



PROCEEDINGS OF THE 11th WORLD RABBIT CONGRESS

Qingdao (China) - June 15-18, 2016

ISSN 2308-1910

Session Reproduction

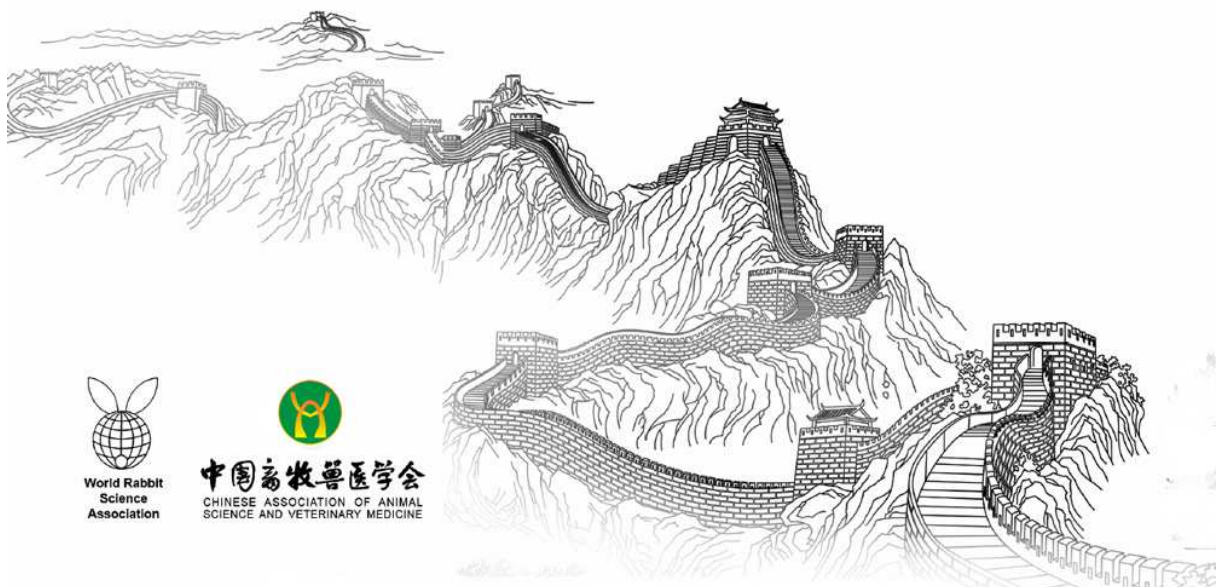
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Full text of the communication

How to cite this paper :

Zahid N., Erkmen T.E., Ejaz A., Emrah İ., Ayşe N.A., Melih A., Nihat T., 2016 - Dietary quercetin might alleviate heat stress-induced testicular histopathological changes in rabbits. *Proceedings 11th World Rabbit Congress - June 15-18, 2016 - Qingdao - China*, 237-240.



DIETARY QUERCETIN MIGHT ALLEVIATE HEAT STRESS-INDUCED TESTICULAR HISTO-PATHOLOGICAL CHANGES IN RABBITS

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ABSTRACT

The present study was designed to examine the effect of quercetin (Que) on testicular damage in summer heat stressed (HS) rabbits. Ten adults male News Zealand rabbits were exposed to summer HS (30-35 °C). After acclimatization, rabbits were divided into two groups (n=5 each group): one group (HS) remained under HS without any treatment and other group (Que) was supplemented with Que (25 mg/kg daily for 8 weeks) along HS. At the end of the treatment, rabbits were slaughtered and testes were processed to observe the histo-pathological changes. In HS group, there was slight hyperemia in interstitial vessels and dilation in lymphatic sinuses testicular stroma and distorted and sparse arrangement of seminiferous tubules contortus was noticed. Increased the degenerative maturing germ cells with swollen cytoplasm and pyknotic nuclei were observed in HS group. In addition, severe degenerative-necrotic sloughed cells were seen in the seminiferous tubules lumen. In contrast, lessened HS-induced testicular histopathologic lesions were noticed in Que compared to HS group. In conclusion, dietary supplementation of quercetin to HS male rabbits might alleviate the HS-induced testicular histopathological changes.

Key words: Heat stress, Quercetin, Rabbit, Testicular histopathology

INTRODUCTION

The lower scrotal temperature (2–8°C) respect the core body temperature provides the optimum environment for spermatogenesis in mammals (Banks et al., 2005). The increment in the scrotal temperature results in disruption of spermatogenesis process that ultimately causes the subfertility (Jung et al., 2005; Paul et al., 2008). Detrimental impingements of HS during the summer months on testicular functions have been documented in different species (Rasooli et al., 2010; Schwalm et al., 2007). Similarly, the negative impact of HS on sexual activity, semen quality (Marai et al., 2003; Roca et al., 2005; Elnagar, 2010) and testicular histological changes (Pie et al., 2012) have been reported in rabbits. The low spermatogonial germ cells in the seminiferous tubules and degeneration of Sertoli and Leydig cells are the main histological dysfunctions following exposure to high thermal stress which further lead to the arrest of the spermatogenesis (Rasooli et al., 2010). In addition, overexpression of apoptosis in spermatogonia and spermatocytes during thermal HS account for poor spermatogenesis process (Kanter et al., 2011).

To combat the thermal HS condition, several dietary alternatives have been acclaimed (Turk et al., 2015a; 2015b; Elnagar, 2010; Zeweil et al., 2013) and it has been observed that dietary supplementation of antioxidants present promising results (Kamboh et al., 2013). In this scenario, different compounds with the antioxidant property such as flavonoids have experimented. Quercetin and rutin, polyphenolic elements derived from citrus fermentation, are known potential antioxidants (Erlund, 2004) and different reports regarding protective effects of quercetin under different stressors on testicular functions are available (Aldemir et al., 2012; 2014; Aktoz et al., 2010; Ciftci et al., 2012; Sonmez et al., 2014). However, there is no single report about the effect of quercetin on testicular histopathology under thermal HS. Therefore, the present study was designed to determine the protective effect of quercetin on summer HS-induced testicular changes in adult male rabbits.

MATERIALS AND METHODS

Animals and housing

Ten adults male white New Zealand rabbits, age between 20 to 24 weeks, 2.9 ± 0.1 kg body weight, were used in the experiment. The experiment has been conducted during the hot summer months (July-September, 2015). During the trial, all the animals were placed in a room with windows exposed to the natural day length and equipped with ventilators. All rabbits from both groups were kept in individual galvanized cages. Daily air temperature ($^{\circ}\text{C}$) and relative humidity (%) were recorded (9:00, 12:00 and 17:00). The average recorded temperature on morning, afternoon and evening were 24.2 ± 0.2 , 35.3 ± 0.3 , and 31.7 ± 0.3 $^{\circ}\text{C}$, respectively. The variations in relative humidity observed at the morning, afternoon and evening were 66.7 ± 1.1 , 41.3 ± 0.8 and 37.6 ± 0.6 , respectively. Rabbits were acclimatized for one week to the environmental conditions before the start of the experiment.

Experimental design

Ten rabbits were fed maintenance diet (150g/d/animal) in pellet form with free access to fresh water. Then, animals were divided into two treatment groups ($n=5$ each group). One group (HS) was offered commercial maintenance diet and remained untreated, while, other group (Que) was fed commercial maintenance pelleted diet supplemented with quercetin (25 mg/kg/day) for 8 weeks (Bhaskar et al., 2013). At the end of the trial, all the rabbits from HS and Que groups were slaughtered. Testes were removed quickly, cleaned the extra surrounding tissues or fat and examined apparently for any lesions. Normal testes were processed further for histopathological findings.

Testicular histopathology

The testes samples were fixed in Bouin's solution for 48 hrs and then, the fixed specimens were dehydrated in alcohol and embedded in paraffin. The embedded tissues were sectioned of 5 μm , deparaffinized and stained with hematoxylin and eosin (H&E). The testicular tissue was examined and evaluated in random order under blindfold conditions with standard light microscopy by a pathologist. In the histopathological examination of testicles, we observed the ten seminiferous tubules with good visibility in a single field using $40 \times$ objective (total one hundred/slide/sample) and any deviation from the normal architecture of seminiferous tubules, germinal and Sertoli cells was recorded. Degenerated/necrotic germ cells, tubular atrophy, the presence of exfoliated germ cells in the lumen and interstitial vacuoles were differentiated by using light microscopy (Olympus BX 51, Japan).

RESULTS AND DISCUSSION

Histological examination of testicular tissues revealed that apparently the interstitial stroma (Leydig cells, vasculature and supporting stroma) appeared normal in both groups. However, there were minor pathological changes in the interstitium of HS groups such as slight hyperemia in interstitial vessels, dilation of lymphatic sinuses and distortion or sparse arrangement of seminiferous tubules contortus (Figure 1B). A large number of degenerative maturing germ cells and diffuse vacuolar degenerated type-B spermatogonia with swollen cytoplasm and pyknotic nuclei were seen in the seminiferous tubules of HS group (Figure 1E). Similarly, hyperchromatic, crescent-shaped primary spermatocytes with early apoptosis (Figure 1B) and apoptotic multinucleated giant cell (Figure 1F) were noticed. In addition, single cell necrosis in primary spermatocytes was observed in seminiferous tubules that characterized by the presence of hypereosinophilic cytoplasm and condensed nuclei (Figure 1D). In HS group, severe degenerative/necrotic sloughed germ cells (immature spermatids, and spermatogonia) were seen in the seminiferous tubules lumen compared to Que group (Figure 1E). The sparse arrangement of seminiferous tubules contortus was also observed in Que group; however, the intensity and frequency occurrence of mentioned lesions was higher in the HS group (Figure 1B).

HS increases the lipid peroxidation and changes the antioxidants enzymes levels (Turk et al., 2015) which in turn manifested in the form of lower defence at the testicular tissue level. Damage and dysfunction of testes during HS occurred due to high lipid peroxidation rate in testicular tissues and spermatogonia or immature spermatids. Several reports are available about HS effect on rabbits semen production and sperm quality (Marai et al., 2003; Roca et al., 2005; Elnagar, 2010); however, a single report shows the adverse influence of HS on testicular histology in rabbits (Pie et al., 2012). Loss of spermatogenic cells or spermatogenic arrest, presence of sloughed germ cells in seminiferous tubules, degenerative alterations in interstitial stroma or

seminiferous tubules, and distortion in germinal epithelium-like changes have been documented as cause to HS when animals were imposed to control or ambient HS (Terim Kapakin et al., 2013; Pie et al., 2012; Rasooli et al., 2010; Yin et al., 1997; Schwalm et al., 2007; Turk et al., 2015a; 2015b).

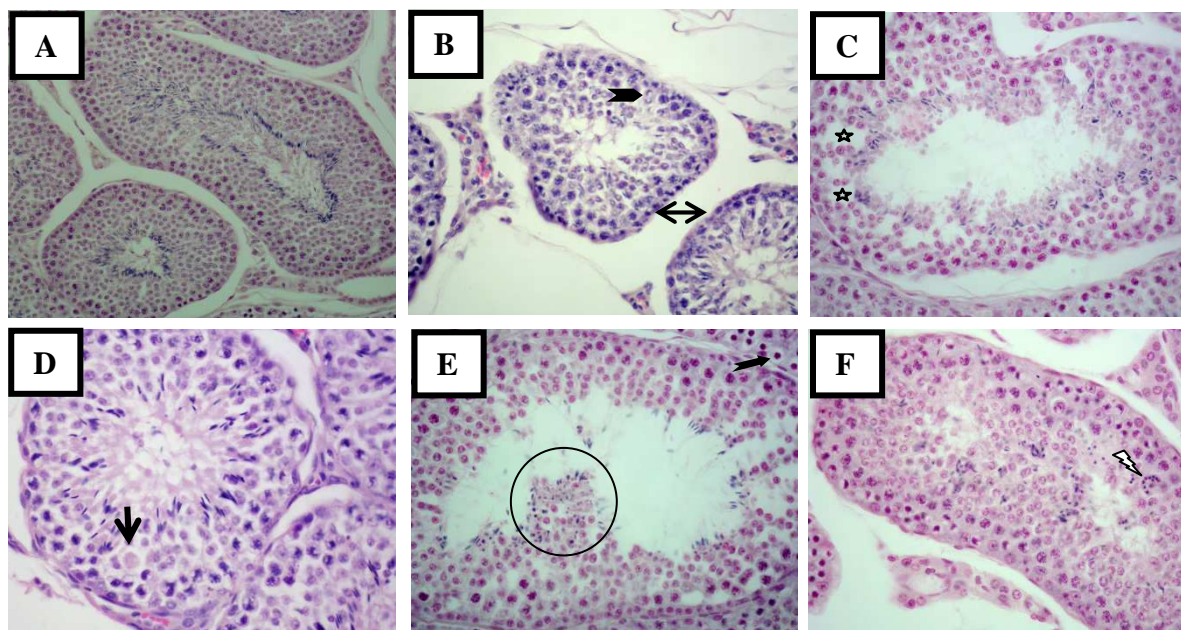


Figure 1: Microscopic view of [A: Que group, 20×] and [B-G: HS group, 40×] testes of HS exposed rabbits. Double arrow (↔) indicate sparse arrangement between the seminiferous tubules, star (★) shows the vacuoles, solid arrow represent the (→) single cell necrosis, chevron arrow (↷) show the crescent-shaped primary spermatocytes and encircled area having the degenerative/necrotic sloughed immature spermatids and spermatogonia, notched arrow (↗) show vacuolar degenerated type-B spermatogonia, apoptotic multinucleated giant cell represented by flash (⚡)

Flavonoids are known phytophenolic compounds to exhibit strong antioxidative capacity. Amongst those, quercetin (3, 3', 4', 5, 7-pentahydroxyflavone) is one of the most popular flavonoids which are widely existing in edible fruits and vegetables. The actions like metal chelation, radicals scavenging, enzyme inhibition and/or induction of the expression of protective enzymes in biological systems represent the high antioxidant capacity of quercetin (Erlund, 2004). Although, the positive impact of dietary quercetin supplementation has been well documented against experimentally induced toxicity on testicular tissues in laboratory animals (Aldemir et al., 2012; 2014; Aktoz et al., 2010; Ciftci et al., 2012; Sonmez et al., 2014). There has been no report about the ameliorative effect of quercetin on HS-induced changes in testicular histopathologic structure. This is the first report of its kind regarding the protective action of quercetin on testicular histopathological changes due to HS. Current findings showed that quercetin dietary supplementation reduced the testicular histopathologic lesions and maintain germ cells population during HS. Quercetin provides the cell protection during stress condition by increasing oxidase and β -glucuronidase activity, decreasing glutathione-S-transferase and by increasing activity as a co-mutagen for 2-acetylaminofluorene (Formica and Regelson, 1995). It appears that the most likely reason for the maintenance of testicular cytoarchitecture in rabbits under HS might be associated with antioxidant and radical scavenging potency of quercetin.

CONCLUSIONS

Current findings clearly evidence that dietary quercetin supplementation minimizes the summer heat stressed testicular histopathological changes in rabbits. The assessment of sperm quality parameters could be beneficial to determine the antioxidant capacity of quercetin supplementation in heat-exposed male.

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