Original Article

Diacerein ameliorates benign prostatic hyperplasia with dysplastic changes in rats via modulation of IL1β and Nrf2/HO1 pathway

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Abstract
Benign prostatic hyperplasia (BPH) is a common disrupting health problem affects elderly men. However, different mechanisms underlying the pathogenesis of BPH are still unclear. For the first time, we tried to explore the role of interleukin 1β (IL1β) antagonist, diacerein (DIA) in BPH and different pathways involved in mediating such effect. BPH was induced using testosterone propionate (TP) (5 mg/kg/day) for 4 weeks subcutaneous injection followed by DIA (50 mg/kg/day) orally for 4 weeks. We evaluated the histopathological changes, prostate weight, dihydrotestosterone (DHT), prostate specific antigen (PSA), and total antioxidant capacity (TAC) in serum. Moreover, malondialdehyde (MDA), heme oxygenase 1 (HO1), nuclear factor erythroid 2–related factor 2 (Nrf2), caspase3, IL1β expressions, and proliferating cell nuclear antigen (PCNA) were measured in tissue specimens. Our results confirmed the induction of BPH by histopathological examination which revealed typical changes of BPH with dysplastic features. Moreover, there are significant increases in prostate weights, DHT, PSA, PCNA, MDA, and IL1β but significant decreases in TAC, HO1, Nrf2 and caspase 3. Interestingly, DIA significantly improved BPH induced histopathological and biochemical changes that is not differing from control group without dysplastic changes. This is mostly attributed to blocking of IL1β, anti-oxidant, apoptotic properties with activation of Nrf2/HO1 pathway.

Key Words: Diacerein, Benign prostatic hyperplasia, Prostatic specific antigen, Proliferating cell nuclear antigen, Interleukin 1β

Introduction
Benign prostatic hyperplasia (BPH) without dysplasia is considered as a noncancerous disorder resulting from the imbalance between two processes; cell proliferation and apoptosis with increased size and number of epithelial and stromal cells complicated with urine retention, dysuria and repeated infection. The key factors in the pathogenesis of the disease are high level of testosterone and dihydrotestosterone (DHT) with stimulation of a vital proliferating agent, proliferating cell nuclear antigen (PCNA) that is responsible for most of hyperplastic disorders such as BPH and endometrial hyperplasia[1,2] accompanied with imbalance between oxidant and antioxidant status, excessive release of free radicals and suppression of apoptosis[3,4].

Oxidative DNA damage is usually preceded by formation of reactive oxygen species (ROS) which is considered as an important mutagenic factor. Antioxidant enzymes as heme oxygenase 1(HO1), nuclear factor erythroid 2–related factor 2 (Nrf2), glutathione peroxidase and superoxide dismutase play a necessary role in preventing the harmful effects of free radicals[5]. Moreover; inflammatory cells like macrophages, T lymphocytes, B lymphocytes, and mast cells are excessively detected in the prostate of BPH patients so chronic inflammation is another contributing factor with profound formation of pro-inflammatory cytokines such as tumor necrosis factor α (TNFα) and interleukins followed by more free radicals release, DNA damage and improper

DNA repair associated with different mutations and rapid cell division[1,2,6,7].

IL1 including IL1β causes the rapid induction of a wide variety of genes encode inflammatory cytokines which stimulate macrophages activation with proliferation and stimulation of T lymphocytes[8] involved in the pathogenesis of different diseases including BPH via NLRP3 inflammasome/caspase1 pathway[8-11].

Diacerein (DIA) is an anthraquinone derivative with non-steroidal anti-inflammatory effect and good efficacy and safety profile in the treatment of osteoarthritis. In addition; DIA has unique biological activities and pharmacological actions including anti-oxidant, anti-inflammatory, apoptotic, anti-catabolic, but pro-anabolic properties. The principal pharmacological effect of DIA is to inhibit the IL1β system and the downstream signaling pathways[12,13]. It inhibits IL1β production, activation and binding with its receptors with suppression of ROS production. Furthermore, it was found that DIA could improve and modulate several pathological conditions including testicular toxicity, breast cancer, psoriasis, endometrial hyperplasia and cervical hyperkeratosis[13]. This is based on its anti-inflammatory properties as it could inhibit neutrophil activation; suppress the release of more cytokines and its antioxidant activity.

There is significant correlation between high levels of IL1β and occurrence of BPH. Also, DIA has potent pharmacological properties that could modulate various pathways of BPH. This forced us to evaluate the possible curative effect of DIA in treating induced BPH and explore the different mechanisms mediate such effect with more impact to study the role of Nrf2/HO1 signaling pathway.

Materials and Methods

Design of the study

Our experiment was conducted on adult male Wistar albino rats aged about eight weeks purchased from the animal house, Giza, Egypt. The average weight of the animals was 250–300 g. The duration of acclimatization for rats was one week and kept in standard housing conditions, 3 rats/cage. Animals received suitable diet (chow and tap water) with 12 h light/dark cycle. Our experiment was conducted in the Pharmacology Department, Faculty of Medicine, Minia University, Egypt. The protocol of our experiment was approved by the faculty board.

Rats were randomly divided into 4 groups (n = 9 each group)

Group I: 1 ml of 1% Carboxymethylcellulose orally for 4 weeks[8].

Group II: TP (5 mg/kg/d) subcutaneous injection for 4 weeks[7].

Group III: TP (5 mg/kg/d) subcutaneous injection for 4 weeks[7] then DIA (50 mg/kg/d orally) for 4 weeks[8].

Group IV: TP (5 mg/kg/d) subcutaneous injection for 4 weeks[7] then left without treatment for 4 weeks.

Collection of samples and storage

At the end of the experiment, each rat was weighed then anesthetized by *i.p*. injection of 20% urethane hydrochloride (1gm/kg). Blood samples were collected from abdominal aorta then centrifuged at 5000 rpm for 15 min (JanetzkiT30 centrifuge, Germany). The sera of the samples were separated and kept at−80°C till used for biochemical analysis. The Ventral lobe of each prostate was washed with saline solution then weighed on Mettler Toledo scale, Switzerland. Part of ventral lobe was homogenized (Glas-Col homogenizer, USA) in ice cold phosphate buffer solution (1:5 wt/vol) for biochemical analysis and centrifuged at 4000 rpm for 20 min at 4 ◦C in cooling centrifuge (Ray Wild TGL-16, Germany) and the supernatant was kept at−80◦C till used. Part of the ventral lobe of each animal was taken and fixed in 10% formalin and embedded in paraffin for histopathological examination.

Chemicals

DIA was obtained from EVA Pharma Company, Egypt and testosterone propionate (TP) was from Cid Company, Egypt. The caspase3 antibody (polyclonal, Ready to use), immunostaining detection kit and quantitative real-time polymerase chain reaction (rt PCR) detection kit were purchased from Thermo Fisher Scientific Inc./Lab Vision (Fermont, USA). ELISA kit of HO1 was purchased from Glory Science Co. Ltd., China and PCNA was from MyBioSource (California, USA). Prostate specific antigen (PSA) and DHT ELISA kits were obtained from US biological life sciences, USA. Total antioxidant capacity (TAC) measuring kit was from Biodiagnostic, Egypt.

Histopathological evaluation

Immediately after sacrifice, part of the ventral lobe of the prostate was dissected. Prostatic tissue was fixed in formalin 10% solution for 24 hours, processed, and embedded in paraffin wax. Five µm serial sections were prepared and stained with hematoxylin and eosin. Assessment of these sections was performed with light microscopy using an Olympus microscope, Japan. The examiner was blinded to the treated groups.

Immunohistochemistry

Five µm thick sections were placed on positively charged slides. Then sections were de-paraffinized with xylene and rehydrated with graded ethyl alcohol. Endogenous peroxidase activity was inactivated by treatment with 3% hydrogen peroxide for 30 minutes then washed in phosphate-buffered saline (PBS) solution. Sections were then boiled in a citrate buffer (pH 6.0) for antigen retrieval.

Sections were also incubated in a humidity chamber overnight by using a cleaved caspase3 primary antibody followed by applying secondary antibody. Sections were washed in PBS and incubated with the streptavidin-biotin complex reagent. 3, 3-diaminobenzidinetetra hydrochloride (DAB) for 5 minutes, then washed in distilled water, counterstained with Mayer's haematoxylin, dehydrated, cleared in xylene, mounted and covered slipped.

Tissue section was screened for positive cells, defined as cells with cytoplasmic staining considered positive when >10% of the cells were positive [14]. The positive cells were counted in randomized non-overlapping 10 high-power fields for each specimen of all groups at magnification ×400. The examiner was blinded to the different treated groups.

Biochemical measurements

Measuring serum TAC, DHT, PCNA and PSA

PCNA, DHT and PSA were measured according to ELISA kit (US biological life sciences, USA). TAC was detected according to the manufacturer's instructions.

Measurement of HO1 in prostate tissue samples

Assessment of tissue HO1 level was detected using a spectrophotometric kit. Results were expressed as µmol/g tissue.

Evaluation of malondialdehyde (MDA) Levels

For evaluating the extent of membrane lipid peroxidation a spectrophotometric method based on the reaction between thiobarbituric acid and MDA was used. The absorbance values were determined at 535 nm using Bechman DU-64 spectrophotometer, USA then the value of blank absorbance was subtracted from the sample value. Standard curve was used to evaluate MDA concentration from the corresponding absorbance by using regression line[15].

rtPCR for the relative quantification of PCNA and Nrf2 gene expression

The SYBR-green technique was used in two-steps reaction. Total RNA of all samples was extracted from the homogenized prostatic tissue using Ribozol solution (Amresco, Solon, USA) according to the manufacturer's instructions.
cDNAs were synthesized by SensiFAST TM cDNA synthesis Kit (Bioline). It was synthesized at 42 °C for 15 min then at 85°C for 5 min followed by cooling on ice. rtPCR was detected using 10 µL of SYBR Green QPCR Mix (SensiFAST™ SYBR ® Lo-ROX Kit, Bioline). The SYBR green data were analyzed by relative quantification to GAPDH (Glyceraldehyde-3-phosphate dehydrogenase) as reference gene.

The sets of primers used were as follows:
GAPDH sense primers: 5′ GTCGGTGTG AACGGATTTG3′, **GAPDH antisense** 5′ CTTGCCGTGGGTAGAGTCAT 3′.
PCNA sense primers: 5′ GAGCAACTTGG AATCCCAGAACAGG 3′, **PCNA antisense** 5′ CCAAGCTCCCCACTCGCAGAAAACT 3′.
Nrf2 sense primers: 5′ CACGGATGATG CCAGCCAG3', **Nrf2 antisense** 5′GCCCGC CCAGAAGTTCAGAGAG3'.
The relative expression level of the gene was calculated by the formula $2^{(-\Delta\Delta Ct)}$ according to Van Guilder et al.,[16]. They were scaled relative to controls and control samples were set at a value of 1. Results of the samples were graphed as relative expression compared with the control.

Western blotting

IL1β expression in prostate tissue was estimated by western blotting method as previously described [17]. Prostate tissue was homogenized in a buffer containing 0.15 M NaCl, 20 mM Tris-HCl (pH 7.5), 0.1% SDS, 0.5% Triton X-100, 1 mM EDTA, and protease inhibitor cocktail (Nacalai Tesque, Inc., Kyoto, Japan) at 1:200 dilution. The total protein was detected then an equal amount of protein from each sample was loaded per lane and separated on a 10 % SDS–PAGE gel. The protein bands were transferred to a nitrocellulose membrane using a semi-dry blotter (Bio-Rad). The blot was probed with primary antibody overnight at 4 °C and diluted 1:500 in the blocking buffer. The used primary antibodies were rabbit polyclonal anti- IL1β antibody (Neo Markers Co., Canada), and mouse monoclonal anti-β-actin antibody (Sigma-Aldrich Co., USA).

Subsequently, incubation of the blot was performed for 1 h at room temperature with alkaline phosphatase (AP)-coupled secondary antibody (anti-rabbit or anti-mouse antibody, respectively), each of them diluted 1:2000 in the blocking buffer. The blot could be blocked by using TBS-T buffer containing 5% skim milk powder for 1 hr at room temperature. Finally, blots were analyzed by using BCIP/NBT (5-bromo-4-chloro-3'-indolyphosphate and nitro-blue tetrazolium) colorimetric detection method. Protein bands were analyzed by using Image-J software (Image J 1.48V, Wayne Rasband National Institutes of Health, USA) and GraphPad Prism (version 6.0; San Diego, California, USA).

Statistical Analysis

One way ANOVA then Tukey's multiple comparison test was used for data analysis. The values of the results were expressed using mean ± SEM. Version 5 of GraphPad Prism software was used for statistical analysis. The difference was considered significant if the P value less than 0.05.

Results

Histopathological evaluation (Fig.1)

Examination of hematoxylin and eosin slides of group I (control group) showed normal prostate architecture, the acini were lined by two layers; inner simple cuboidal epithelium and outer flattened cells (Fig.1.A). In group II,IV (TP given groups), exhibited proliferation of glandular epithelium and the acini showed many long branched papillary folds with fibrovascular cores. The stroma showed congested blood vessels and inflammatory cells infiltration. Some foci of epithelial stratification with hypertrophy of the lining, crowding, nuclear pleomorphism (Fig.1.B,D). Treatment with DIA (50 mg/kg/d) for 4 weeks to BPH induced group (group III) showed marvelous improvement in the histological appearance of the prostatic acini. The epithelial height reduced and the intraluminal papillae diminished (Fig.1.C).

Immunoexpression of cleaved caspase3 (Fig 2.A)

Group I (control) showed negative expression (Fig 2. A). (Fig 2.B &D) Group II,IV (BPH induced group) showed negative expression. (Fig 2.C) Group III (BPH induced group+DIA treatment) showed positive immunoexpression**.**

Semiquantitative analysis of cleaved caspase3 (Fig.2.B)

Results showed significant increase in cleaved caspase 3 immunoexpression in BPH treated group with DIA (group III) in comparison with group II, IV.

Evaluation of prostate weight, PCNA, serum DHT and PSA

Group II, IV group showed significant increases in prostate weight, PCNA, serum DHT and PSA compared to control group (group I). However, co-administration of DIA (group III) showed significant decreases in the measured parameters compared to group II, IV and insignificant from control group (group I) (table 1).

Table (1): Evaluation of prostate weight, PCNA, serum DHT and PSA

Group	Prostate/mg Weight/mg	DHT pg/ml	PSA pg/ml	PCNA ng/ml
Group I	330.6.±13.09^{bd}	1.7±0.09^{bd}	2.8±0.11^{bd}	0.3±0.10^{bd}
Group II	494.8±17.33^{ac}	2.6±0.16^{ac}	4.3±0.20^{ac}	8.7±0.40^{ac}
Group III	348.3±15.34^{bd}	1.7±0.10^{bd}	2.6±0.16^{bd}	5.19±0.40^{bd}
Group IV	477.0±15.23^{ac}	2.3±0.09^{ac}	4.4±0.20^{ac}	7.5±0.42^{ac}

Values are representation of 9 observations in each group as means ± S.E.M. Results are considered significantly different when $P < 0.05$. [a] Significant difference compared to group I, [b] Significant difference compared to group II, [c] significant difference compared to group III, [d] significant difference compared to group IV.

Effect of DIA on prostatic tissue MDA, HO1 level and serum TAC

Group II,IV showed significant increase in MDA level but significant decrease in prostatic tissue HO1 and serum TAC in comparison with normal control group (group I). Co-administration of DIA (Group III) had significant decrease in MDA levels but significant increase in HO1 and serum TAC in comparison with Group II,IV (table2).

Table (2): Effect of DIA on MDA, TAC, HO1 in induced BPH

Group	TAC	HO1	MDA
Group I	1.8±0.17^{bd}	2.8±0.12^{bd}	36.44±1.8^{bd}
Group II	0.5±0.04^{ac}	0.87±0.05^{ac}	54.22±2.4^{ac}
Group III	1.5±0.14^{bd}	2.5±0.15^{bd}	32.8±1.7^{bd}
Group IV	0.6±0.05^{ac}	0.8±0.04^{ac}	54.8±1.8^{ac}

Values are representation of 9 observations in each group as means ± S.E.M. Results are considered significantly different when $P < 0.05$. [a] Significant difference compared to group I, [b] Significant difference compared to group II, [c] significant difference compared to group III, [d] significant difference compared to group IV.

rtPCR for the relative quantification of PCNA, Nrf2 gene expressions.

Group II,IV showed significant increase in PCNA but significant decrease in Nrf2 gene expression in comparison with group I. Administration of DIA (50 mg/kg/d) for 4 weeks to BPH induced group (group III) showed a significant decrease in PCNA but significant increase in Nrf2 gene expression compared to group II,IV (Fig.3,4).

Evaluation of IL1β by western blotting

Group II,IV showed significant increase of IL1β expression in comparison with group I. Administration of DIA to BPH induced group (group III) showed a significant decrease in IL1β compared to group II,IV (Fig.5).

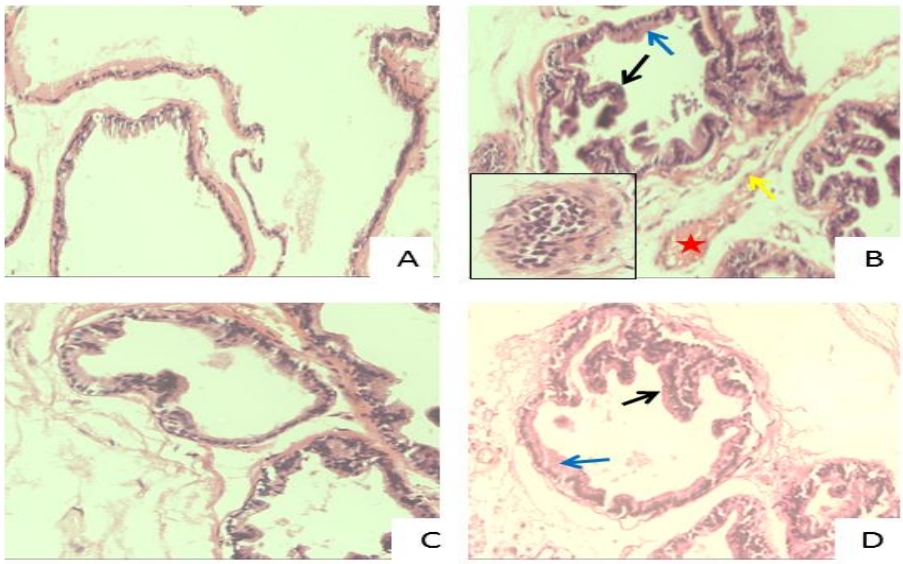

Figure.1
Examination of hematoxylin and eosin slides of group I (control group) showed that normal prostate architecture, the acini were lined by two layers; inner simple cuboidal epithelium and outer flattened cells (Fig.1.A) (X200). In group II,IV (TP given groups), exhibited proliferation of glandular epithelium with stratification and hypertrophy of the lining (blue arrow) and the acini showed many long branched papillary folds with fibrovascular cores (black arrow). The stroma showed congested blood vessels (asteroid) and inflammatory cells infiltration (yellow arrow). Other foci exhibited cellular crowding and nuclear pleomorphism (insets) (Fig.1.B,D) (X200). Treatment with DIA (50 mg/kg/d) for 4 weeks to BPH induced group (group III) showed marvelous improvement in the histological appearance of the prostatic acini. The epithelial height reduced and the intraluminal papillae diminished (Fig.1.C) (X200).

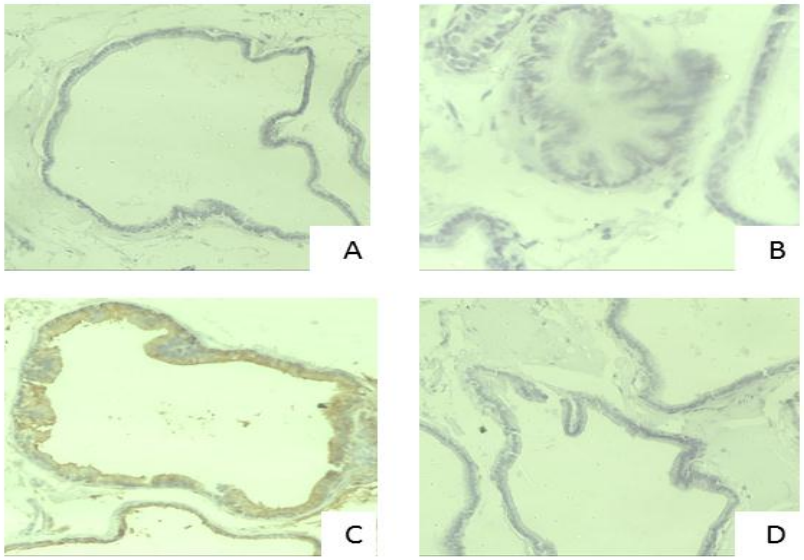

Figure2.A: Immunoexpression changes of cleaved caspase3
Immunohistochemical expression of cleaved caspase3 in (Fig 2. A) group I (control) showed negative expression (X200). (Fig 2. B &D) Group II,IV (benign prostatic hyperplasia induced group) showed negative expression (X200). (Fig 2. C) Group III (benign prostatic hyperplasia induced group+diacerein treatment) showed positive immunoexpression (X200).

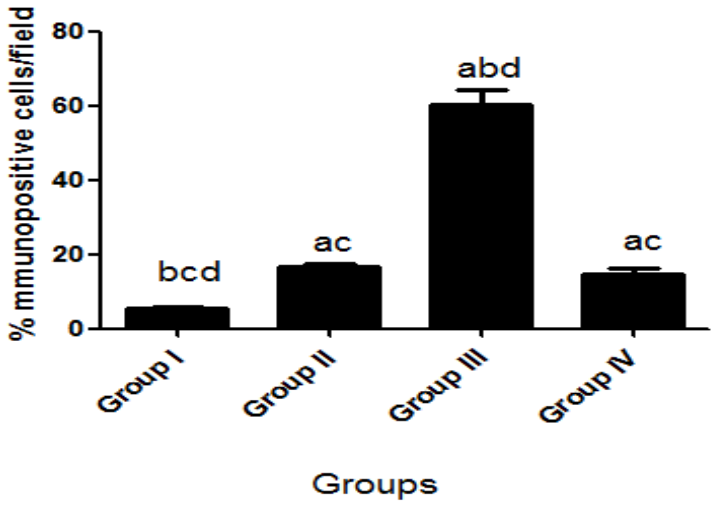

Figure 2.B: Semiquntitive analysis of cleaved caspase3
Benign prostatic hyperplasia (BPH)+diacerein (DIA) group had significant increase of its immunoexpression compared to benign prostatic hyperplasia (BPH) group.

Fig.3

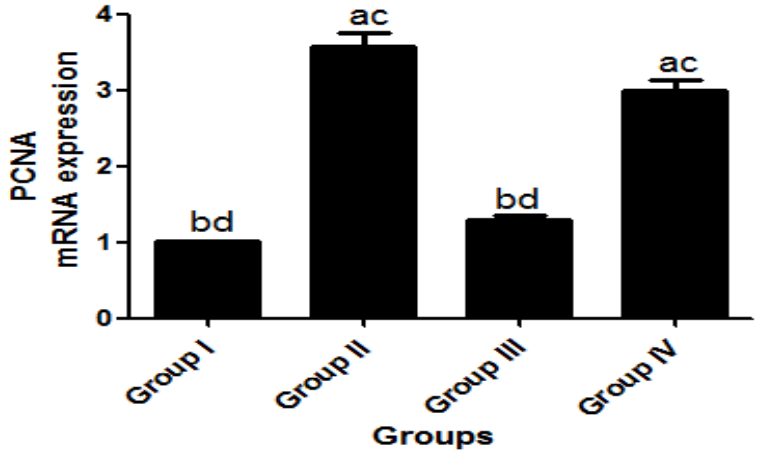

Figure 3:
proliferating cell nuclear antigen (PCNA) gene expression
It significantly increased in benign prostatic hyperplasia (group II,IV) compared to control group (group I). However, in benign prostatic hyperplasia (BPH)+ diacerein (DIA) treated group (group III) significantly decreased compared to benign prostatic hyperplasia group (group II).

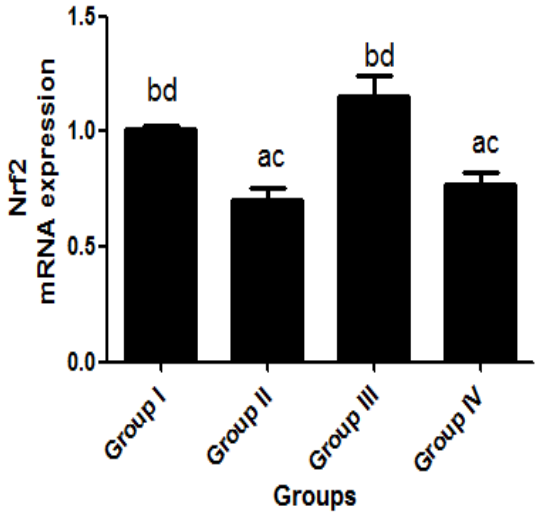

Figure 4: nuclear factor erythroid 2–related factor 2 (Nrf2) gene expression changes
It significantly decreased in benign prostatic hyperplasia (group II,IV) compared to control group (group I). Benign prostatic hyperplasia (BPH)+ diacerein (DIA) treated group (group III) had significant increase of its gene expression compared to benign prostatic hyperplasia group (group II).

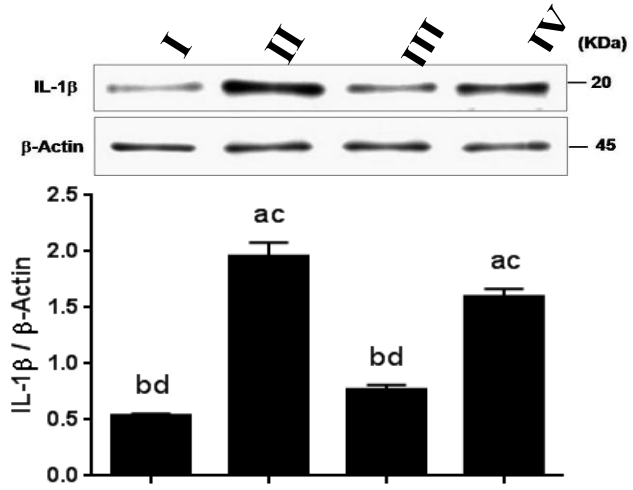

Figure 5: evaluation of interleukin 1β by western blotting
It significantly elevated in benign prostatic hyperplasia (group II,IV) compared to control group (group I). Diacerein (DIA)+benign prostatic hyperplasia (BPH) treatment (group III) showed significant decrease of its levels compared to benign prostatic hyperplasia group (group II).
Values represent 9 observations in each group as means ± S.E.M. Results are considered significantly different when $P < 0.05$. [a] Significant difference compared to group I, [b] Significant difference compared to group II, [c] significant difference compared to group III, [d] Significant difference compared to group IV.

Discussion

BPH is the most frequent, progressive lesion among the elderly men characterized by massive stromal and glandular tissue hyperplasia leading to enlarged prostate associated with lower urinary tract symptoms including hesitancy, feeling of incomplete bladder emptying, poor intermittent stream, and irritative symptoms such as urgency, increased frequency, and nocturia[4,7,18].

We aimed in current study to explore the possible curative effect of DIA in induced BPH using TP in rats. Our results revealed occurrence of BPH as shown in the histopathological features of TP given group. Furthermore, there are significant elevations of prostate weights, DHT, PSA, MDA, IL1β and PCNA with decreased Nrf2 gene expression, serum TAC, caspase3 and HO1 levels.

Interestingly, administration of DIA with BPH (group III) effectively improved BPH induced changes which was observed in form of decreasing prostate weights, MDA, DHT, PSA, PCNA but increasing Nrf2 gene expression, TAC and HO1 levels. Furthermore, histological examination revealed that DIA treatment could normalize and markedly improve BPH as it could elevate cleaved caspase3 immuno-expression indicating stimulation of apoptosis and diminish IL1β expression with suppression of the inflammatory process.

Prostate enlargement and increasing its weight is considered as a vital marker for detecting and evaluating BPH. In our results, TP given group significantly increased prostate weight and these results are in line with previous studies[7,18,19]. However, DIA plus BPH could significantly diminish prostate weight indicating the improvement of BPH. The most effective prostatic hormone in developing BPH is DHT that is formed from testosterone in presence of 5α-reductase enzyme[20]. Moreover; growth factors have a major role in developing cell growth, differentiation and proliferation[21]. PCNA is another proliferative marker and plays an important role in developing different physiological and pathological processes[22]. This is found in our model that showed marked increase in DHT level and PCNA gene expression in BPH induced group. PSA is a protein formed by normal prostate cells, participates in dissolution process of the seminal fluid and plays an essential role in fertility. Increasing the amount of PSA could be detected in prostate diseases as prostatitis, BPH, cancer as found in current model of BPH with precancerous changes and could be supported by previous studies[23,24].

Oxidative stress and excessive production of ROS occurs due to the imbalance between body reductants and oxidants. These harmful agents are associated with oxidative DNA damage and constitute an important mutagenic and carcinogenic factor. However, antioxidants could scavenge these free radicals and prevent tissue damage [25, 26]. One of the most effective antioxidant pathways is Nrf2-ARE transcriptional pathway. It regulates different genes that control the gene expression of a group of critical proteins involved in detoxification and elimination of ROS. Activation of Nrf2 is associated with release of different antioxidants as GPx and and HO1. The letter is the key enzyme in heme salvage pathway and removes the pro-oxidant free heme and converts it to carbon monoxide and bilirubin which is considered as an antioxidant molecule but carbon monoxide is a potent vasodilator and anti-inflammatory molecule. Unfortunately, excessive release of free radicals is accompanied by depletion of HO1[27]. Furthermore, membrane lipid peroxidation occurs due to the reaction of $O^{2\bullet-}$ with lipid to form lipid peroxides. This is followed by β-oxidation and MDA formation which is the most important indicator for detecting the state of membrane lipid peroxidation and cell damage[28-30].

In our study we found marked decrease of HO1, Nrf2 levels and serum TAC but increase in MDA levels in BPH group due to induction of oxidative stress with excessive formation of ROS. These results are in accordance with different previous studies [4,18,28]. However, administration of DIA to BPH group showed marked improvement and normalization of the measured oxidant and antioxidant parameters indicating the protective effect of DIA against the formation of ROS and its anti-oxidant properties that is in agreement with other models[7, 31].

Occurrence of BPH is associated with dys-regulation of androgens, increased cell proliferation and decreased prostate cell

apoptosis. This is detected in our model that found significant decrease of caspase3 (apoptotic marker) in group II,IV. The same was found in previous studies[32-35]. Group III (BPH+DIA) showed increase in caspase3 immunoexpression with induction of the apoptotic process, decreasing the overgrowth of prostate tissue, preservation and protection of the prostate. These findings are in line with previous models that confirmed the apoptotic effect of DIA[30,36].

Inflammatory cytokines are the key factor mediating tumor microenvironment due to excessive infiltration of tumor cell autocrine signaling, immune cell paracrine secretion and promote precancerous and cancer cell survival with disease progression. One of the most essential pro-inflammatory cytokines is IL1β which promotes tumor angiogenesis, metastasis and bone colonization with further stimulation of other cytokines, oxidative stress and inflammation. These pathological effects of IL1β are found in BPH, cancer prostate, breast cancer and endometrial hyperplasia[13,36,37].

Inflammatory cytokines are highly involved in development and progression of many inflammatory disorders including atypical excessive cell proliferation and tumors. DIA inhibits several cytokines especially IL1β, IL6/STAT which are the key pathways involved in several precancerous and cancerous disorders as prostate cancer, breast cancer, endometrial hyperplasia and chondrosarcoma. In addition, there is an emerging evidence explained that DIA has anti-proliferative and antitumor properties supporting the potential recent uses of DIA as an anticancer agent. Our data revealed that DIA has anti-inflammatory, anti-proliferative, apoptotic and antioxidant effects [13,30,31,34-37] and these properties of DIA explain its curative effect in treating BPH with dysplastic changes. We think that our study paves the way for others to evaluate usage of DIA in BPH patients.

Conclusion

Diacerein is an interleukin 1β antagonist markedly improved the pathological changes associated with induced benign prostatic hyperplasia with dysplastic changes in rats. Further studies are highly recommended to evaluate the possible role of diacerein in treating benign prostatic hyperplasia patients.

Conflicts of Interest: none

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Authors' contribution:

Dr. Marwa selected the point, performed the experimental part, wrote and sent the manuscript. Dr. Maram performed and wrote the histopathology and immunohistochemical part and revised the manuscript as a whole. Dr. Asmaa performed and wrote the part of western blotting and revised the manuscript.

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References

1- Ishola I., Anunobi C., Yemitan K., Afolayan O., Durojaiye T. Potential of Moringa oleifera in the Treatment of Benign Prostate Hyperplasia: Role of Antioxidant Defence Systems. Med Princ Pract. 2018; 27(1):15-22.

2- Sarbishegi M., Khani M., Salimi S., Valizadeh M., Aval F.S. Antiproliferative and antioxidant effects of Withania Coagulans extract on benign prostatic hyperplasia in rats. Nephrourol Mon. 2016; 8 e33180.

3- El-Ashmawy N.E., Khedr E.G., El-Bahrawy H.A., Helmy N.N. Modulatory Effect of Silymarin on Apoptosis in Testosterone -Induced Benign Prostatic Hyperplasia in Rats. Pathol Oncol Res. 2020; 26(3):1947-1956.

4- Liu M.Y., Chi N.J., Feng J., Shi X.P., Wen A.D. Ruyi Jinhuang Plaster suppresses BPH by reducing the expressions of P38, JNK2, NF-BP65 and STAT3 in the prostate tissue of rats. Zhonghua Nan Ke Xue. 2018; 24(12):1116-1121.

5- Duru R., Njoku O. and Maduka I. Oxidative Stress Indicators in Patients with Prostate Disorders in Enugu, South-East Nigeria. BioMed Research International. 2014; Volume 2014, Article ID 313015, 6 pages.

6- Modugno F., Ness R.B., Chen C., Weiss N.S. Inflammation and endometrial cancer:

a hypothesis. Cancer Epidemiol. Biomarkers Prev. 2005; 14:2840–2847.
7- Al-Trad B., Aljabali A., Al Zoubi M., Shehab M., Omari S. Effect of gold nanoparticles treatment on the testosterone-induced benign prostatic hyperplasia in rats. Int J Nanomedicine. 2019; 14:3145-3154.
8- Refaie M.M.M., El-Hussieny M. The role of interleukin-1b and its antagonist (diacerein) in estradiol benzoate-induced endometrial hyperplasia and atypia in female rats. Fundam Clin Pharmacol. 2017; 31(4):438-446.
9- Yang X., Yuan L., Xiong C., Yin C., Ruan J. Abacopteris penangiana exerts testosterone-induced benign prostatic hyperplasia protective effect through regulating inflammatory responses, reducing oxidative stress and anti-proliferative. J Ethnopharmacol.2014;157:105-113
10- Kim S.S., Kim J.H., Han I.H., Ahn M.H., Ryu J.S. Inflammatory Responses in a Benign Prostatic Hyperplasia Epithelial Cell Line (BPH-1) Infected with Trichomonas vaginalis. Korean J Parasitol. 2016; 54(2):123-32.
11- Fouad A.A., Abdel-Aziz A.M., Hamouda A.A.H. Diacerein Downregulates NLRP3/ Caspase-1/IL-1β and IL-6/STAT3 Pathways of Inflammation and Apoptosis in a Rat Model of Cadmium Testicular Toxicity. Biol Trace Elem Res. 2020; 195(2):499-505.
12- Basha S.Z., Mohamed G.A., Abdel-Naim A.B., Hasan A., Abdel-Lateff A. Cucurbitacin E glucoside from Citrullus colocynthis inhibits testosterone-induced benign prostatic hyperplasia in mice. Drug Chem Toxicol. 2019;12:1-11.
13- Almezgagia M., Zhangb Y., Hezame K., Shamsan E., Gamaha M., Al-shaebic F., Abbas A, Shoaibb M, Saifd B, Hana Y, Jiaa R, Zhanga W. Diacerein: Recent insight into pharmacological activities and molecular pathways. Biomedicine & Pharmacotherapy.Volume 131, 2020, 11059.
14- Zhang H.Y., Meng X., Du Z.X. et al., Significance of survivin, caspase-3, and VEGF expression in thyroid carcinoma. Clin. Exp. Med. 2009; 9:207–213.
15- Mihara M. and Uchiyama M. Properties of thiobarbituric acid-reactive materials obtained from lipid peroxide and tissue homogenate. Chem.Pharm.Bull. 1983; 31 (2): 605-611.
16- Van Guilder H.D., Vrana K.E., Freeman W.M. Twenty-five years of quantitative PCR for gene expression analysis. Biotechniques. 2008; 44 (5): 619-626.
17- Ewees M.G., Messiha B.A.S., Abdel-Bakky M. S., Bayoumi A. M. A., and Abo-Saif A. A.Tempol, a superoxide dismutase mimetic agent, reduces cisplatin-induced nephrotoxicity in rats. Drug Chem Toxicol. 2018;1-8.
18- Vafa A., Afzal S.M., Barnwal P., Rashid S., Shahid A., Alpashree, Islam J., Sultana S. Protective role of diosmin against testosterone propionate-induced prostatic hyperplasia in Wistar rats: Plausible role of oxidative stress and inflammation. Hum Exp Toxicol. 2019:960327119889655.
19- An Y.J., Lee J.Y., Kim Y., Jun W., Lee Y.H. Cranberry Powder Attenuates Benign Prostatic Hyperplasia in Rats. J Med Food. 2020; 23(12):1296-1302.
20- Nicholson T.M. and Ricke W.A. Androgens and estrogens in benign prostatic hyperplasia: past, present and future. Differentiation. 2011; 82:184–199.
21- Ciarmela P., Islam M.S., Reis F.M., Gray P.C., Bloise E., Petraglia F., Vale W., Castellucci M. Growth factors and myometrium: biological effects in uterine fibroid and possible clinical implications. Hum Reprod Update. 2011; 17:772–790.
22- Ammar A.E., Esmat A., Hassona M.D., Tadros M.G., Abdel-Naim A.B., Guns E.S. The effect of pomegranate fruit extract on testosterone-induced BPH in rats. Prostate. 2015; 75(7):679-92.
23- Ishola I.O., Anunobi C.C., Tijani K.H., Afolayan O., Udokwu V.U. Potential of telmisartan in the treatment of benign prostatic hyperplasia. Fundam Clin Pharmacol. 2017; 31(6):643-651.
24- Ishola I.O., Tijani H.K., Dosumu O.O., Anunobi C.C., Oshodi T.O. Atorvastatin attenuates testosterone-induced benign prostatic hyperplasia in rats: role of peroxisome proliferator-activated receptor-γ and cyclo-oxygenase-2. Fundam Clin Pharmacol. 2017; 31(6):652-662.
25- Abdel-Naim A.B., Neamatallah T., Eid B.G., Esmat A., Alamoudi A.J., Abd El-Aziz G.S., Ashour O.M. Methoxyestradiol Attenuates Testosterone-Induced Benign

Prostate Hyperplasia in Rats through Inhibition of HIF-1α/TGF-β/Smad2 Axis. Oxid Med Cell Longev. 2018: 4389484.

26- Ye W., Zhu S., Liao C. et al. Advanced oxidation protein products induce apoptosis of human chondrocyte through reactive oxygen species-mediated mitochondrial dysfunction and endoplasmic reticulum stress pathways. Fundam. Clin.Pharmacol. 2017; 31: 64–74.

27- Refaie M.M.M., El-Hussieny M., Bayoumi A.M.A., Shehata S. Mechanisms mediating the cardioprotective effect of carvedilol in cadmium induced cardiotoxicity. Role of eNOS and HO1/Nrf2 pathway. Environ Toxicol Pharmacol. 2019; 70:103198.

28- Eid B.G.and Abdel-Naim A.B. Piceatannol AttenuatesTestosterone-Induced Benign Prostatic Hyperplasia in Rats by Modulation of Nrf2/HO-1/NFκB Axis. Frontiers in Pharmacology. 2020; 11: 614897

29- Rofaeil R.R., Kamel M.Y., Abdelzaher W.Y. Different effects of selective b1-adrenoceptor antagonists, nebivolol or atenolol in acetaminophen-induced hepatotoxicity of rats. Fundam. Clin. Pharmacol. 2017; 31 165–173.

30- Abdel-Aziz A.M., El-Tahawy N.F.G., Abdel Haleem M.A.S., Mohammed M.M., Ali A.I., Ibrahim Y.F. Amelioration of testosterone-induced benign prostatic hyperplasia using febuxostat in rats: The role of VEGF/TGFβ and iNOS/COX-2. Eur J Pharmacol. 2020; 889:173631.

31- Abdel-Gaber S.A., Mohammed R.K., Refaie M.M.M. Mechanism mediating the protective effect of diacerein in ischemia-reperfusion-induced testicular injury in rats. Life Sci. 2018; 209:57-62.

32- Shin S.J., Lee K.H., Chung K.S., Cheon S.Y., An H.J. The traditional Korean herbal medicine Ga-Gam-Nai-Go-Hyan suppresses testosterone-induced benign prostatic hyperplasia by regulating inflammatory responses and apoptosis. Exp Ther Med. 2017; 13 (3):1025-1031.

33- Morgia G., Micali A., Rinaldi M., Irrera N., Marini H., et al., Survivin and NAIP in Human Benign Prostatic Hyperplasia: Protective Role of the Association of Serenoa repens, Lycopene and Selenium from the Randomized Clinical Study. Int J Mol Sci. 2017; 18 (3). pii: E680.

34- Sayed R.H., Saad M.A., El-Sahar A.E. Dapoxetine attenuates testosterone-induced prostatic hyperplasia in rats by the regulation of inflammatory and apoptotic proteins. Toxicol Appl Pharmacol. 2016; 311: 52-60.

35- Bharti R., Dey G., Ojha P.K., Rajput S., Jaganathan S.K., Sen R., Mandal M. Diacerein-mediated inhibition of IL-6/IL-6R signaling induces apoptotic effects on breast cancer. Oncogene. 2016; 35(30): 3965-3975.

36- Abd-Ellatif R.N., Hegab II, Atef M.M., Sadek M.T., Hafez Y.M. Diacerein protects against glycerol-induced acute kidney injury: Modulating oxidative stress, inflammation, apoptosis and necroptosis. Chem Biol Interact. 2019; 306:47-53.

37- Thomas-Jardin S.E., Kanchwala M.S., Jacob J., Merchant S., Meade R.K., Gahnim N.M., Nawas A.F., Xing C, and Delk N.A. Identification of an IL-1-induced gene expression pattern in AR+ PCa cells that mimics the molecular phenotype of AR− PCa cells. Prostate. 2018; 78(8): 595–606.