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Original Research

In vitro and in vivo androgen regulation of *Dendropanax morbiferus* leaf extract on late-onset hypogonadism

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Abstract: Late-onset hypogonadism (LOH) is associated with advancing age and is caused by a deficiency in serum testosterone levels. The aim of this study was to examine the effect of a *Dendropanax morbiferus* H.Lév. leaf extract (DME) on LOH using TM3 cells and aging male rats as *in vitro* and *in vivo* models, respectively. The *in vitro* effects of DME on testosterone levels and 3β-hydroxysteroid dehydrogenase (3β-HSD) protein expression in TM3 cells were analyzed. In the *in vivo* experiments, DME was orally administered to rats at three doses (50, 100, and 200 mg/kg/day) for 4 weeks. DME significantly increased the testosterone levels and 3β-HSD protein expression in TM3 cells. The DME groups showed significantly increased levels of androgenic hormones such as testosterone and dehydroepiandrosterone sulfate. The sex hormone-binding globulin production was significantly lower in the DME groups than that in the control group, while the neurohormone levels in the hypothalamic–pituitary–gonadal axis markedly increased. No significant differences were observed in the glutamic pyruvic transaminase, glutamic oxaloacetic transaminase, and prostate-specific antigen levels among the DME and control groups. The triglyceride and low-density lipoprotein cholesterol levels were significantly lower, while the high-density lipoprotein cholesterol levels were significantly higher in the DME groups than those in the control group. The latency time in the rotarod, treadmill, and swimming tests increased with the DME treatment. Furthermore, the sperm counts in the epididymis markedly increased. These results suggest that DME can be effectively used to alleviate the symptoms of LOH.

Key words: Late-onset hypogonadism; *Dendropanax morbiferus*; TM3 Leydig cells; Testosterone; Hypothalamic–pituitary–gonadal axis.

Introduction

Late-onset hypogonadism (LOH) is defined as a clinical and biochemical syndrome associated with the advancing age and characterized by typical symptoms and a deficiency in serum testosterone levels (1). In men, serum testosterone levels gradually decline by 0.4–2.6% per year after the age of 40 (2-5). This decline has been associated with parallel declines in the bone mass, muscle mass/strength, physical function, and sexual function (4,6). It has also been shown that LOH can result in a significant deterioration of the quality of life and adversely affect the function of multiple organs (1,4). The anterior pituitary gland releases two hormones, known as gonadotropins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH); these hormones act on the gonads in men and women. Secretion of gonadotropins by the anterior pituitary gland is stimulated by gonadotropin-releasing hormone (GnRH), which is produced by neurosecretory cells of the hypothalamus. FSH acts on Sertoli cells to stimulate spermatogenesis, and LH stimulates the secretion of androgens by Leydig cells (7). Androgens such as testosterone are steroids that diffuse locally into seminiferous tubules to promote spermatogenesis. Sex steroids are produced from adrenal precursors, dehydroepiandrosterone (DHEA) and DHEA-sulfate. DHEA, an inactive prohormone, is produced by the adrenal glands from cholesterol. DHEA and DHEA-sulfate are interconvertible by sulfatases and sulfotransferases, respectively. In peripheral tissues, hydroxysteroid dehydrogenases (HSDs) convert DHEA to androstenedione and androstenediol, which are precursors of testosterone.

Dendropanax morbiferus H.Lév. is a subtropical, broad-leaved, evergreen tree belonging to the Araliaceae family (8). Several parts of *D. morbiferus* are used in traditional medicine for treating migraines and dysmenorrhea (9). *D. morbiferus* is an endemic species in South Korea and has also been cultivated on the Jindo Island, in Suncheon city, and on the Wando Island. A crude extract from leaves of *Dendropanax arboreus* (Araliaceae) has been shown to exhibit cytotoxic activity against the HepG2, A431, H411E, and L1210 tumor cell lines but was not toxic against normal hepatocytes (10). *D. morbiferus* extracts (DMEs) have been reported to show various biological activities, including immune activation against cancer cells, as well as antioxidant (11,12) and antidiabetic (13). In addition, polyacetylene compounds isolated from *D. morbiferus* have been reported to exhibit an anticomplement activity (14), and rutin has been reported to protect human dopaminergic cells against rotenone-induced cell injury (15). Moreover, an essential oil from this plant has been reported to exhibit an antiatherogenic activity in rats (16). Howe-

ver, the effects of naturally occurring compounds on LOH have not been investigated in detail.

We previously reported that an aqueous extract of *D. morbiferus* leaves affected the sexual behavior of male rats (17). Based on these results, we evaluated the ameliorating effect of DME on LOH using TM3 Leydig cells and aging male rats as *in vitro* and *in vivo* models, respectively. The ameliorating effect of DME was evaluated using TM3 Leydig cells and rotarod, treadmill, and swimming retention tests, histological analysis, and biomarker analysis in aging male rats.

Materials and Methods

Preparation of Dendropanax morbiferus extracts

D. morbiferus was obtained from Jindo, Jeollanamdo (South Korea), washed three times with distilled water, and dried at 45 °C in a drying oven for 48 h. The plant material (100 g) was extracted with 2 L of distilled water at 100 °C for 3 h. The residue was removed by filtration through a 150-mm mesh. The filtrate was evaporated to 16 °Bx and freeze-dried to obtain a DME powder. The dried extract was stored at 4 °C.

Qualitative analysis of flavonoids in DME

DME flavonoids were analyzed by high-performance liquid chromatography (HPLC) using a Waters 2998 HPLC system (USA), equipped with a ZORBAX Eclipse XDB-C18 column (5 μm, 250 mm × 4.6 mm internal diameter; Agilent, USA). The mobile phase was composed of solvents A (0.1% formic acid) and B (methanol), and the flow rate was 1.0 mL/min. The following mobile phase gradient was employed: 0–8 min, 35% A, isocratic; 8–38 min, a linear gradient from 35 to 50% A; 38–40 min, from 50 to 100% A; 40–50 min, from 100 to 50% A; and 50–55 min, a linear gradient from 50 to 35% A. The column was then washed and reconditioned. The sample injection volume was 10 μL. The signal was detected at a wavelength of 370 nm at 40 °C, which was the optimum temperature for this HPLC separation.

Cell culture and testosterone levels

TM3 Leydig cells, a nontumorogenic cell line derived from mouse testes (18), were purchased from the Korean Cell Line Bank (Seoul, South Korea). This cell line responds to LH by increasing testosterone production and secretion through mechanisms. Cells were grown in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum and 1% penicillin/streptomycin. For all experiments, early-passage cells were grown to 80–90% confluence. To investigate the changes in testosterone levels in TM3 cells treated with the sample extract, cells were seeded at a density of 1 10^5 cells/well in 24-well plates. After 24 h of incubation. To induce oxidative cellular stress, the cells were treated with hydrogen peroxide (H_2O_2). The cells were treated with various concentrations of DME (1, 3, 10, 30, and 100 μg/mL), in the presence or absence of 200 μM H_2O_2, for 24 h, and then the supernatants were collected.

Testosterone concentrations were measured in cell culture media and in the serum of rats using a testosterone EIA kit (ENZO Life Sciences, Farmingdale, NY, USA), according to the manufacturer's instructions.

3β-HSD expression

TM3 cells were lysed in RIPA buffer [50 mM Tris–HCl, 150 mM NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS), 1 mM phenylmethanesulfonyl fluoride, 1 mM dithiothreitol, and 2 mg/mL each of leupeptin and aprotinin]. The lysate was centrifuged at 12,000 rpm for 10 min. The supernatant was collected, and the total protein was quantified using the Bradford reagent. The lysates (50 μg of protein) were subjected to SDS–polyacrylamide gel electrophoresis. The separated proteins were transferred to polyvinylidene difluoride membranes (Millipore, Billerica, MA, USA), and then the blots were blocked in 5% skim milk in Tris-buffered saline containing 0.1% Tween 20 (TBST) for 1 h at room temperature. The blotted proteins were probed with an anti-3β-HSD antibody overnight at 4 °C. The blot was washed three times with TBST at 15-min intervals, then incubated with a goat anti-mouse IgG (Cell Signaling Technology) for 1 h, and washed again three times with TBST. Finally, protein bands were detected using an enhanced chemiluminescence western blotting detection system (Pierce, Rockford, IL, USA).

Experimental design

Male Sprague–Dawley rats (6–7-month-old, weighing 590–620 g) were purchased from Samtako (Osan, South Korea). The rats were housed in plastic cages at a controlled temperature (23 ± 2 °C) and humidity (50 ± 5%), with 100% fresh HEPA-filtered air and a 12-h light–dark cycle (lights on from 08:00 to 20:00). All experimental procedures were conducted in accordance with the guidelines for the care of experimental animals and approved by the Institutional Animal Care and Use Committee of the Jeollanamdo Institute of Natural Resources Research (approval number JINR-1611). A safety evaluation for a 2-week single oral dose toxicity test was conducted by a certified testing organization, the Korea Testing & Research Institute (approval number TBH-1651, 1652), according to the Korean Food and Drug Administration Guideline for Toxicity Testing of Pharmaceuticals.

The rats were divided into four groups, five rats in each. These groups were treated as follows: no administration of DME (control), 50 mg/kg DME (DME 50), 100 mg/kg DME (DME 100), and 200 mg/kg DME (DME 200). DME was dissolved in distilled water and orally fed to the rats using a feeding needle, daily for 4 weeks. The rats were provided *ad libitum* access to a basal diet and water during the experiment.

After 4 weeks of DME treatment, the body weights of the rats were measured, and the animals were anaesthetized. Then, the testes, seminal vesicles, epididymis, and prostate glands were carefully removed, along with vital organs such as the liver, kidney, and spleen, and weighed immediately.

Rotarod test

The rats were placed on a standard five-lane accelerating rotarod (Jeung Do Bio & Plant, Seoul, South Korea) and tested twice at an accelerating speed of 20 rpm for 5 min or until they fell off. The retention times were recorded weekly. On the testing day, each rat was submitted to two trials with an interval of 10 min.

Treadmill test

The rats were subjected to a treadmill exercise on a motorized rodent treadmill (Jeung Do Bio & Plant) after the completion of the 4-week treatment period. The rats were individually placed into a treadmill lane at a 15° incline, and the electric shock (2 mA) frequency and intensity were set for 30 min. The treadmill was started at 6 m/min for 3 min, and then the speed was gradually increased at a rate of 9, 12, 15, and 18 m/min at 3-min intervals. The time until the rats were exhausted was measured. The rats in an exhausted state could not run for more than 10 s.

Swimming retention test

A swimming retention test was performed to evaluate the physical function of the rats, and the results were recorded throughout the 4-week treatment period. The swimming exercise was performed in a plastic barrel filled with water maintained at 32–36 °C. The time from the start of swimming until the rats were exhausted and sank below the water surface for 10 s was recorded. The rats were immediately rescued from water to avoid drowning.

Biomarker analysis

At the end of the experimental period, the rats were fasted for 12 h. Blood was collected from the abdominal artery, and serum was obtained by centrifugation of the blood at 4,000 rpm for 15 min.

Testosterone levels were measured at 0 and 4 weeks. Testosterone and LH levels were measured in the serum using testosterone and LH enzyme-linked immunosorbent assay (ELISA) kits (ENZO Life Sciences), respectively, according to the manufacturer's instructions. The serum SHBG level was measured using an SHBG ELISA kit (Cloud-Clone Corp., Wuhan, Hubei, China). Serum DHEA-sulfate, GnRH, FSH, and inhibin levels were measured using rat DHEA, GnRH, FSH, and INHA ELISA kits (FineTest, Wuhan, Hubei, China), respectively. Serum prostate-specific antigen (PSA) was measured using a rat PSA ELISA kit (CUSABIO, USA) according to the manufacturer's instructions. Total cholesterol (TC), triglycerides (TGs), high-density lipoprotein (HDL) cholesterol, glutamic oxaloacetic transaminase (GOT), and glutamic pyruvic transaminase (GPT) were measured in the serum using commercial assay kits (DRI-CHEM 4000i; Fujifilm, Tokyo, Japan). The serum low-density lipoprotein (LDL) cholesterol value was calculated using the Friedwald formula (19) as follows: LDL cholesterol = TC − (HDL cholesterol + TG/5). The atherogenic index (AI) was calculated as follows: AI = (TC − HDL cholesterol)/HDL cholesterol. The cardiac risk factor (CRF) was calculated as follows: CRF = TC/HDL cholesterol.

Histological analysis

After 4 weeks of treatment, the rats were anaesthetized; the testis was excised, then fixed in Bouin's solution (Sigma, St. Louis, MO, USA), embedded in paraffin, and 5-µm sections were cut using a microtome. The sections were deparaffinized in xylene, dehydrated in a graded alcohol bath, and stained with acidified hematoxylin and eosin. Finally, they were examined under an optical microscope.

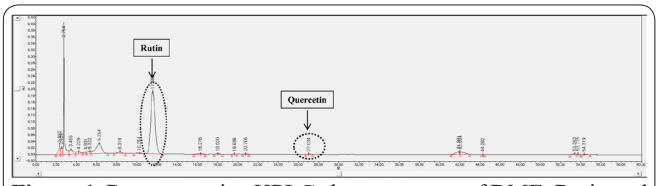

Figure 1. Representative HPLC chromatogram of DME. Rutin and quercetin were detected in DME at 11.64 and 27.11 min, respectively.

Statistical analysis

The results are expressed as the mean ± standard error of the mean (SE). The data were statistically evaluated using a one-way analysis of variance, followed by Duncan's multiple comparison test, using the SPSS software. Statistical significance was defined as $P < 0.05$.

Results

Standardization of DME

DME was standardized based on the rutin and quercetin contents, determined by HPLC. The contents of the two main flavonoids of DME were determined based on standard curves, and their retention times were consistent with those of the standards. The rutin and quercetin retention times in DME were typically near 11.64 and 27.11 min, respectively (Figure 1).

Testosterone levels in TM3 cells

Treatment with DME at concentrations of 10, 30, and 100 µg/mL significantly increased the testosterone levels in TM3 cells to 129.5 ± 11.2, 135.0 ± 6.9, and 141.4 ± 8.2%, respectively ($P < 0.05$), of that in the untreated control (Figure 2a). The protective effect of DME against H_2O_2-induced damage was also examined. As shown in Figure 2b, the testosterone level signifi-

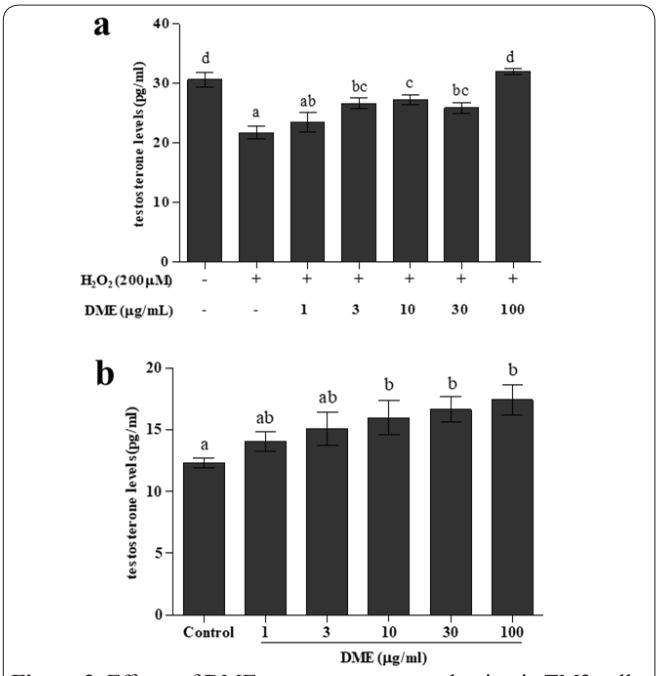

Figure 2. Effects of DME on testosterone production in TM3 cells. TM3 cells were treated for 24 h with various concentrations of DME (**a**) alone and (**b**) in the presence of H_2O_2. The values are the means ± SE. Different letters above the bars indicate significant differences, as determined by the Duncan's multiple comparison test ($P < 0.05$).

cantly decreased in the H_2O_2-treated TM3 cells compared with that in the control group. However, treatment of cells with 3, 10, 30, and 100 µg/mL DME significantly increased the testosterone levels compared with that in the H_2O_2-treated cells.

3β-HSD expression in TM3 cells

Leydig cells, which are testosterone-producing cells located in the interstitial compartment of a mammalian testis, support spermatogenesis in seminiferous tubules. 3β-HSD expression is a prerequisite for testosterone production by the TM3 cell line, derived from mouse Leydig cells, and DME significantly upregulated the 3β-HSD expression (Figure 3).

Body and organ weights

We further determined whether DME caused acute toxicity in rats. There were no significant toxicological changes in the following parameters, due to DME, during the experimental period (data not shown): the number of deaths, clinical signs, body weights, and necropsy findings.

The weekly body weights of the aging rats treated with DME for 4 weeks are shown in Figure 4. In the control group, the body weights did not change during the first week of treatment but gradually increased from week 2 until the end of the experiment. The body weights of the rats in the DME 50 and 100 groups gradually increased over the experimental period. In the DME 200 group, the mean body weight gradually decreased over the experimental period (from 603.6 ± 24.2 g in the first week to 592.1 ± 12.1 g in the fourth week). However, compared with the control group, the DME groups did not show significant differences in the body weights.

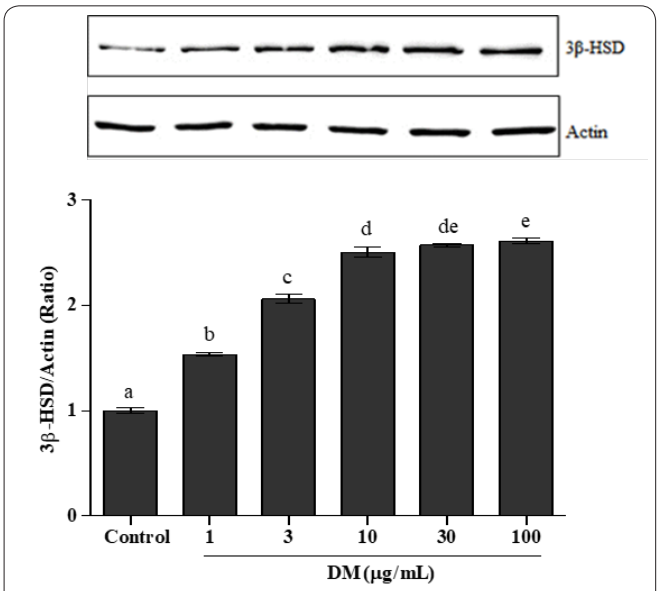

Figure 3. Effects of DME on protein expression of 3β-HSD, determined in TM3 cell lysates by western blotting. Densitometric analysis was performed using the ImageJ software. The data are representative of three independent experiments. The values are the means ± SE. Different letters above the bars indicate significant differences, as determined by the Duncan's multiple comparison test ($P < 0.05$).

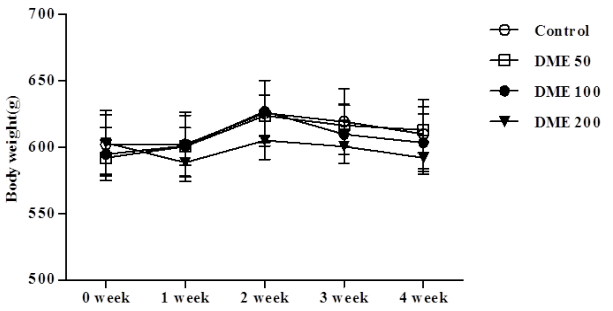

Figure 4. Effects of DME on body weights of aging male rats. Control, no administration of DME; DM 50, 50 mg/kg DME; DME 100, 100 mg/kg DME; and DME 200, 200 mg/kg DME administered daily. The values are the means ± SE.

Seven different body organs were weighed from each rat. As shown in Table 1, no significant differences were observed in the weights of the testis, epididymis, seminal vesicle, prostate, liver, kidney, and spleen among the groups.

Physical tests

We investigated the effects of DME on the aging male rats using rotarod tests. The latency to fall time on the rotarod increased in the DME group compared with that in the control group (Figure 5a). Oral administration of DME for 4 weeks significantly increased the retention time compared with that in the control group.

Treadmill tests were performed to evaluate the effects of DME on the adaptation process of rat skeletal muscles to long-term physical strength. The running time on the treadmill significantly increased, from approximately 5 to 12 min. in the DME 200 group, indicating more than a 100% improvement (Figure 5b).

The DME-treated rats also tended to show an increased swimming time. However, the differences in the swimming time between the control and DME-treated groups were not statistically significant (Figure 5c).

Androgen levels

We also investigated the effects of DME on serum testosterone levels in the aging male rats. As shown in

Table 1. Organ weights of aging male rats.

Groups	Testes (g)	Epididymis (g)	Seminal vesicle (g)	Prostate (g)	Liver (g)	Kidney (g)	Spleen (g)
Control	4.34 ± 0.17	0.76 ± 0.01	1.96 ± 0.19	1.27 ± 0.08	18.29 ± 0.63	3.86 ± 0.23	1.04 ± 0.07
DME 50	4.32 ± 0.14	0.73 ± 0.04	1.98 ± 0.10	1.23 ± 0.08	19.18 ± 0.75	3.96 ± 0.16	1.09 ± 0.06
DME 100	4.44 ± 0.05	0.77 ± 0.02	2.07 ± 0.25	1.11 ± 0.05	18.37 ± 1.85	3.82 ± 0.19	0.98 ± 0.02
DME 200	4.37 ± 0.20	0.73 ± 0.03	1.92 ± 0.22	1.25 ± 0.14	18.17 ± 0.58	3.79 ± 0.12	1.01 ± 0.08

Control, no administration of DME; DME 50, 50 mg/kg DME; DME 100, 100 mg/kg DME; and DME 200, 200 mg/kg DME per day. The values are the mean ± SE.

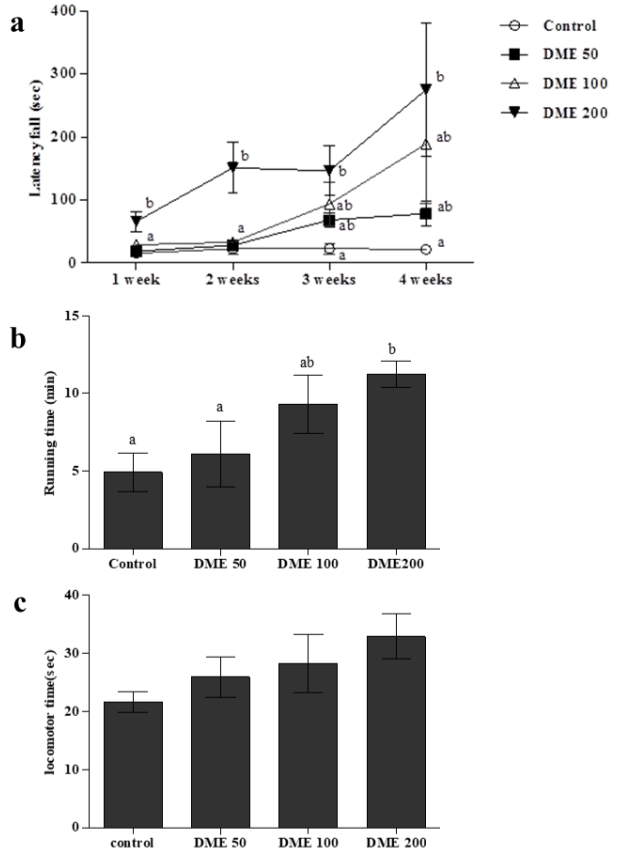

Figure 5. Effects of DME on physical activity of aging male rats in (**a**) a rotarod test; (**b**) a treadmill test; and (**c**) a swimming test. Control, no administration of DME; DME 50, 50 mg/kg DME; DME 100, 100 mg/kg DME; and DME 200, 200 mg/kg DME administered daily. The values are the means ± SE. Different letters above the bars indicate significant differences, as determined by the Duncan's multiple comparison test ($P < 0.05$).

Figure 7a, the testosterone levels before the administration (week 0) were 249.79 ± 85.34, 250.68 ± 64, 247.00 ± 59.25, and 252.00 ± 40.00 pg/mL in the control, DME 50, DME 100, and DME 200 groups, respectively. Oral administration of 50, 100, and 200 mg/kg DME for 4 weeks significantly increased the serum testosterone levels, to 741.19 ± 141.31, 834.64 ± 86.91, and 858.94 ± 144.56 pg/mL, respectively, compared with that in the control group (307.76 ± 82.71 pg/mL).

SHBG is a glycoprotein that binds to, transports, and inhibits the function of testosterone. When testosterone is bound to SHBG, it cannot be used by the body. To investigate the effects of DME on serum SHBG levels in the aging male rats, DME (50, 100, and 200 mg/kg) was administered daily for 4 weeks. After 4 weeks, the SHBG levels were significantly lower in the DME groups than that in the control group (Figure 7b).

On the other hand, the DHEA concentrations in the DME 50, 100, and 200 groups (1.76, 6.35, and 8.60 pg/mL, respectively) were significantly higher after 4 weeks of treatment than that in the control group (0.72 pg/mL; Figure 7c). As shown in Figure 6a, the testosterone levels were also significantly higher in the DME-treated groups than that in the control group. These results suggest that DME influences the production of DHEA, a precursor of testosterone.

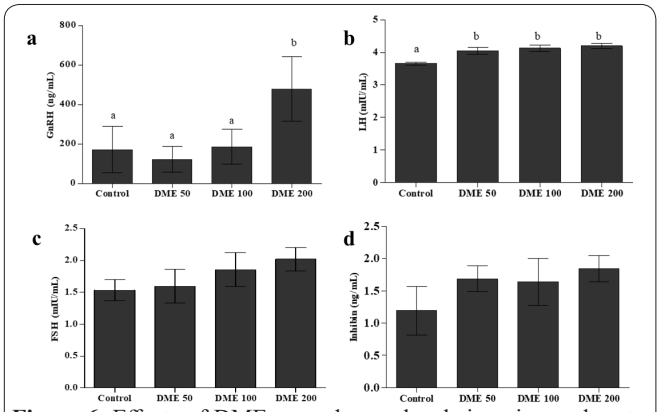

Figure 6. Effects of DME on androgen levels in aging male rats. Control, no administration of DME; DME 50, 50 mg/kg DME; DME 100, 100 mg/kg DME; and DME 200, 200 mg/kg DME. The values are the means ± SE. Different letters above the bars indicate significant differences, as determined by the Duncan's multiple comparison test ($P < 0.05$).

Neuronal hormones

LH and FSH, produced by the anterior pituitary lobe, are necessary for maintaining testosterone levels. An increase in LH and FSH levels elevates testosterone levels. Therefore, the effects of DME on neuronal hormones were also measured in the aging male rats (Figure 6). As shown in Figure 7b, the serum LH levels (4.05 ± 0.11, 4.13 ±0.10, and 4.20 ± 0.08 mIU/mL) in the DME group were higher than those in the control group (3.65 ± 0.05 mIU/mL). FSH and inhibin levels did not significantly differ among the treatment and control groups (Figure 6c and 6d).

Additionally, the effects of DME were measured on the GnRH levels in the aging male rats. As shown in Figure 7a, the GnRH levels in the DME 200 group (478.54 ± 164.40 mIU/mL) were higher than those in the control group.

These data suggest that the age differentially influences the control mechanisms of FSH and LH secretion. The observed simultaneous increases in FSH, LH, GnRH, and inhibin levels due to DME administration suggest that it may exerts its effects through the hypo-

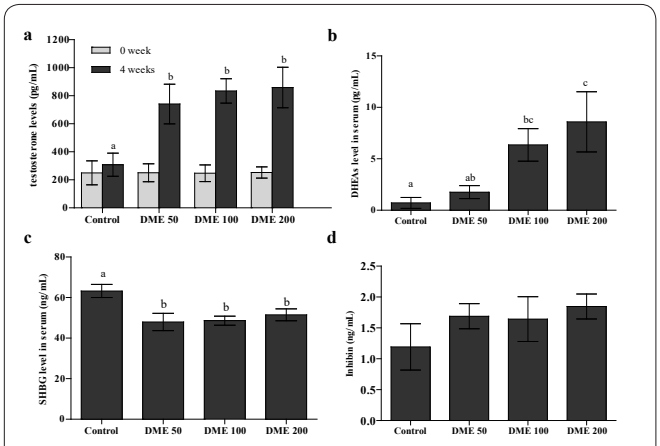

Figure 7. Effects of DME on the levels of neuronal hormones in aging male rats: (**a**) GnRH, (**b**) LH, (**c**) FSH, and (**d**) inhibin. Control, no administration of DME; DME 50, 50 mg/kg DME; DME 100, 100 mg/kg DME; and DME 200, 200 mg/kg DME administered daily. The values are the means ± SE. Different letters above the bars indicate significant differences, as determined by the Duncan's multiple comparison test ($P < 0.05$).

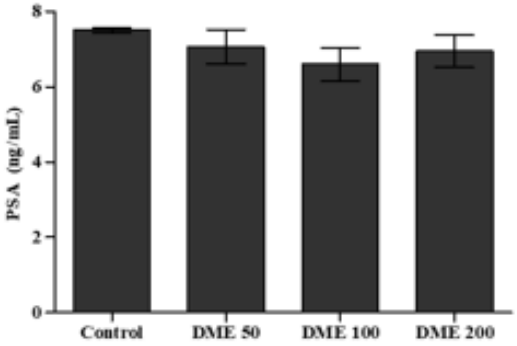

Figure 8. Effects of DME on PSA levels in aging male rats. Control, no administration of DME; DME 50, 50 mg/kg DME; DME 100, 100 mg/kg DME; and DME 200, 200 mg/kg DME administered daily. The values are the means ± SE.

thalamic-pituitary-testicular axis.

Serum lipid profiles and safety markers

As shown in Figure 8, the serum PSA levels did not significantly differ among the treatment and control groups. However, oral administration of DME decreased the TG and LDL cholesterol levels compared with those in the control group. The serum level of HDL cholesterol did not significantly differ among the treatment and control groups (Table 2).

GOT and GPT are important cellular metabolic enzymes used as sensitive indicators of hepatotoxic effects. In this study, the hepatic GPT and GOT levels in the DME-treated rats showed no significant differences with those in the control. These results indicate that the different doses of DME did not cause hepatotoxicity. There were no significant differences in CRF and AI among the groups either.

Histological analysis

Histological examination of the testes from these aging rats revealed degeneration of spermatogonia cells, lining seminiferous tubules. The changes in the tissue structure of seminiferous tubules in the control group, such as the basement membrane thickening and reduction in seminiferous cells, indicated age-related damage to seminiferous tubules and associated cells. The DME groups showed increased testicular spermatogenesis and slight improvement in the damaged steroidogenic cells compared to the control (Figure 9).

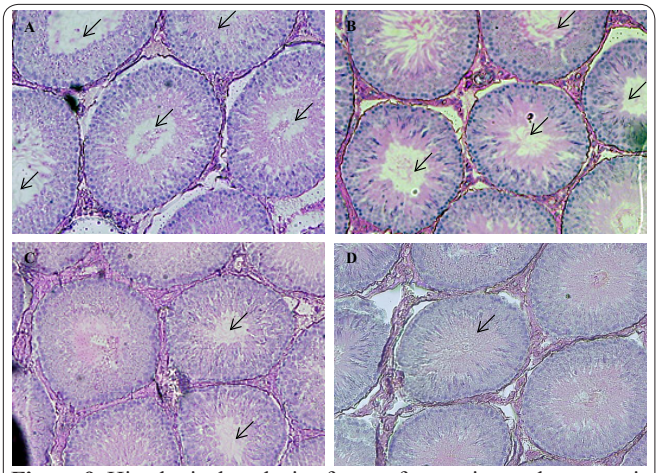

Figure 9. Histological analysis of testes from aging male rats, stained with hematoxylin and eosin. (**A**) Control group (no administration of DME); (**B**) DME 50 mg/kg group; (**C**) DME 100 mg/kg group; (**D**) DME 200 mg/kg group. Magnification 100×

Discussion

Late-onset hypogonadism is associated in aging men with a decrease in testosterone levels (20**)**, leading to a loss of the skeletal muscle mass and a decreased locomotive capacity, as well as to depression, oligozoospermia, and sperm inactivation. Leydig cells are continuously exposed to various harmful effects, particularly from the middle age onward. These cells sustain gradual and progressive damage, along with age-related loss of physiological function. Many components of metabolic syndrome, such as obesity, hypertension, dyslipidemia, impaired glucose regulation and insulin resistance are also present in hypogonadal men (21**)**. The results of the present study indicate that DME may have a significantly positive effect on testosterone production in TM3 cells and the sustained production of testosterone. The DM group showed increased spermatogenesis in the testes. The serum testosterone level is linked to the production of active sperms. It can be suggested that the enhanced activity and health of Leydig cells together with the enhanced serum testosterone levels induced by DM treatment underlie the improved spermatogenesis. DME influences the production of DHEA, a precursor of testosterone. DHEA appears to serve as a prohormone that can be rapidly metabolized in target tissues to produce biologically active steroids, including androstenedione,

Table 2 Effects of *Dendropanax morbiferus* leaf extract (DME) on lipid profiles and safety markers in aging male rats.

Parameters	Control	DME 50	DME 100	DME 200
Total cholesterol (mg/dL)	124.75 ± 14.00^{a}	106.25 ± 6.05^{a}	105.00 ± 8.17^{a}	99.6 ± 6.51^{a}
Triglycerides (mg/dL)	159.20 ± 27.2^{b}	132.40 ± 17.45^{b}	117.00 ± 11.58^{ab}	92.20 ± 13.54^{a}
HDL cholesterol (mg/dL)	55.25 ± 5.42^{a}	61.50 ± 3.33^{a}	62.40 ± 2.08^{a}	65.24 ± 3.71^{a}
LDL cholesterol (mg/dL)	25.18 ± 2.66^{b}	20.63 ± 2.25^{ab}	19.10 ± 1.65^{ab}	16.75 ± 1.25^{a}
CRF	0.83 ± 0.05^{a}	0.73 ± 0.13^{a}	0.72 ± 0.11^{a}	0.74 ± 0.09^{a}
AI	1.83 ± 0.05^{a}	1.73 ± 0.12^{a}	1.72 ± 0.11^{a}	1.74 ± 0.09^{a}
GOT (U/L)	66.20 ± 2.75^{a}	60.60 ± 3.11^{a}	60.40 ± 4.06^{a}	60.00 ± 3.83^{a}
GPT (U/L)	25.40 ± 1.86^{a}	24.80 ± 1.85^{a}	23.40 ± 1.44^{a}	23.20 ± 0.58^{a}

The values are expressed as the mean ± SE. Different superscript letters indicate significant differences, as determined by the Duncan's multiple comparison test ($P < 0.05$). Control, no administration of DME; DME 50, 50 mg/kg DME; DME 100, 100 mg/kg DME; and DME 200, 200 mg/kg DME per day. CRF, cardiac risk factor; AI, atherogenic index; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase.

testosterone, and estradiol (22). Testosterone enhances the physical locomotion capacity (23). Notably, the increased testosterone levels observed in DME-treated groups in this study were associated with significant enhancements of locomotor activities. FSH stimulates spermatogenesis in Sertoli cells, and LH stimulates the synthesis and release of testosterone by Leydig cells (24). The DME groups showed increased spermatogenesis in the testes. Thus, it can be suggested that the improved spermatogenesis reflects an enhanced activity and health of Leydig cells. Testosterone promotes germ cell differentiation and active maturation (25, 26).

Testosterone therapy has long been considered contraindicated in men with prostate cancer, and PSA has been widely used as a tumor marker for screening and follow-up in patients with prostate cancer (27). Moreover, it serves as a safety marker for testosterone therapy. SHBG is a glycoprotein that binds to, transports, and inhibits the function of testosterone. When testosterone is bound to SHBG, it cannot be used by the body. The SHBG protein is perceived as maintaining the hormonal balance in the body. The present results indicated that DME might play a significant role in the positive regulation of SHBG levels. In men, aging of the reproductive system is characterized by primary testicular failure and secondary (hypothalamic/pituitary) testicular dysfunction. In male rats, the age-associated reproductive decline is partially attributed to the reduced GnRH secretion. Hypothalamic *Gnrh* gene expression is decreased in aging male rat models (28), suggesting that a reduction in the GnRH synthetic capacity may contribute to age-associated declines in gonadotropin secretion and reproductive function. Inhibin B is produced by Sertoli cells in response to FSH and appears to reciprocally inhibit FSH secretion because serum inhibin B and FSH levels are negatively correlated in healthy men, men with testicular disorders, and those with gonadotropin deficiency undergoing GnRH treatment (29,30). These data suggest that age differentially influences the mechanisms controlling FSH and LH secretion. The simultaneous increases in FSH, LH, and GnRH levels, observed in this study, suggest that DME affects the hypothalamic–pituitary–testicular axis.

Our data revealed a significant increase in androgenic hormones such as testosterone and DHEA following the treatment with DME. Since the testosterone levels also significantly increased in the DME-treated groups, these results suggested that DME promoted the production of DHEA, a precursor of testosterone. The level of physical strength was also increased by DME treatment. Furthermore, the SHBG level was significantly downregulated, while the levels of GnRH, LH, FSH, and inhibin were increased by DME treatment. However, no significant differences in PSA, GOT, or GPT were observed among the groups. The levels of TGs and LDL-cholesterol significantly decreased after DME administration. In conclusion, the present study provides evidence that DME improves various indicators associated with LOH and shows the potential to be used as a natural agent for treating LOH.

Sexual behavior is dependent on the normal functioning of the hypothalamic-pituitary-gonadal axis (31). The enhancement of libido may be due to an increase in the anterior pituitary hormone and serum testosterone levels, which in turn stimulates sexual behavior (32, 33). Recently, we reported that an aqueous DME affected the sexual behavior of male rats (17).

There are some limitations to this study. First, the mechanisms underlying the effects of DME remain to be explored. Second, the candidate chemicals that regulate the ameliorating effect of DME on LOH are unknown. Therefore, further studies are required to elucidate the underlying mechanism and identify the principal components of DME.

In this study, we demonstrated a significant increase in androgenic hormones such as testosterone and DHEA following the treatment with DME. DME significantly increased the testosterone level and 3β-HSD protein expression in TM3 cells. The latency times in rotarod, treadmill, and swimming tests with rats were also increased by DME treatment. Furthermore, SHBG levels were significantly downregulated, whereas those of neurohormones such as GnRH, LH, FSH, and inhibin were increased by DME treatment. However, no significant differences were observed in GOT and GPT among the groups. Similarly, the PSA levels in the DME groups were not significantly different from that in the control group. The levels of TGs and LDL cholesterol were significantly decreased by DME administration. In conclusion, the data obtained in this study provide evidence that DME enhances various indicators associated with TDS and can potentially be used as a natural substance for treating LOH.

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Interest Conflict

The authors declare that they have no competing interests.

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