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Preventive Effects of *Monascus* on Androgen-Related Diseases: Androgenetic Alopecia, Benign Prostatic Hyperplasia, and Prostate Cancer

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Supporting Information

ABSTRACT: Androgen-related diseases impair the well-being of many aging men. Unfortunately, the medications used to treat these diseases have many side effects. Therefore, there is a significant need for the development of novel drugs to treat androgen-related diseases. In this study, we investigated the effects of *Monascus* cursory extraction (M-CE) on androgen-related diseases, including androgenetic alopecia (AGA), benign prostatic hyperplasia (BPH) and prostate cancer. We found that M-CE suppressed baldness in male B6CBAF1/j mice. Furthermore, M-CE decreased PSA levels, indicating a protective effect of M-CE on testosterone-induced hyperplasia. M-CE also significantly decreased tumor volume and tumor incidence in an *N*-methyl-*N*-nitrosourea (MNU)/testosterone-induced rat prostate cancer model and markedly decreased dihydrotestosterone (DHT) but not testosterone. Additionally, PCNA expression was decreased in the prostate of rats treated with M-CE. These results suggest that M-CE could be a new potential therapeutic candidate for the treatment of androgen-related diseases.

KEYWORDS: *Monascus, androgenetic alopecia, benign prostate hyperplasia, prostate cancer*

INTRODUCTION

Red mold rice (RMR) is primarily composed of nonglutinous rice, mycelium of *Monascus* and byproducts of their fermentation. RMR is a traditional spice that is consumed throughout Asia.[1] It is produced by fermenting the food fungus, a *Monascus* species, with steamed rice, and it has also been used to produce wines and other fermented food products.[2] *Monascus*-fermented products have many functional secondary metabolites, including monacolin K, citrinin, ankaflavin, and monascin. Many studies have shown that these secondary metabolites possess anti-inflammatory, antioxidative, cholesterol-lowering effect, and antitumor activities.[3−7] Recently, it was reported that RMR significantly reduced the tumor volumes of androgen-dependent and androgen-independent prostate xenograft tumors compared with animals receiving vehicle alone. One of the potential underlying mechanisms is through decreased gene expression of androgen-synthesizing enzymes (HSD3B2, AKR1C3, and SRD5A1).[8] In addition, our laboratory recently found that monascuspiloin, a yellow pigment isolated by our group from *Monascus pilosus* M93-fermented rice, reduced the viability and enhanced the radiation sensitivity of human prostate cancer cells.[6,7] On the basis of these findings, RMR may serve as a potential botanical candidate for the treatment of androgen-related diseases and therefore warrants further investigation.

Androgens and the androgen receptor (AR) are required for expression of the male phenotype. However, androgenetic alopecia (AGA) and benign prostatic hyperplasia (BPH) are androgen-dependent entities that respond to the inhibition of 5α-reductase.[9] Previous studies have demonstrated that AGA affects up to 70% of men and 40% of women at some point in their lifetime.[10] Furthermore, androgens effect the growth of scalp and body hair in humans. AGA is induced via the activation of AR in hair follicles by dihydrotestosterone (DHT).[11] Because DHT is produced from testosterone by 5α-reductase, this enzyme plays a key role in the DHT-mediated effects of BPH and AGA.[12] Currently, two FDA-approved drugs are available for AGA therapy: oral finasteride, at a dose of 1 mg per day, and a topical solution of minoxidil.[13] Finasteride, a competitive inhibitor of 5α-reductase, inhibits the conversion of testosterone to DHT. The rationale for the use of finasteride to treat AGA in men is based on the presence of increased 5α-reductase activity and DHT levels in individuals with balding scalps.[14] However, finasteride is contraindicated in women who are or may become pregnant because 5α-reductase inhibitors may cause malformation of the external genitalia of male fetuses. Minoxidil promotes hair growth by increasing the duration of the anagen phase, whereas the mechanism of action for minoxidil on hair growth is poorly understood. Unfortunately, the efficacy of minoxidil is variable and temporary, making it difficult to predict treatmenet success on an individual basis.[15] Thus, there is a need for the development of novel drugs for the treatment of AGA.

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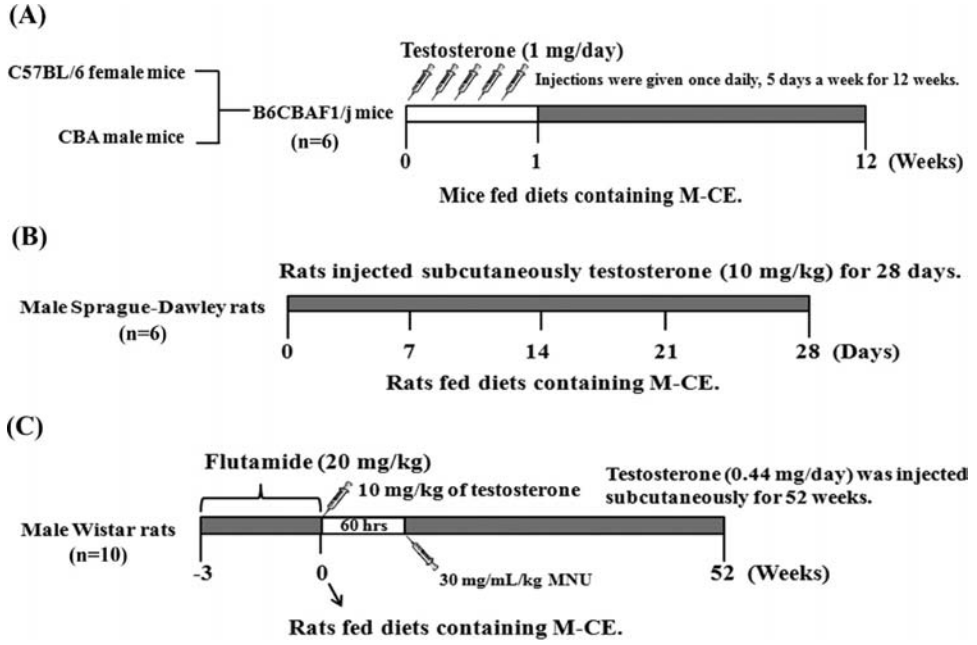

Figure 1. Schematic representation of experimental protocol showing treatment schedules for the animal models of androgenetic alopecia (A), benign prostatic hyperplasia (B), and prostate cancer (C). Details of the experimental protocols are described under Materials and Methods.

Benign prostatic hyperplasia (BPH) is a disease that impairs the well-being of many aging men. Although its precise molecular etiology remains unclear, androgens have been implicated in the development and maintenance of BPH. Accumulating evidence linking androgens and androgen receptor pathways to BPH has been reported.[16] The major androgen within the prostate is DHT. Previous studies have demonstrated that DHT and 5α-reductase are highly associated with prostate cancer.[17] BPH and prostate cancer are accompanied by uncontrolled tissue growth, during which the tissue microenvironment becomes hypoxic, thereby stimulating angiogenesis.[18] The main pharmacological agents for BPH are 5α-reductase inhibitors, which regulate the levels of 5α-DHT, and α-adrenergic blockers.[19] The inhibition of 5α-reductase activity can reduce the risk of prostate cancer development, slow tumor progression, and even treat the existing disease.[17] The current treatment methods for prostate cancer include chemotherapy, radiotherapy, and/or surgery.[20] However, both chemotherapy and radiation therapy are largely ineffective, and metastatic disease frequently develops even after potentially curative surgery. Due to the limited treatment options that are available for this disease,[21] novel methods for the treatment of prostate cancer need to be developed.

Our current study examined the effects of *Monascus* cursory extraction (M-CE) on androgen-related diseases, including AGA, BPH, and prostate cancer. Furthermore, we investigated whether the beneficial effects of M-CE are mediated through the reduction of testosterone and DHT levels in vivo.

MATERIALS AND METHODS

Microorganism and Preparation of *Monascus* Cursory Extraction (M-CE). *M. pilosus* M93 was obtained from BCRC, FIRDI (Hsinchu, Taiwan) and maintained on potato dextrose agar (PDA; Difco). The strain was plated onto PDA plates and cultivated at 25 °C for 7 days. The spores were then washed from the PDA plate using sterile water, and the concentration of the resulting spore suspension was adjusted to 1×10^6/mL. Following the spores enrichment step, 1 mL of spore suspension were inoculated into 250 mL flasks containing 50 mL of RGY medium (3% rice starch, 7% glycerol, 1.2% polypeptone, 3% soybean powder, 0.1% $MgSO_4$, and 0.2% $NaNO_3$) and cultivated with shaking (150 rpm) at 25 °C for 3 days to obtain the mycelium broth of M93. For the production of RMR, 50 450-mL glass bottles, each containing 75 g of rice and 75 mL of deionized water, were sterilized for 20 min at 121 °C. M93 mycelium broth (7.5 mL) and RGY medium (7.5 mL) were added to each bottle. The bottles were then incubated at 25 °C for 21 days (7.5 mL of RGY medium was added to each bottle on the 10th day of incubation), and the contents were then lyophilized to remove water. The RMR (1 kg) was extracted using 95% ethanol (3 L) at 25 °C for 24 h, and the ethanol was removed by vacuum-drying to obtain the M-CE.

AGA Model with B6CBAF1/j Hybrid Mice. The AGA model, previously described by Sundberg et al, uses B6CBAF1/j mice, a hybrid cross between C57BL/6 female and CBA male mice.[22] The B6CBAF1/j male mice were bred and housed five per cage at 24 ± 2 °C and 50 ± 10% relative humidity and were subjected to a 12 h light/12 h dark cycle. The animals were acclimatized for 1 week prior to the start of experiments and fed a Purina chow diet and water ad libitum. All experiments using mice were performed according to the guidelines of our institute (*Guide for Care and Use of Laboratory Animals*, National Cheng Kung University). The animal experimental protocol listed below was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC). Twelve-week-old male AGA mice were injected daily with testosterone (Fluka Chemical, Germany) at a dose of 1 mg/day; testosterone was suspended in an aqueous carboxymethylcellulose (CMC) vehicle. Injections were given once daily, 5 days a week for 12 weeks. Animals in the control group received injections of testosterone and were fed a diet that did not contain M-CE (group 1). The M-CE-treated groups were injected with testosterone and fed diets containing 0.03% M-CE (group 2), 0.1% M-CE (group 3), or 0.3% M-CE (group 4). The finasteride-treated mice (group 5) were injected with testosterone and given oral finasteride (0.5 mg/day) (Merck & Co., Inc., USA) (Figure 1A). Body weights were recorded once per week and were used as an indicator of systemic toxicity from the treatments. After sacrifice, the mouth skin was fixed with 10% formalin and embedded in paraffin for histological analysis.

BPH Rat Model. The BPH model was previously established by Li et al.[18] Male Sprague–Dawley rats (6–8 weeks old) were acquired from the animal center of the National Cheng Kung University

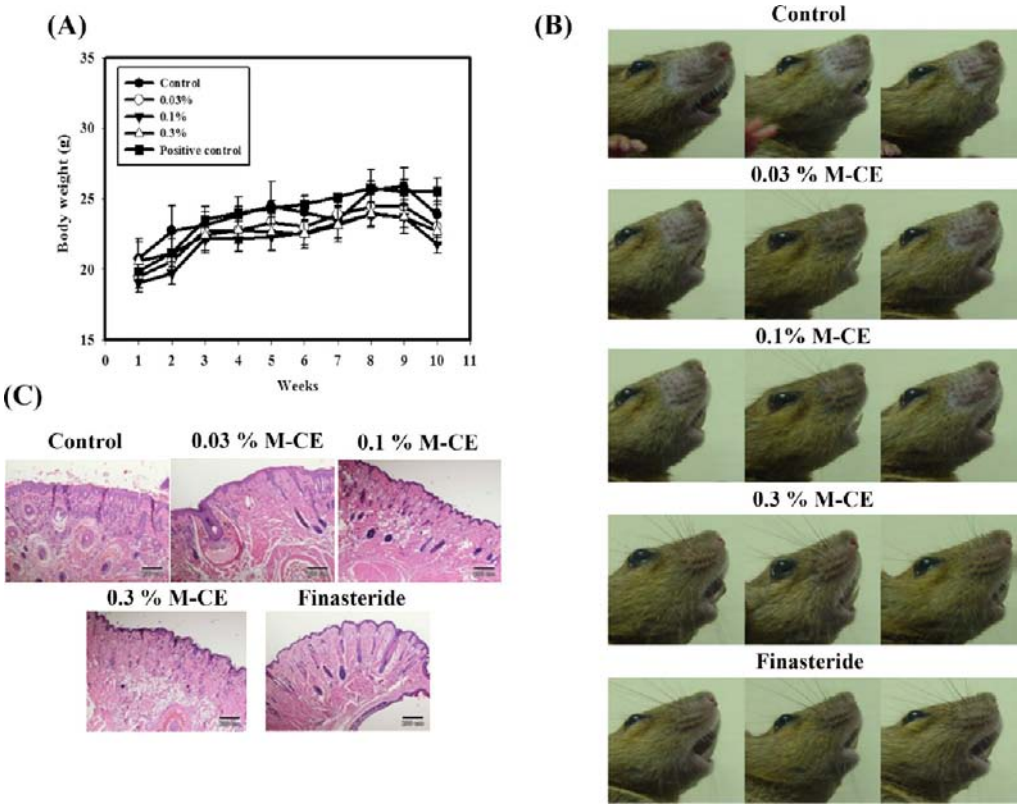

Figure 2. Ingestion of M-CE suppressed AGA in male B6CBAF1/j mice: (A) body weight in B6CBAF1/j mice measured weekly; (B) photographs of AGA mice in each group; (C) histological observation of H&E-stained cross sections from each group (×100 objective magnification).

Medical College. The rats were randomly divided into seven groups (n = 6): (1) normal group, which was injected subcutaneously (sc) with the vehicle, CMC; (2) control group, which was injected subcutaneously with testosterone (10 mg/kg) for 28 days; and (3) positive control group, which was injected subcutaneously with testosterone (10 mg/kg) and finasteride (5 mg/kg) for 28 days. The M-CE groups were injected subcutaneously with testosterone (10 mg/kg) for 28 days and fed diets containing 0.2% M-CE (4), 0.5% M-CE (5), 1% M-CE (6), or 3% M-CE (7) for 28 days (Figure 1B). Body weights were recorded weekly during the experiment. All animals were sacrificed after the final treatment. Whole prostates were immediately removed, weighed, fixed with 10% formalin, and embedded in paraffin for histological analysis. The serum was separated by centrifugation and stored at −80 °C.

Prostate Tumor Induction. The prostate tumor animal model was established according to the method reported by Narayanan et al. with minor modifications.[23] Male Wistar rats (6−8 weeks old) were acquired from the animal center of the National Cheng Kung University Medical College. After quarantine, all rats in the experimental groups received a daily oral dose of flutamide (Sigma Chemical Co., USA) at a dosage of 20 mg/kg for 21 consecutive days to inhibit androgen synthesis. The rats received a single sc injection of 10 mg/kg of testosterone in corn oil one day after the final dose of flutamide. Sixty hours after the first dose of testosterone, the rats in designated experimental groups received a single intravenous (iv) injection of 30 mg/mL/kg of *N*-methyl-*N*-nitrosourea (MNU) (Sigma Chemical Co., USA). Testosterone (0.44 mg/day) was injected sc for 52 weeks. Dietary administration of 0.2% M-CE or 0.5% M-CE was initiated on day 21, after the carcinogen treatment. The dietary regimens for the experimental rats were continued until the termination of the study at the end of 52 weeks (Figure 1C). Body weights were recorded every month. At the end of the study, the whole prostates were weighed and the numbers of tumor nodules counted. The prostate tissue was then fixed with 10% formalin and embedded in paraffin for histological analysis. The serum was separated by centrifugation and stored at −80 °C.

Histological Analysis. The tissues were fixed in 10% formalin (in normal saline). After 3 days, the tissues were sectioned using a microtome and stained with hematoxylin and eosin (H&E) for histological analyses. The slides were observed under a microscope, and the images were recorded.

Immunohistochemical (IHC) Staining Analysis. Paraffin-embedded tissue sections (4 μm) were dried, deparaffinized, and rehydrated. Following microwave pretreatment in citrate buffer (pH 6.0; for antigen retrieval), the slides were immersed in 3% hydrogen peroxide for 20 min to block the activity of endogenous peroxidase. After intensive washing with PBS, the slides were incubated overnight at 4 °C with an anti-PCNA antibody (proteinTech Group, USA). The sections were then incubated with a secondary antibody for 1 h at room temperature, and the slides were developed using the STARR TREK Universal HRP detection kit (Biocare Medical, Concord, CA, USA). Finally, the slides were counterstained using hematoxylin. Each slide was scanned at low power (×100).

Measurement of Prostate-Specific Antigen (PSA). The level of PSA in the serum was quantified using a USCNLIFE Rat prostate-specific antigen ELISA kit (Uscn Life Science Inc., USA) according to the manufacturer's protocol. Briefly, 100 μL of serum sample was added into the appropriate wells and then incubated for 2 h at 37 °C. The liquid of each well was removed, and 100 μL of Detection Reagent A working solution was added. After 1 h of incubation at 37 °C, the wells were washed three times, and then 100 μL of Detection Reagent B working solution was added for 30 min at 37 °C. The wells were washed five times, and 90 μL of Substrate Solution was added to each well for 15−25 min at 37 °C; then, 50 μL of Stop Solution was added. Plates were analyzed with an ELISA reader (Emax, Molecular Devices, Sunnyvale, CA, USA) at 450 nm. On the basis of the standard curve, the concentrations of PSA in each sample were determined.

Biochemistry Tests. Whole blood samples from treated mice were collected by intracardiac puncture and centrifuged at 2000g for 20 min

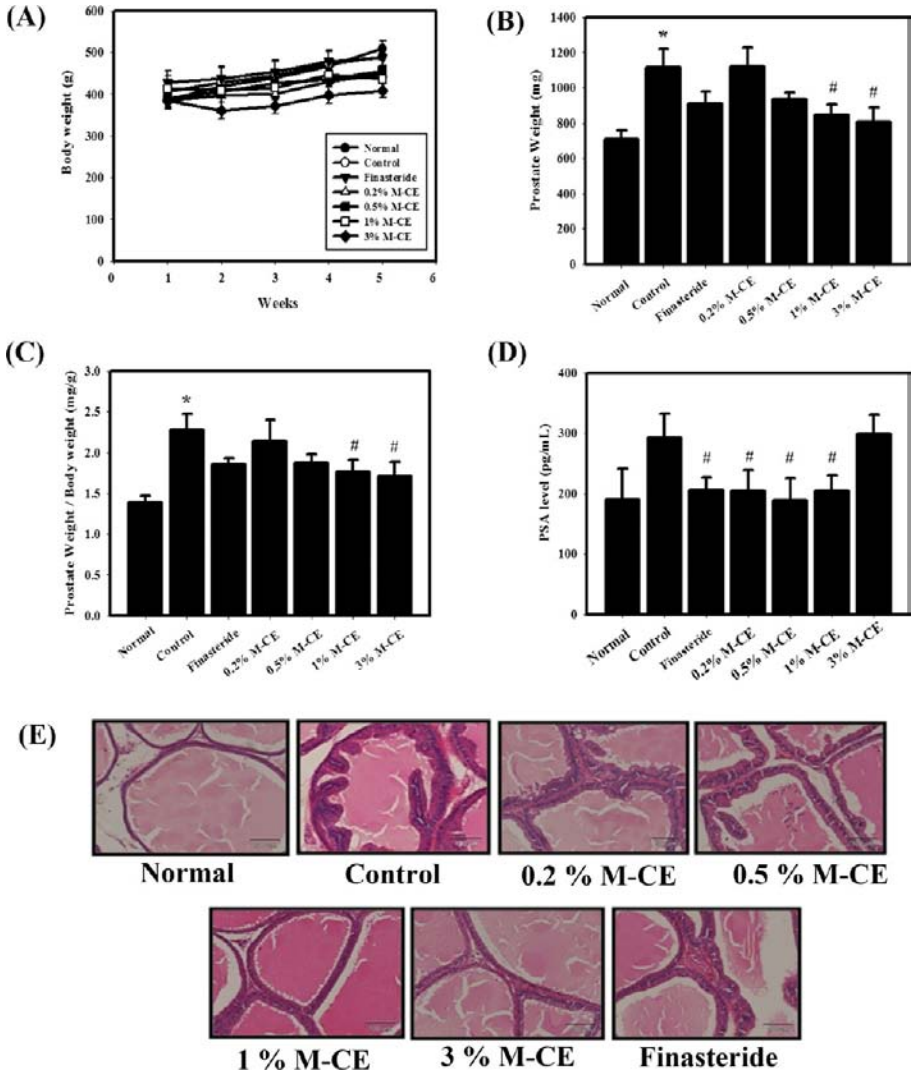

Figure 3. Ingestion of M-CE suppressed BPH in male Spraque–Dawley rats: (A) body weight measured weekly and Effects of M-CE on prostate weight (B) and prostatic/body weight ratio (C) in Spraque–Dawley rats; (D) PSA levels measured by ELISA [(*) $p < 0.05$, normal versus control; (#) $p < 0.05$, versus control]; (E) histological examination of H&E-stained prostate tissue (×100 objective magnification).

to separate the serum. A biochemistry evaluation included the quantification of glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), glucose, high-density lipoprotein cholesterol (HDL-C), triglyceride (TG), and total cholesterol (TCHO). The biochemical parameters hepatic function (GOT and GPT), total amount of fatty substances (TG and TCHO), and lipoprotein (HDL-C) have been analyzed.

Measurement of Serum Testosterone and Dihydrotestosterone (DHT) Concentration. The testosterone level from individual animals in each group was measured using a testosterone EIA kit (Cayman Chemical Co., Ann Arbor, MI, USA) according to the manufacturer's protocol. In brief, 50 μL of the testosterone standard or serum, 50 μL of the testosterone AChE tracer, and 50 μL of the testosterone antiserum were added to the wells of an ELISA plate containing 100 μL of EIA buffer. The optical density was read using an ELISA reader at 405 nm. For DHT level determination, the DHT ELISA kit from BioVendor (Czech Republic) was used. Fifty microliters of the serum and conjugate working solution was added to the wells and then incubated for 1 h at room temperature. The plates were washed three times, and 150 μL of TMB substrate was added into each well and incubated for 10–15 min at room temperature. After addition of the 50 μL stopping solution, the plates were read on an ELISA reader at 450 nm.

Statistical Analysis. The data are expressed as the mean ± SE. Statistical significance was determined using Student's t test for comparison between the means or a one-way analysis of variance with a post hoc Dunnett's test.[24] Differences were considered to be significant when p was <0.05. In addition, the Kolmogorov–Smirnov (K–S) test was used to confirm all of our raw data have a normal distribution.

RESULTS

Effect of M-CE on Alopecia in Male B6CBAF1/j Hybrid Mice (AGA Model). To investigate the effect of M-CE on the specific androgen-related baldness process, we treated male AGA mice for 12 weeks with M-CE and gave concomitant injections of testosterone at a dose of 1 mg/day. None of the treatment regimens resulted in loss of body weight (Figure 2A). As shown in Figure 2B, the control group experienced hair loss in the mouth area. M-CE and finasteride treatments suppressed baldness in male B6CBAF1/j mice. Furthermore, many anagen hair follicles were observed in the M-CE and finasteride groups, whereas only a few anagen hair follicles were observed in the mouth area of mice in the control group (Figure 2C).

Effect of M-CE on Prostate Weight and PSA Levels in the BPH Rat Model. As shown in Figure 3A, none of the treatment regimens caused >10% of body weight loss, except the 3% M-CE group. The prostate weight is an important indicator of BPH. The control group showed a significant increase in prostate weight and prostatic/body weight ratio compared with the normal group (Figure 3B,C). As expected, the finasteride-treated group showed a significant reduction in prostate weight and prostatic/body weight ratio compared with the control group. Furthermore, the prostate weights and prostatic/body weight ratios of 1 and 3% M-CE-treated rats were lower than those of controls. In clinical studies, serum PSA levels are abnormally elevated in patients with prostate cancer, BPH, and inflammatory conditions of the prostate.[25] After 4 weeks of testosterone treatment, serum PSA levels were significantly increased in the control group (Figure 3D). The finasteride-treated group showed decreased PSA levels. The 0.2, 0.5, and 1% M-CE treatments also caused a decrease in PSA levels, which suggests a protective effect of M-CE on testosterone-induced hyperplasia.

To evaluate the growth of the prostate, we measured the prostate epithelial cell layer thickness. The rat prostate histology can be observed in Figure 3E, showing epithelial cell layers and the stromal spaces of the prostates, in which those of the control group were larger than those from the normal group. Moreover, the M-CE-treated group had a reduction in the thickness of the epithelial cell layers and stromal spaces of the prostate compared with the control group, which is similar to the results observed for the finasteride-treated group.

Influence of M-CE on the Incidence and Volume of Tumors in the MNU/Testosterone-Induced Rat Prostate Cancer Model. It has been reported that sequential administration of antiandrogen (cyproterone acetate), chemical carcinogen (MNU), and androgen (testosterone) induces a high incidence of prostate cancer in male Wistar rats.[26] We used this model to evaluate the chemopreventive effects of M-CE on the incidence and volume of prostate tumors. As shown in Table 1, the tumor incidence in the positive control group found in our current study was 67%. The rats fed diets containing M-CE showed a significant decrease in tumor incidence compared with the controls. In addition, the M-CE groups showed a significant decrease in tumor volume compared with the control group; treatment with 0.2 and 0.5% M-CE reduced the inhibition efficacy of tumor volume by 58 and 64%, respectively. These results indicate that M-CE effectively reduces the incidence and size of tumors in the rat prostate cancer model.

Table 1. Influence of M-CE on Incidence and Volume of Tumors in Wistar Rats Treated with *N*-Methyl-*N*-nitrosourea (MNU) + Testosterone (T)

	control	0.2% M-CE	0.5% M-CE
chemical carcinogen	MNU	MNU	MNU
hormone treatment	T	T	T
chemopreventive agent	none	0.2% M-CE	0.5% M-CE
no. of animals evaluated	15	15	16
no. of rats with tumor per group	10	6	7
incidence of tumor (%)	67	40	43
tumor volume per rat[a] (mm^3)	116.5 ± 2.03	48.5 ± 16.3*	41.1 ± 9*
inhibition of tumor volume per rat (%)		58	64

[a]Tumor volume (mm^3) was calculated using the formula $V = (0.52 \times \text{long diameter} \times \text{short diameter}^2)$. Values are the mean ± SE per rat. (*) $p < 0.05$ by Student's t test compared with control group.

Effect of M-CE on Prostate Weight, Testosterone and DHT Levels, and Biochemical Factors in the MNU/Testosterone-Induced Rat Prostate Cancer Model. The health and physical activity of the rats in each group remained normal until the day of sacrifice after 52 weeks of treatment with M-CE. An overall weight gain was observed in the normal group of rats compared with other experimental groups (Figure 4A). Although a small decrease in the body weight was observed among the rats in all M-CE-treated groups, there was no sign of treatment-related toxicity. Furthermore, the prostate weight and prostatic/body weight ratio were increased significantly in the control group when compared with the normal group (Figure 4B,C). Dietary administration of 0.2 and 0.5% M-CE for 52 weeks resulted in a significant decrease in the prostate weight and prostatic/body weight ratio compared with the control group. As shown in Figure 4D, none of the M-CE treatments changed the levels of GOT, GPT, or HDL-C. The serum glucose level of 5% M-CE-treated rats was significantly lower than that of the control group. In addition, the control group showed a significant increase in serum TCHO compared with the normal group, and the serum TG and TCHO levels were significantly decreased in the 2 and 5% M-CE groups.

In rat prostates, whereas the control group had greater levels of testosterone and DHT than the normal group, the 0.5% M-CE-treated group had markedly decreased levels of DHT, but not testosterone (Figure 5A,B). A histological examination of prostate tissue sections from the normal group showed normal ventral prostate architecture with connective tissue between the acini; the epithelial tubules were thin and condensed around the acini and tubules of the gland. In the control group, we observed high-grade prostate intraepithelial neoplasia (PIN) with cribriform, micropapillary architecture, and compression of the prostatic epithelium. The cell number in the PIN was markedly increased, and cell density with sparse cytoplasm was also observed. Simultaneous M-CE treatment resulted in a few dysplastic and hyperplastic nodules, and the stroma was very loosely organized. The prostates from the M-CE group had a normal appearance, with epithelial tubules lining the lumen secretions (Figure 5C). We also evaluated expression patterns of a cell proliferation marker,[27] proliferating cell nuclear antigen (PCNA), in the prostate using IHC staining (Figure 5C). We found that PCNA expression was decreased in the prostates of rats treated with M-CE compared with the controls.

DISCUSSION

The most common form of human hair loss is AGA. It affects at least 50% of men by the age of 50 years and 50% of women by the age of 60 years. It is more obvious in men and often manifests itself a decade earlier in men than in women.[28] Currently, only a few medications are used to treat AGA. Among these, 5α-reductase inhibitors, finasteride and dutasteride, are used to treat androgen-related disorders.[29] Unfortunately, these treatments have several side effects, such as impotence, abnormal sexual function, testicular pain, impairment of muscle growth, and severe myopathy.[30] Here, we demonstrate for the first time that a natural product, M-CE, suppresses baldness in male B6CBAF1/j mice with an efficacy

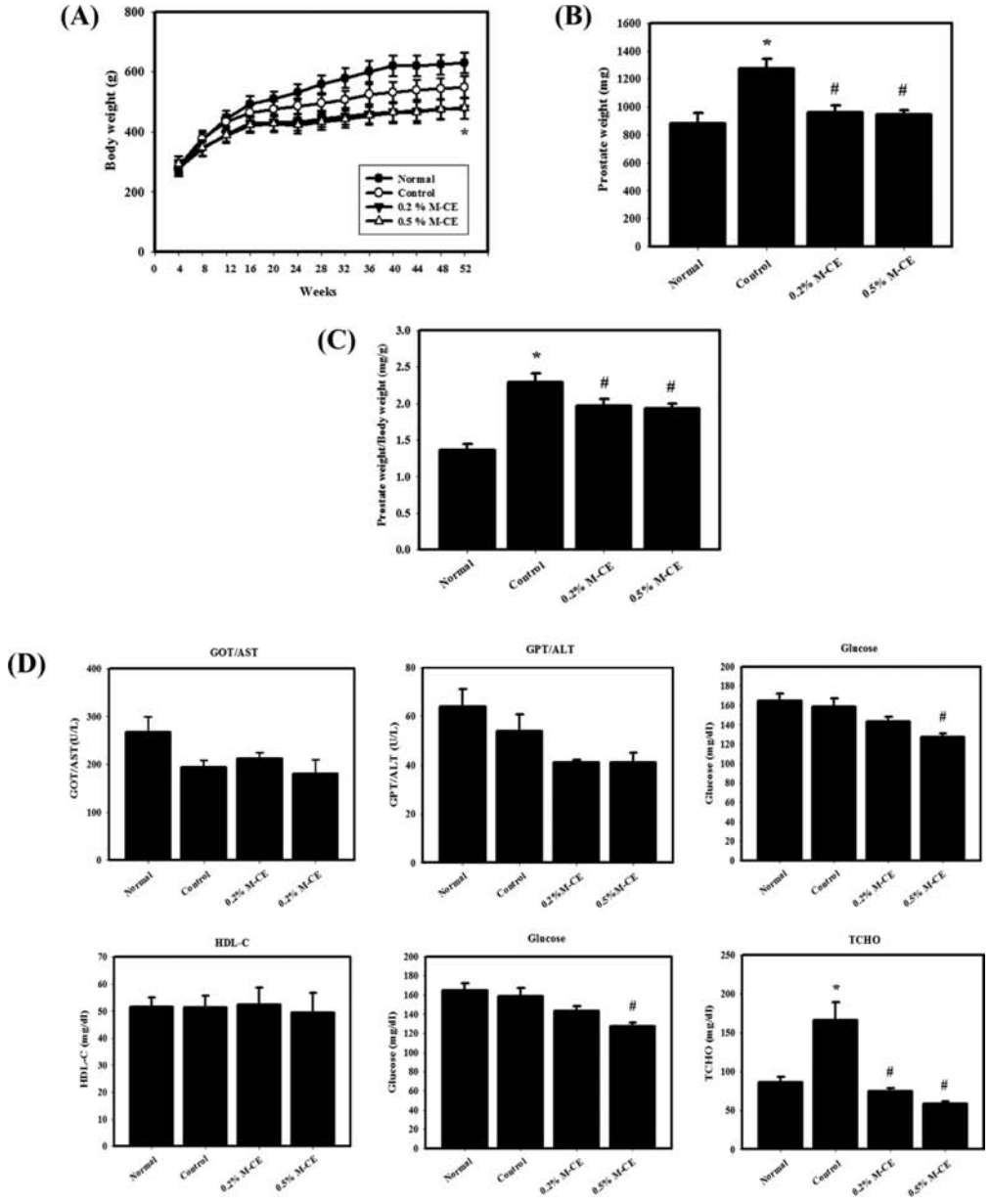

Figure 4. Effect of M-CE on prostate weight, testosterone, DHT levels, and biochemistry tests in the MNU/testosterone-induced rat prostate cancer model: (A) body weight in Wistar rats measured monthly; (B) effects of M-CE on prostate weights (B) and prostatic/body weight ratio (C) in Wistar rats [(∗) $p < 0.05$, normal group versus controls; (#) $p < 0.05$, control versus M-CE]; (D) effects of M-CE on serum GOT, GPT, glucose, HDL-C, TG, and TCHO [(∗) $p < 0.05$, normal group versus controls; (#) $p < 0.05$, control group versus M-CE].

similar to that of finasteride. The histological evaluation of hair follicles showed that animals given dietary M-CE had more anagen hair follicles than those in the control group. Moreover, there was no sign of toxicity accompanying M-CE treatment (Figure 2). Despite these findings, the detailed mechanism underlying the inhibition of hair loss by M-CE treatment in the AGA model remains unclear and warrants further investigation.

Both AGA and BPH are androgen-dependent diseases involving testosterone and DHT; the enzyme 5α-reductase, which transforms testosterone into DHT, plays a key role. Recently, it has been reported that AGA, which can manifest decades before the onset of BPH, may serve as an early marker of prostate abnormalities.[9] In our study, in addition to the inhibition of AGA, we also found that M-CE reduced the thickness of epithelial cell layers and the prostate weight (Figure 3). As reported previously, PSA is a protein produced by the cells of the prostate gland, and serum PSA levels are abnormally elevated in patients with prostate cancer, BPH, and inflammatory conditions of the prostate.[31] Therefore, a decrease in PSA levels indicates protective effects against the inflammatory conditions and hypertrophy of the prostate that are induced by testosterone. We demonstrated that testosterone treatment increased PSA levels and that M-CE treatment significantly decreased PSA levels, indicating protective effects against BPH (Figure 3D).

Testosterone and DHT play a key role in the development of male reproductive organs.[32] Although testosterone is the most abundant serum androgen, DHT is the main prostatic androgen. DHT has 2−5 times higher binding affinity for the AR than testosterone and has a 10-fold higher potency in inducing AR signaling than testosterone.[33] DHT plays an important role in several human diseases, including BPH and

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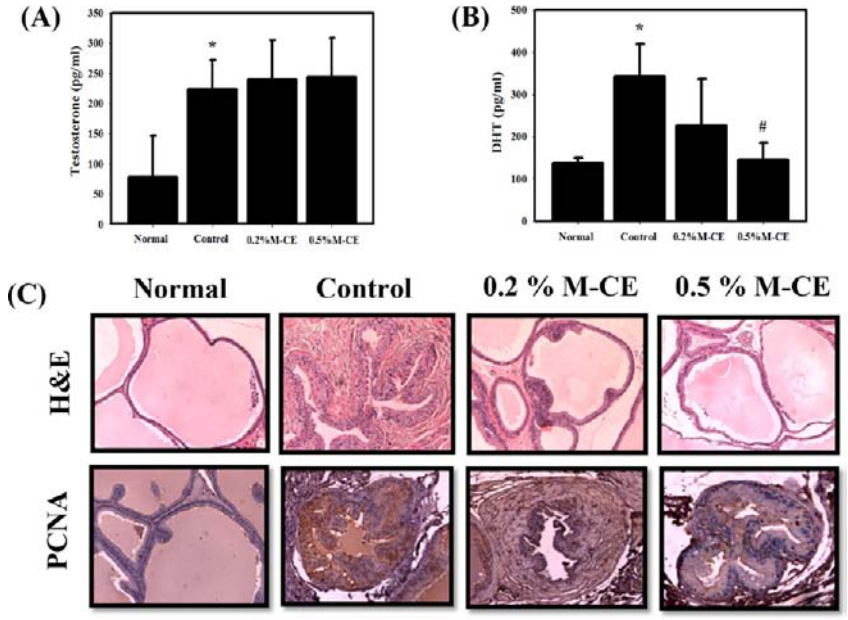

Figure 5. Effects of M-CE on testosterone (A) and DHT (B) levels in serum [(∗) $p < 0.05$, normal group versus control group; (#) $p < 0.05$, control group versus M-CE group]; (C) IHC and H&E staining of the prostate tissue. IHC was used to determine the expression levels of PCNA (×100 objective magnification).

prostate cancer.[32,33] The results presented in this study clearly show that M-CE suppressed MNU/testosterone-induced prostate tumor incidence (Table 1) and decreased the prostate weight and prostatic/body weight ratio (Figure 4). Furthermore, M-CE markedly decreased DHT but not testosterone (Figure 5). In addition, PCNA expression was decreased in the prostate of rats treated with M-CE compared with the controls (Figure 5). These results indicate that M-CE may be useful in prostate cancer prevention.

RMR has been used as a food additive and as an herbal medication in China for centuries. It has been reported that RMR inhibits cholesterogenesis and that its components, red pigments, have strong antioxidant properties.[34] In addition, monacolin compounds, which are formed by *Monascus* during the fermentation process, cause a reversible competitive inhibition of the microsomal hydroxymethyl-glutaryl coenzyme A (HMG-CoA) reductase, thus preventing the reduction of HMG-CoA into mevalonic acid and the formation of cholesterol.[35] Here, we found that long-term dietary administration of M-CE decreased serum glucose, TG, and TCHO in rats (Figure 4D), which is consistent with those reports. Furthermore, M-CE-treated groups caused only a mild decrease in the body weight (Figure 3A,D). Several studies, including ours, have also assessed the anticancer activity of RMR in different types of cancer such as colon, breast, lung, and prostate cancer.[3,6] We recently reported that monascuspiloin (an analogue of the *Monascus* pigment monascin) effectively inhibits the growth of human prostate cancer cells in vitro and in vivo.[6] The underlying mechanisms of monascuspiloin-induced cell death (autophagy and apoptosis) in prostate cancer cells may be through the inhibition of the PI3K/Akt and the activation of the AMPK signaling pathways. Additionally, we recently reported that monascuspiloin enhanced the radiation sensitivity of human prostate cancer cells by stimulating endoplasmic reticulum stress and inducing autophagy.[7] In our current study, M-CE markedly decreased tumor incidence and tumor volume in the MNU/testosterone-induced rat prostate cancer model (Table 1), further demonstrating the chemopreventive potential of M-CE.

Taken together, our results indicate that M-CE suppressed baldness and restored the growth of hair follicles in male B6CBAF1/j mice. Thus, M-CE could be used as an agent for the treatment of AGA. Moreover, oral administration of M-CE in a BPH rat model significantly decreased the prostate size, prostatic hyperplasia, and PSA levels in the serum and prostate, demonstrating for the first time that M-CE may have the potential to prevent prostate cancer through the inhibition of DHT. Thus, M-CE has the potential for use in the context of prevention and/or treatment of androgen-related diseases.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The qualitative and quantitative profile of M-CE and chemical composition of M-CE. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

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Notes

The authors declare no competing financial interest.

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