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Icariin Ameliorates Metabolic Syndrome-Induced Benign Prostatic Hyperplasia in Rats

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Abstract

Metabolic syndrome (MetS) is an immense health issue that causes serious complications in aging males with MetS. Icariin (ICA) is a flavonol glycoside that exerts a plethora of pharmacological effects. The present investigation tested the potential of ICA to ameliorate benign prostatic hyperplasia (BPH) induced by MetS in rats. Animals were allocated to 5 groups in which the first and second groups were kept on water and regular food pellets. MetS was induced in the third, fourth and fifth groups by keeping the animals on high fructose and salt diets for twelve consecutive weeks. These groups were given vehicle, ICA (25 mg/kg) and ICA (50 mg/kg) respectively. MetS was confirmed by the apparent increase in rats' weight, accumulation of visceral fat, insulin resistance and dyslipidemia. This was accompanied by signs of BPH including increased prostate weight, prostate index and histopathological alterations. Treating the animals with both doses of ICA significantly ameliorated the raise in weight and index of the prostate as well as prostate histopathology. In addition, ICA significantly decreased cyclin D1 expression, up-regulated *Bax* and down-regulated *Bcl2* mRNA expression. ICA prevented lipid peroxidation, reduced glutathione (GSH) depletion and catalase (CAT) exhaustion, which further lowered inflammatory markers of the prostate such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α). Moreover, ICA prevented the decrease in prostate content of phosphorylated 5′-adenosine monophosphate (AMP)-activated protein kinase (pAMPK). In conclusion, ICA protects against MetS-induced BPH. This is due to its antiproliferative, proapoptotic, antioxidant and anti-inflammatory activities as well as the activation of AMPK.

Introduction

Benign prostatic hyperplasia (BPH) is a debilitating condition seen in males during the 4^{th} decade of life. BPH incidence increases with aging targeting 70–80% in those older than 80 years. (Madersbacher et al. 2019). BPH may induce lower urinary tract symptoms (LUTS) like voiding and storage symptoms, and dribbling (Yin et al. 2015; Zou et al. 2016). Apart from age, other factors involved in the pathogenesis of BPH include androgen disorders, ethnicity and metabolic syndrome (MetS) (Stroup et al. 2012; Kopp 2018). The later was reported as a great risk for BPH development and progression of LUTS (Zou et al. 2016; Ngai et al. 2017; Wu et al. 2019). MetS is a medical condition, with characteristic signs of obesity, dyslipidemia, elevated blood pressure and insulin resistance (Yin et al. 2015). Links between MetS and BPH/LUTS remain unclear, however, many findings indicate that chronic inflammation has a critical role (Ngai et al. 2017). Other reported mechanisms that contribute to the development of BPH include, disturbed sex hormones, inflammations and hyperinsulinemia (Ricke et al. 2011). In addition, it has been found that decreased 5′-adenosine monophosphate (AMP)-activated protein kinase (AMPK) activity is a risk factor associating MetS to BPH (Vanella et al. 2014). This is evidenced by reports highlighting activation of AMPK as a mediator of the protective activity of drugs as metformin (Mosli et al. 2015).

Icariin (ICA) is a flavonol glycoside obtained from the traditional herb *Epimedium koreanum* Nakai (Lu et al. 2014). ICA exhibits a variety of therapeutic activities, including improvement of sexual dysfunction (Makarova et al. 2007), modulation the immune system (He et al. 1995), and amelioration of

cardiovascular dysfunction (Xu and Huang 2007), Moreover, ICA has been evidenced to possess antiproliferative (Wang et al. 2015; Huang et al. 2019), antiinflammatory (Wang et al. 2020) and antioxidant (Yoon et al. 2021) actions. Interestingly, ICA has been shown to exhibit an inhibitory function in a cell model of BPH by upregulating MicoRNA-7 (Han et al. 2019). In addition, ICA has been shown to combat MetS in rats via activation of AMPK (Aljehani et al. 2020). Hence, the goal of this investigation was to study the role of ICA in a rodent model of MetS-induced BPH.

Material And Methods

Chemicals

Icariin (ICA) >98% was purchased from Xi'an Nate Biological Technology, China. Chemicals were of highest grade (>98%).

Animals and grouping

Forty male Wistar rats weighing (190–240 g) were bought from the animal house at the Faculty of Pharmacy, King AbdulAziz University, Saudi Arabia. They were maintained at 23 ± 2 °C under a 12 h dark/light cycle. Ethical issues were revised and approved by the Research Ethics Committee, the Faculty of Pharmacy, King Abdulaziz University (Ref. PH-114-40).

Animal treatment

After recoding the initial body weights, animals in Group 1 (control A) and Group 2 (control + ICA 50 mg/kg) were kept on water and standard food. 12 week feeding of pellets containing 3% sodium chloride and 10% fructose water incited MetS in Groups 3, 4, and 5 (Abdallah et al. 2016; Azhar et al. 2020). At week 8, animals in Groups 3, 4 & 5 were orally dosed 1% carboxymethyl cellulose (CMC) in normal saline, ICA (25 mg/kg in 1% CMC suspension), and ICA (50 mg/kg in 1% CMC suspension), respectively, daily until the end of week 12. All oral doses were administered at 10 ml/kg and were given for five days per week. Doses were chosen after a pilot study as well as based on previous experiments (Ding et al. 2018; Liu et al. 2018). At the conclusion of the study, each animal was weighed, anesthetized using 50 mg/kg ketamine and retro-orbital plexus was used for collecting blood. Then, animals were decapitated. Ventral prostates were rapidly dissected out and weighted. The remaining prostate tissues underwent fixation in 10% neutral formalin for histology studies and maintained for future analysis at -80 °C.

Assessment of MetS-related parameters

Blood sera were used to determine Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), triglycerides (TG) and low-density lipoproteins (LDL-C) as elucidated previously in our laboratory (Aljehani et al. 2020).

Weight and Prostate Index

Following prostate dissection, prostate weights were taken and calculation of the prostate index was performed per rat as a ratio of prostate weight to body weight times 1,000.

Histopathological Examination

Wax blocks of the fixed prostatic tissues were sectioned 5 µm thick. This was followed by hematoxylin and eosin (H&E) of the deparaffinized and rehydrated sections. The software Image J (1.46a, NIH, United States) was used to determine prostate glandular epithelial heights.

Assessment of oxidative status markers

After homogenization of prostate tissues in cold phosphate-buffered saline (50 mM, pH 7.5). Homogenates were employed to measure the levels of SOD, total protein, MDA, GSH using ELISA kits (cataloged under numbers 10009055, 703002 and 707002 respectively, Cayman Chemical, Ann Arbor, MI, USA).

Immunohistochemical investigation

Ethyl alcohol was utilized for deparaffinization and re-hydration of prostate sections. This was followed by a 10 min boiling of the sections in citrate buffer (pH 6.0). Then, sections were kept in 5% bovine serum albumin in tris buffered saline (BSA/TBS) for 2 hours. Primary antibodies, anti-cyclin D1 (ab16663), anti-IL-6 (ab271269) and anti-TNF-α (ab205587) (ABCAM, Cambridge, UK) were used for incubation at 4^{o}C for 12 h. The slides were washed using TBS and further incubated with a biotinylated secondary antibody (Ant-rabbit HPRD-DAB, Catalog # CTS005, R&D systems, MN, USA). Images were analyzed and quantified from at least three rats using Image J software (Image J, 1.46a, NIH, USA).

Bax and Bcl-2 analysis by real-time polymerase chain reaction (RT-qPCR)

An ultrasonic probe was used to homogenize prostate tissue. RNA extraction was performed with an extraction kit for nucleic acid (NucleoSpin 740955, Macherey-Nagel GmbH & Co. KG, Duerin, Germany). RNA concentration was measured using a spectrophotometer (dual-wavelength Beckman, Spectrophotometer, USA). cDNA was then synthesized using Reverse Transcription Kit (4368814, Applied Biosystems, Foster City, CA, USA). Amplification was done using Taq PCR Master Mix Kit (catalog # 201443, Qiagen, Valencia, CA, USA). The primers used had the following sequences: Bax sense primer, CCTGAGCTGACCTTGGAGCA and the corresponding antisense primer GGTGGTTGCCCTTTTCTACT, Bcl2 sense primer TGATAACCGGGAGATCGTGA and the corresponding antisense primer AAAGCACATCCAATAAAAAGC, as well as the reference gene β-actin sense primer TCCGTCGCCGGTCCACACCC and the corresponding antisense primer TCACCAACTGGGACGATATG. Results were shown in the cycle threshold (Ct). PCR product specificity was undertaken with PCR melt curve analysis and relative quantitation (RQ) of various genes to β-actin was using the equation $2^{-\Delta\Delta Ct}$.

Assessment of phosphorylated AMP-activated protein kinase (pAMPK)

Prostate content of phosphorylated AMP-activated protein kinase (pAMPK) was determined by ELISA kit (MyBioSource, San Diego, USA).

Statistical analysis

All data are shown as mean ± SD. Comparisons were done with one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Analyses were completed with IBM SPSS® ver 25 (SPSS Inc., Chicago, IL, USA). A value of $p < 0.05$ indicated significance in differences.

Results

Effects of ICA on metabolic syndrome-related parameters

Table 1 indicates that keeping rats on this regimen to induce MetS was successfully achieved and this was evidenced by the significant elevations in rats' weight, visceral fat weight, HOMA-IR and concentrations of TG and LDL-C as compared to both the control and the control + ICA 50 mg/kg groups. However, ICA administration significantly prevented the rise in these parameters. In particular, the higher dose of ICA (50 mg/kg) ameliorated increased weight gain, visceral fat weight, HOMA-IR, serum TG and serum LDL-C by 41.7, 30.8, 51.4, 39.4 and 27.6% relative to MetS group, respectively.

Effects of ICA on prostate weigh and prostate index

As indicated by results in Table 2, MetS induction resulted in a major augmentation of prostate weight and index by 150% and 65% respectively, relative to the corresponding controls. Animals treated with ICA at 25 and 50 mg/kg decreased prostatic weights by 30% and 48% and the prostatic index by 22% and 33% relative to the MetS group respectively.

Histopathological investigation

Histopathological investigation of control prostates (Fig 1A) and normal animals given ICA 50 mg/kg (Fig 1B) showed normal histology of acini that were lined by cuboidal to low columnar epithelium. Prostate sections obtained from MetS animals showed markedly hyperplastic acini characterized by frequent inward folding of the epithelial layer forming intraluminal projections. The hyperplastic epithelium appeared as stratified layers of cells filling the acinar lumen. Some examined sections showed expansion of the interstitial tissue with edema and infiltrated inflammatory cells (Fig 1C). However, prostate sections of animals administered ICA (25 and 50 mg/kg) displayed few hyperplastic growth as indicated by average acini and stroma and less inflammatory cell infiltration in a dose-related manner (Figs 1D and 1E). Glandular epithelial height determination confirmed this finding. ICA at both 25 and 50 mg/kg prevented raised epithelial heights by 41% and 51%, respectively relative to MetS rats (Fig 1F).

ICA anti-proliferative activity

A. Cyclin D1

The expression of cyclin D1 protein was evaluated using immunohistochemical technique. Both controls, C and C + 50 mg/kg, had moderately stained cells (Fig 2A & 2B). Induction of MetS resulted in a higher intensity of stained cells which suggests a higher cyclin D1 expression compared with controls (Fig. 2C). At the same time, ICA treatment (25 and 50 mg/kg) decreased cyclin D1 immunostaining relative to the MetS group dose-dependently (Figs 2D and E). The intensity of cyclin D1 was quantified by a densitometer. Results in Fig. 2F indicate that ICA (25 and 50 mg/kg) lowered the expression of cyclin D1 by 38% and 63%, as compared to MetS group respectively.

B. Bax and Bcl-2 mRNA expression

Experimental induction of MetS caused a marked reduction in *Bax* mRNA level by 31% compared with controls. While, ICA (low and high doses) resulted in an up-regulation of *Bax* mRNA expression by 16 and 28%, respectively (Fig 3A). In addition, MetS caused a profound rise in *Bcl2* mRNA expression, 126% relative to controls. ICA (25 and 50 mg/kg) down-regulated the expression of *Bcl2* by 16% and 42% respectively (Fig 3B).

Oxidative stress markers

Table 2 shows that MDA level was increased by 123% while CAT activity and GSH content were reduced by 43 and 49% in comparison to controls. However, ICA (25 and 50 mg/kg) administration to MetS animals caused an improvement in the oxidative status. The rise in MDA as a marker of lipid peroxidation was inhibited by 40% and 52% respectively. In addition, ICA (50 mg/kg) significantly reversed CAT exhaustion and GSH depletion and enhanced their values by 45% and 86% as compared to MetS group, respectively.

Immunohistochemical evaluation of IL-6 and TNF-α expression in the prostate

Data in Fig 4 indicate that 12 week feeding of high-fructose high-salt diet increased inflammatory markers IL-6 and TNF-α by 166%, and 173% respectively in comparison to controls. However, ICA (25 and 50 mg/ kg) protected against raised IL-6 expression by 19% and 43% respectively. Similarly, both doses (25 and 50 mg/kg) inhibited increased TNF-α protein expression by 27% and 44% respectively compared to MetS values.

Effect of ICA on prostatic content of pAMPK

Experimental induction of MetS significantly decreased the prostatic content of pAMPK by 53% as compared to control values 25 or 50 mg/kg ICA administration significantly prevented the drop in pAMPK content and enhanced the values by 94% and 112% respectively relative to MetS rats (Fig 5).

Discussion

The aim of this investigation was to assess the protection by ICA in MetS-induced BPH in rats. The use of MetS model of BPH has the advantage of connecting the disease to metabolic disorders (Zhang et al.

2021). These include central obesity, insulin resistance and dyslipidemia (Vignozzi et al. 2016). In fact, the link between BPH and MetS in humans is well recognized (Ozden et al. 2007; Xiong et al. 2021). In the current work, MetS was achieved by keeping rats on high-fructose drinking water and high-salt diet. MetS was confirmed by the observed accumulation of visceral fat, increased body weight gain, insulin resistance as dyslipidemia. ICA successfully ameliorated most of the hallmarks of MetS. This has been previously published (Aljehani et al. 2020). Additionally, MetS was accompanied by BPH as indicated by increased prostate weight and prostate index. BPH was also confirmed histologically as there was a significant increase in glandular epithelia height. This agrees with several experimental investigations linking different parameters of MetS to BPH (Ribeiro et al. 2006; Aaron-Brooks et al. 2019). ICA at both doses (25 and 50 mg/kg) significantly prevented the rise in prostate weight and the development of BPH. This is in consistent with an in vitro study that highlighted ICA's ability to stop growth and migration of and the prostate epithelial cells RWPE-1 and the BPH-1 epithelial cells (BPH-1). (Han et al. 2019). Interestingly, ICA low dose (25 mg/kg) caused no significant decreased weight gain suggesting that the protective effects of ICA against development of BPH is be independent of the observed amelioration of signs of MetS.

The inhibitory effects of ICA against MetS-induced hyperplasia of prostatic tissue can be linked to its antiproliferative activities. This is evidenced by the observed inhibition of cyclin D1, the enhancement of Bax as well as inhibition of Bcl2 mRNA expression. This gains support by several reports highlighting the antiproliferative and anticancer activities of ICA. The compound has been proven to inhibit the growth of many cell lines including leukemia (Lin et al. 2004), Leyding (Wang et al. 2011), esophageal (Gu et al. 2017), colon (Tian et al. 2018) and ovarian (Alhakamy et al. 2020) cancer cells. In addition, the compound icaritin, which is related in structure is known to inhibit growth and proliferation of te PC-3 (prostate carcimoma cells) (Huang et al. 2007). Moreover, the antiproliferative effects of ICA in cervical cancer cells promoted of apoptosis as evidenced by down-regulation of Bcl2 and c-Myc as well as the upregulation of Bax and P53 (Li et al. 2021). Also, ICA has been reported to trigger apoptosis via inhibition of SIRT6/NF-κB in triple-negative breast cancer cells (Song et al. 2020) and increase response of human colon cancer cells to apoptosis induced by TRAIL (Kim et al. 2020).

Oxidative stress was postulated to be involved in the pathogenesis of MetS (Mahjoub and Masrour-Roudsari 2012). MetS is characterized by a disturbed balance between synthesis and deactivation of reactive oxygen species (Roberts and Sindhu 2009). This has been previously reported in an experimental model of MetS prompted by high fructose and high salt feeding (Aljehani et al. 2020). Our data indicated MetS inducted caused increased oxidative stress in prostatic tissues as seen by the buildup of lipid peroxides, GSH depletion and CAT exhaustion. The occurrence of BPH as a result of oxidative stress was proven and well-established (Udensi and Tchounwou 2016). Our data indicated that ICA ameliorated all markers of oxidative stress in the prostate implicating the antioxidant role of ICA in preventing the development of BPH. These data are also in consistent with ICA's reported antioxidant effects (He et al. 2020). The chemical structure of ICA plays a pivotal role in its antioxidant properties. ICA is a prenylated flavonol that can stabilize reactive oxygen species exerting antioxidation (Li et al. 2015).

Many justifications were suggested to elucidate the basis of MetS. Low-grade chronic inflammation has been considered as a main factor of MetS (De Ferranti and Mozaffarian 2008). Indeed, MetS is widely accepted as an inflammatory condition (Reddy et al. 2019). The findings of this study consolidate these explanations as MetS rats showed elevated expression of levels of IL-6 and TNF-α. According to many reports, there is a strong link between BPH pathophysiology and inflammation as well as inflammatory mediators (McClinton et al. 1990; Theyer et al. 1992; Madersbacher et al. 2019; Devlin et al. 2020). ICA successfully prevented the rise in the inflammatory markers IL-6 and TNF-α. This agrees with the reported anti-inflammatory activities of ICA (Wu et al. 2011) and lends an acceptable explanation for its observed beneficial actions in prostatic tissues. To deepen our understanding of the anti-inflammatory effects of ICA, its impact on pAMPK was investigated in the prostatic tissues. Our data indicated that MetS induction also meant a lower content of active AMPK. However, treatment of animals with ICA caused amelioration of pAMPK in prostates. This is consistent with a previous study in our laboratory (Aljehani et al. 2020). AMPK regulates diverse metabolic and physiological processes and is dysregulated in several chronic diseases (Jeon 2016). In particular, BPH has been shown to involve lower contents of active AMPK (Vanella et al. 2014). Activation of AMPK has been proposed to counteract pro-inflammatory mediators (Green et al. 2011; Lim et al. 2015; Zhang et al. 2018). In conclusion, ICA affords protection against MetS-induced BPH in rats. A partial explanation of the observed effects could be due to its antiproliferative, proapoptotic, antioxidant and antiinflammatory activities as well as activation of AMPK.

Declarations

Ethics approval and consent to participate: All animal handling procedures were approved and supervised by the Research Ethics Committee, the Faculty of Pharmacy, King Abdulaziz University (Ref. PH-114-40).

Consent for publication: Not applicable

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interest: The authors declare no competing interests

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Author contribution Conceptualization: Abeer Aljehani, and Mohammed Nasrullah: Performing experimental part and data collection. Thikryat Neamatallah and Basma. Eid: Supervision of experiments, statistical analysis and writing first draft. Nawal Albadr and Ashraf Abdel-Naim: Designing the set of experiments and revising the manuscript.

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Tables

Table 1: Effect of icariin on metabolic syndrome due to high-fructose and high salt feeding in rats

	Weight gain (%)	Visceral fat (g)	HOMA-IR	TG (mg/dL)	LDL-C (mg/dL)
Control (C)	51.9 ± 8.1	10.3 ± 2.1	2.51 ± 0.46	88.23 ± 7.45	24.56 ± 2.84
C + ICA 50 mg/kg	50.6 ± 4.7	9.5 ± 2.2	2.60 ± 0.35	90.45 ± 8.81	26.62 ± 3.11
MetS	103.3 [a,b] ± 12.8	19.1 [a,b] ± 2.6	5.79 [a,b] ± 0.59	195.56 [a.b] ± 17.40	38.43 [a,b] ± 3.18
MetS + ICA 25 mg/kg	87.1 [a,b] ± 8.1	15.5 [a,b,c] ± 1.9	3.39 [a,b,c] ± 0.55	135.84 [a,b,c] ± 11.70	28.34 [c] ± 2.16
MetS + ICA 50 mg/kg	60.2 [c,d] ± 7.5	13.2 [c] ± 1.9	2.81 [c] ± 0.25	118.45 [a,b,c] ± 9.25	27.80 [c] ± 2.34

Results are shown as Mean ± SD

a Significantly different from control (C) group at $p < 0.05$

b Significantly different from C + ICA 50 mg/kg group at $p < 0.05$

c Significantly different from MetS group at $p < 0.05$

d Significantly different from MetS + ICA (25 mg/kg) group at $p < 0.05$

Table 2: Effect of icariin on prostate weight and prostate index in MetS-induced BPH in rats.

	Final body weight (g)	Prostate weight (mg)	Prostate index X 10^3
Control (C)	312 ± 14.8	249.6 ± 34.5	0.80 ± 0.11
C + icariin (50 mg/kg)	316 ± 8.2	254.6 ± 32.5	0.80 ± 0.105
Metabolic syndrome (MetS)	431 [a,b] ± 23.8	624.5 [a,b] ± 75.8	1.45 [a,b] ± 0.15
MetS + icariin (25 mg/kg)	385 [a,b,c] ± 22.5	434.6 [a,b,c] ± 52.7	1.13 [a,b,c] ± 0.15
MetS + icariin (50 mg/kg)	331 [c,d] ± 18.3	323.6 [c,d] ± 41.1	0.97 [c] ± 0.11

Results are shown as Mean ± SD

a Significantly different from control (C) group at $p < 0.05$

b Significantly different from C + ICA 50 mg/kg group at $p < 0.05$

c Significantly different from MetS group at $p < 0.05$

d Significantly different from MetS + ICA (25 mg/kg) group at $p < 0.05$

Table 3: Icariin's effect on oxidative status in MetS-induced BPH rats

	MDA (nmol/mg protein)	GSH (nmol/mg protein)	CAT (U/mg protein)
Control (C)	0.64± 0.07	1.62 ± 0.15	0.96 ± 0.07
C + icariin (50 mg/kg)	0.61 ± 0.07	1.58 ± 0.14	0.94 ± 0.08
Metabolic syndrome (MetS)	1.43 ^{a,b} ± 0.15	0.83 ^{a,b} ± 0.07	0.55 ^{a,b} ± 0.06
MetS + icariin (25 mg/kg)	0.86 ^{a,b,c} ± 0.09	1.28 ^{ab,c} ± 0.18	0.62 ^{a,b} ± 0.05
MetS + icariin (50 mg/kg)	0.69 ^{c,d} ± 0.05	1.55 ^{c,d} ± 0.16	0.80 ^{a,b,c,d} ± 0.08

Results are shown as Mean ± SD

a Significantly different from control (C) group at $p < 0.05$

b Significantly different from C + ICA 50 mg/kg group at $p < 0.05$

c Significantly different from MetS group at $p < 0.05$

d Significantly different from MetS + ICA (25 mg/kg) group at $p < 0.05$

Figures

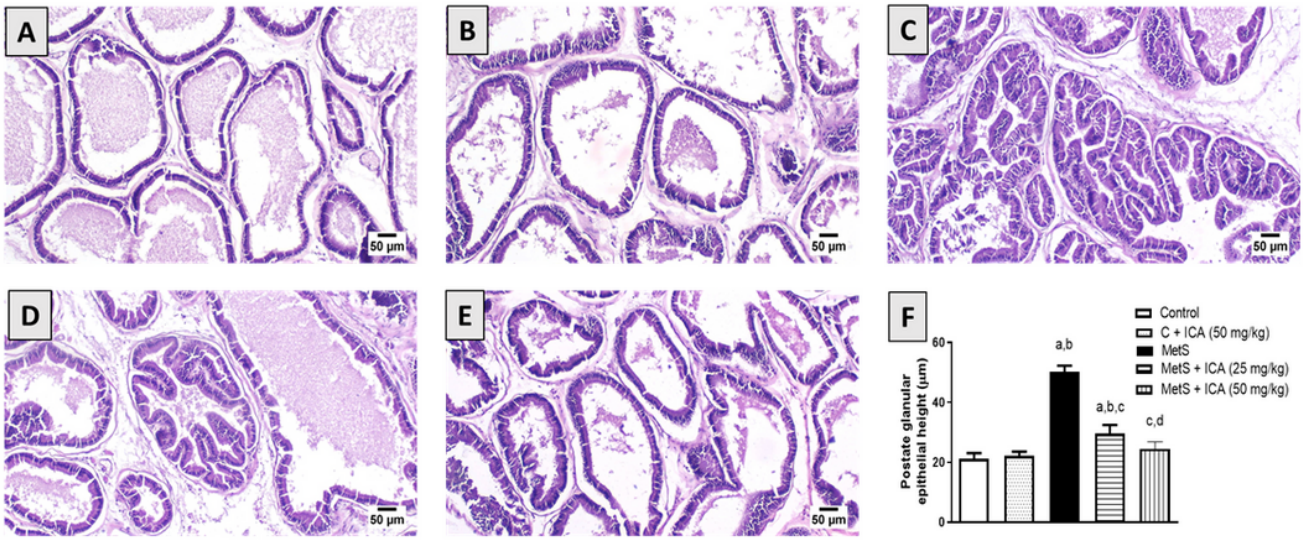

Figure 1

Photomicrograph of prostate sections stained with Hematoxylin-eosin. A: Section from control rat (C) with normal histoarchitecture of the prostate gland. B: Section from C + ICA 50 mg/kg rat. C: Section from MetS rat showing increased epithelial thickness and hypertrophy D: Section from MetS animals administered ICA (25 mg/kg) showing a small decrease in hyperplasia and hypertrophy. E: Section from MetS animals administered ICA (50 mg/kg) with an obvious decrease in hyperplasia and hypertrophy, E: Measurement of prostate glandular epithelial height in the different treatment groups. Results are shown as mean ± SD. a: significantly different from control (C), b significantly different from C + ICA 50 mg/kg, c: significantly different from MetS, d: significantly different from MetS + ICA (25 mg/kg).

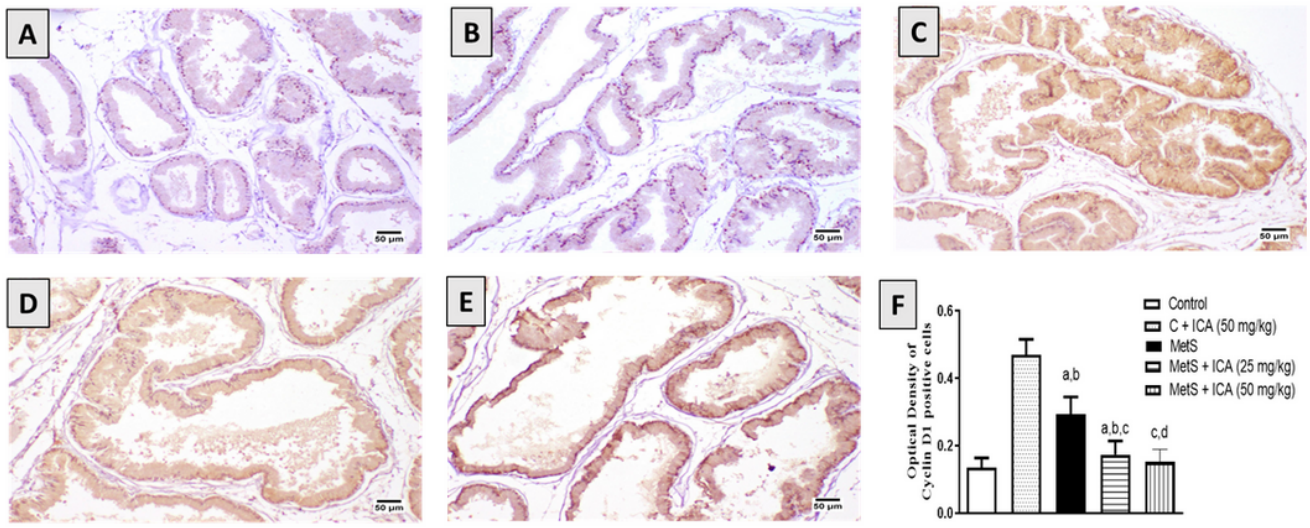

Figure 2

Photomicrographs of prostate sections immune-stained for cyclin D1. A: Section from control rat (C) B: Section from C + ICA 50 mg/kg rat. C: Section from MetS rat with extensive cyclin D1 positive cells D: Section from MetS rat treated with ICA (25 mg/kg) E: Section from MetS rat treated with ICA (50 mg/kg) F: The effect of ICA treatment on expression of cyclin D1 in the prostate. Results are shown as mean ± SD. a: significantly different from control (C), b significantly different from C + ICA 50 mg/kg, c: significantly different from MetS, d: significantly different from MetS + ICA (25 mg/kg).

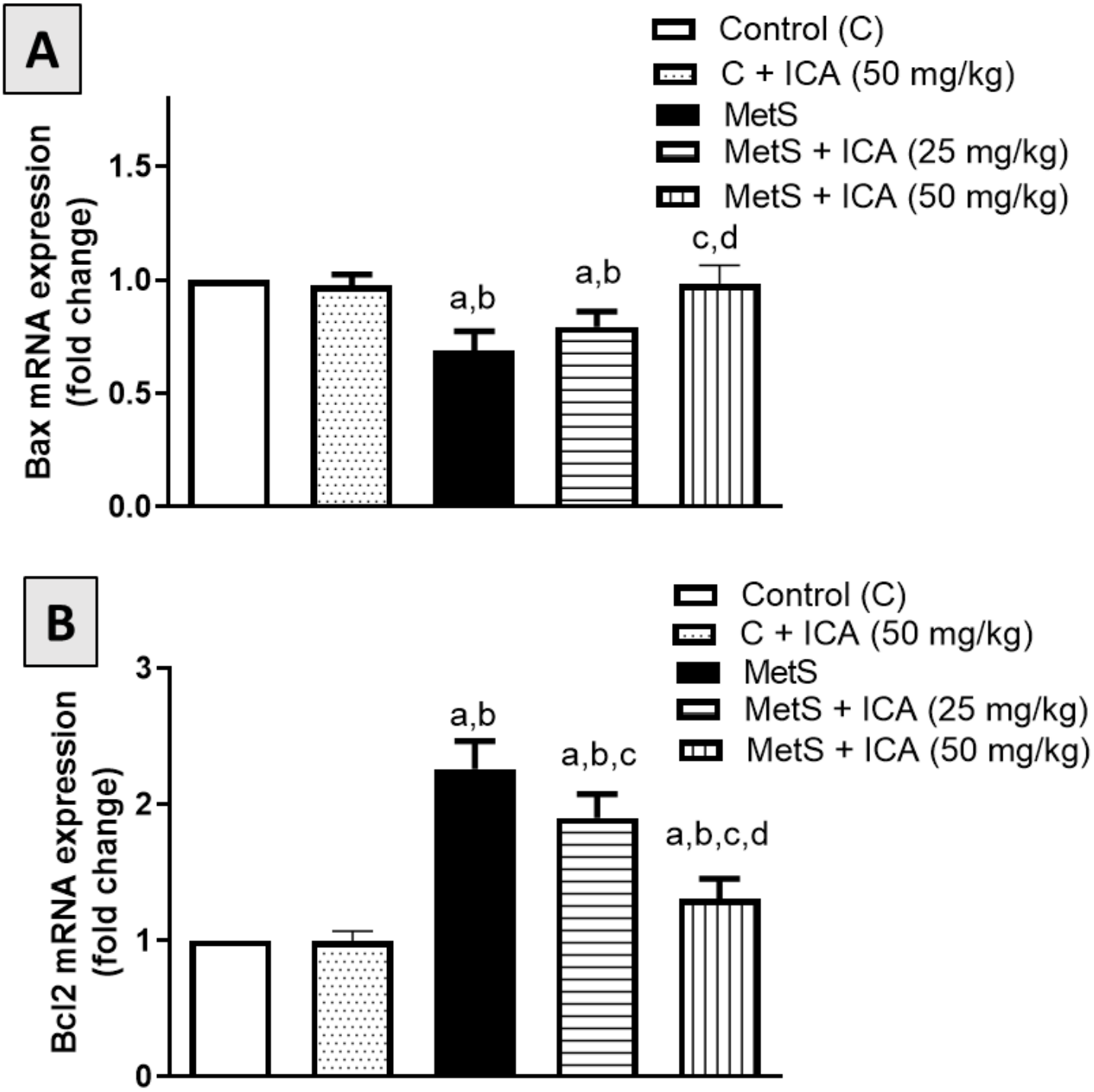

Figure 3

qRT-PCR relative quantification of Bax (Panel A) and Bcl-2 (Panel B) mRNA levels in prostates. Results are represented as mean ± SD. a: significantly different from control (C), b significantly different from C + ICA 50 mg/kg, c: significantly different from MetS, d: significantly different from MetS + ICA (25 mg/kg).

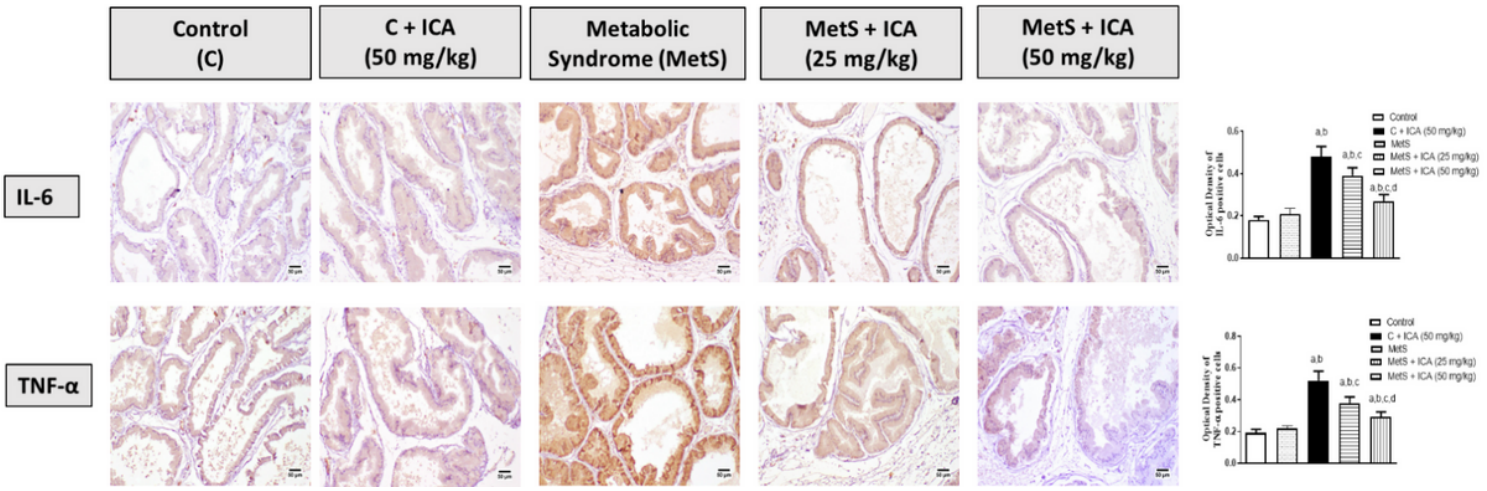

Figure 4

Photomicrographs of prostate sections immune-stained for IL-6 and TNF-α. Results are represented as mean ± SD. a: significantly different from control (C), b significantly different from C + ICA 50 mg/kg, c: significantly different from MetS, d: significantly different from MetS + ICA (25 mg/kg).

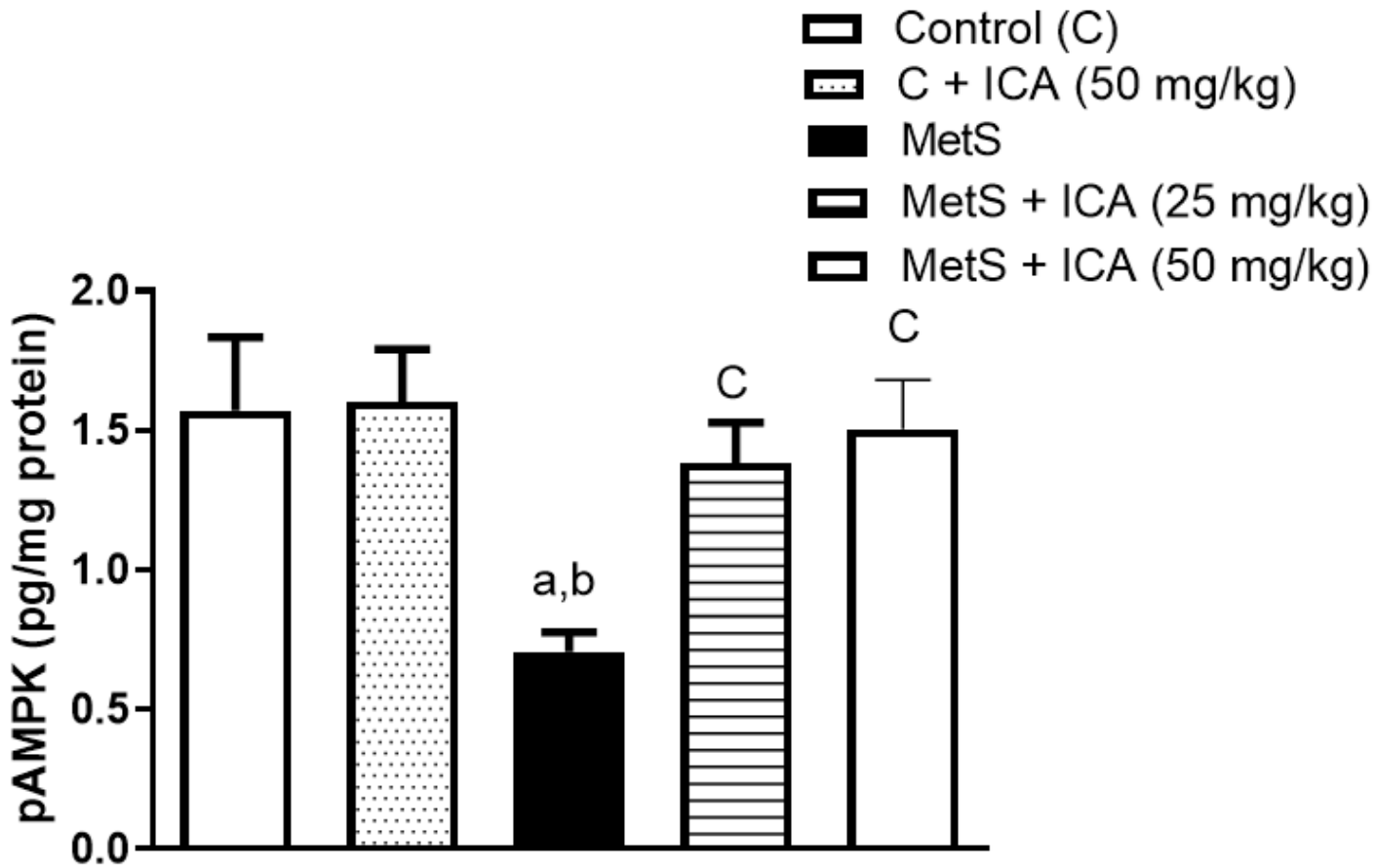

Figure 5

Effect of ICA on prostatic pAMPK content in metabolic syndrome (MetS) rats. Results are represented as mean ± SD. a: significantly different from control (C), b significantly different from C + ICA 50 mg/kg, c: significantly different from MetS, d: significantly different from MetS + ICA (25 mg/kg).