Cordycepin Attenuates Testosterone-Induced Benign Prostatic Hyperplasia in Rats via Modulation of AMPK and AKT Activation

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Abstract: Benign prostatic hyperplasia (BPH) is a disease that commonly affects elderly men. Cordycepin is an adenosine analog with a wide range of pharmacological activities including antiproliferative and prostatic smooth muscle relaxant effects. This study was designed to assess the actions of cordycepin in testosterone-induced BPH in rats. Animals were divided into six treatment groups: control, cordycepin-alone (10 mg/kg), testosterone-alone (3 mg/kg), cordycepin (5 mg/kg) + testosterone, cordycepin (10 mg/kg) + testosterone, and finasteride (0.5 mg/kg) + testosterone. Treatments were continued daily, 5 days a week, for 4 weeks. Cordycepin significantly prevented the increase in prostate weight and prostate index induced by testosterone. This was confirmed by histopathological examinations. Cordycepin antiproliferative activity was further defined by its ability to inhibit cyclin-D1 and proliferating cell nuclear antigen (PCNA) expression. In addition, cordycepin exhibited significant antioxidant properties as proven by the prevention of lipid peroxidation, reduced glutathione diminution, and superoxide dismutase exhaustion. This was paralleled by anti-inflammatory activity as shown by the inhibition of interleukin-6, tumor necrosis factor-α, and nuclear factor-κB expression in prostatic tissues. It also enhanced apoptosis as demonstrated by its ability to enhance and inhibit mRNA expression of Bax and Bcl2, respectively. Western blot analysis indicated that cordycepin augmented phospho-AMP-activated protein kinase (p-AMPK) and inhibited p-AKT expression. Collectively, cordycepin has the ability to prevent testosterone-induced BPH in rats. This is mediated, at least partially, by its antiproliferative, antioxidant, anti-inflammatory, and pro-apoptotic actions in addition to its modulation of AMPK and AKT activation.

Keywords: cordycepin; benign prostatic hyperplasia; testosterone; AMPK; AKT

1. Introduction

Benign prostatic hyperplasia (BPH) is characterized by enhanced multiplication of prostatic stromal and epithelial cells. PBH is a common disease within the elderly male population [1]. According to several reports, the incidence of BPH rises with age [2]. BPH symptoms are thought to affect about half of all men over the age of 60 [3]. Symptoms of BPH can significantly and negatively impact the quality of life of affected men [4]. These symptoms of BPH include decreased urinary flow and urinary incontinence, nocturia, dribbling, and difficulty initiating flow [5]. The pathogenesis of BPH has been linked
to oxidative stress, chronic inflammation, and disturbed apoptosis [6,7]. The two main medical treatments for BPH are 5α-Reductase inhibitors and α-adrenergic blockers. These drugs, on the other hand, can have negative impacts on ejaculation, cognitive functions, and mental health [8,9]. Phytotherapies have emerged as an acceptable and additional option for BPH management because they are effective, less expensive, and well-tolerated [10].

The *Cordyceps* genus contains over 400 species, including *Cordyceps sinensis* and *Cordyceps militaris*, which have been utilized as a herbal and tonic medicine in China for thousands of years. They were used for a variety of health problems such as kidney and lung diseases and impotence [11]. Additionally, *Cordyceps militaris* has been widely utilized clinically for the management of chronic fatigue syndrome, chronic bronchitis, and diabetic nephropathy [11]. Cordycepin is an active compound found in the *Cordyceps militaris* caterpillar fungus [12]. Chemically, it is identical in structure to adenosine, however, cordycepin only lacks one 3′-hydroxyl group of the five-membered ribose ring [13]. Cordycepin has a wide range of pharmacological effects, the most studied of which are its antitumor [14], antioxidative [15], and anti-inflammatory properties as it suppresses the production of pro-inflammatory mediators in different cells [11]. Interestingly, it has been suggested that adenosine has antiproliferative activity via adenosine A3 receptor stimulation in several types of cancer [16,17]. Activation of this receptor was even found to inhibit prostate carcinoma cell growth [18]. Moreover, adenosine agonists have the potential to alleviate prostate smooth muscle contraction. Experimentally, electrical field stimulation of the contractile responses in isolated rat prostates was found to be attenuated by the activation of presynaptic A1 adenosine receptors [19]. Furthermore, α1 adrenoceptor-mediated responses of human prostatic stromal cells have been found to be inhibited by the stimulation of post-junctional A2A adenosine receptors [20]. Therefore, the current study was designed to assess the potential of cordycepin to attenuate BPH induced by testosterone in rats.

2. Materials and Methods

2.1. Chemicals

Cordycepin (>98%) was purchased from Salus Nutra Inc. (Xi’an, China). Testosterone enanthate was obtained as a kind gift from Chemical Industries Development Co. (CID), Giza, Egypt. Finasteride was obtained from Sigma-Aldrich (St. Louis, MO, USA). The chemicals used in this investigation were of the highest analytical quality.

2.2. Animals

Experimental work involving animals was conducted according to the approved procedures by the Faculty of Pharmacy’s Research Ethics Committee, King Abdulaziz University (Ref # PH-1443-23). Male Wistar rats (200–230 g) of 10-week of age were obtained from the animal facility of Faculty of Pharmacy, King Abdulaziz University. The rats were maintained under an air-conditioned environment (20–24 °C), alternating 12-h light/12-h dark cycles, and ad libitum feeding with a standard diet and water. The animals were acclimatized for 1 week to our experimental facility before the start of experiments.

2.3. Toxicity Study

To assess the acute toxicity of cordycepin based on the OECD Test Guideline 423, animals were administered an oral cordycepin dose of 2000 mg/kg. After that, the rats were observed closely during the first hour and regularly for the following 24 h of administration. As all animals survived, the experimental protocol was carried out again utilizing three additional rats.

2.4. Experimental Design

Thirty-six male rats were separated into 6 groups randomly (6 animals per group). Group 1 (control) was subcutaneously (s.c.) administered normal saline (10 mL/kg, p.o.) and corn oil (1 mL/kg). Group 2 (cordycepin) was administered cordycepin dissolved in normal saline (10 mg/kg p.o.) and corn oil (s.c.). Group 3 (testosterone) was given
normal saline (10 mL/kg, p.o.) and testosterone (3 mg/kg dissolved in corn oil, s.c.). Group 4 (testosterone + cordycepin 5 mg/kg) was administered cordycepin (5 mg/kg, p.o.) and testosterone (5 mg/kg s.c.). Group 5 (testosterone + cordycepin 10 mg/kg) was administered cordycepin (10 mg/kg, p.o.) and testosterone (5 mg/kg s.c.). Group 6 (positive control) was given finasteride (0.5 mg/kg p.o.) and testosterone (5 mg/kg s.c.). Administration of oral doses was carried out before s.c. injections by 1 h. Treatment of animals was administered once daily every 5 days per week for 4 consecutive weeks. All specified doses and treatment schedules were according to a pilot study. Prostate tissues from each animal group were harvested immediately after the sacrifice and 72 h after the last treatment. Parts of the prostatic ventral lobes were kept in a 10% neutral buffered formalin for histopathology and immunohistochemistry studies. The remaining tissues were flash-frozen in liquid nitrogen and stored at −80 °C for assessment of mRNA expression and biochemical markers.

2.5. Prostate Index and Weight

Prostate weights were registered after dissection, and hence the prostate index was calculated relative to the total body weight of the animal by dividing the prostate weight by the total body weight before multiplying by 1000.

2.6. Histopathological Examination

Paraffin sections of 4 µm were obtained from the fixed prostate tissues. Following de-paraffinization and rehydration, the paraffin sections were stained with hematoxylin and eosin (H&E). The height of the prostate glandular epithelia was determined using Image J software (Image J, 1.46a, NIH, Bethesda, MD, USA).

2.7. Oxidative Status Markers Assessment

Phosphate-buffered saline containing 50 mM potassium phosphate (ice-cooled, pH 7.5) was used for the homogenization of prostate tissues to determine malondialdehyde (MDA), glutathione (GSH), and superoxide dismutase (SOD) levels (Cat # 10009055, 703002 and 707002, Cayman Chemical, Ann Arbor, MI, USA, respectively). Total protein was assessed by a BCA protein assay kit (Cat # 355526, MyBioSource, Inc., San Diego, CA, USA).

2.8. Immunohistochemical Analyses

Tissue sections were deparaffinized, rehydrated with ethanol, and boiled in citrate buffer (pH 6.0) for 10 min. After a two-hour incubation in tris buffered saline (TBS) with 5% bovine serum albumin (BSA), the sections were immersed overnight at 4 °C with the following primary antibodies: rabbit monoclonal anti-cyclin D1 (Cat # ab16663), proliferating cell nuclear antigen (PCNA, Cat # ab92552), polyclonal anti-IL-6 (Cat # ab9324), monoclonal anti-TNF-α (Cat # ab220210), and polyclonal anti-NFκB (p65, Cat # ab194726, Cambridge, UK). After that, the slides were flushed with TBS and the primary antibodies were detected using Mouse- and Rabbit Specific-HRP/DAB (ABC) Detection IHC kit according to the manufacturer instructions (Cat # ab64264, ABCAM, Cambridge, UK). Image analysis of at least 3 sections per rat was conducted using Image J analysis software (Image J, 1.46a, NIH, Bethesda, MD, USA).

2.9. Analysis of mRNA Expression of Bax and Bcl2

An ultrasonic probe was used for homogenization of prostate tissues before extracting RNA using NucleoSpin RNA Mini kit (Macherey-Nagel GmbH & Co. KG, Duerin, Germany). The purity (A260/A280 ratio) and concentration of the extracted RNA was assessed using a spectrophotometer (Beckman, Brea, CA, USA) before carrying out the reverse transcription step using High-Capacity cDNA Reverse Transcription Kit (Cat # 4368814, Applied Biosystems, Foster City, CA, USA). For real-time polymerase chain reaction (qPCR), SYBR™ Green PCR Master Mix kit (Cat # 4309155, Applied Biosystems, Foster City, CA, USA) was utilized with the following forward primers Bax, Bcl2,
and β-actin: 5′CCTGAGCTGACCTTGGAGCA, 5′GATAACCGGGAGATCGTGA, and 5′TCCGTCGCCGGTCCACACCC, respectively [21]. The reverse primers for Bax, Bcl2, and β-actin: 5′GGTGGTTGCTCTTCTTCTACT, 5′AAAGCACTCCAATAAAAAGC, and 5′TCACCAACTGGGACGATATG, respectively [21]. Data analysis was according to the ∆∆CT method in which the data were normalized to β-actin readings [22].

2.10. Protein Expression of Bax, Bcl2, Total & Phosphor-AKT, and Total and Phosphor-AMPK by Western Blot

The prostates were homogenized by incubation in ice-cold radioimmunoprecipitation assay (RIPA) buffer for 30 min containing phosphatase and protease inhibitor cocktails. Following centrifugation at 3000 rpm for 30 min at 4 °C, the supernatant was used for the assay of protein content by a BCA protein assay kit (Cat # 355526, MyBioSource, Inc., San Diego, CA, USA). The protein lysates (80 μg/lane) were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. After the electrophoresis step, the separated proteins were transferred onto an activated polyvinylidene difluoride membrane (Cat # ab133411, Abcam, Cambridge, UK). The membranes were blocked by 5% non-fat milk in TBST for 1 h at room temperature before overnight incubation with the following primary antibodies: anti-Bax antibody (Cat # sc-23959) and anti-Bcl2 antibody (Cat # sc-7382) from Santa Cruz Biotechnology (Dallas, TX, USA), and anti-pan-AKT antibody (Cat # ab8805), anti-AKT (phospho T308) antibody (Cat # ab38449), anti-AMPK alpha antibody (Cat # ab32047), anti-AMPK alpha 1/2 (phospho T183/T172) antibody (Cat # ab133448), and anti-β actin antibody (Cat # ab6276) from Abcam (Cambridge, UK). After washing, the membranes were incubated for 1 hour at room temperature with HRP-conjugated secondary antibody (Cell Signaling Technology, Danvers, MA, USA) before membrane development using ECL Substrate kit (Cat # ab133406, Abcam, Cambridge, UK). Membrane visualization and protein quantification were performed using ChemiDoc MP Imaging System with Image Lab Software (Bio-Rad Laboratories, Dubai, United Arab Emirates) and Image J (1.46a, NIH, Bethesda, MD, USA), respectively.

2.11. Statistical Analysis

Data are reported as mean ± SD. For statistical analysis with multiple comparisons using GraphPad Prism version 8.1. (GraphPad, La Jolla, CA, USA), one-way ANOVA with Tukey’s post-hoc test was utilized and a p-value of less than 0.05 is considered statistically significant.

3. Results

3.1. Acute Toxicity Assessment

After 24 h of oral administration of cordycepin at 2000 mg/kg, there were no deaths detected among the three male rats utilised in this study. Hence, a following study was performed on three additional rats utilising the same experimental protocol, which also led to no deaths observed in the tested animals.

3.2. Prostate Weights and Indices

 Principally, cordycepin at both doses tested was safe and well-tolerated as it resulted in no mortality or significant changes in prostate weights. In contrast, testosterone enan-

thate significantly increased prostate weight and index by 178.2% and 153.0%, respectively, relative to the control value (Table 1). However, coadministration with cordycepin at both 5 and 10 mg/kg led to a substantial decrease in prostate weight by 32.2% and 45.2% and prostate index by 29.5% and 41.5%, respectively, compared to rats that received testosterone alone. It was also noticeable that cotreatment of rats with finasteride at 0.5 mg/kg significantly decreased the prostate weight and index by 52.5% and 48.4% associated with testosterone administration.
Table 1. Effect of cordycepin on prostate weight and index in testosterone-treated rats.

| Treatment                        | Final Body Weight (g) | Prostate Weight (mg) | Prostate Index ($\times 10^3$) |
|----------------------------------|-----------------------|----------------------|-------------------------------|
| Control                          | 245 ± 20.2            | 281 ± 30.1           | 1.15 ± 0.16                   |
| Cordycepin (COR 10 mg/kg)        | 250 ± 21.2            | 305 ± 32.5           | 1.23 ± 0.19                   |
| Testosterone (T; 3 mg/kg)        | 272 ± 30.8            | 782 $^{ab}$ ± 81.4   | 2.91 $^{ab}$ ± 0.48           |
| T + COR (5 mg/kg)                | 260 ± 22.8            | 530 $^{a,b,c}$ ± 58.3| 2.05 $^{a,b,c}$ ± 0.26        |
| T + COR (10 mg/kg)               | 255 ± 26.2            | 428 $^{a,b,c,d}$ ± 46.7| 1.70 $^{a,c,d}$ ± 0.31        |
| T + FIN (0.5 mg/kg)              | 251 ± 27.2            | 371 $^{a,c,d}$ ± 32.5| 1.50 $^{a,c,d}$ ± 0.23        |

Data ($n = 6$) are expressed as mean ± SD. COR = cordycepin, T = testosterone, FIN = finasteride. a, b, c, or d: statistically different ($p < 0.05$) from control, cordycepin, testosterone or T + COR (5 mg/kg), respectively.

3.3. Histopathological Examination

Microscopic examination of the prostate gland from the control and cordycepin-alone groups revealed normal histology of acini that were lined by cuboidal to low columnar epithelium with occasional papillary infoldings (Figure 1A,B). However, testosterone injection resulted in hyperplasia of prostatic acini that was characterized by frequent inward folding of the epithelial lining forming intraluminal projections. The hyperplastic cells appeared cuboidal to tall columnar with granular eosinophilic cytoplasm. Some examined sections showed expansion of the interstitial tissue with edema and inflammatory cell infiltration (Figure 1C). Regarding cordycepin (5 mg/kg)-treated animals, moderate improvement was detected in the examined sections as several acini showed hyperplasia of the epithelial lining accompanied by fewer areas of interstitial inflammatory regions (Figure 1D). The highest protection was observed in cordycepin (10 mg/kg)-treated animals as acinar epithelia appeared almost normal. Only fewer sections showed the epithelial lining hyperplasia with intraluminal projections (Figure 1E). The positive control group has obvious improvements against testosterone hyperplasia with almost normal prostatic architecture. Few acini showed desquamated epithelial cells into the acinar lumen admixed with eosinophilic tissue debris and inflammatory cells infiltration (Figure 1F).

Figure 1. Cont.
was capable of significantly ameliorating the substantial increment of cyclin D1 and PCNA-positive cells associated with administering testosterone alone. Interestingly, finasteride proliferation rate (Figure 2C). As shown in Figure 2D,E, the coadministration of cordycepin (5 mg/kg) + testosterone-treated group and (E) cordycepin (10 mg/kg) + testosterone-treated group showing moderate and high restoration of almost normal prostate histology, respectively; and (F) finasteride + testosterone group is without apparent acini hyperplasia. (G) Graphic presentation of prostate glandular epithelial height in the different treatment groups. Data are presented in the bar chart (n = 6) as mean ± SD. COR = cordycepin, T = testosterone, FIN = finasteride. a, b, c, or d: statistically different (p < 0.05) from control, cordycepin, testosterone or T + COR (5 mg/kg), respectively.

3.4. Proliferation Markers

Immunohistochemical analysis of the key regulators of cell proliferation cyclin-D1 and PCNA revealed an average number of positive cells for either of them in the control group (Figure 2A). Similarly, cordycepin resulted in no significant changes in the number of stained cells compared to the control group (Figure 2B). However, testosterone significantly induced a noticeable increase in the number of stained cells, indicating an elevated proliferation rate (Figure 2C). As shown in Figure 2D,E, the coadministration of cordycepin was capable of significantly ameliorating the substantial increment of cyclin D1 and PCNA-positive cells associated with administering testosterone alone. Interestingly, finasteride resulted in comparable findings to cordycepin at 10 mg/kg (Figure 2F). Densitometry data in Figure 2 revealed that cordycepin (10 mg/kg) significantly reduced the expression of cyclin D1 and PCNA by 48.8% and 31.7%, respectively.
3.5. Oxidative Stress Markers

The protective activity of cordycepin against oxidative stress induced by testosterone in prostatic tissues was also evaluated. Table 2 shows that testosterone exposure significantly elevated the levels of MDA, a lipid peroxidation marker, by around 3.5 folds while markedly depleting the content of GSH and reducing the activity of SOD by 64.0% and 71.4%, respectively, relative to the control values. Interestingly, treatment with cordycepin at 5 mg/kg and 10 mg/kg significantly attenuated the testosterone-induced increase in MDA levels by 33.0% and 42.1%. Moreover, cordycepin significantly ameliorated GSH depletion and SOD exhaustion by both doses tested. In a similar vein, finasteride was effective in ameliorating testosterone-induced oxidative stress as indicated by the MDA and GSH content and SOD activity levels.

Table 2. Effect of cordycepin on oxidative status in prostatic tissues of testosterone-treated rats.

|                      | MDA (nmol/mg Protein) | GSH (mmol/mg Protein) | SOD (U/mg Protein) |
|----------------------|-----------------------|-----------------------|--------------------|
| Control              | 18.77 ± 2.10          | 89.00 ± 9.73          | 8.3 ± 0.91         |
| Cordycepin (COR 10 mg/kg) | 17.11 ± 1.86        | 97.41 ± 10.2          | 8.8 ± 0.98         |
| Testosterone (T; 3 mg/kg) | 65.10 ± 7.53         | 32.17 ± 3.41          | 2.37 ± 0.30        |
| T + COR (5 mg/kg)    | 43.61 ± 4.51          | 66.31 ± 7.05          | 6.53 ± 0.68        |
| T + COR (10 mg/kg)   | 37.71 ± 4.05          | 75.52 ± 6.34          | 6.62 ± 0.72        |
| T + FIN (0.5 mg/kg)  | 27.52 ± 2.3           | 80.45 ± 8.7           | 8.6 ± 0.96         |

Data (n = 6) are expressed as mean ± SD. COR = cordycepin, T = testosterone, FIN = finasteride. a, b, c, d or e: statistically different (p < 0.05) from control, cordycepin, testosterone, T + COR (5 mg/kg) or T + COR (10 mg/kg), respectively.

3.6. Inflammatory Markers

Further immunohistochemical analysis was carried out to examine the inflammatory state of prostatic tissues in cordycepin-treated rats following testosterone administration (Figure 3). Testosterone significantly stimulated the expression of IL-6, while cordycepin treatment significantly ameliorated this increased expression of IL-6 by 13.2% and 54.2% at 5 mg/kg and 10 mg/kg, respectively. Furthermore, the expression of TNF-α and NF-κB were also induced with testosterone and these expression levels were markedly attenuated by cordycepin treatment at 5 mg/kg by 15.7% and 17.5%, respectively. Cordycepin at 10 mg/kg caused even further reduction in TNF-α expression by 49.7% and NF-κB expression by 40.5% compared to testosterone alone (Figure 3).

3.7. Expression of Bax and Bcl2

Cordycepin’s antiapoptotic activity was examined according to Bax and Bcl2 mRNA and protein expression in prostatic tissues of testosterone-treated rats. As shown in Figure 4A,D, testosterone markedly decreased the mRNA and protein expression of the pro-apoptotic protein Bax, respectively. Yet, cordycepin significantly attenuated this decrease in the expression of Bax at all doses tested in this study. With regards to the antiapoptotic protein Bcl2, testosterone significantly increased its mRNA and protein expression, however, cordycepin significantly attenuated these changes at 5 mg/kg and 10 mg/kg (Figure 4B,E). Finasteride also showed significant anti-apoptotic activity, as indicated by the induced expression of Bax and the reduced expression of Bcl2 relative to the testosterone-alone group (Figure 4).
Figure 3. Effect of cordycepin on the expression of IL-6, TNF-α, and NF-κB as determined by immunohistochemistry in prostatic tissues of testosterone-treated rats. (A) Control, (B) cordycepin, (C) testosterone, (D) testosterone + cordycepin 5 mg/kg, (E) testosterone + cordycepin 10 mg/kg, and (F) testosterone + finasteride. Data (n = 6) presented in the bar charts are mean ± SD. COR = cordycepin, T = testosterone, FIN = finasteride. a, b, c, or d: statistically different from control, cordycepin, testosterone, or T + COR (5 mg/kg), respectively (p < 0.05).

Figure 4. Cordycepin effect on prostatic expression of Bax (A) and Bcl2 (B) mRNAs and proteins (C) in testosterone-treated rats. Lanes I, II, III, IV, V, and VI represent control, cordycepin, testosterone, testosterone + cordycepin 5 mg/kg, testosterone + cordycepin 10 mg/kg, and testosterone + finasteride, respectively. Densitometric data of Bax and Bcl2 protein expression are demonstrated in bar charts (D,E), respectively. Data presented in the charts are mean ± SD. COR = cordycepin, T = testosterone, FIN = finasteride. a, b, c, d or e: significantly different (p < 0.05) from control, cordycepin, testosterone, T + COR (5 mg/kg) or T + COR (10 mg/kg), respectively.
3.8. Expression of p-AKT and p-AMPK

Finally, the phosphorylated and total protein forms of AMPK and AKT were analyzed to study the cellular targets driving the beneficial effects of cordycepin that were observed throughout this study. As can be seen in Figure 5A, testosterone treatment led to a noticeable decrease in p-AMPK concomitant with an increase in p-AKT expression. However, cordycepin treatment at doses of 5 mg/kg and 10 mg/kg administered to testosterone-challenged animals resulted in significant amelioration of p-AMPK inhibition as well as p-AKT enhanced expression. The 5-α reductase inhibitor finasteride also enhanced p-AMPK expression and inhibited p-AKT protein content associated with testosterone administration (Figure 5B,C).

Figure 5. Effect of cordycepin on prostatic protein expression of p-AKT and p-AMPK (A) in testosterone-treated rats. Lanes I, II, III, IV, V, and VI represent control, cordycepin, testosterone, testosterone + cordycepin 5 mg/kg, testosterone + cordycepin 10 mg/kg, and testosterone + finasteride, respectively. Densitometric data of p-AKT and p-AMPK expression presented in bar charts (B,C) are mean ± SD (n = 6). COR = cordycepin, T = testosterone, FIN = finasteride. a, b, c, d or e: significantly different (p < 0.05) from control, cordycepin, testosterone, T + COR (5 mg/kg) or T + COR (10 mg/kg), respectively.

4. Discussion

BPH is one of the most prevalent benign tumors in men, with increasing prevalence after 40 years of age [23]. Uncontrolled cell proliferation, oxidative stress, disturbed cell apoptosis, and inflammation participate in the development of BPH [24–26]. Symptoms of BPH such as lower urinary tract symptoms can negatively influence the quality of life of affected men. The main available approaches for pharmacological management of this disease involve using adrenergic α-1 antagonists and 5-α reductase inhibitors [27]. However, it is known that these pharmacological management options may cause intolerable side effects including postural hypotension, impotence, and gynecomastia, which can affect patient compliance [28,29]. Cordycepin is a nucleoside analog that is commonly isolated from the fermentative fluid and fruiting bodies of the entomopathogenic fungus Cordyceps militaris [30]. Cordycepin has several biological and pharmacological properties reported in the literature including the regulation of steroidogenesis, cell proliferation, inflammation, and apoptosis through various signaling pathways [31–34]. Hence, our study was conducted to investigate the potential of cordycepin to mitigate BPH induced by testosterone in rats.

In this study, it was observed that treatment with cordycepin for 4 weeks significantly attenuated increased prostate weights and indices associated with testosterone administration. Furthermore, histopathological examination confirmed these results as cordycepin cotreatment was associated with almost normal acinar epithelia. In accordance with these findings, it was reported that Cordyceps militaris fruiting body extract tended to inhibit the increase in prostate weight induced by the administration of testosterone over 30 days [35]. Our findings are also in line with the established antiproliferative activity of cordycepin against various cells, including those of the prostate [36,37]. This can be explained by the ability of cordycepin to activate the A3 adenosine receptor, and its activation is associated with the inhibition of prostate cancer cell proliferation [16–18].
Several studies also highlight a major role of oxidative stress in the pathogenesis of testosterone-induced BPH [38]. Interestingly, our findings demonstrated that cordycepin possesses significant antioxidant activity as evidenced by the reduced lipid peroxidation and antioxidant enzyme exhaustion. This is in harmony with previous findings reporting the significant antioxidant activity of cordycepin [39–41]. In addition, these results are parallel with several reports correlating the anti-oxidation of many compounds with protection against PBH [42–44].

Accumulating evidence indicates a significant role of inflammation in the development of testosterone-induced BPH [45]. In this study, cordycepin demonstrated significant anti-inflammatory activity supported by the decreased expression of IL-6, TNF-α, and NF-κB in the prostatic tissues of testosterone-challenged rats. These findings gain support from previous reports highlighting a significant anti-inflammatory activity of cordycepin mediated through suppression of NF-κB signaling pathways [41,46–48]. Oxidative stress can trigger certain inflammatory pathways via activating transcription factors including NF-κB, which in turn control the gene expression of proinflammatory mediators such as TNF-α [49–51]. Hence, the decreased expression of inflammatory mediators associated with cordycepin could be partly due to its considerable antioxidant activity.

Prostatic inflammation can lead to stromal and glandular hyperplasia by disturbing the balance between cell proliferation and cell death in the prostate [52]. In the same vein, testosterone is known to induce pro-proliferative and anti-apoptotic changes in cycline-D1, PCNA, Bax, and Bcl2 mRNA content in the prostate gland [8]. In this study, cordycepin at both tested doses significantly ameliorated these pro-proliferative and anti-apoptotic changes induced by testosterone administration. These findings are in harmony with those in the literature describing the antiproliferative activity of cordycepin against prostate cells and other types of cells that involve regulating the expression of cyclin-D1, PCNA, Bax, and Bcl2 [37,53–56].

Interestingly, cordycepin was noted to inhibit cell proliferation by modulating AKT signaling in several types of cancer, including prostate cancer [57–61]. It is known that AKT signaling plays a significant role in BPH development as its overexpression is associated with an increased prostate size [62,63]. AKT signaling activation is also associated with the dysregulation of cell death by reducing the expression of Bax and inducing the expression of Bcl-2 [64]. Hence, the potential of cordycepin to inhibit AKT activity in BPH was explored in this study. It was found that testosterone alone significantly induced the expression levels of p-AKT in rat prostate. This is in line with reported findings that PI3K/AKT activation is essential for cell proliferation in testosterone-induced BPH [65]. Remarkably, cordycepin in the current study significantly attenuated this rise in p-AKT expression in the prostatic tissues of testosterone-treated rats. Moreover, cordycepin caused significant amelioration of testosterone-induced decrease in the prostatic expression of p-AMPK. Similar findings were reported in several studies where cordycepin has been shown to activate AMPK and inhibit AKT activation [58–61]. In this regard, it has been demonstrated that BPH involves lower levels of p-AMPK, and its activation has been proposed to counter-modulate the signaling of pro-inflammatory mediators [66–69]. This is also in harmony with previous findings demonstrating the beneficial effects of targeting the PI3K/AKT pathway in BPH [63,70–72]. The suggested mechanism of actions of cordycepin are illustrated in Figure 6.
Interestingly, cordycepin was noted to inhibit cell proliferation by modulating AKT signaling in several types of cancer, including prostate cancer [57–61]. It is known that AKT signaling plays a significant role in BPH development as its overexpression is associated with an increased prostate size [62,63]. AKT signaling activation is also associated with the dysregulation of cell death by reducing the expression of Bax and inducing the expression of Bcl-2 [64]. Hence, the potential of cordycepin to inhibit AKT activity in BPH was explored in this study. It was found that testosterone alone significantly induced the expression levels of p-AKT in rat prostate. This is in line with reported findings that PI3K/AKT activation is essential for cell proliferation in testosterone-induced BPH [65]. Remarkably, cordycepin in the current study significantly attenuated this rise in p-AKT expression in the prostatic tissues of testosterone-treated rats. Moreover, cordycepin caused significant amelioration of testosterone-induced decrease in the prostatic expression of p-AMPK. Similar findings were reported in several studies where cordycepin has been shown to activate AMPK and inhibit AKT activation [58–61]. In this regard, it has been demonstrated that BPH involves lower levels of p-AMPK, and its activation has been proposed to counter-modulate the signaling of pro-inflammatory mediators [66–69]. This is also in harmony with previous findings demonstrating the beneficial effects of targeting the PI3K/AKT pathway in BPH [63,70–72].

The suggested mechanism of actions of cordycepin are illustrated in Figure 6.

5. Conclusions
Overall, cordycepin offers protection against testosterone-induced BPH in rats. This protective activity can be at least partially explained by its antiproliferative, antioxidant, anti-inflammatory, and pro-apoptotic actions as well as its modulation of AMPK and AKT activation.

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