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Original Article

Effects of apigenin on scrotal heat-induced damage in the mice testis

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Abstract: To investigate the effect of apigenin on scrotal heat-induced damage in mice, 8-week-old ICR mice were divided into five groups: (I) control group; (II) heat-treat (H) group; (III) H + apigenin 10 mg/kg; (IV) H + apigenin 20 mg/kg; (V) H + apigenin 50 mg/kg. Serum testosterone and inhibin B levels by immunoassays were evaluated. The related oxidative damage markers were determined by kit and histopathological examination was also performed. The results showed that the administration of apigenin was shown a significant effect on the recovery of testis function. When compared with the H group, the H + apigenin groups showed a significant increase in testosterone and inhibin B levels. Superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activity increased after treated by apigenin, but malondialdehyde (MDA) reduced. Histopathological examination further verified the effects of apigenin on scrotal heat-induced damage mice. In conclusion, the results of the present study indicate for the first time the ability of apigenin in attenuation of heat-induced testicular damage, apigenin therapy may be useful as a suppressor of degenerative effects following testicular hyperthermia.

Keywords: Apigenin, testis, antioxidant, scrotal hyperthermia, mice

Introduction

Scrotal temperature and fertility are closely associated and the temperature of the testis is a few degrees lower than the abdomen, this cooler temperature is essential for testis to maintain a suitable environment for spermatogenesis [1-3]. Many studies have documented the adverse effects of heat stress on the human testis [4]. Seeking an effective therapeutic to solve or prevent the problem of scrotal heat-induced sterility is urgently.

Oxidative stress play a fundamental role in the regulation of apoptosis, it widely involved in the pathophysiologies of male infertility [5, 6]. Previous studies have reported that scrotal temperatures above the normal range caused oxidative stress and germ cell loss in testis, which lead to male subfertility [7, 8]. Oxidative stress caused by numerous factors including elevated temperature and so on, germ cell apoptosis caused by heat-induced oxidative stress is a potential mechanism to impair spermatogenesis. Pharmaceutical agents with antioxidant features may be beneficial for scrotal heat-induced sterility.

Apigenin (4, 5, 7-trihydroxyflavone), a less toxic and non-mutagenic flavones subclass of flavonoids, commonly found in parsley and dried flowers of chamomile as well as in other plants, fruits, vegetables, herbs, and spices [9, 10]. Apigenin has many pharmacological activities, such as anticancer, antiviral, antibacterial, antioxidant, anti-apoptosis, and anti-inflammatory [11-13]. To the best of our knowledge the beneficial effects of antioxidant pharmaceutical agent were limited in heat-induced sterility. Therefore, this study was set to clarify whether the harmful effects of testicular hyperthermia could be prevented by chronic administration of apigenin and it could accelerate the recovery process of mouse testis following heat exposure.

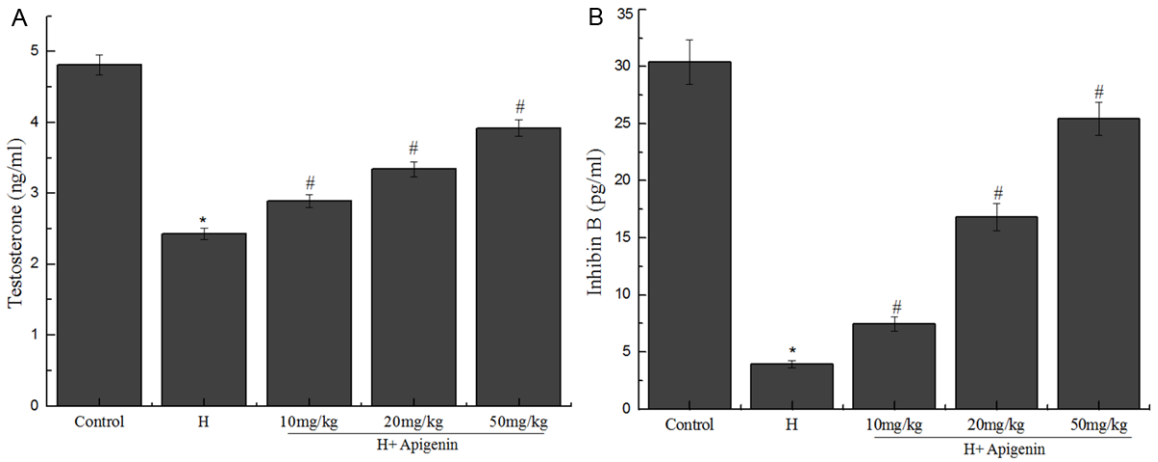

Figure 1. Effects of apigenin on serum cytokine levels in mice. Apigenin markedly increases the serum levels of testosterone (A) and inhibin B (B) in the mice underwent heat-treat. Data were shown as mean ± SD. $^{*}P < 0.05$ vs. control group, $^{\#}P < 0.05$ vs. H group.

Materials and methods

Materials

Apigenin was obtained from Chengdu Must Biotechnology Co., Ltd. (Chengdu, China), the purity of the chemical was more than 98.0%.

Animals

Male Institute of Cancer Research (ICR) mice (8 weeks old) were obtained from the Experimental Animal Center of Suzhou Aiermaite technology Co. Ltd. (SPF grade, Certificate No. SCXK20140007). The mice were housed in a room under temperature (23 ± 2°C), with a relative humidity of 50 ± 10%, 12 h light/dark cycle and free access to water and food.

Ethics statements

All animal experiments were performed in accordance with the Institutional Animal Care Committee of Yuhuangding hospital.

Experimental procedure

Mice were randomly assigned to five groups: (I) control group; (II) heat-treat (H) group; (III) H + apigenin 10 mg/kg; (IV) H + apigenin 20 mg/kg; (V) H + apigenin 50 mg/kg. Each group consisted of 10 mice.

Testicular hyperthermia was induced as described previously [14]. In short, after mice anesthesia, their testes were immersed once in a thermostatically controlled water bath at 43°C for 30 min once daily for six consecutive days. Control mice were treated in the same way, but kept at 22°C. After scrotal hyperthermia, animals were dried, examined for any redness or injury to the scrota, and then returned to their cages. The mice in the apigenin treatment groups (groups III, IV and V) received apigenin by gavage, once daily for 35 days respectively. I, II groups received physiologic saline instead.

Serum cytokines analysis

The mice were anesthetized with 10% chloral hydrate (300 mg/kg), the blood sampling was centrifuged 3,000×g for 10 min at 4°C. The supernatant was collected and serum testosterone and inhibin B levels were measured by enzyme-linked immunosorbent assay (ELISA) kits (Nanjing Jiancheng Co., Nanjing, Jiangsu, CHN).

Biochemical analysis of antioxidant status

The testes were homogenized in physiological saline solution and centrifuged at 3, 000×*g* for 10 min at 4°C. The supernatant was collected and quantitatively assayed for the levels of MDA, SOD and GSH-Px. The detection of these substances used enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer instructions (Nanjing Jiancheng Co., Nanjing, Jiangsu, CHN).

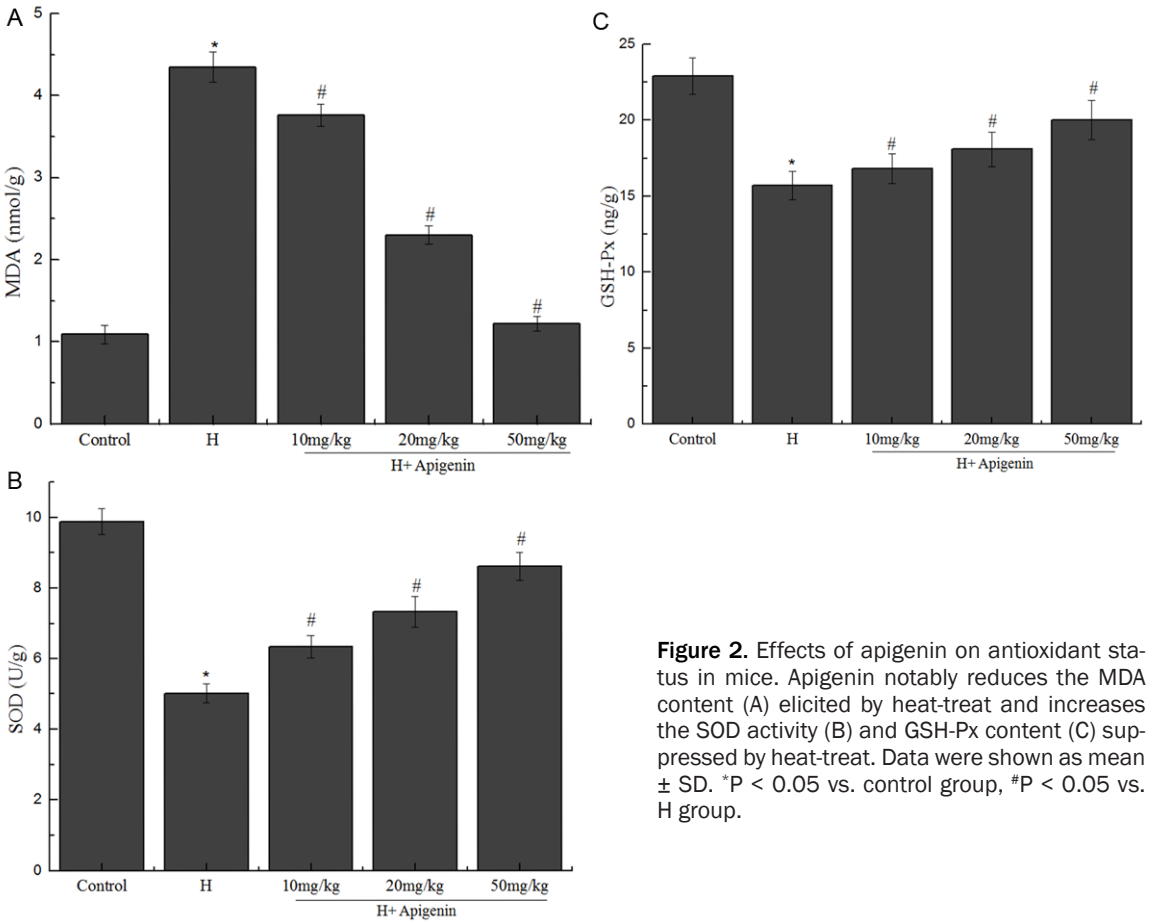

Figure 2. Effects of apigenin on antioxidant status in mice. Apigenin notably reduces the MDA content (A) elicited by heat-treat and increases the SOD activity (B) and GSH-Px content (C) suppressed by heat-treat. Data were shown as mean ± SD. *P < 0.05 vs. control group, #P < 0.05 vs. H group.

Histopathological evaluation

Testes from each animal were prepared for histological study. Following fixation of testicular sections in 4% paraformaldehyde solution overnight, the specimens were embedded in paraffin wax and then cut into 5-μm thick sections. Finally, the sections were stained with hematoxylin and eosin (H&E) and examined under a light microscope.

Statistical analysis

Statistical analysis was performed by using SPSS 17.0 and data were reported as the mean ± SD. Differences between groups were analyzed by one-way analyses of variance (ANOVA) followed by Scheffe test. P < 0.05 was considered to indicate statistically significant.

Results

Effect of apigenin on serum testosterone and inhibin B level in mice

Differences in testosterone concentrations in serum among groups are shown in **Figure 1A**. Serum testosterone level decreased in mice treated with hyperthermia compared to the control group (P < 0.05), while it increased in H + apigenin groups as compared to the H group (P < 0.05, respectively).

As shown in **Figure 1B**, serum inhibin B levels decreased significantly after heat treatment (P < 0.05). The inhibin B levels were higher in the H + apigenin groups than the H group (P < 0.05, respectively).

Effect of apigenin on testicular malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activity in mice

As shown in **Figure 2**, scrotal hyperthermia resulted in significant increases in MDA levels in the testicular tissues when compared with the control group (P < 0.05). The antioxidant activities of SOD and GSH-Px enzymes after scrotal hyperthermia was significantly lower when compared with the control (P < 0.05, respectively). On the contrary, administration of

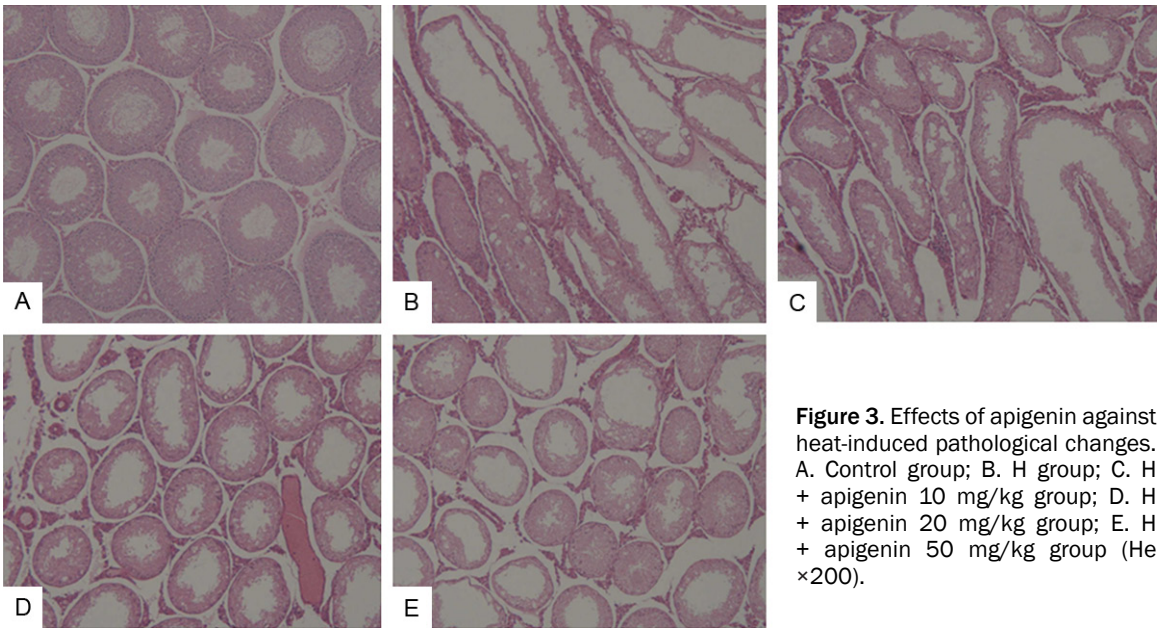

Figure 3. Effects of apigenin against heat-induced pathological changes. A. Control group; B. H group; C. H + apigenin 10 mg/kg group; D. H + apigenin 20 mg/kg group; E. H + apigenin 50 mg/kg group (He ×200).

apigenin to mice resulted in a significant reduction in testicular MDA contents ($P < 0.05$) and a significant increase in testicular SOD and GSH-Px content ($P < 0.05$, respectively) as compared to the H group.

Effect of apigenin on testicular histopathological changes in mice

Figure 3 demonstrates photomicrographs that illustrate the histological changes in the mouse testis of different treatment groups. On histopathological examination, testes of control mouse showed normal morphology and spermatogenesis, containing abundant amounts of spermatids and sperm in the lumen (**Figure 3A**). On the contrary, H group showed remarkable degenerative changes, necrosis and disorganization of the histological structure of seminiferous tubules. Germinal epithelial cells were separated from each other and from the tubular basement membrane. There was also desquamation of germinal cells and consequent appearance of irregular spaces in the epithelium (**Figure 3B**). Administration of apigenin to scrotal heat treated mice significantly prevented scrotal hyperthermia-induced testicular injury, where normal histological pattern, preserved diameter of seminiferous tubules, and normal spermatogenic series and spermatids formation were revealed. In addition, the thickness of basement membrane, sertoli cells and interstitial Leydig cells appeared intact and normal (**Figure 3C-E**).

Discussion

The present study examined the protective effects of apigenin on heat-induced testicular damage in the mice. The results of our experiment demonstrated that the deleterious effects of testicular hyperthermia can be improved obviously by apigenin administration.

An increase in scrotal temperature can cause the production of poor quality spermatozoa, germ cell depletion, and male infertility [15, 16]. Previous study demonstrated a sharp reduction in testis weight and collapsed tubules in mice subjected to scrotal heat [17]. In this study, we established scrotal heat model through exposure of the mouse scrotal testis at 43°C for 30 min. Our research shows scrotal heat treatment could reduce the concentrations of testosterone and inhibin B in serum, which are the serum markers of spermatogenesis. However, apigenin markedly increases the serum levels of testosterone and inhibin B in the mice underwent heat-treat.

Mammalian testes are highly susceptible to oxidative stress. Previous research has suggested that high concentrations of ROS play an important role in the pathophysiology of human spermatozoa [18]. ROS is a product of biological aerobic metabolism, ROS formation is always accompanied by an up-regulation of the antioxidant enzyme system, which protects tissues against damage caused by excessive ROS via

the scavenging activity of enzymes such as SOD and GSH-Px. SOD is an enzyme that is extensively used as a biochemical indicator of pathological states associated with oxidative stress [19]. GSH-Px acts as an enzymatic antioxidant both intracellularly and extracellularly in conjunction with various enzymatic processes that reduce hydrogen peroxide (H_2O_2) and hydroperoxides [20]. MDA is the end-product of the oxygen-derived free radicals and lipid oxidation, which reflects the damage caused by ROS [21]. Testicular oxidative stress is affected by the balance between ROS production and this scavenging system. In our study, MDA concentrations were significantly increased after heat treatment, concomitantly with the decrease in the SOD and GSH-Px activities. However, apigenin could significant ameliorate these abnormalities. These results indicated that scrotal heat induced oxidative stress in the testes and apigenin treatment could inhibition heat induced oxidative damage.

Histopathological examination of testis in the current study emphasized the biochemical parameters and confirmed the deleterious effects of testicular hyperthermia on the mouse testis. Our studies showed that nandrolone decanoate administration to mice reduced the number and size of Leydig cells, enhanced cytoplasmic vacuolisation, elicited lipid droplets deposition, increased lysosomes in sertoli cells, and reduced the length of seminiferous tubules. Apigenin administration in the present work has completely abolished scrotal heat-induced damage on the histopathological level.

In conclusion, our results provide evidence that testicular heating can lead to male sterility and oxidative stress occurred in heated testes. We also demonstrated that apigenin could attenuate heat-induced testicular damage in mouse model. The mechanisms of action for apigenin include inhibiting oxidative injuries. Our findings suggest that apigenin therapy may be useful as a suppressor of degenerative effects following testicular hyperthermia.

Disclosure of conflict of interest

None.

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