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Inhibitory effects of mangosteen *(Garcinia mangostana)* on testosterone-induced benign prostatic hyperplasia in rats

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ABSTRACT

Mangosteen (*Garcinia mangostana L.*) is famous as the queen of fruits and is considered a natural health-promoting dietary supplement. Recently, mangosteen fruit has received a great deal of attention due to its therapeutic properties in treating different diseases throughout the world. However, scientific studies in Egypt on the effects of mangosteen in vivo are very rare. This work aims to assess the protective effect of mangosteen fruit extract on three doses (1, 2 and 3ml/kg b.w) on benign prostatic hyperplasia induced by testosterone in male rats. Thirty-six rats weighing between 190-200g, were divided into six groups (6 rats for each); one served as the normal control group (N.C) (-ve), BPH (+ve), BPH+ zinc (20 mg/kg b.w), BPH + MFE_1 (1ml/kg b.w), BPH + MFE_2 (2ml/kg b.w) and BPH + MFE_3 (3ml/kg b.w), To induce BPH, rats were injected with testosterone (5 mg/kg b.w) daily. On the 29th all rats were sacrificed, and their serum and prostate were analyzed. The results showed that mangosteen with different doses (1,2 and 3ml/kg b.w) significantly diminished the development of benign prostatic hyperplasia by decreasing (prostate volume, prostate weight, prostate weight index, and testosterone levels) and increasing the levels of LH, FSH, and total protein in serum in all protective groups which received different doses of MFE in comparison with BPH (+ve) untreated. In conclusion, mangosteen fruit extract at different levels has some significant protective effects on reproductive functions in male rats.

Keywords: Mangosteen fruit; Zinc supplement; Prostate volume; BPH; Testosterone

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INTRODUCTION

Benign prostatic hyperplasia (BPH) is one of the top popular hazards of ageing in males, affecting 42% of propulsion well over their 50 years and more than 80% of octogenarians **(De Nunzio *et al.*, 2016).** BPH is associated with the slowly progressive enlargement of glands and stromal cells, which leads to an increase in the size of the prostate **(Al-Trad *et al.*, 2017).** Hyperplasia of the prostate can blockade the urethra, causing acute urinary troubles such as dysuria, a weak urinary stream, hindrance in bladder outlet, urinary frequency and no bladder emptying **(Jeon *et al.*, 2017).** Due to the rising occurrence of benign prostatic hyperplasia, there has been considerable interest in the control and management of BPH disease to slow down related complications.

To date, numerous medical drugs can be used in the treatment of benign prostatic hyperplasia (BPH) but with adverse effects like erectile dysfunction, which is still a big challenge **(Bullock and Andriole 2006 and Traish *et al.*, 2011).** Given the above, searching for constituents that effectively diminish the development of BPH and are linked with confirmed safety and no occurrence of adverse impacts is deeply needed. Herbal remedies derived from bioactive compounds may supply an alternative source to treat BPH without any harmful side effects. One of the powerful tropical fruits is mangosteen (*Garcinia mangostana L*) which belongs to the family Clusiaceae, is grown in Southeast Asian nations, and is recognized as the queen of fruits. Mangosteen fruit is dusky red or purple, smooth, and has a white pulp that is edible with a sweet aroma, a slight acidity and a pleasant flavour (**Febrina *et al*., 2018).** The fruit has become one of the most important agricultural plants in different parts of the world especially in the Middle East, owing to its high value **(Aizat *et al.*, 2019).** Recently, the extract of mangosteen has been used as a high quality food or beverage, that promotes and boosts general health through numerous activities, such as anti-diabetic **(Abdallah *et al.*, 2017),** Anti-cancer (**Nakagawa *et al*., 2007; Fukuda *et al.*, 2017; Mohamed *et al.*, 2017 and Wu *et al.*, 2017),** anti-microbes **(Nanasombat *et al.*, 2018 and Nittayananta *et al.*, 2018),** hepatoprotective **(Wang *et al.*, 2018),** eye protection **(Yang *et al.*, 2018),** neuroprotective **(Jaisin *et al.*, 2018),** cardiovascular protection **(Fang *et al.*, 2018),** anti-inflammatory potential (**Chen *et al*., 2008 and Fu *et al.*, 2018**), perhaps due to containing a variety of bioactive compounds **(El-Seedi *et al.*, 2009, 2010; Ovalle-Magallanes *et al.*, 2017 and Tousian *et al.*, 2017).** In addition, various studies demonstrated that the extract of mangosteen fruit display to possess high antioxidant activities (**Weecharangsan *et al*., 2006 and Chatatikun and Chiabchalard 2017).**

Therefore, the present study aims to hypothesize the potential protective impact of mangosteen fruit extract at three different levels (1, 2 and 3ml/kg b.w) against the damage induced by testosterone in male rats with benign prostatic hyperplasia.

MATERIALS AND METHODS

Plant material and extract preparation: mangosteen (*Garcinia mangostana* L) fruit was obtained from a local market, Giza, Egypt. The extraction of the mangosteen fruit was prepared according to the method of **(Charles *et al.*, 1993).**

Animals: Thirty-six white albino rats (Sprague-Dawley strain), 190-200 g provided from the National Research Center, Cairo, Egypt. Feeds on the basal diet (growers) were obtained from the National Research Center **(NRC, 1995).**

Chemicals and reagents: zinc (Octozinic©), capsules produced by October Pharma S.A.E and contain 110 zinc sulphate heptahydrate. Biochemical kits were purchased from Alkan Co. for Chemicals and Biodignostics , Dokki, Egypt.

Quality assessment of the extract: The extract was undergoing high performance thin layer chromatography (HPTLC) examinations **(Meena *et al.*, 2010).**

Induction of BPH: A subcutaneous injection of testosterone (5 mg/kg) daily was used for 28 days to induce benign prostatic hyperplasia in rats **(Veeresh Babu *et al.*, 2010).**

Experimental Design:

After an acclimatization period of one week, 36 male rats were randomly divided into 6 groups (*n* = 6) and treated for 28 consecutive days as follows:

N.C: Normal group control, fed on a basal diet.

BPH: Fed on the basal diet and served as an untreated group (+ve).

BPH + Zinc: Fed on the basal diet and treated with zinc (20 mg/kg body weight), dissolved in distilled water and given to rats by oral intubations according to **Paget and Barnes (1964)**.

BPH + MFE$_1$: Fed on the basal diet and orally received mangosteen extract (1ml/kg.bw).

BPH + MFE$_2$: Fed on the basal diet and orally received mangosteen extract (2ml/kg.bw).

BPH + MFE$_3$: Fed on the basal diet and orally received mangosteen extract (3ml/kg.bw).

Body weight was measured weekly during the study. On the 29th day, blood was collected from retro orbital plexus and animals were sacrificed. Immediately, the prostate gland and bladder were dissected and weighed and various parameters were measured.

Prostate weight to body weight ratio: the prostate weight to body weight ratio was calculated by dividing the prostate weight by the animal's body weight for the individual study group.

Blood samples and biochemical analysis: Blood samples were collected and centrifuged at 2000×*g* for 20 min to obtain serum for further analyses.

Total protein in prostate: Prostate glands were dissected and homogenates were made in phosphate buffer solution (0.01 M sodium phosphate buffer, pH 7.4, containing 0.14 M NaCl) at a ml volume/g gland wet weight ratio of 4:1. Homogenates were centrifuged at 13,000×*g* for 20 min and the supernatant collected **(Shin *et al.*, 2012).** The supernatant was used as a source of proteins and the concentration was determined by a modified biuret end point assay method.

Hormonal assay: The concentration of serum testosterone was assessed by the method of radioimmunoassay using commercial kits (Diagnostic Products Co, Los Angeles, USA). The hormone labeled with iodine-125 was used as a radioactive marker. Samples were run in the same assay to avoid inter-assay variation. The intra-assay variation was 5.5% for testosterone. The sensitivity of the testosterone assay was 4 ng/dl. The concentrations of LH and FSH were determined based on a solid-phase enzyme-linked immune-absorbent assay as described by **Uotila *et al.*, (1981).**

Antioxidants parameters: content of total antioxidants capacity (TAC), and superoxide dismutase (SOD) activity were determined according to **Cao *et al.*, (1993) and Nishikimi *et al.*, (1972)** and malondialdehyde (MDA) was measured by **Uchiyama and Mihara (1978).**

Statistical analysis: The obtained data were statistically analyzed using computerized SPSS (Statistic Program Sigmastat, Statistical Soft-Ware, SAS Institute, Cary, NC). The effects of different treatments were analyzed by a one-way ANOVA (analysis of variance) test using Duncan's multiple range test and $p<0.05$ was used to indicate significance between different groups according to **Snedecor and Cochran (1967)**.

RESULTS AND DISCUSSION

HPTLC analyses of mangosteen fruit extract (MFE)

The quality assessment of mangosteen extract was assessed using high-performance-thin-layer chromatography (HPTLC) as illustrated in Fig. 1. The retention times of p-hydroxybenzoic acid, *m*-Hydroxybenzoic acid, 4-Amino-benzoic, protocatechuic acid, sinapic acid, 3, 4 dihydroxymandelic, Cyanidin-3-glucoside, and P-conmaric were 6.9, 9.3, 13.3, 15.9, 17.5, 19.3, 20.1 and 21.3 min, respectively. These findings are in parallel with those obtained by **Azima *et al.*, (2017)** who reported that mangosteen fruit contained plenty of phenolic compounds like benzoic acid derivatives are recognized to possess antioxidative and anti-inflammatory properties (**Lin *et al.*, 2009 and Ortega-García and Peragón 2010)**.

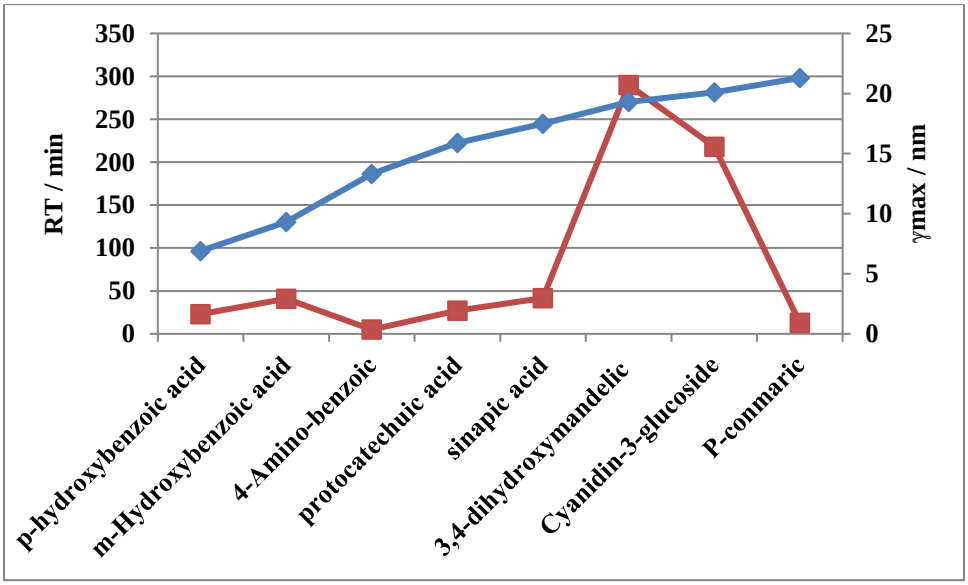

Figure.1. Phenolic compounds present in mangosteen fruit extract

Effect of MFE on different levels of nutritional indicators in the normal and BPH groups

The final body weight was measured after 4 weeks (Table 1). The final body weight in N.C (-ve), BPH (+ve), BPH+ zinc, BPH+MFE_1, BPH+MFE_2, and BPH+MFE_3 groups was increased compared with the initial body weight and recorded (27.80 %, 20.22, 22.76 %, 26.54, 24.90 and 26.15 %, respectively). There is a significant difference among the BPH (+ve) and BPH+ zinc groups compared with the normal control (N.C). BWG and FER displayed a significant decrease in BPH (+ve) rats compared with the N.C group. Oral administration of mangosteen fruit extract

with different doses showed significant improvement in BWG and FER in comparison with BPH (+ve), but showed non-significant changes in feed intake among different groups.
In the current work, benign prostatic hyperplasia induced by testosterone caused a reduction in final weight, BWG, and FER. The significant decline in BWG in BPH rats is in parallel with the findings of (**Kim *et al*., 2013 and Mohamed *et al.*, 2016).**

Table 1. Effect of MFE with different levels on nutritional parameters in each group.

Groups / Parameters	N.C (-ve)	BPH (+ve)	BPH+ zinc	BPH+ MFE_1 (1 ml)	BPH+ MFE_2 (2 ml)	BPH+ MFE_3 (3ml)
Initial	195.12± 9.42 [a]	197 ± 9.03 [d]	196.10 ±9.13 [a]	195.75± 9.21 [b]	199.02± 9.43 [b]	199.10± 9.10 [b]
Final	270.25± 18.44 [a]	246.93± 13.17 [c]	253.9± 18.98 [b]	266.46± 17.91 [a]	265.01± 17.05 [a]	269.62± 17.44 [a]
Feed Intake (g/day)	16.85± 1.84 [a]	15.73± 1.17 [a]	15.99± 1.45 [a]	16.21± 1.22 [a]	16.21± 1.22 [a]	16.21± 1.22 [a]
BWG (g)	74.72± 8.42 [a]	45.88± 5.13 [d]	61.42.± 8.13 [a]	62.75± 7.91 [b]	68.65± 7.44 [b]	70.80± 7.52 [b]
FER	0.0894± 0.001 [a]	0.059± 0.003 [d]	0.065± 0.005 [c]	0.074± 0.004 [b]	0.074± 0.004 [b]	0.074± 0.004 [b]

Data are expressed as mean ±SD, Mean values in each column having different superscripts a, b, c, denote significant difference, BWG: Body weight gain, FER: Food efficiency ratio, N.C: Normal control, BPH: Benign prostatic hyperplasia, MFE: Mangosteen fruit extract.

Effect of MFE with different levels in prostate weight, prostate volume, prostate weight index, and wet weight index of the prostate in the normal and BPH rats.

Table 2 shows the prostate weight, prostate volume, prostate weight index and wet weight index of the prostate in normal and BPH rats. After 28 days of testosterone injection to induce benign prostatic hyperplasia, the weight of the prostate was increased in (+ve) compared to N.C (-ve) rats. Data indicated that the BPH disease occurred after successive injections of testosterone. Moreover, after consumption of the three doses of MFE, MFE_3 was the most effective treatment as prostate weight declined by 38.1% compared with the (+ve) untreated group. The rise in the weight of prostate in BPH rats in the current work is in concord with other findings obtained by **(Kim *et al*., 2013 and Yang *et al*., 2014)** as they mentioned that induction of BPH in rat's model is linked with an increase in prostate size. Furthermore, administration of MFE with different doses causes a reduction in prostate volume, prostate weight index and wet weight index of the prostate in rats compared with the BPH (+ve) untreated group.

Table 2. Prostate weight, prostate volume, prostate weight index, wet weight index of prostate and increase in prostate weight in BPH rats.

Groups / Parameters	N.C (-ve)	BPH (+ve)	BPH+ zinc	BPH+ MFE_1 (1 ml)	BPH+ MFE_2 (2 ml)	BPH+ MFE_3 (3 ml)
prostate weight (g)	0.62± 0.03 ^{c}	1.05± 0.07 ^{a}	0.82± 0.11^{b}	0.78± 0.05 ^{b}	0.70± 0.2 ^{b}	0.65± 0.05 ^{b}
Prostate volume (cm^2)	0.42± 0.11 ^{c}	0.80± 0.12 ^{a}	0.55± 0.04^{b}	0.50± 0.12 ^{b}	0.52± 0.2 ^{b}	0.55± 0.04 ^{b}
prostate weight index (g/g*1000)	2. 64± 0.17 ^{a}	4.64± 0.13 ^{d}	4.18± 0.21^{c}	3.76± 0.32 ^{b}	3.34± 0.37 ^{b}	2.94± 03.12 ^{b}
Wet weight index of prostate (mg/100gB.W)	59.24± 6.87 ^{d}	173.19± 14.83 ^{a}	75.65± 8.005 ^{c}	84.044± 9.34 ^{b}	79.04± 8.58 ^{b}	82.33± 8.21 ^{b}
Increase in prostate weight (%)	___	100	75. 65± 8.005 ^{c}	84.044± 9.34 ^{b}	79.04± 8.58 ^{b}	82.33± 8.21 ^{b}

Data are expressed as mean ±SD, Mean values in each column having different superscript a, b, c,.. denote significant difference, N.C: Normal control, BPH: Benign prostatic hyperplasia, MFE: Mangosteen fruit extract

Effect of MFE with different levels on testosterone, LH, FSH, and total protein in the normal and BPH groups

Figure 2 shows the serum testosterone concentrations of different experimental groups. Testosterone levels were significantly higher in the (+ve) untreated group compared with N.C rats. In addition, testosterone concentration was significantly diminished after consumption of mangosteen extracts in BPH groups in comparison with BPH (+ve). Oral administration of MFE with different levels showed a significant decrease of serum testosterone concentration compared with the BPH (+ve) untreated. LH level of (+ve) group was significantly decreased compared with the N.C group. whole BPH groups receiving treated levels of MFE showed a significant increase in serum LH concentration compared with the BPH (+ve), and the protected groups MFE_2 and MFE_3 showed non-significant with normal control (-ve) rats. Mangosteen fruit extract at the high dose (3ml/kg b.w) showed the highest increase in LH level followed by treated MFE_2 compared with BPH (+ve) group. The increase in testosterone level in BPH (+ve) rats in the current work is in harmony with previous studies obtained by **(Yang *et al*., 2014 and Mohamed *et al*., 2016)** they stated that the BPH induction in rat model causes an increase in testosterone concentration. The reduction in FSH level that observed after testosterone induction is agreement with reports of **(Crawford *et al*., 2014)** who reported that dysfunction of (FSH) the follicle-stimulating hormone plays an important role in the progression of abnormal growth of prostate in benign prostatic hyperplasia (BPH) disease. In addition, **Zeng *et al*., (2012)** mentioned that there is a positive correlation between FSH and LH sex hormone levels and aging as well as with international Prostate Symptom Score**.**

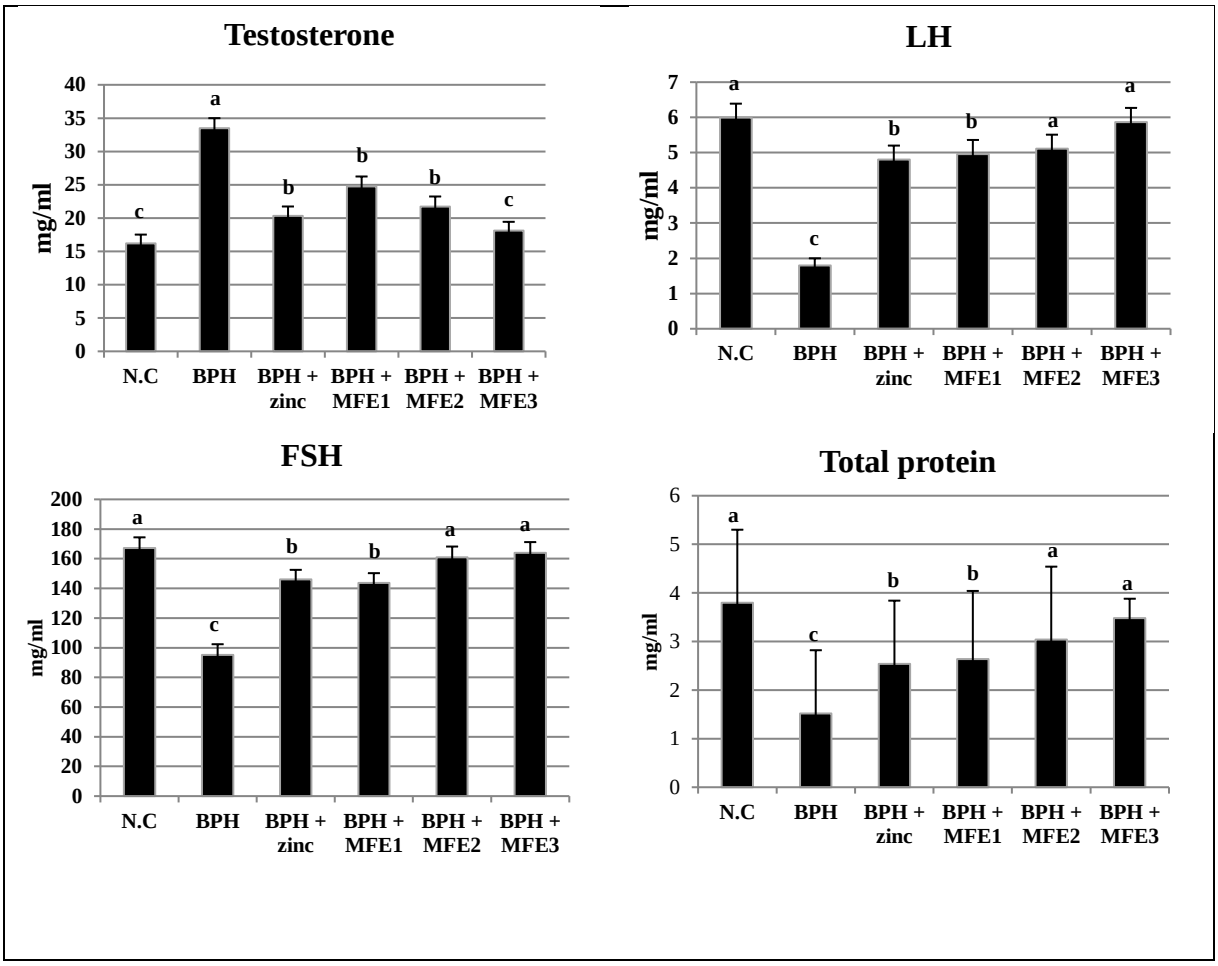

Figure 2. Effect of MFE with different levels on the concentrations of testosterone, LH, FSH, and total protein in each group. Mean values in each bar having different superscripts (a, b, c..) denote significant differences, N.C: Normal control, BPH: Benign prostatic hyperplasia, MFE: Mangosteen fruit extract.

Effect of MFE with different levels on SOD, TAC and MDA levels in normal and BPH groups

The alterations occurring in MDA levels in the normal and BPH groups of rats are shown in (Figure 3). MDA level as a marker of lipid peroxidation was significantly raised in the (+ve) untreated group compared with the N.C (-ve). This rise in MDA concentration and reduced SOD and TAC activities demonstrated that BPH is linked with a significant increase in lipid peroxidation and oxidative stress which are induced by testosterone in rats. Administration of different doses of MFE to BPH groups caused a significant decline in MDA concentration. The MFE_3 group is considered as the most favorable protective factor in decreasing MDA levels and increasing SOD and TAC levels. Hence, a non-significant difference of MDA was observed in MDA, SOD, and TAC levels in MFE3 protected group as compared to N.C (-ve) normal control group. Interestingly, MFE enhanced antioxidant defense mechanisms.

Srivastava and Mittal (2005) indicated that the reduction in antioxidant activities owing to free radicals. As they stated, the imbalance of oxidant-antioxidant may be one of the major causes accountable for BPH progress. The generation of free radicals as occurred by raising MDA concentration in patients with BPH could be one of the reasons, associated with the development

of cancer. Moreover, **Aydin *et al,* (2006)** and **Aryal *et al*., (2007)** adduced that peroxidation of lipids increases after BPH induction and antioxidants activities are reduced. However, the enhancement in antioxidant defense activities after treatment with MFE in agreement with other findings obtained by **(Karim *et al*., 2018)** who reported that phytochemical components of mangosteen caused the nephroprotective effect by reducing MDA level. In addition, (**Liu *et al*., 2018)** as they reported that bioactive constituents presented in mangosteen increase antioxidant enzyme (SOD). Furthermore, various studies demonstrated that the extract of mangosteen has high antioxidant activities (**Weecharangsan *et al*., 2006 and Chatatikun and Chiabchalard 2017).**

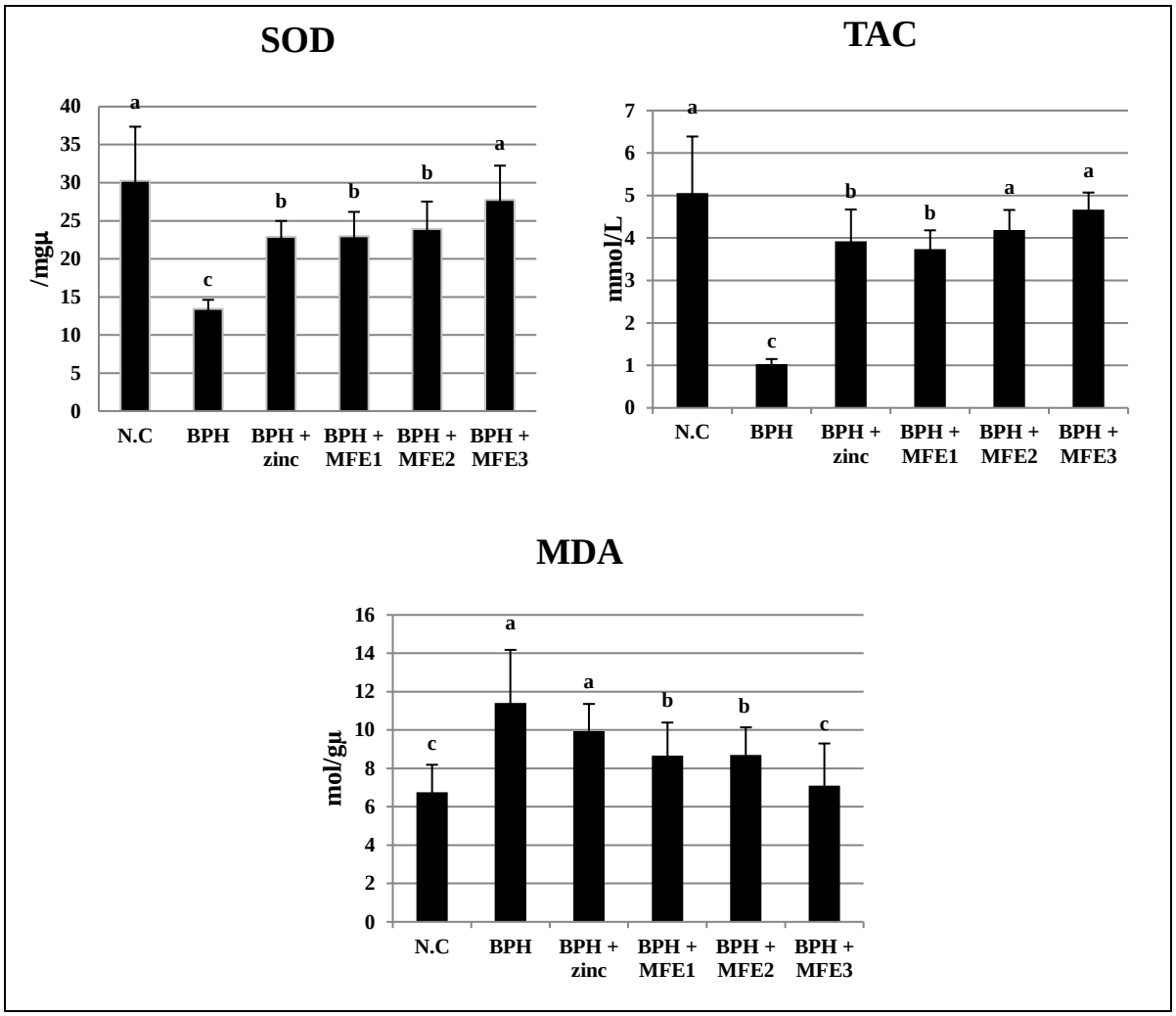

Figure 3. Effect of MFE with different levels on SOD, TAC and MDA levels in normal and BPH groups, Mean values in each bar having different superscript (a, b, c..) denote significant difference, N.C: Normal control, BPH: Benign prostatic hyperplasia, MFE: Mangosteen fruit extract, TAC: total antioxidant capacity, SOD: superoxide dismutase, MDA: malondialdehyde

CONCLUSION

Findings from this study showed that mangosteen fruit extract (MFE) with different levels, especially the high dose of (3ml/kg b.w) protected benign prostatic hyperplasia (BPH) induced by testosterone and the mechanisms possibly through enhancing total antioxidant capacity, and restoration of SOD activity and decline of MAD levels. Thus, mangosteen fruit extract could be a

potential bioactive therapeutic agent in the management and control of BPH in men. Further studies with isolated constituents are recommended for better knowledge of the complete mechanism of mangosteen fruit in benign prostatic hyperplasia.

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