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Research Article

Effect of *Cuscuta reflexa* Extract in Mitigating Testosterone-Induced Benign Prostatic Hyperplasia in Rats: Targeting Inflammation and Oxidative Stress

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Abstract

Background: Benign prostatic hypertrophy (BPH) is a significant health issue in the aging male population. BPH prevalence is on the rise, due to an increase in modifiable metabolic risk factors. The development of BPH has been linked to inflammation, cell proliferation, and oxidative stress. ***Objective***: This study aimed to investigate the therapeutic potential of *Cuscuta reflexa* (*CR*) on testosterone-induced BPH in male Wistar rats. ***Methods***: For four weeks, the rats were given an injection of testosterone propionate (3 mg/kg/day) to cause BPH. During the study, they were also given either CR ethanolic extract (400 mg/kg/day) or finasteride (5 mg/kg/day) along with the testosterone injection. ***Results***: There was a significant rise in the prostate index, a rise in the prostate-specific antigen (PSA), and changes in the tissue that are typical of BPH after testosterone administration. Moreover, testosterone prompted an increase in inflammatory markers (interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and transforming growth factor-beta (TGF-β) and an increase in nuclear factor-κB (NF-κB) and oxidative stress indicators (increase in malondialdehyde (MDA) and decrease in glutathione (GSH)). However, intervention with *CR* extract effectively mitigated these changes, possibly via anti-inflammatory and antioxidative properties. ***Conclusions***: These findings highlight the potential of *CR* extract as adjunctive therapies for BPH, offering an approach for targeting inflammation and oxidative stress. Additionally, long-term studies are necessary to assess the safety and efficacy of Cr extract in managing BPH.

Keywords: Benign prostatic hyperplasia, *Cuscuta reflexa*, Finasteride, Testosterone.

التأثير المحتمل لمستخلص نبتة الحامول المنعكس في التخفيف من تضخم البروستات الحميد المُحفَّز بالتستوستيرون في الجرذان من خلال استهداف الالتهاب والإجهاد التأكسدي

الخلاصة

الخلفية: يمثل تضخم البروستات الحميد مشكلة صحية كبيرة لدى الذكور مع تقدم العمر. وتشير البيانات إلى أن انتشار تضخم البروستات الحميد في تزايد، ويعزى ذلك إلى زيادة عوامل الخطر الأيضية القابلة للتعديل. وقد ارتبط تطور تضخم البروستات الحميد بالالتهاب، تكاثر الخلايا، والإجهاد التأكسدي. **الهدف**: التحقق من الإمكانات العلاجية لنبات الحامول المنعكس *Cuscuta reflexa (CR)* في علاج تضخم البروستات الحميد المُحفَّز بالتستوستيرون في ذكور جرذان ويستار. **الطرائق**: تم إعطاء الجرذان التستوستيرون بروبيونات (3 ملغ/كجم/يوم) لمدة أربعة أسابيع لتحفيز تضخم البروستات الحميد، مع علاجها بمستخلص الحامول المنعكس الإيثانولي (400 ملغ/كجم/يوم) أو الفيناستراید (5 ملغ/كجم/يوم) بالتزامن مع حقن التستوستيرون طوال مدة الدراسة. **النتائج**: أظهرت النتائج زيادة كبيرة في مؤشر البروستات، وارتفاع في مستويات مستضد البروستات النوعي (PSA)، وتغيرات نسيجية مرضية مميزة تشير إلى تضخم البروستات الحميد بعد إعطاء التستوستيرون. بالإضافة إلى ذلك، تسبب التستوستيرون في زيادة في علامات الالتهاب (إنترلوكين-6، عامل نخر الورم-ألفا، عامل النمو المحول-بيتا، وزيادة في عامل نووي κB ، بالإضافة إلى تغير في مؤشرات الإجهاد التأكسدي (زيادة في المالونداييهايد وانخفاض في الجلوتاثيون). ومع ذلك، أدى التدخل بمستخلص *CR* إلى التخفيف الفعال من هذه التغيرات، وذلك على الأرجح من خلال خصائصه المضادة للالتهاب والأكسدة. **الاستنتاجات**: تسلط هذه النتائج الضوء على الإمكانات العلاجية لمستخلص الحامول المنعكس كعلاج مساعد لتضخم البروستات الحميد، حيث يوفر نهجًا يستهدف الالتهاب والإجهاد التأكسدي. هناك حاجة إلى مزيد من الأبحاث لتوضيح الآليات الكامنة وراء هذه التأثيرات العلاجية والتحقق من صحة هذه النتائج في الدراسات السريرية.

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INTRODUCTION

Benign prostatic hyperplasia is a common urological disorder among men. It is associated with increased proliferation of prostatic epithelial and stromal cells, resulting in increasing their size and number and enlargement of the prostate [1,2]. BPH etiology is believed to be associated with hormonal changes in aging men [3,4]. It's age-related; nevertheless, other factors, including metabolic syndrome, inflammation, hormonal alterations, and growth factors, contribute to its development and regulation [4]. Until now the precise pathophysiology remains unknown, but the androgen system and androgen receptor are involved.

In addition, metabolic factors and prostatic inflammation are being identified as contributors to benign prostate enlargement and LUTS [4-7]. 5α-reductase inhibitors, such as finasteride, stop androgen signaling by stopping testosterone from changing into its active form, DHT. This lowers the levels of DHT, which shrinks an enlarged prostate and stops the disease from getting worse to BPH [8,9]. It is often difficult to achieve satisfactory efficacy with a single drug in LUTS-BPH patients, and patients often discontinue treatment due to the side effects [10]. Herbal remedies are used all over the world for the treatment of numerous human disorders. Furthermore, natural products are known to play an important role in the treatment of chronic disorders [11]. In recent years, phytochemicals originating from vegetables, fruits, and tea have gained significant interest for researchers [12]. *Cuscuta*, commonly known as dodder, is a genus of the Convolvulaceae family, which contains about 170 species. Phytochemical investigations have confirmed the existence of biologically active moieties like flavonoids, phenolics, alkaloids, saponins, tannins, and fatty acids [13]. *Cuscuta reflexa* is a perennial parasitic herb characterized by slender, elongated yellow stems that belong to the Convolvulaceae family. It is distributed in tropical regions in South Asian countries like Pakistan and India [11,14]. CR is a treasured medicinal herb extensively utilized in traditional medical systems for the treatment of numerous disorders [15]. Various pharmacological studies have revealed the use of this herb as an antibacterial, antioxidant [16], anticholinergic, antihistaminic, and antihypertensive agent [17] and for the treatment of several illnesses, including alopecia, diabetes, tumors, and urinary infections [14,18-20]. CR is known to contain a number of alpha-glucosidase inhibitory compounds. A new flavanone, reflexin, tetrahydrofuran derivatives, and coumarin have been isolated from the stems of the plant. Methanol extracts of the stem reportedly demonstrated anti-steroidogenic and antibacterial activities [21]. It has been documented for its antioxidant properties and its suppression of the 5α-reductase enzyme in prior research [14,20]. This study aims to investigate the therapeutic potential of *Cuscuta reflexa* (CR) on testosterone-induced BPH in male Wistar rats.

METHODS

Chemicals

Testosterone propionate was obtained from Aspen Pharmacare Holdings, Germany; finasteride powder from Hangzhou Hyper Chemicals, China; and *CR* was obtained as a dried herb from Jadibootikart, a raw ayurvedic store, India. Assistant Professor Dr. Sukeyna Abaas Aliwy then authorized the identity of the plant at the Department of Biology, College of Science, University of Baghdad (voucher number: 393, date: 14-Feb.-2024).

Extraction of Cuscuta reflexa

The stems of dried *CR* were ground to get the coarse powder. The powder had been extracted with 95% ethanol using Soxhlet's apparatus for 8 hrs., according to the method done by Patel *et al*. [22] with slight modification, then filtered and dried using a rotary evaporator. The weight of the extract was 15g for each 250 g of the herb powder (yield 6% w/w).

Study design

In this randomized controlled trial study, male Wistar rats weighing between 200 and 250 g, obtained from the animal house of the Biotechnology Research Center, Al-Nahrain University, Baghdad/Iraq, were housed under controlled conditions, maintaining a temperature of 22 ± 2 °C with alternating 12-hour light and dark cycles. The animals were given seven days to acclimate before the experiment began at the same facility. Rats had been randomly allocated into groups, kept in cages at the animal house of the College of Pharmacy, Al-Farahidi University, Baghdad, Iraq, with free access to diet and water. Thirty-two rats were subdivided randomly into four groups, each group containing eight rats (n=8 per group). All treatments had been given once daily in the morning, 5 days/week for four consecutive weeks. The study design was shown in Figure 1.

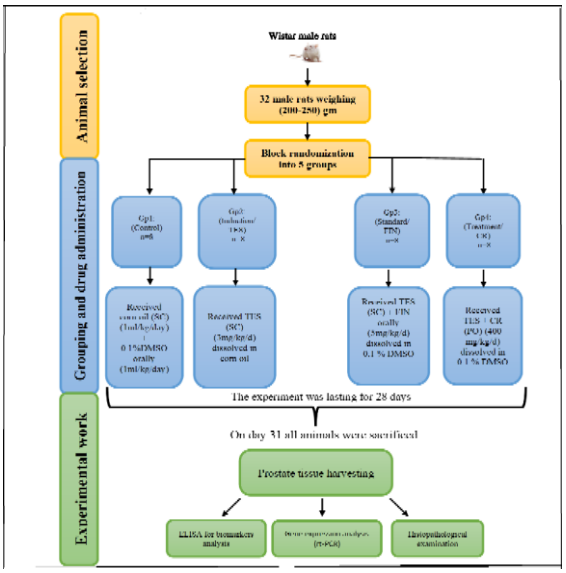

Figure 1: Flow chart of the study.

Regarding the allocation of rats, Group I, the control group (CON), received corn oil (1 ml/kg/day) SC and 0.1% dimethyl sulfoxide (DMSO) (1 ml/kg/day) orally; Group II, the induction group (TES), received 3 mg/kg/day testosterone (TES) SC dissolved in corn oil for 4 weeks to induce BPH [23]; Group III, the finasteride group, received 5 mg/kg/day finasteride orally [23] dissolved in 0.1% DMSO concurrently with testosterone as described above and served as the standard group; and Group IV, the *Cuscuta reflexa* group (*CR*), received 400 mg/kg/day *CR* ethanolic

extract [24] orally dissolved in 0.1% DMSO concurrently with testosterone as described above.

Tissue sampling and processing

At the end of the experiment, rats were anesthetized by ketamine/xylazine (10%) IM injection 72 h after the last SC injection; the abdomen was dissected by a midline incision, then the prostates were isolated for morphological assessment and weighing. All efforts were made to minimize animal suffering. Regarding ELISA assessment, each 100-200 mg of tissue was mixed with chilled PBS and minced into small pieces, then homogenized by the homogenizer machine; the homogenate was centrifuged for 20 minutes at 4°C and 2000 rpm in a cold centrifuge, and the supernatant was used. Regarding RT-PCR analysis, 20 mg of prostate tissue was added to 1 ml of TRIzol solution, frozen, homogenized via the homogenizer machine, and then centrifuged.

Assessment of prostate weight and index

The rats under anesthesia were weighed. Subsequently, prostate tissues were excised and weighed individually. The prostate index was determined by dividing the prostate weight by the total body weight of the rat [25].

Assessment of biochemical markers

The supernatant of tissue homogenate was collected and assessed for level TNF-α using ELISA kits from Cloud-Clone Corp., USA, Cat.No. The IL-6 and TGF-β were using ELISA kits from BT Lab, China, Cat.No. E0135Ra and Cat.No. E0778Ra, respectively. The supernatant was separated, and the reduced glutathione (GSH) and malondialdehyde (MDA) were assessed. The tests were conducted using kits from Cloud-Clone Corp., USA, Cat.No. CEA294Ge and Cat.No. CEA597Ge, respectively. Additionally, the supernatant was used to assess the levels of PSA using ELISA kits from Cloud-Clone Corp., USA, Cat.No. #SEA151Ra.

RT-qPCR analysis of NF-κB gene expression

We used the cDNA synthesis kit, and the Trans Start® Top-green qPCR Super Mix kit (TransGen Biotech., China) to do the experiment. To look at the data, we used the ΔΔCt method [26]. Results were presented as relative fold changes compared to the gene expressions of the control group (Table 1).

Table 1: Primers used for RT-qPCR analysis

Type of primer	Sequence
GAPDH primer	F: 5′ - CGGGTTCCTATAAATACGGACTG-3′ R: 5′ - CCAATACGGCCAAATCCGTTC-3′
NF-κB primer	F: 5′ -AAGACAAGGAGCAGGACATG-3′ R: 5′ -AGCAACATCTTCACATCCC-3′

Histopathological examination

Hematoxylin and eosin (H&E)-stained slides were utilized for routine histopathological assessment. ImageJ software (National Institutes of Health, USA) was utilized to assess epithelial thicknesses.

Ethical consideration

The study was approved by the Research Ethics Committee at the College of Medicine, Al-Nahrain University, following approval from the Institutional Review Board (IRB) committee (animal ethical approval number: 202207175, date: 6th November 2022).

Statistical analysis

The data collected from several groups were analyzed statistically by using GraphPad Prism 9.5.1 software (GraphPad Software, San Diego, CA). The data analysis utilized one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. The data were presented as the mean value ± standard deviation (SD), and the statistical significance was determined at $p< 0.05$.

RESULTS

The levels of prostate index in rats injected with TES were significantly elevated compared with rats in the control group, which suggested that TES successfully induced BPH. Nevertheless, administration of *CR* extract led to a significant decrease in prostate index compared to the BPH group ($p< 0.05$) (Figure 2).

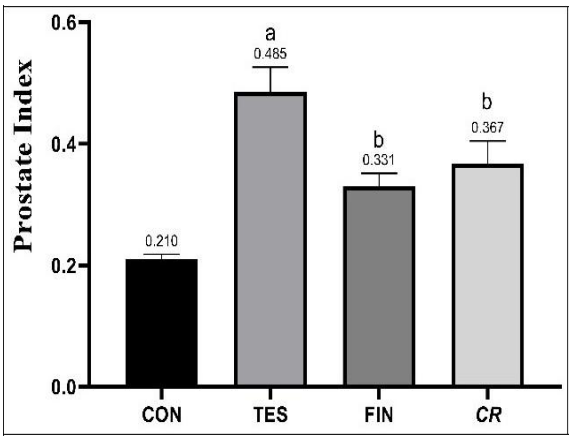

Figure 2: Effect of *CR* on prostate weight index. The data are expressed as mean ± SD and analyzed using one-way ANOVA, followed by Tukey's multiple comparisons test. Significant differences were denoted as follows: [a] significantly different compared to the CON group ($p < 0.05$), [b] significantly different compared to the BPH group ($p<0.05$).

As shown in Figure 3, compared with the control group, TES significantly increased the levels of IL-6, TNF-α, and TGF-β, respectively ($p< 0.05$). However, treatment with CR extract resulted in a significant reduction in these markers compared to the TES group ($p < 0.05$).

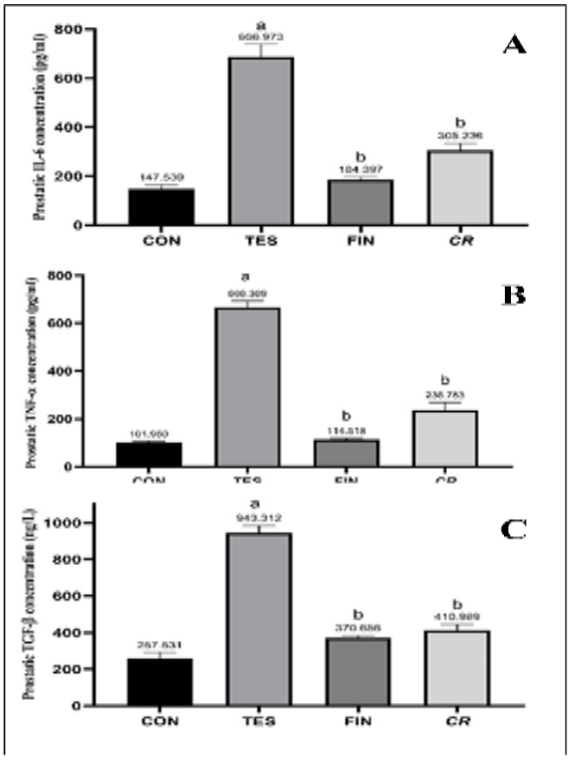

Figure 3: Effect of CR on inflammatory markers in prostatic tissue. **A)** IL-6; **B)**TNF-α; and **C)** TGF-β. The data are expressed as mean±SD and were analyzed using one-way ANOVA, followed by Tukey's multiple comparisons test. Significant differences were denoted as follows: [a] significantly different compared to the CON group (p<0.05), [b] significantly different compared to the BPH group (p<0.05).

The BPH group revealed a significant decrease in GSH and a significant elevation in tissue content of MDA compared to the control group (p< 0.05). The CR group resulted in a significant increase in GSH and a significant reduction in the MDA marker compared to the TES group (p< 0.05), as shown in Figures 4A and 4B, respectively.

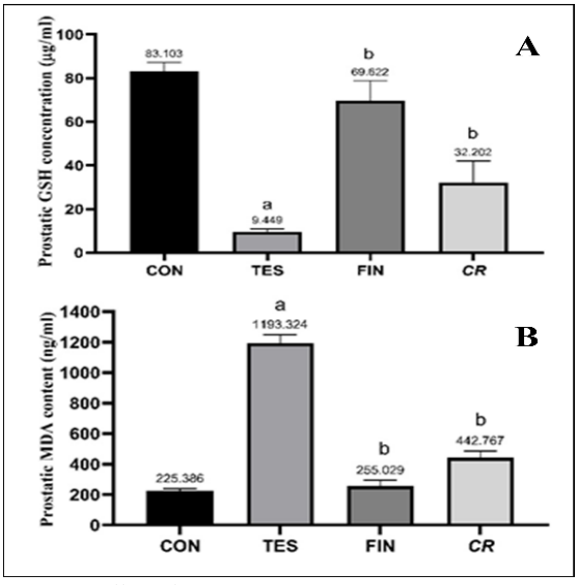

Figure 4: Effect of *CR* on oxidative stress markers in prostatic tissue. **A)** GSH; and **B)** MDA. The data are expressed as mean±SD and were analyzed using one-way ANOVA, followed by Tukey's multiple comparisons test. Significant differences were denoted as follows: [a] significantly different compared to the CON group (p<0.05), [b] significantly different compared to the BPH group (p<0.05).

The concentration of prostate tissue PSA was significantly higher in the BPH group than in the control group (p< 0.05). However, the rats that received *CR* revealed a significant reduction in prostatic PSA as shown in Figure 5.

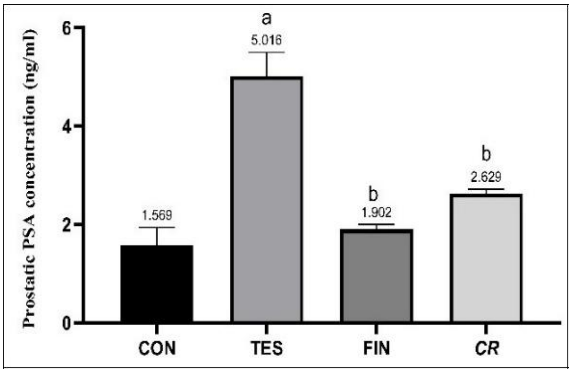

Figure 5: Effect of *CR* on PSA marker in prostatic tissue. The data are expressed as mean±SD and were analyzed using one-way ANOVA, followed by Tukey's multiple comparisons test. Significant differences were denoted as follows: [a] significantly different compared to the CON group (p<0.05), [b] significantly different compared to the BPH group (p<0.05).

The gene expression of NF-κB was significantly higher in the induction group (p< 0.05). Nevertheless, the administration of *CR* extract showed a significant reduction as compared to the BPH group (p< 0.05) (Figure 6).

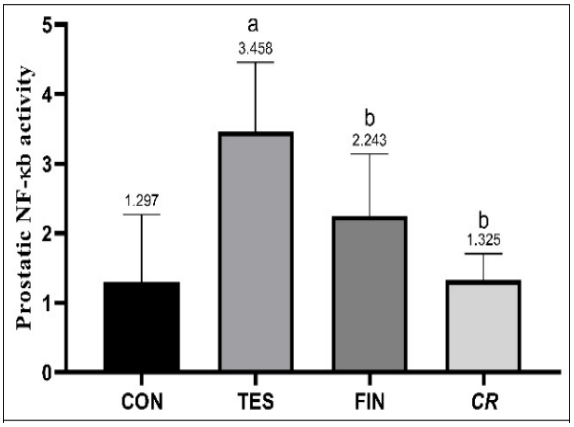

Figure 6: Effect of *CR* on gene expression of NF-κB in prostatic tissue. The data are expressed as mean±SD and were analyzed using one-way ANOVA, followed by Tukey's multiple comparisons test. Significant differences were denoted as follows: [a] significantly different compared to the CON group (p<0.05), [b] significantly different compared to the BPH group (p<0.05).

A histopathological examination of prostate tissue was carried out to find out more about how the CR ethanolic extract affected BPH caused by testosterone in rats. Figure 7A shows a tissue section from a rat in the control group. It shows a normal prostate gland (green arrow) that is lined with stroma (black arrow) and a doubled cell layer of epithelial cells (red arrow) and basal cells (yellow arrow). A part of the testosterone group showed clear hyperplasia of glands (more of them) (green arrow) and multilayering of epithelial cells (black arrow) (Figure 7B). The finasteride group has the right number of glands, and the black arrow shows that the glands are lined with mild multilayering of epithelial cells. Additionally,

Figure 7D shows that the CR group section exhibits mild gland hyperplasia along with moderate multilayering (green and black arrows). The thickness of glandular epithelium is illustrated in Figure 7E.

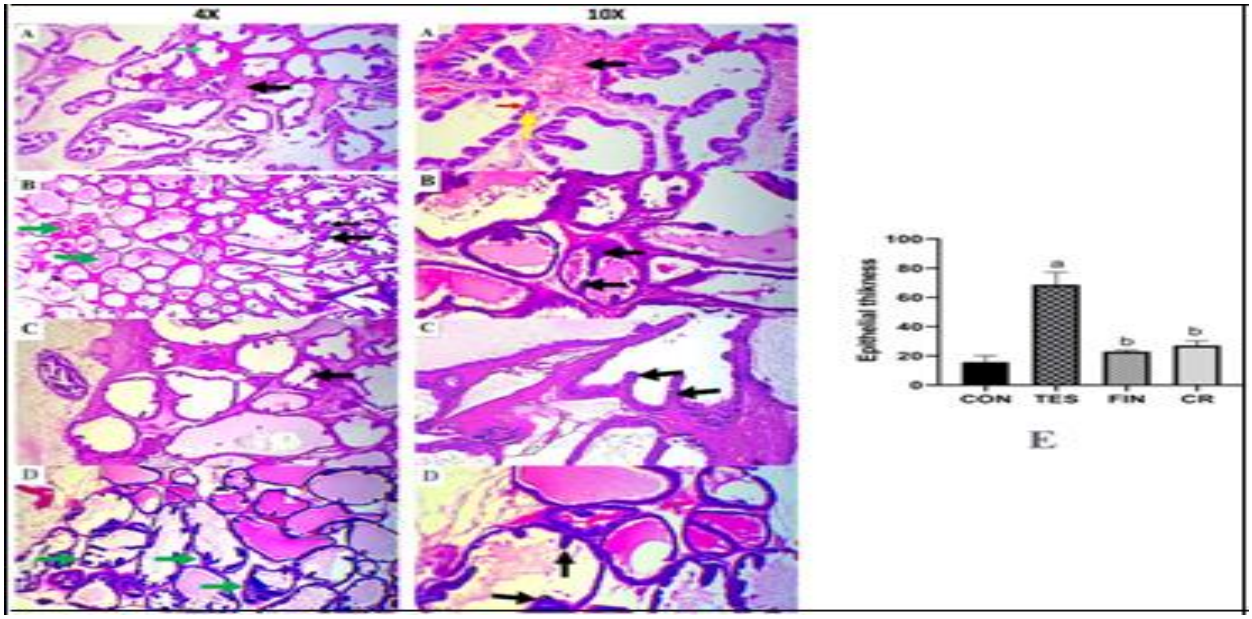

Figure 7: Histopathological sections of rat prostatic tissue: **A)** control group, **B**) BPH group, **C**) finasteride (standard) group, **D**) *CR* treatment group; H&E stain (4X & 10X), and **E**) Prostate glandular epithelial thickness.

DISCUSSION

Benign prostatic hyperplasia is a type of prostate adenoma that is shown by the growth of stromal and epithelial cells in the transitional zone of the prostate gland [27]. Notably, herbal medicines have been used extensively to treat ailments and improve health. This corresponds with the heightened global interest and desire for safe, economical, and efficacious medicinal approaches [27,28]. The goal of the study was to investigate the therapeutic potential of CR ethanolic extract in reducing testosterone-induced BPH. Notably, this research is regarded as the first of its kind in Iraq, focusing on the impact of new agents aimed at treating BPH, which have been experimentally developed in rats. This study provided more information on the biochemical and pathological changes connected to BPH caused by testosterone. It confirmed the results of earlier studies [23,29–31]. The current investigation documented prostate hyperplasia after testosterone injection, as indicated by significant increases of the prostate index, PSA levels, histopathological examination, and measurement of epithelial thickness. These findings were confirmed by revealing characteristics indicative of BPH. Nevertheless, pretreatment with CR extract reduced the severity of all these effects. Analogous results have been documented in prior research about testosterone, and the alleviating impacts were ascribed to investigational protective medications that operated via distinct mechanistic pathways [1,23,29,31]. The contribution of the androgen system to the development of BPH is associated with DHT and the resulting inflammation of the prostate. Several research studies from animal models have shown that testosterone therapy can contribute to BPH [6,7,32]. The research by Pandit *et al.* [14] showed that the petroleum-ether extract of CR had strong hair-growing properties by stopping androgen-induced alopecia and increasing hair growth. It can be posited that one mechanism through which *CR* extract may mitigate BPH, like finasteride, is by the inhibition of 5α-reductase. Several studies have revealed that BPH development is attributed to chronic inflammation, hormonal imbalance, metabolic syndrome, and age [3,33,34]. Moreover, its pathogenesis may link to oxidative stress and imbalance between cell proliferation and apoptosis [23]. Cytokines prostatic inflammatory cells such as interleukins (IL-1β, IL-6) and TNF-α may stimulate proliferation in both epithelial and stromal compartments, thereby enhancing the prostatic tissue proliferation and potentially mediating prostate volume enlargement under inflammatory conditions in patients with BPH [35-37]. The IL-6 is a pleiotropic pro-inflammatory cytokine that facilitates acute phase reactions, hematopoiesis, and specific immunological responses [38]. TNF-α is a key pro-inflammatory cytokine that stimulates inflammation through many pathways [39]. In the current research, in the BPH group there was an elevation in the level of IL-6 and TNF-α, while with treatment with *CR* extract there was a significant decrease in these cytokine levels. Inconsistent with Rho *et al*. [29], who found that testosterone injection for rat models increased the expression levels of IL-6, IL-1β, and TNF-α, while in the treatment groups there was a significant reduction in such cytokine levels. This is supported by the approved anti-inflammatory effects of aqueous and alcoholic extracts of *CR* stems that were shown to have significant anti-inflammatory activity against a paw edema model in a rat model [40]. The TGF-β plays a crucial role in the development of cell growth and immune function. It had been reported to be upregulated in BPH samples, and their overexpression can induce inflammation and fibrosis in a murine model [41,42]. Ficarra *et al*. [43]

suggested a theory, which revealed that interleukins, like IL-6, stimulate androgen receptors and promote further trans-differentiation leading to TGF-β production induction. NF-kB is one of the major transcription factors that have a role in many cellular processes, such as cell growth, apoptosis, and inflammation. Its activation is stimulated by other proinflammatory mediators, especially IL-6, and is known to promote prostate carcinogenesis progression of BPH [44,45]. Since the activated NF-κB is involved in numerous stages of the inflammatory-proliferative process as described by Baeuerle and Henkel [46], therefore, inhibition of NF-κB activation may be a promising strategy to reduce inflammation in BPH. Thus, all these data strongly show that the NF-κB pathway plays a crucial role in BPH development and *CR* extract may have a protective effect against inflammation. Also, the *CR* group showed a significant reduction in PSA levels when compared to the BPH group, suggesting its protective effects on hypertrophy of the prostate induced by testosterone. The oxidative marker MDA can change the innate immune system's inflammatory response through several signaling pathways, such as NF-κB [47]. Stems collected from different *Cuscuta* species were analyzed for quantity of phenolic and flavonoid content and their antioxidant capacity [48]. An earlier study revealed that the water extract of *CR* possesses anti-cancer and anti-inflammatory activities via downregulation of TNF-α and COX-2 genes. Also, it was able to block the binding of NF-κB to its DNA binding motifs, making it evident that the extract downregulates TNF-α and COX-2 via NF-κB inhibition [49]. Treatment with *CR* extract significantly restored the oxidative stress markers that may be contributing to its antioxidative activity. In consistence with Savitha *et al*. [48] and Sharma *et al*. [20], who investigated the antioxidant activity of *CR* stems and showed that ethanol extract had an antioxidant effect, which aligns with the current study. All the previous results, along with histopathological investigation, demonstrated that the *CR* extract could have promised an antiproliferative effect against BPH.

Study limitations

It is worth noting that the present study used single doses; it would be optimal if multiple dosages were utilized; however, to minimize the cost and reduce the number of animals used, a single dose was chosen, serving as a preliminary study for future research. Although animal models offer significant insights into human diseases, they may not be completely valuable insights into human diseases; they may not completely stand for the complexity of BPH in humans. The study did not include clinical data from human participants. Despite the results in the rat model being promising, further research is needed to determine the safety and efficacy of *CR* extract in human subjects.

Conclusion

The study's results show that CR ethanolic extract may be useful as a medicine to treat testosterone-induced BPH in animals by reducing inflammation, protecting cells from damage, and stopping cell growth. CR extract offers promising avenues for the management of BPH. Further research is warranted to validate these findings in clinical settings and elucidate the underlying mechanisms driving the observed therapeutic effects.

Conflict of interests

The authors declared no conflict of interest.

Funding source

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Data sharing statement

Supplementary data is available from the corresponding author upon reasonable request.

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