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Morchella esculenta Intensifies Testosterone Production and Spermatogenesis in Male Mice with High-Fat Diet-Induced Obesity by Enhancing Antioxidant Capacity

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Abstract: Obesity has become a major health concern worldwide and is strongly correlated with various comorbidities including reproductive dysfunction. Therefore, this study aimed to explore the effects of *Morchella esculenta* (ME) in improving reproductive dysfunction caused by high-fat diet-induced obesity. Male C57BL/6J mice were randomly divided into control, obese, and ME-treated obese groups. The total duration of high-fat diet feeding was 24 weeks, and over the last 8 weeks, the ME-treated groups received low-dose (100 mg/kg) and high-dose (500 mg/kg) ME extract for 5 days of each week. Biochemical markers, hormones, liver, testicular histology, and semen quality were analyzed. The activities of the testicular antioxidants and the products of lipid peroxidation were measured. The protein expression levels of apoptosis-, autophagy-, and inflammation-related markers were also surveyed. The HFD-fed mice had abnormal sex hormone levels, poor sperm quality, and destroyed testicular structure, which were significantly restored following ME treatment. Despite having no effect on apoptosis-related markers, decreased activities of antioxidants in obese mice were notably ameliorated. Furthermore, the levels of the inflammation-related markers NF-κB and IL-6 were altered by ME treatment. In conclusion, ME has therapeutic effects on reproductive dysfunction caused by diet-induced obesity, which may be partially due to improving antioxidant capacity and inhibiting inflammation. ME might be useful as a complementary treatment for male infertility.

Keywords: high-fat diet; obesity; male infertility; *Morchella esculenta*; spermatogenesis

1. Introduction

Obesity, as excessive body fat accumulation due to continued energy imbalance, has been a notable health burden since the 1980s [1], and currently, 38% of the global population is classified as overweight or obese. Furthermore, it is expected that by 2035, 27% of the world population will be overweight and another 24% will be obese [2]. An increasing projected prevalence of obesity has been

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reported in Asia countries [3,4]. Moreover, the Nutrition and Health Survey in Taiwan (NAHSIT) revealed that the prevalence of overweight and obesity increased rapidly from 1993–1996 to 2013–2016 (33.2% to 50.7%), and approximately one in two men and one in three women were overweight or obese [5].

Obesity-associated comorbidities include type 2 diabetes, cardiovascular disease, cancers, osteoarthritis, cognitive decline, and infertility [6]. Studies have shown that overweight and obese men have higher rates of abnormal sperm quality and hormone levels [7,8] and higher reproductive failure rates, such as pregnancy loss and decreased pregnancy rates following artificial reproductive technology treatment [9,10]. Male overweight and obesity even adversely impact the health of their offspring, with an increased risk of metabolic disorders and effects on neurologic function and respiratory function [11].

Obesity and obesity-induced metabolic changes, such as elevated insulin levels and lipid profiles, alter various mechanisms involved in male reproduction, including the hypothalamic–pituitary–gonadal axis, testosterone biosynthesis, spermatogenesis, oxidative stress, apoptosis and inflammation [12,13]. Abnormal autophagy is another mediator of obesity's downstream effects that was recently found to function in spermatogenesis and testosterone biosynthesis [14]. Furthermore, oxidative stress-mediated mechanisms contribute to nearly 50% of infertility cases. The accumulation of reactive oxygen species triggers inflammation, apoptosis, and autophagy, leading to abnormalities of sperm function and infertility [15,16].

Due to its delicious taste and widely distribution across temperate regions, including Europe, North America, and Asia, *Morchella esculenta* (ME), has been one of the most popular edible mushrooms [17]. In the past few decades, with the understanding of the health benefits associated with mushroom consumption, studies on mushrooms have greatly expanded. According to modern studies, ME and its bioactive ingredients, such as organic acids, phenolic compounds and polysaccharides, exert health-promoting, antiobesogenic, antioxidant, antimicrobial, anti-inflammatory, and antitumor properties [18–21]. In addition, oral feeding of ME extract has been reported to have a protective effect against reproductive impairments in a heavy metal toxicity-induced rat model [22]. Because its potential actions on male infertility caused by diet-induced obesity are not completely understood, the goal of this study was to identify the effects of ME treatment on testicular and sperm function using a high-fat diet-induced obese animal model and to investigate its roles in redox imbalance, apoptosis, inflammation, and autophagy in the testis.

2. Materials and Methods

2.1. Animals and Treatment

The animal experiments and protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of the National Defense Medical Center (Taipei, Taiwan), and animal care was conducted in accordance with the guidelines. C57BL/6J mice (weighing 20–25 g, aged 8 weeks) were obtained from the National Laboratory Animal Center (Taipei, Taiwan) and housed in the animal center of the National Defense Medical Center. Mice were kept in plastic cages (2–3 per cage) under a regular 12 h light/dark cycle, 23 ± 2 degrees Celsius and 55 ± 5% humidity, and provided a standard rodent chow diet (LabDiet 5010; 13% energy from fat, 58% from carbohydrates and 29% from protein) or high-fat diet (Research Diets D12451; 45% energy from fat, 35% from carbohydrates and 20% from protein) and water *ad libitum*. Mice were assigned to the control group (Control, $n = 8$) or diet-induced obesity group (DIO, $n = 24$) for 16 weeks after acclimation. Then, the DIO group was divided into three groups ($n = 8$ per group), and all groups were given vehicle or doses of ME by oral gavage 5 days a week for 8 weeks: control (vehicle), DIO (vehicle), LME (low-dose ME: 100 mg/kg), and HME (high-dose ME: 500 mg/kg).

ME powder was prepared and provided by Grape King Bio Ltd. (Taoyuan, Taiwan), and the safety of the ME powder was tested [23]. Briefly, the mycelium was isolated from the fresh fruiting body and maintained on potato dextrose agar (PDA) slants at 25 degrees. After 7 days, 1 cm^3 ME was

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removed from the PDA and transferred to a 2 L flask containing 1 L synthetic culture medium (composed of 2% sucrose, 2% soybean powder, 0.3% yeast extract, 0.05% KH_2PO_4, and 0.05% $MgSO_4$, adjusted to pH 5) at 25 °C for 5 days with shaking at 120 rpm. This fermentation process was then scaled up from a 2 L shake flask to 200 L fermenters for 5 days. After fermentation with a controlled pH of 5, agitation at 50 rpm, and temperature at 25 °C, the whole broth was harvested, lyophilized, ground to a powder, and stored in a desiccator at room temperature. For aqueous and ethanolic extract preparation, the freeze-dried ME powder was suspended at 1:20 w/v in water and ethanol (95%). Briefly, the aqueous suspension mixture was boiled at 121 degrees for 15 min, while the ethanolic suspension mixture was sonicated in a bath sonicator for 1 h. Afterward, both suspensions were passed through filter papers and concentrated through a rotary evaporator or freeze dryer. The nutrient content of the dried ME powder revealed that the crude carbohydrate, protein, and lipid contents were 39%, 39%, and 17%, respectively, with 4% moisture and 0.3% ash [23]. The total polysaccharides in the ME powder was determined using phenol-sulfuric acid reaction [24], and the content was 11.5%.

2.2. Serum Analyses

Mice were euthanized, and blood was collected by heart puncture at the end of the experiment. Serum was separated from blood samples using centrifugation and sent to the National Laboratory Animal Center for measurements of total cholesterol (TC), triglyceride (TG), and glucose levels. Serum insulin (10-1247-01; Mercodia, Uppsala, Sweden), follicle-stimulating hormone (MBS2507988; MyBioSource, San Diego, CA, USA), luteinizing hormone (MBS2514287; MyBioSource), and testosterone (No. 582701; Cayman, Ann Arbor, MI, USA) levels were determined using commercially available kits. The homeostasis model assessment for the insulin resistance (HOMA-IR) was calculated by using the following formula: HOMA-IR = [fasting serum glucose (mg/dL) × insulin (mIU/L)] /405 [25].

2.3. Assessments of Sperm Motility, Sperm Count, and Sperm Morphology

Spermatozoa were collected from the vas deferens after weighing, and an evaluation of sperm motility was instantly performed. The diluted sample was loaded in a Neubauer chamber (Marienfield-Superior, Lauda-Königshofen, Germany) and placed on the microscope stage, and the percentage of motile cells was visually calculated. The sperm count was assessed using an automated cell counter (Bio-Rad, Redmond, WA, USA) by inserting the counting slide filled with sample. In terms of sperm morphology, smear samples were fixed in methanol, stained with 2% eosin solution, washed with ethanol, dried at room temperature, and observed under a light microscope, and the percentage of normal sperm morphology was calculated.

2.4. Histology

Liver and testis tissues were cleaned in cold phosphate-buffered saline and fixed with 10% formalin. Then, the tissues were sent to the pathology division of the Cardinal Tien Hospital (New Taipei City, Taiwan) for processing, paraffin embedding, sectioning into 4-μm slices and staining with hematoxylin and eosin. The stained slides were observed at appropriate magnification under a light microscope (Leica, Wetzlar, Germany) and analyzed using ImageJ.

2.5. Testicular Redox Status

Total protein was extracted from the testis tissues and used to measure the activities of testicular antioxidants, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). In addition, the formation of malondialdehyde (MDA) in the testis was also measured with commercial ELISA kits following the manufacturer's protocols (Catalog number: 706002, 707002, 703102 and 10009055, respectively, for SOD, CAT, GPx and MDA; Cayman).

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2.6. Western Blotting

Proteins were separated using sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred from the gel to a polyvinylidene difluoride membrane. The membrane was blocked with 5% nonfat dry milk blocking buffer and incubated in different dilutions of primary antibody at 4 °C overnight. The membrane was washed in TBST 3 times for 10 min, incubated with a dilution of secondary antibody, and rewashed in TBST 3 times for 10 min. Before detection with a chemiluminescent imaging system, the membranes were soaked in ECL reagent for 1 min. The following primary antibodies were used: anti-caspase 9 (#9508, 1:1000 dilution; Cell Signaling Technology, MA, USA), anti-Bax (#2772, 1:1000 dilution; Cell Signaling Technology), anti-Bcl-xl (ab32370, 1:1000 dilution; Abcam, MA, USA), anti-caspase 3 (#9662, 1:750 dilution; Cell Signaling Technology), anti-NF-κB (E381, 1:1000 dilution; Abcam), anti-TNF-α (ab1793, 1:1000 dilution; Abcam), anti-IL6 (sc-48402, 1:500 dilution; Santa Cruz Biotechnology, CA, USA), anti-Beclin 1 (sc-48341, 1:1000 dilution; Santa Cruz Biotechnology), anti-LC3B (sc-271625, 1:1000 dilution; Santa Cruz Biotechnology), anti-P62 (sc-48402, 1:1000 dilution; Santa Cruz Biotechnology), and anti-β-actin (A5316, 1:10000 dilution; Sigma, MO, USA). The following secondary antibodies were used: anti-mouse (sc-2005, 1:5000 dilution; Santa Cruz Biotechnology) and anti-rabbit (sc-2054, 1:4000 dilution; Santa Cruz Biotechnology).

2.7. Statistical Analyses

All data are presented as the mean ± SD. After assessing heterogeneity among the groups with the Bartlett's test, one-way ANOVA and Welch's ANOVA were used to examine data from multiple groups, followed by Tukey's honestly significant difference (HSD) test and Dunnett's T3 test, and results were considered significant at $p < 0.05$.

3. Results

3.1. Body Weight, Weight Gain, Food Intake, Energy Intake, and Weight of Reproductive Organs

Compared to the control, the groups fed a high-fat diet (DIO, LME, HME) had significantly higher body weight and weight gain (Figure 1a). These groups also had a lower food intake but a greater daily energy intake because of consuming a diet with a high calorie density (Figure 1b,c). Although there were no differences in the testis and epididymis weights among the four groups, the vas deferens weight of the HME group was significantly higher than that of the LME group (Figure 1d). Furthermore, the DIO, LME, and HME groups had an increased fat mass compared with the control group (Figure 1e).

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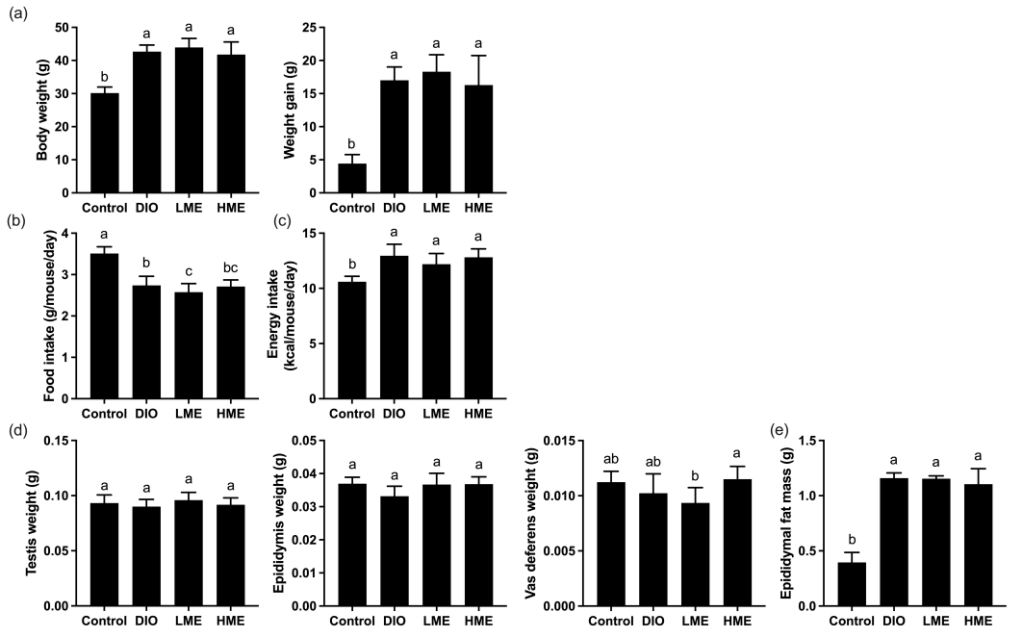

Figure 1. (**a**) Body weight, weight gain, (**b**) food intake, (**c**) energy intake, (**d**) reproductive organs, and (**e**) epididymal fat mass in control, obese, LME-treated obese, and HME-treated obese mice. Values are presented as the mean ± SD (n = 8 per group). Bars in (**a**,**c**,**d**) with letters (**a**,**b**) presented statistical significance ($p < 0.05$) based on one-way ANOVA with Tukey's HSD test; in (**b**,**e**) with letters (**a**–**c**) based on Welch's ANOVA with Dunnett's T3 test. LME, low-dose ME; HME, high-dose ME.

3.2. Effects of ME Treatment on Serum Biochemical Parameters in Obese Mice

A 24-week high-fat diet resulted in elevated serum glucose, insulin, and total cholesterol with reduced triglyceride levels. Mice consuming a high-fat diet and receiving low-dose ME treatment had similar results, and high-dose ME treatment led to a slightly decreased glucose level and a significant decrease in insulin between the LME and HME groups was found. Furthermore, high-dose ME had a significantly lower HOMA-IR (Figure 2).

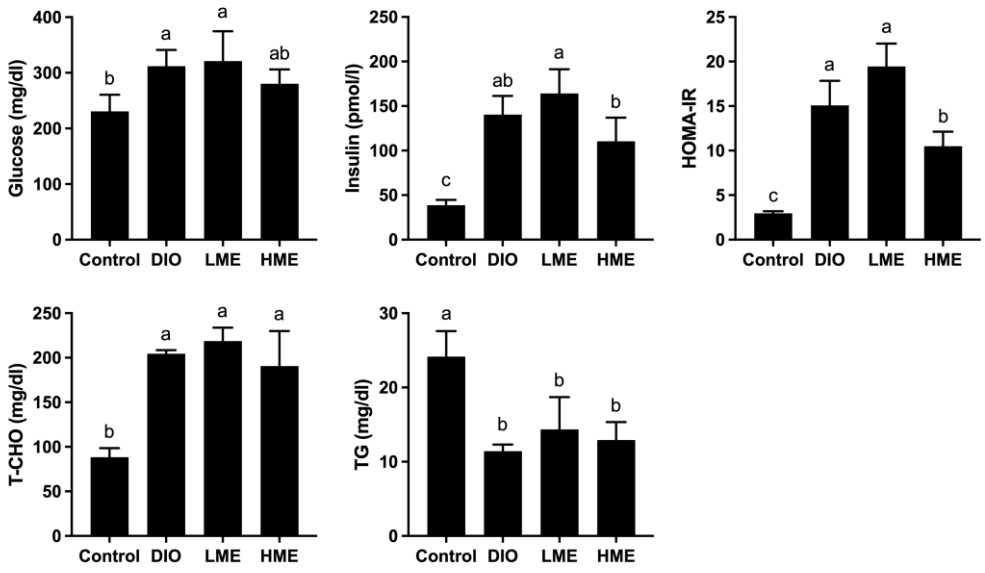

Figure 2. Serum glucose, insulin, HOMA-IR, total cholesterol, and triglyceride levels in control, obese, LME-treated obese, and HME-treated obese mice. Values are presented as the mean ± SD (n = 6 per group). Bars with

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letters (**a**–**c**) presented statistical significance ($p < 0.05$) based on one-way ANOVA with Tukey's HSD test. HOMA-IR, homeostasis model assessment of insulin resistance; LME, low-dose ME; HME, high-dose ME.

3.3. Effects of ME Treatment on Liver Weight, Liver Histology, and Percentages of Hepatic Lipid Droplet Area in Obese Mice

The liver weights of the DIO and LME groups were markedly higher than that of the control group. High-dose ME treatment decreased the liver weight when compared to the DIO group and had no significant difference when compared to the control group. In terms of liver histology, microvesicular and macrovesicular steatosis was observed in the groups consuming a high-fat diet. The steatosis in the HME group was less severe than that in the DIO group, with significantly less lipid droplet accumulation (Figure 3).

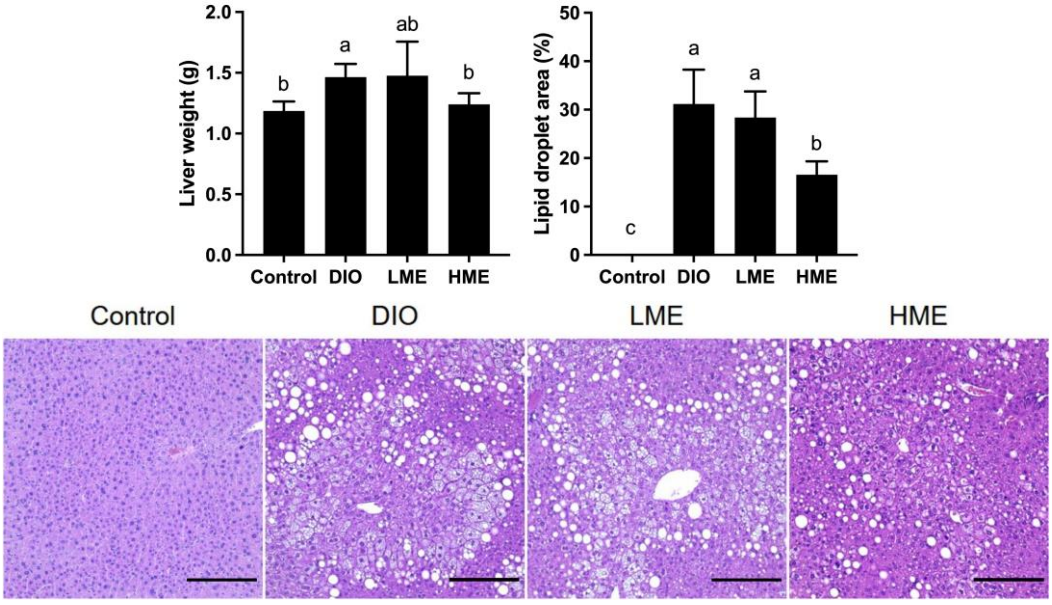

Figure 3. Liver weight, percentages of hepatic lipid droplet area, and liver histology in control, obese, LME-treated obese, and HME-treated obese mice. All scale bars are 100 μm. Values are presented as the mean ± SD (n = 8 per group). Bars with letters (**a**–**c**) presented statistical significance ($p < 0.05$) based on Welch's ANOVA with Dunnett's T3 test. LME, low-dose ME; HME, high-dose ME.

3.4. Effects of ME Treatment on Sperm Quality and Serum Hormones in Obese Mice

Obese mice exhibited significantly lower percentages of sperm motility and normal morphology. Following low-dose and high-dose ME treatment, these mice showed significantly increased sperm motility and normal sperm morphology in comparison to the obese mice. However, the two parameters were still significantly different between the control and LME groups, and a notably decreased sperm motility was observed between the control and HME groups. Additionally, there was no significant difference in sperm count among the four groups (Figure 4a). The serum testosterone level was significantly lower in the DIO group than in the control group. Meanwhile, the serum level of the upstream hormone LH was significantly reduced, with a decreasing trend toward the FSH level. Treatment with low-dose and high-dose ME normalized the blood testosterone level and showed an increasing trend in the FSH level. The LH level was slightly reversed in the LME group, with a more obvious change than in the HME group (Figure 4b).

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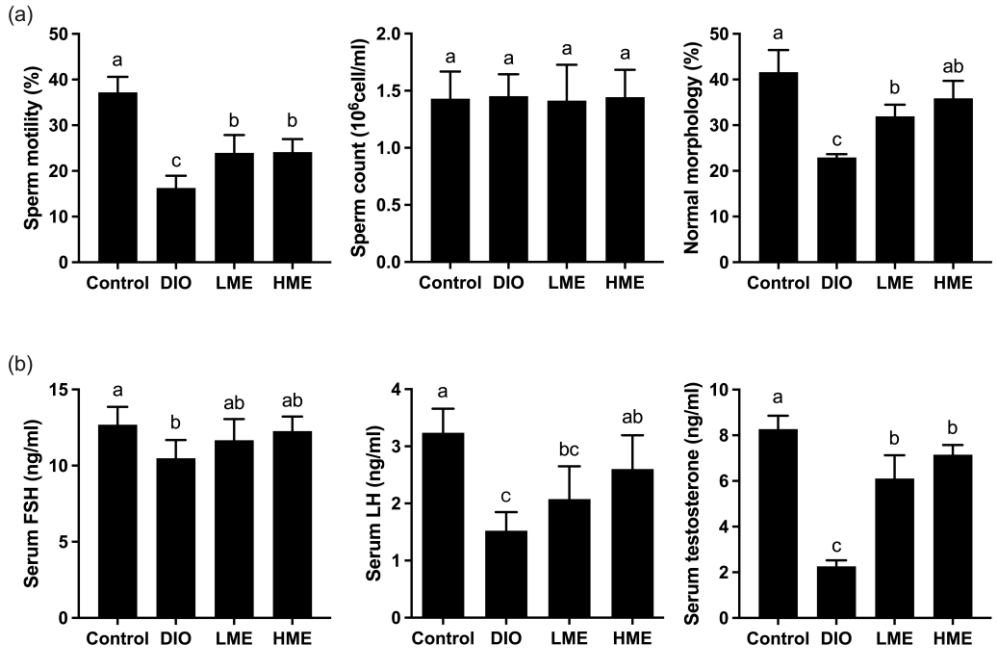

Figure 4. (**a**) Sperm quality and (**b**) FSH, LH, and testosterone levels in control, obese, LME-treated obese, and HME-treated obese mice. Values are presented as the mean ± SD with (**a**) $n = 8$; (**b**) $n = 6$ per group. Bars with letters (**a**–**c**) presented statistical significance ($p < 0.05$) based on one-way ANOVA with Tukey's HSD test. FSH, follicle-stimulating hormone; LH, luteinizing hormone; LME, low-dose ME; HME, high-dose ME.

3.5. Effects of ME Treatment on Spermatogenesis in Obese Mice

Damaged spermatogenesis, characterized by a degenerated and vacuolized seminiferous tubular lumen and decreased numbers of spermatids, was observed in obese mice. Meanwhile, the mean testicular biopsy score (MTBS) was calculated following the Johnsen score on a scale of 1 to 10 with corresponding descriptions of testicular histological criteria, and the results showed that the DIO group's MTBS was significantly lower than that of the control group. In contrast, high-dose ME treatment markedly improved the damaged structure of the testis and mean biopsy score, and low-dose ME treatment slightly restored the mean biopsy score. However, the mean diameter of seminiferous tubules (MSTD), calculated as the average of the two longest horizontal and vertical diameters, was not significantly different among the four groups (Figure 5).

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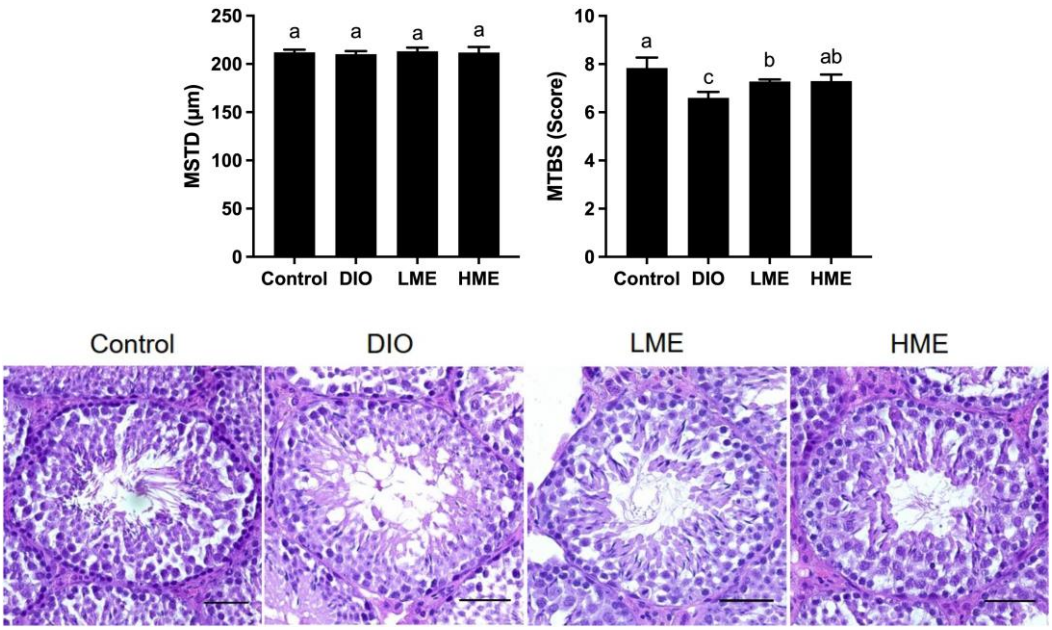

Figure 5. Testicular histology and evaluations of MSTD and MTBS in control, obese, LME-treated obese, and HME-treated obese mice. All scale bars are 50 μm. Values are presented as the mean ± SD (n = 8). Bars with letters (**a**–**c**) presented statistical significance ($p < 0.05$) based on one-way ANOVA with Tukey's HSD test. MSTD, mean seminiferous tubule diameter; MTBS, mean testicular biopsy score; LME, low-dose ME; HME, high-dose ME.

3.6. Effects of ME on Testicular Antioxidants and MDA Content in Obese Mice

The testicular antioxidative activities of SOD, CAT, and GPx were markedly decreased in obese mice compared to control mice. Meanwhile, a markedly increased MDA level in obese mice was found. Notably, both low-dose and high-dose ME treatments significantly reversed the trend, elevated activities of testicular antioxidants, and reduced the MDA level, suggesting that ME treatment attenuated redox hemostasis in obese mice (Figure 6).

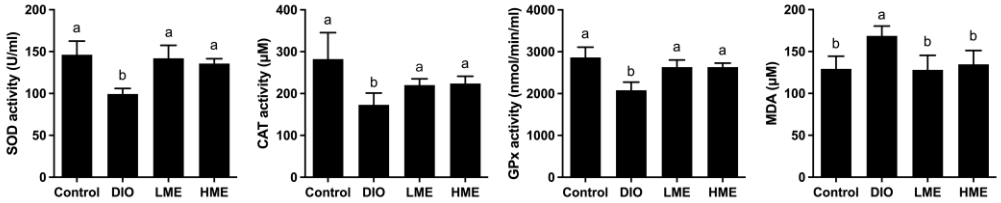

Figure 6. Activities of SOD, CAT, and GPx and MDA content in the testes of control, obese, LME-treated obese, and HME-treated obese mice. Values are presented as the mean ± SD (n = 6). Bars with letters (**a**–**b**) presented statistical significance ($p < 0.05$) based on one-way ANOVA with Tukey's HSD test. SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase; MDA, malondialdehyde; LME, low-dose ME; HME, high-dose ME.

3.7. Effects of ME on the Expression of Apoptosis-Related Markers in Obese Mice

The ratio of Bax/Bcl-xl and the expression levels of caspase 9, cleaved caspase 9, and cleaved caspase 3 were significantly higher in the testes of the DIO group. The LME and HME groups had significantly decreased expression levels of cleaved caspase 9 compared to the DIO group, and these changes were also significant compared to the control group. In addition, ME treatment had a lowering trend in caspase 9 expression but no effect on cleaved caspase 3 expression (Figure 7).

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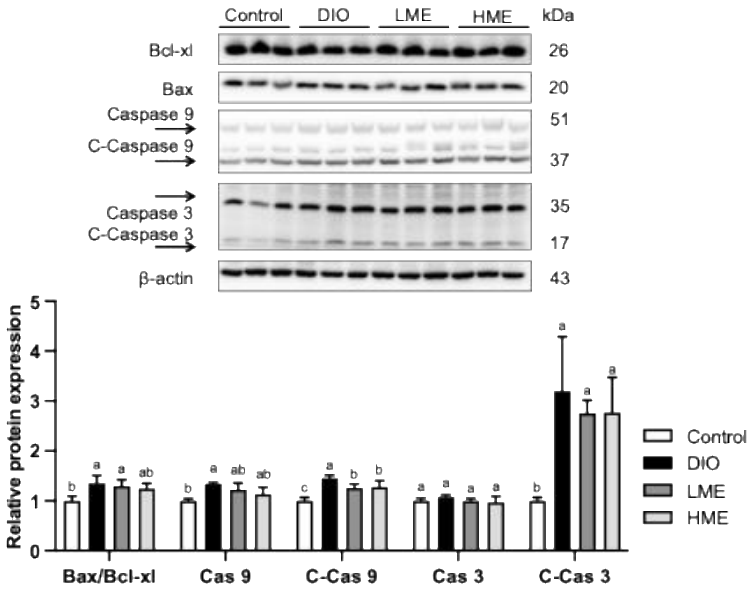

Figure 7. Protein expression of apoptosis-related markers in the testes of control, obese, LME-treated obese, and HME-treated obese mice. Values are presented as the mean ± SD (n = 6). Bars with letters (**a**–**c**) presented statistical significance ($p < 0.05$) based on one-way ANOVA with Tukey's HSD test. LME, low-dose ME; HME, high-dose ME.

3.8. Effects of ME on the Expression of Inflammation-Related Markers in Obese Mice

NF-κB p50 subunit expression was notably elevated in the DIO group, and different doses of ME treatment attenuated its increase to a notable extent. The high-dose ME treatment lowered IL-6 expression when compared to the DIO and low-dose ME treatment groups. However, changes in the protein expression of TNF-α were not significant (Figure 8).

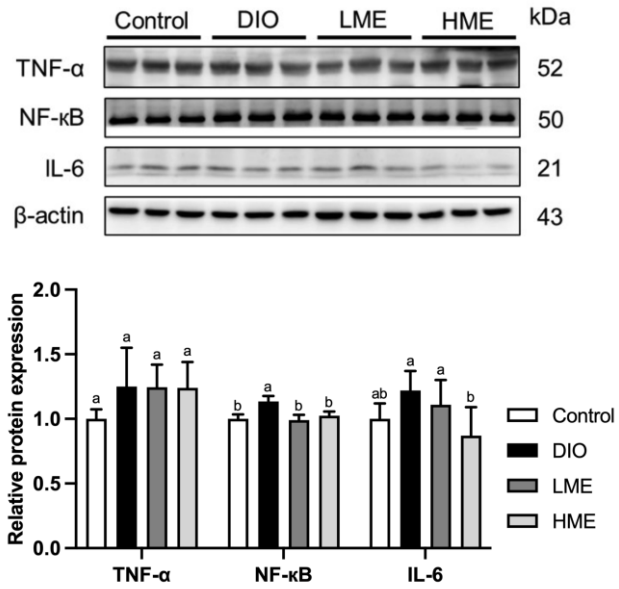

Figure 8. Protein expression of inflammation-related markers in the testes of control, obese, LME-treated obese, and HME-treated obese mice. Values are presented as the mean ± SD (n = 6). Bars with letters (**a**–**b**) presented statistical significance ($p < 0.05$) based on one-way ANOVA with Tukey's HSD test. LME, low-dose ME; HME, high-dose ME.

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3.9. Effects of ME on the Expression of Autophagy-Related Markers in Obese Mice

Overexpression of Beclin 1, P62, and LC3B-II was observed in the testes of obese mice, while different doses of ME treatment decreased Beclin 1 expression. The protein expression of P62 in the low-dose ME-treated group was lower than in the DIO group. A slightly reduced LC3B-II expression was also found in the high-dose ME-treated group but it showed no significant difference compared to the DIO group or the control group. (Figure 9).

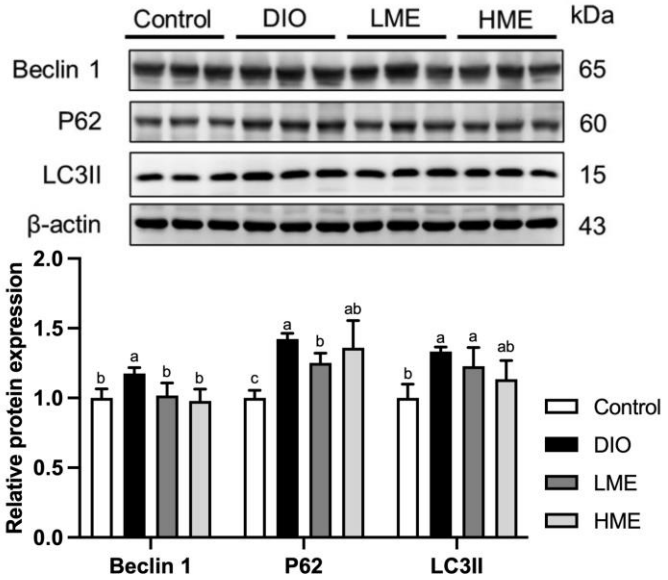

Figure 9. Protein expression of autophagy-related markers in the testes of control, obese, LME-treated obese, and HME-treated obese mice. Values are presented as the mean ± SD (n = 6). Bars with letters (**a**–**c**) presented statistical significance ($p < 0.05$) based on one-way ANOVA with Tukey's HSD test. LME, low-dose ME; HME, high-dose ME.

4. Discussion

This study established a mouse model of HFD-induced obesity, and obese mice presented higher weight gain, epididymal fat mass, and metabolic markers, including serum glucose, insulin, and total cholesterol levels, as expected. However, obese mice fed low-dose ME in this study showed no significant changes in the previously mentioned parameters, whereas those fed high-dose ME showed slightly reduced glucose and insulin levels, along with lower HOMA-IR, indicating an improvement in insulin resistant. The mild regulation of glucose and insulin may be the result of a long-term HFD that impairs structure and secretory functions of β-cells, as suggested by Yi et al [26]. Furthermore, ME-derived polysaccharides (200, 400, and 600 mg/kg) could notably improve hyperglycemia and insulin sensitivity in a diabetic mice model, possibly through binding glucose and decrease absorbable glucose level in the intestine [27].

In addition, obese mice have lower serum TG concentrations, whether receiving ME treatment or not, which may be explained by elevated hepatic TG accumulation because of HFD feeding that enhances fatty acid uptake into the liver [28]. H&E-stained images of the liver have revealed higher lipid accumulation in obese mice. Nevertheless, in this study, the hepatoprotective effect was scarcely noticeable in the low-dose ME treatment but was more apparent in the high-dose ME-treated obese mice, with a significantly lower liver weight and decreased lipid droplet accumulation compared to the vehicle-treated obese mice. Meng et al. established an alcohol-induced liver injury mice model, and both 400 and 800 mg/kg doses of ME fruit bodies demonstrated comparable efficacy in alleviating serum markers of hepatocellular damage, while the 200 mg/kg dose had no significant effect, which is consistent with the findings of the present study. The lack of dose-dependency in ME treatment

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may be attributed to the common characteristic of natural product, in which nutritional components and bioactive ingredients possess beneficial effects by modulating various signaling pathways rather than targeting a specific mechanism [29].

Although the mice in this study exhibited weight gain and visceral fat with aberrant metabolic parameters, ME treatment significantly improved sperm motility and the percentage of normal morphology. The process of spermatozoa production was also analyzed. Because the spermatogenic cycle in mice is normally approximately 35 days [30], 8 weeks of supplementation should be enough to correct obesity-derived abnormal spermatozoa production and indeed improve spermatogenesis, with better arranged sperm cells and fewer vacuoles found in ME-treated mice. Besides, the serum testosterone level was notably reduced in obese mice and restored following ME treatment. Iqbal reported that the use of ME extract helps restore testis and epididymal morphology, as well as testosterone production in cadmium-induced male rats, and inferred a promoting effect of ME on hypothalamic–pituitary–gonadal (HPG) axis [22]. The HPG axis, which comprises of GnRH, FSH, and LH, regulates production of testosterone and sperm. FSH affects Sertoli cells, which in turn control germ cell survival, and LH stimulates testosterone synthesis in Leydig cells [31]. FSH and testosterone could inhibit proliferation–apoptosis blocks during spermatogenesis and help spermatogonia differentiate into spermatozoa, as well as the maintenance of the blood-testis barrier integrity [32]. In this study, both doses of ME had a slightly increasing trend in FSH level in the obese mice that had no significance compared with lean and obese mice but exhibited an attenuated obesity-induced reduced LH level.

Various lines of evidence suggest that oxidative stress is one of the most important factors responsible for male infertility [33,34]. Wu et al. reviewed and reported the antioxidant properties of ME, including scavenging free radicals, increasing the activities of antioxidant enzymes, and decreasing the formation of lipid peroxidation [35]. Similarly, ME-treated obese mice in this study had elevated activities of SOD, CAT, and GPx with reduced MDA content. Bioactive compounds of ME, including phenolic compounds, polysaccharides, organic acids and tocopherols, have been reported strong antioxidant properties based on in vivo and in vitro studies [18]. Besides, Tian et al. suggested that the polysaccharides play a key role in the antioxidant ability of ME [36], and are abundantly present in the ME powder (11.5%). Inflammation-, apoptosis-, and autophagy-mediated factors were also analyzed to enhance discussions of male infertility [14,15,37]. The anti-inflammatory effects of ME observed in other animal disease models [29,38] were linked to the NF-κB signaling, and the protein expression of p50 subunit and IL-6 in this study as lowering t. Rehman et al. demonstrated that ME-derived polysaccharide downregulated the TLR4 signaling pathway and inactivated NF-κB and proinflammatory cytokines, and restore intestine integrity damaged by HFD-induced obesity [39]. In addition, ME may exert anti-inflammatory effects by regulating the arachidonic acid metabolic pathway (upstream of NF-κB), prostaglandin endoperoxide synthetase activity and prostaglandin biosynthesis (downstream of NF-κB), as shown in a molecular docking and network pharmacology study [20].

Oxidative stress due to ROS accumulation triggers apoptosis and autophagy in germ cells [40], as supported by an increased Bax/Bcl-xl ratio and elevated expression of caspases in obese mice. However, unlike other studies [41,42], obese mice in this study had higher protein expression of Beclin 1, P62, and LC3B-II. Beclin 1 and LC3 are general markers indicating activation of autophagy, while P62 indicates the inhibition of autophagy, revealing inconsistent signs of autophagy. ME treatment significantly lowered cleaved caspase 9 and Beclin 1 expression but had no significant effects on other markers involved in apoptosis and autophagy, and high-dose ME exhibited minor improvements compared to low-dose ME. Jeong et al. stated that, despite having inconspicuous impacts on apoptosis and autophagy, caspase 9 is not only the upstream mediator of apoptosis but also a mediator of autophagy [43].

Furthermore, the markers analyzed in this study are principal but not comprehensive, such as the lack of detection of DNA fragmentation as the late stage of apoptosis, monitoring of

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inflammation, apoptosis, and autophagy in the testis via immunofluorescence or immunohistochemistry.

5. Conclusions

In summary, ME could be a potential therapeutic option for obesity-induced reproductive impairments since it restores sperm quality and hormone levels, and high-dose exerts better impacts. The possible mechanism of ME action may involve the modulation of imbalance redox status and inflammation. Future research could focus on the anti-oxidant and anti-inflammatory related pathways of ME.

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