in PBS, treated with streptavidin peroxidase (10 min), washed in PBS and treated with DAB. The slides were counterstained with hematoxylin. In each section, 20 seminiferous tubules were examined for NOX2-positive cells. Seminiferous tubules with three or more NOX2-positive cells were notified as the percentage of 20 tubules.

All stained sections were evaluated with a photomicroscope (Olympus BX51) and photographed with digital camera (Olympus DP72).

2.10. Statistical Analysis

Biochemical and histological results were estimated using a one-way analysis of variance. Post-hoc testing was completed with Tukey's multiple comparisons test and the data were conveyed as mean ± standard error of the mean (SEM). Significance of differences were used as $p< 0.05$. Analysis was calculated by an instant statistical analysis package (Prism 6.0 GraphPad Software, San Diego, CA, USA).

3. RESULTS

3.1. Body and Testis Weight Results

The body weight of rats in the HF ($p< .001$) and HF+APC ($p< .05$) groups significantly increased compared to the rats in the control group at the end of the study (Figure 1). Testis weight was 1.77±0.15 g in control group, 1.78±0.11 g in HF group and 1.83±0.14 g in HF+APC group. This value was not significantly different among the experimental groups.

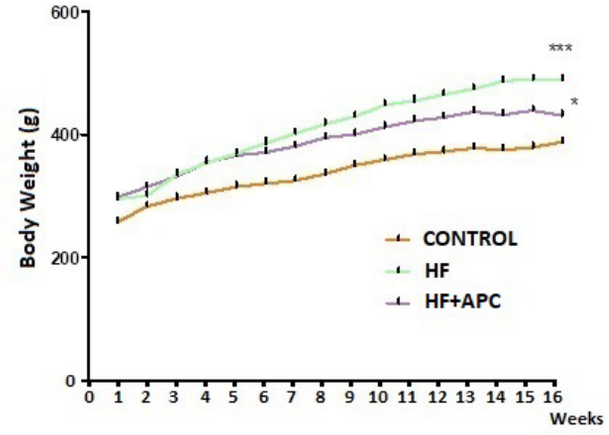

Figure 1. *Body weights of control, HF and HF+APC groups. * P<0.05 and *** P< 0.001 in comparison with control group.*

3.2. Cholesterol, Triglyceride and HDL Values

Serum cholesterol ($p< .001$) and triglyceride ($p< .01$) levels were higher in the HF group than the control group. However, cholesterol ($p< .001$) and triglyceride ($p< .05$) levels decreased in APC treated HF group compared to HF group. Furthermore, HDL level reduced in HF ($p< .001$) and HF+APC ($p< .05$) groups compared to the control group. This value increased in HF+APC group ($P<0.05$) compared to HF group (Figure 2).

3.3. Leptin, Estrogen, Testosterone, LH and FSH Values

Serum leptin ($p< .001$), estrogen ($p< .01$) and testosterone ($p< .001$) levels were higher in the HF group than the control group. These leptin ($p< .001$), estrogen and testosterone ($p< .001$) values were lower in the HF+APC group compared to the HF group. LH level ($p< .01$) decreased in the HF group in comparison with the control group and this value increased

in the HF+APC group (p< .05) in comparison with the HF group. Although the FSH level decreased in the HF group in comparison with control group and increased in comparison with the HF+APC group, these findings were not statistically significant (Figure 3).

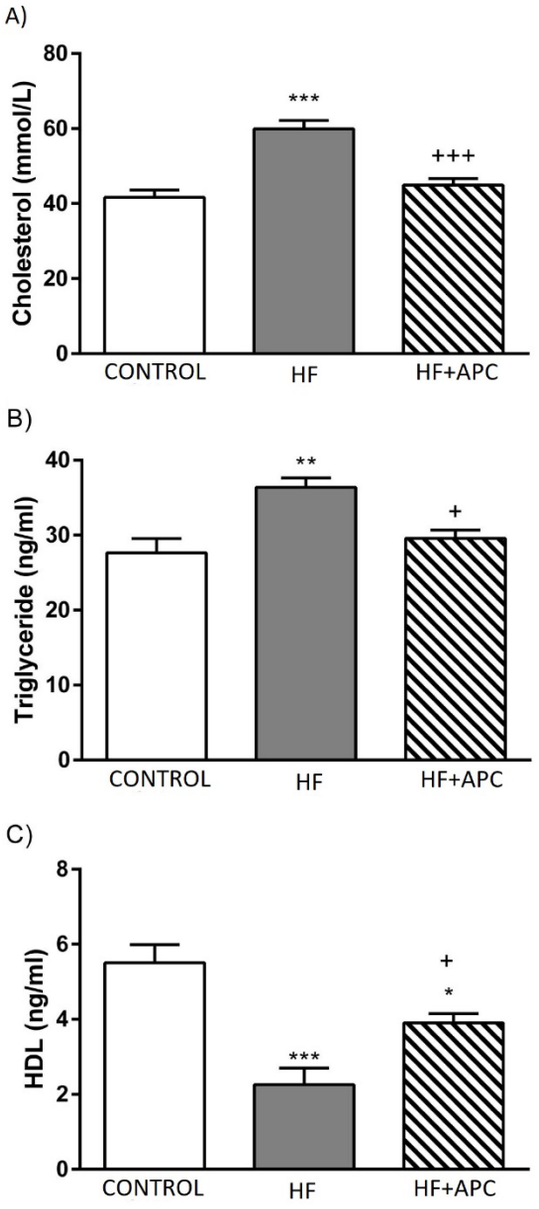

Figure 2. *Cholesterol (A), triglyceride (B) and HDL (C) levels in the control, HF and HF+APC groups. * P<0.05, ** P<0.01 and *** P<0.001 in comparison with control group, + P<0.05 and +++ P<0.001 in comparison with HF group.*

3.4. MDA, 8-OHdG, GSH, SOD and MPO Values

Testicular MDA (p< .001), 8-OHdG (p< .01) and MPO (p< .01) levels increased and GSH (p< .001) and SOD (p< .05) levels decreased in the HF group in comparison with the control group. But, MDA (p< .001), 8-OHdG (p< .05) and MPO (p< .01) levels decreased and GSH level (p< .01) increased in the HF+APC group in comparison with the HF group (Figure 4).

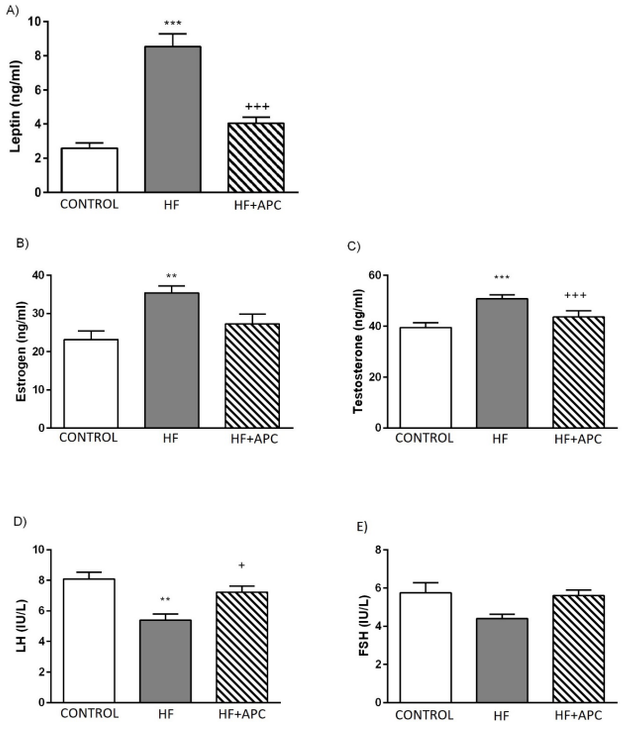

Figure 3. *Leptin (A), estrogen (B), testosterone (C), LH (D) and FSH (E) levels in the control, HF and HF+APC groups. ** P<0.01 and *** P<0.001 in comparison with control group, + P<0.05 and +++ P<0.001 in comparison with HF group.*

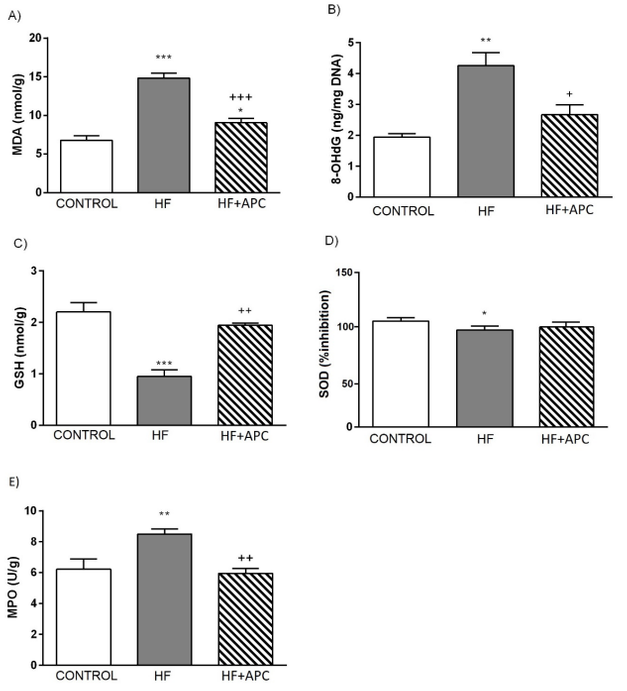

Figure 4. *MDA (A), 8-OHdG (B), GSH (C), SOD (D) and MPO (E) levels in the control, HF and HF+APC groups. * P<0.05, ** P<0.01, *** P<0.001 in comparison with control group. + P<0.05, ++ P<0.01, +++ P<0.001 in comparison with HF group.*

3.5. Sperm Parameter Results

Normal spermatozoa and spermatozoa with tail, neck and head abnormalities were observed in all of the experimental groups. The number of epididymal spermatozoa (p< .01) and sperm motility (p< .05) reduced in the HF group compared to the control group. Although the number of epididymal spermatozoa and sperm motility increased in the HF+APC group in comparison with the HF group, these findings were not statistically significant. Spermatozoa with normal morphology decreased in the HF and HF+APC groups (p< .05), and spermatozoa with tail defects (p< .05) increased in the HF group compared to the control group (Figure 5).

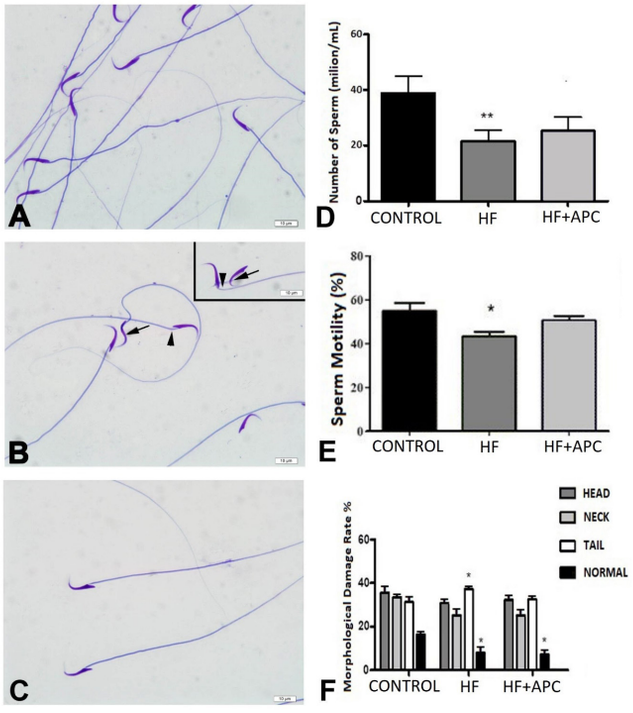

Figure 5. *Representative light micrographs of spermatozoa morphology (A-C), number of spermatozoa (D), sperm motility (E) and sperm morphologic damage ratio (F) in the control, HF and HF+APC groups. Spermatozoa with normal morphology and spermatozoa with head (arrow), neck (arrowhead) and tail abnormalities are seen in the control (A), HF (B) and HF+APC (C) groups. Diff-Quick staining. Original magnification: 1000x. Scale bar: 10µm. * P<0.05, ** P<0.001 in comparison with control group.*

3.6. Histopathological Results

Normal seminiferous tubules morphology were present in the control group. The HF group had many degenerated seminiferous tubules, decreased number of spermatogenic cells, many dilatations among the germinal epithelial cells, and many immature germ cells in the lumen. The HF+APC group had significantly more normal seminiferous tubules, but there were some dilatations among the spermatogenic cells and immature sperms in luminal region. Seminiferous tubule diameter (p< .001), germinal epithelium thickness (p< .001) and histopathologic Johnsen's score (p< .01) decreased in the HF group compared to control group. These seminiferous tubule diameter (p< .05), germinal epithelium thickness (p< .001) and histopathologic Johnsen's score (p< .01) increased in the HF+APC group compared to the HF group (Figure 6).

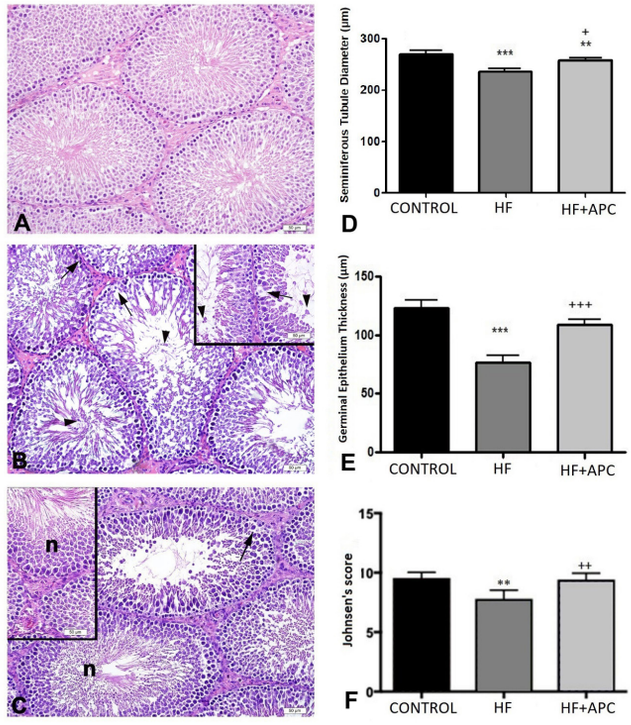

Figure 6. *Representative light micrographs of testis samples (A-C), seminiferous tubule diameter (D), germinal epithelium thickness (E) and Johnsen's histopathological scores (F) of the control, HF and HF+APC groups. Normal germinal epithelium of seminiferous tubules is present in the control group (A). Dilatations (arrow) among the germinal epithelial cells and cellular debris (arrowhead) in lumen are seen in the HF group (B). Normal seminiferous tubules (n) and dilatation of germinal epithelium in some area (arrow) are seen in the HF+APC group (C). Scale bar: 50 µm. ** P<0.01, *** P<0.001 in comparison with control group, $^{+}$P<0.05, $^{++}$ P<0.01 and $^{+++}$ P<0.001 in comparison with HF group.*

3.7. PCNA Immunohistochemistry Results

PCNA-positive cells in the epithelium of seminiferous tubules were seen in all of the experimental groups. Proliferative index was lower in the HF group (p< .01) compared to the control group and this parameter was higher in the HF+APC group (p< .05) compared to the HF group (Figure 7A_1-D_1).

3.8. TUNEL Analysis Results

TUNEL-positive cells were present in all of the experimental groups, but the number of TUNEL-positive cells increased in the HF group compared to the control and HF+APC groups. Apoptotic index was higher in the HF group compared to the control group (p< .01), this parameter was lower in HF+APC group (p< .05) compared to HF group (Figure 7A_2-D_2).

3.9. NOX2 Immunohistochemistry Results

NOX2-positive cells were seen in the interstitial area and germinal epithelium in all of the experimental groups, but the number of NOX2-positive cells increased in the HF group compared to the control and HF+APC groups. NOX2 – positive cells increased in the HF group compared to the control group ($p< .01$) and decreased in the HF+APC group ($p< .05$) compared to the HF group (Figure 7A_3-D_3).

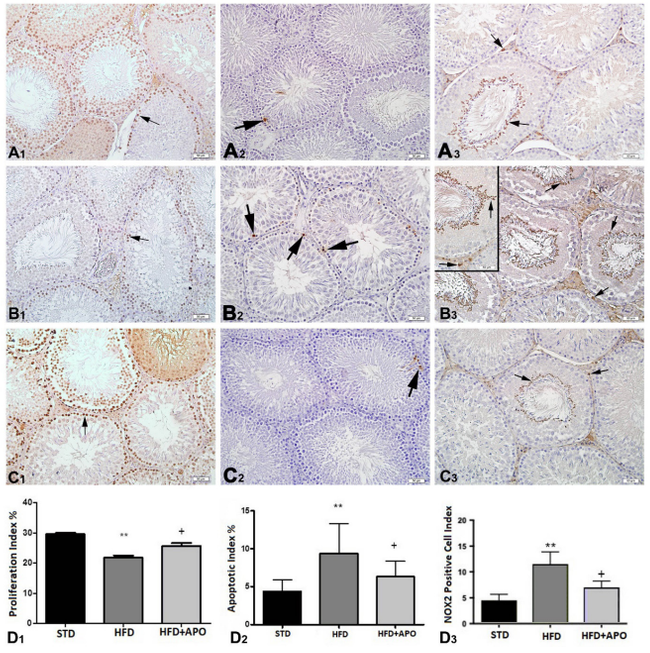

Figure 7. *Representative light micrographs of PCNA immunostained (A_1-C_1), TUNEL-stained (A_2-C_2) and NOX2 immunostained (A_3-C_3) testis samples, proliferation index (D_1), apoptotic index (D_2) and NOX2-positive cell ratio (D_3) in the control, HF and HF+APC groups. PCNA – positive (arrow), TUNEL – positive (arrow) and NOX2 – positive (arrow) cells in seminiferous tubules are seen in the control (A_1-A_3), HF (B_1 – B_3) and HF+APC (C_1 – C_3) groups. Scale bar: 50 µm. ** P<0.01, in comparison with control group, + P<0.05 in comparison with HF group.*

4. DISCUSSION

In this study, increase of body weight, changes in serum lipid and hormone profiles were demostrated in the HF group compared to the control rats. The biochemical results showed increased oxidative stress parameters and decreased endogeneous antioxidant levels in the HF group compared to the control group. Moreover, altered sperm paramaters, increased degenerated seminiferous tubules with apoptotic and NOX2-positive cells and decreased proliferative cells in seminiferous tubules were found in the HF group compared to the control group. All these HF-induced alterations in lipid and hormone profiles, sperm parameters, testicular oxidative stress and histological damage findings were ameliorated by APC administration.

Obesity is related to the increase of white adipose tissue in the body and BMI over 30 kg/m^2. As BMI increases, the rate of infertility increases in men (19). Decrease in semen quality was observed 3 times more in obese men then in men with normal weight (20). Moreover, decrease of testis weight was reported in obese rats (21). Also, decrease of sperm concentration (22) and sperm motility were shown in obese patients (23), and alteration of epididymal sperm parameters (24), teratozoospermia and degeneration of testis morphology (25) were shown in obese animals. Parallel with the previous studies, in the present study, an increase in body weight, the decrease in number, motility and normal sperms were observed in the HF group. Although testis weight was not different amoung the groups, decreased the diameter and epithelial thickness of seminiferous tubules and increased of Johnsen's score was observed in the HF group. APC treatment ameliorated these sperm parameters and testicular morphological damage in the HF-fed rats via its antioxidative activity.

Obesity is related with an increase of blood free fatty acids and the accumulation of fat in the white adipose tissue (1). Increased blood glucose, serum cholesterol, triglyceride, and LDL levels and decreased HDL level were shown in obese animals (8,26,27). An increase in free fatty acid stimulates the activation of NOX in the body cells, ultimately causing oxidation and formation of free radicals (8). Similar to the previous studies, an increase of cholesterol and triglyceride levels and decrease of HDL level were observed in the serum of HF fed rats, and APC treatment ameliorated lipid profiles in the serum of HF-fed rats via its regulatory effects on oxidative stress.

Leptin is one of the important factors affecting weight gain in relation to appetite control. Increased serum leptin level was observed in obese infertile patients in both sexes (28). Increased leptin level and decreased fertility rate was reported in obese mice (29). It was shown that increased leptin level had harmful effects on sperm production in obese male (30). Similarly, Yi et al. (2017) showed an increase in serum leptin, leptin mRNA and protein levels in testis, and sex hormone dysregulation in mice fed with HF (31). Obesity may also adversely affect the male reproductive endocrine system which regulates the reproductive function. It has been reported that obesity alters the sex hormone levels such as increased estrogen level and decreased LH, FSH and testosterone levels (32). Increased estrogen level alters the production of LH, FSH and testosterone levels (33). Similar to the previous studies, increased leptin and estrogen levels and decreased LH and FSH levels were shown in the HF group in this study. Although increased testosterone level was observed in the HF group, degenerated testis morphology and altered sperm parameters were seen in the HF group. Since spermatogenesis is a complex event, it is regulated not only by testosterone level but also from the other hormones and Sertoli cells regulatory function. Therefore, the role of Leydig and Sertoli cells in HF-induced obesity may consider in further studies on this subject. HF-induced obesity in rats might have caused alteration of sexual hormone levels which regulates the spermatogenesis and APC treatment

may regulate the hormone producing cells activity via anti-oxidative activity.

Positive correlation between the oxidative stress, and obesity have been reported previously. Also, increased reactive oxygen and nitrogen species levels and decreased antioxidant defences in obese patients were observed (4). Oxidative stress is quite associated with cumulative injury to the body caused by free radicals that are weakly neutralized by antioxidants, and oxidative damage is worsened by reduction of SOD, catalase (CAT) and glutathione S-transferase activities (34). A decrease in SOD activity and an increase in MDA level induce oxidative stress and lead to cellular damage in testis of obese rats (25). Aditionally, it was noted that, ROS formation causes sperm membrane damage which reduces the motility and ability to fuse with the oocyte and directly sperm DNA damage (1). Plasma MDA values were found higher in obese individuals than non-obese group. It has been shown that the MDA/SOD ratio is higher in obese individuals compared to normal individuals (35). Recent studies have shown the increased MDA level and decreased SOD activity and GSH level in testis of obese rats (8,36). Moreover, increased MDA level and decreased SOD and CAT activities were shown in the testis of obese rats with intake of high-fructose corn syrup (37). In this study, increased MDA and decreased GSH and SOD levels were observed in the testis of HF-fed rats. Increased oxidative stress may cause testicular degereneration with decrease in diameter and germinal epithelium thickness of seminiferous tubules and proliferative cells and increase in NOX2 immunpositive and apoptotic cells. We suggest that APC treatment reversed these oxidative and histopathological parameters in testis samples of HF-fed rats via regulating of oxidative stress.

Obesity causes formation of ROS through NOX activation in the adipose tissue (38). APC, an inhibitor of NOX2, has a role for the inhibition of NOX-induced ROS production. Also, APC stimulates the g-glutamylcysteine synthethase which effects the synthesis of GSH (39). It has been shown that the serum and hepatic MDA levels were higher and hepatic SOD, glutathione-peroxidase and CAT activities and GSH level were lower in the HF-fed mice and APC treatment amelioriated the oxidant/antioxidant parameters in both serum and hepatic tissues in these mice (40). Moreover, it was shown that, administration of 16 mg/kg APC for 4 weeks reduced oxidative stress and apoptotic cells in testicular tissue in diabetic rats (14). Additionally, it was shown that, administration of both 20 mg/kg and 50 mg/kg doses of APC decreased oxidative stress parameters and testicular damage in ischemia/reperfusion induced toxicity (15). Administration of 25 mg/kg APC reduced oxidative stress, testicular damage, apoptosis and sperm parameters in cisplatin induced testis toxicity (12) and administration of both 20 mg/kg and 50 mg/kg doses of APC reduced oxidative stress parameters, testicular damage and apoptosis in methotrexate-induced testis toxicity (13). Parallel to the previous studies, increase of MDA level, apoptotic and NOX2-positive cells and decrease of GSH and SOD level in HF group were observed, while APC (25 mg/kg) administration ameliorated all these oxidative stress parameters, testicular damage and sperm parameters via the inhibiting of NOX activation.

Obesity-induced inflammatory responses and their relation with the generation of free oxygen radicals have been demonstrated previously (4,35). Activation of MPO, a heme protein synthesized by neutrophils, is associated with the obesity-induced inflammation. It was observed that the number of neutrophils in circulatory blood are rised in obese individuals and type 2 diabetic patients. Moreover, studies showed that there is a positive correlation between MPO activation and metabolic disorders and MPO plasma levels are higher in these patients (41,42). Additionally, it has been reported that obesity-induced testicular inflammation causes disruption of spermatogenesis and steroidogenesis (35). Recent studies have shown the anti-inflammatory effects of APC in ischemia/reperfusion – (15), cisplatin – (12) and methotrexate-induced (13) testis damage. In this study, oxidative parameters such as MDA and 8-OHdG levels, NOX2-positive cells as well as MPO activity in HF-fed rats were increased, APC treatment to the HF-fed rats reversed the MPO activity to the control level.

Apoptosis is related to many diseases including obesity. The germ cell population during spermatogenesis is regulated by apoptosis, however increased apoptotic activity may lead to male infertility (43). It was reported that HF caused an increase of apoptotic cells in the testis (8,21). The 8-OHdG measurement is one of the most commonly used method to determine oxidative DNA damage. Increased DNA fragmentation in testis and spermatozoa were reported in obese men (34). Additionally, it was reported that apoptotic cell death and 8-OHdG level were increased in various oxidative testicular damage models (5,8,12-15). Moreover, it has been noted that various antioxidant compounds including APC has neutralizing effect on harmful free radical compounds as well as preventive effect on the apoptotic processes in these oxidative testicular damage models (8,12-15). Parallel to the previous studies, increase of testicular apoptotic cells and 8-OHdG level together with increase of NOX2-positive cells and decrease of proliferative cells were observed in HF-fed rats, APC treatment reversed this apoptotic cell death and oxidative cell damage to the level of standard diet-fed rats.

5. CONCLUSION

In conclusion, HF-induced obesity caused changes of lipid and hormone profiles in serum, changes of sperm parameters, increase of oxidative, inflammatory and apoptotic activity and decrease of proliferative cells and endogeneous antioxidants in rat testis. APC treatment might have ameliorated obesity-induced functional and morphological testis damage by inhibition of oxidative stress, apoptosis, inflammation and regulation of oxidant/antioxidant balance. Additionally, the efficacy of APC as a therapeutic agent was approved in many studies and also in our study, suggesting its testis damage reducing effect in HF-induced obesity. However, limitations of our study were that we did not study the proinflammatory and apoptotic markers, in the testis and oxidative parameters

in the epididymis. Therefore, the molecular mechanisms involved in effects of apocynin on high fat diet-induced testis damage require further studies.

Acknowledgements: *The authors would like to thank Dr. Dilek Akakın for comments of our manuscript.*
Funding: *This study was financially supported by Marmara University Scientific Research Project Committee (SAG-C-YLP-131.217.0655).*
Conflicts of interest: *The authors declare that they have no conflict of interest.*
Ethics Committee Approval: *This study was approved by the Animal Care and Ethical Committee for Experimental Animals at Marmara University.*
Peer-review: *Externally peer-reviewed.*
Author Contributions:
Research idea: F.E.
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Analysis of data for the study: İ.E., M.K.K., B.C., B.E., G.Ş., F.E.
Interpretation of data for the study: F.E., G.Ş.
Drafting the manuscript: F.E, M.K.K., G.Ş.
Revising it critically for important intellectual content: F.E.
Final approval of the version to be published: İ.E., M.K.K., B.C., B.E., G.Ş., F.E.

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How to cite this article: Hersek İ, Köroğlu MK, Coşkunlu B, Ertaş B, Şener G, Ercan F. Apocynin Ameliorates Testicular Toxicity in High-Fat Diet-Fed Rats by Regulating Oxidative Stress. Clin Exp Health Sci 2023; 13: 75-83. DOI: 10.33808/clinexphealthsci.1035133