Research Article

"Quercetin Impairs the Reproductive Potential of Neonatal Male Rats"

Reda H. ElMazoudy[1,2*], Nema A. Mohamed[1], Azza A. El-massry[1], Fatma R. Abdelsadek[1]
[1]Zoology Department, Faculty of Science, Alexandria University, Alexandria, Egypt.
[2]Biology Department, College of Science in Dammam-Girls, University of Dammam, Dammam, KSA.
*Corresponding author's E-mail: redaelmazoudy@hotmail.com

ABSTRACT

The effects of quercetin on the reproductive system, parameters of oxidant/antioxidant status and metabolism in neonatal male rats were investigated. An insignificant increase in body weights at QR (10 and 20 mg/kg) and an insignificant decrease at QR (40 mg/kg) as compared with the control group. There were no statistical differences in organ weights between the experimental and the control groups. QR (10 and 20 mg/kg) had no effect on malondialdehyde (MDA), while, QR (40 mg/kg) effectively increased the MDA levels. Quercetin (10 and 20 mg/kg) increased catalase (CAT) and superoxide dismutase SOD, while, QR (40 mg/kg) caused a significant decrease in these enzymes. The hypolipedemic effect of QR was pronounced at all doses specially 40 mg/kg. Testosterone and LH levels were significantly increased in QR (10 and 20 mg/kg), while, QR (40 mg/kg) significantly decreased testosterone and LH levels as compared to other groups. However, there was no significant change in FSH at all doses of QR. The doses 10 and 20 mg/kg caused an insignificant increase in sperm count, motility and viability, while, QR (40 mg/kg) caused a significant decrease in spermatozoa count. QR (40 mg/kg) significantly decreased the sperm motility and increased the total abnormal sperm numbers. The testis sections of QR (10 and 20 mg/kg), showed nearly normal histological architecture of the seminiferous tubules. QR (40 mg/kg) revealed a remarkable wide degenerative interstitial tissue. Based on the present results, the use of quercetin as alternative drug for treatment of male infertility should be considered in low doses.

Keywords: Quercetin, sex hormones, semen analysis, neonatal rats.

INTRODUCTION

Quercetin has a long history of traditional medicinal use in many countries. Researchers focus on these naturally occurring substances and being a subject to many rigorous scientific and clinical studies[1].

Quercetin has drawn attention for its remarkable scope of health benefits and its putative beneficial effects in the prevention and treatment of disease, which make quercetin a leading compound for developing new and effective food supplement and a promising ingredient of so-called functional food or medicines[2].

Quercetin biopharmacological properties may offer promising options for the development of more effective chemopreventive and chemotherapeutic strategies because of its powerful antioxidant and free-radical scavenging properties[3]. It has been shown that quercetin has highly potent antioxidant and cytoprotective effects in preventing apoptosis and oxidative damage induced by cadmium in both *in vivo* and *in vitro* studies[4].

Quercetin's antioxidant and physicochemical properties enable it to counteract the damaging effects of oxidation caused by ROS or other types of free radicals in many types of tissues and cells[5], which contributes to the genesis of atherosclerosis, diabetes, ischemic heart disease, heart failure, and hypertension[6].

A review of the literature shows that quercetin treatment has been associated with selective antiproliferative effects and induction of cell death, probably through an apoptotic mechanism, in breast or other cancer cell lines but not in normal cells[7].

Quercetin has exhibited anticancer potential against a wide range of cancers[8]. Quercetin has also been shown to enhance the anti-cancer effects of several chemotherapeutic drugs[9]. Quercetin treatment can improve memory and learning ability as well as cognitive ability in neonate rats with hypoxic ischemic brain damage[10]. It can prevent memory dysfunction in streptozotocin-diabetic rats[11].

Also, it has been reported that quercetin has neuroprotective effect[12]. Several studies have reported that antioxidants in diet can protect DNA and other important molecules from oxidation and damage[13], and can improve sperm quality and consequently increase fertility rate in men[14]. Therefore, the role of nutritional and biochemical factors in reproduction and sub-fertility treatment is very important.

The aim of the current study is to evaluate the effect of quercetin on the reproductive system of neonatal male rats. Therefore, testosterone, FSH and LH levels in serum were investigated; histopathological changes in their testis and sperm quality were assessed. Also, the present work investigates the effect of quercetin as natural antioxidant on biochemical parameters including lipid peroxidation product malondialdehyde (MDA) and activity of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) and also investigates its effect on lipid profile.

MATERIALS AND METHODS

Chemicals

Quercetin Preparation

Quercetin powder was obtained from Sigma Chemical Company (St. Louis, MO, USA). It was dissolved in corn oil and freshly prepared each week.

Animals

The present study was carried out on 80 healthy weanling immature growing male rats of Wistar strain about one month age. The weight range was from 50-60 g. Rats were purchased from Faculty of Medicine, Alexandria University, Alexandria, Egypt. Animals were housed in the usual stainless steel cages (43 cm × 30 cm × 20 cm). The cages were cleaned daily. Male rats were maintained in a well-ventilated animal house under standard laboratory conditions on a photo schedule 12h light/12h darkness cycle in a temperature-controlled room at 22 ± 3 °C, relative humidity: 55 ± 10 %. The animals were provided with a balanced animal diet obtained commercially and had free access to drinking water *ad libitum*. The animals were acclimatized to the animal house condition for two weeks prior to commencing the experiment. Throughout the experiments, animals were processed according to the suggested international ethical guidelines for the care of laboratory animals and all experimental procedures were approved by the Animal Care and Use Committee of Alexandria University, Egypt.

Animal Treatment Schedule

Dose Selection for Treatment and Experimental Procedures

The dose of quercetin was chosen on the basis of LD_{50}. The LD_{50} of quercetin given orally was 160 mg/kg bw in rats[15]. Therefore, the initial dose of quercetin chosen for the present study is 10 mg/kg bw equivalent approximately to 1/16 of LD_{50}. After two weeks of acclimation animals were randomly divided into four equal groups; each group containing 20 rats as follows.

Group I (Control)

Rats given the vehicle and used as control.

Group II (QR I)

Rats given 10 mg/kg quercetin (1/16 LD_{50}).

Group III (QR II)

Rats given 20 mg/kg quercetin (1/8 LD_{50}).

Group IV (QR III)

Rats given 40 mg/kg quercetin (1/4 LD_{50}).

Male rats were administered quercetin or vehicle orally by gavage once a day using a metallic needle. The doses of quercetin were administered in the morning between (8.00–9.00 am) and the experimental duration was continued for 28 days.

Male Observations

The rats were monitored daily for health status and examined for signs of toxicity throughout the experimental periods.

Measurement of Body weight and Organ weights

The animals were weighed and values obtained were recorded as initial weights at the beginning of the experiment and every week till necropsy. After 24 h of final dose, the end of the treatment period, the rats were anesthetized with diethyl ether and killed by cervical dislocation. Testes and epididymides of control and experimental groups were collected, cleared of adhering connective tissue and weighed.

Blood Sample Collection

Blood samples were collected from dorsal aorta via sterile injector and centrifuged at 3000 g for 5 min. Serum was separated and then stored at -20 °C until biochemical and hormonal analyses.

Biochemical Estimations

Measurement of Serum Malondialdehyde (MDA), Superoxide Dismutase (SOD) and Catalase (CAT)

The levels of MDA, SOD and CAT in serum were estimated by colorimetric methods using commercial kits.

Biochemical Estimations of Lipid Profiles

The levels of total cholesterol (T.Ch)[16], triglycerides (TG)[17], and high density lipoprotein cholesterol (HDL-C)[18] in the serum were estimated by colorimetric methods using commercial Kits.

The concentration of the VLDL-C was calculated by using the following formula:

Concentration of VLDL-Cholesterol (mg/dl) = Triglycerides / 5.

The concentration of the LDL-C level was calculated by using the following formula

Concentration of LDL-C (mg/dl) = TC — HDL-C — (VLDL-C)

Serum Hormone Analysis Assay

Serum levels of testosterone[19], LH and FSH[20], were measured by the immunoassay for the quantitative determination in serum; the ElectroChemiLuminescence Immunoassay (ECLIA) is intended for use on Elecsys and cobas immunoassay analyzers kit (Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim). All the used Kits were purchased from bio-diagnostic company, 29 Tahreer St., Dokki, Giza, Egypt.

Sperm Quality Analysis

Sperm Collection

The right cauda epididymides of each rat were finely minced by anatomical scissors in 1 mL of isotonic saline in

a Petri dish. It was completely squashed by a tweezers for 2 min, and then allowed to incubate at room temperature for 4 h to provide the migration of all spermatozoa from epididymal tissue to fluid. After incubation, the epididymal tissue-fluid mixture was filtered via strainer to separate the supernatant from tissue particles. The supernatant fluid containing all epididymal spermatozoa[21].

Sperm Count

The epididymal sperm count was determined with a hemocytometer using a modified method described by Türk[22]. Briefly, one drop of the supernatant fluid containing all epididymal spermatozoa was transferred to both counting chambers of an Improved Neubauer (Deep 1/10 mm, LABART, Darmstadt, Germany) and allowed to stand for 5 min. The sperm cells in chambers were evaluated as million sperm cells per ml of suspension under 200x magnification using light microscope. Epididymal sperm counts were expressed as $\times 10^6$/mL.

Sperm Motility

One drop of the suspension was placed on a slide, covered by 24x24 mm cover slips and evaluated under light microscope. Sperm motility was categorized into "motile" or "immotile". The percentage of forward progressive sperm motility was evaluated visually at 400X using a light microscope with heated stage by Sönmez[23]. Motility estimation was performed from three different fields in each sample. The mean of the three successive estimations was used as the final motility score.

Sperm Morphology

After the evaluation of the epididymal sperm motility, one drop was prepared for the sperm morphology analysis. One drop of the suspension was taken and smeared on glass slides. To determine the percentage of morphologically abnormal spermatozoa, the sperm suspension stained with eosin–nigrosin (1.67% eosin, 10% nigrosin and 0.1 M sodium citrate) smears were prepared on slides, air-dried and made permanent. The slides were examined under the microscope using 100 x objectives and oil immersion. Two thousand sperms were evaluated on each slide and results were recorded as a percentage of abnormal sperm for each slide. Abnormal heads and tails were evaluated using the criteria by Okamura[24].

Sperm Viability Test

Viability study was assessed using the eosin/nigrosin stain. The staining was performed with one drop of fresh suspension into two drops of staining solution on a microscope slide. Using another slide; a smear was made and allowed to dry. Unstained (intact) and red to pink-colored (with damaged membranes) spermatozoa were counted under the microscope using oil immersion. Pink-stained dead sperm were differentiated from unstained live sperm, and their numbers were recorded. The viability of sperm was expressed as the percent of viable spermatozoa[25].

Histopathology of the Testis

To determine the changes in testicular and spermatogenic cells, testis tissues were dissected out. The removed testicular tissues were fixed in Bouin's solution for 48 h, and then they were dehydrated by upgrading from 30 to 100% series of ethyl alcohol and then to xylene each for 1 h, embedded in paraffin wax. Paraffin blocks were sectioned at 5 µm thickness. The sections were then stained with hematoxylin and eosin. Microscopic fields were selected randomly for the evaluation of histopathological alterations of seminiferous tubules. The slides were examined with a light microscope[26]. Permanent preparations of testicular tissues were histologically examined and photographed using a digital camera (Canon Virginia Inc., Newport News, VA, USA) mounted on a light microscope (Carl Zeiss Inc., Jena, Germany).

Statistical Analysis

Data were presented as mean ± Standard Deviation (S.D.). Data were analyzed using SPSS/PC software package program. The degree of significance was set at $P < 0.05$. The significance was calculated using one-way analysis of variance (ANOVA) to determine the differences among the groups and followed by a multiple comparison procedure to calculate the significance.

RESULTS

Morbidity, Mortality and Clinical signs of Toxicity

There were no mortality or morbidity during the course of the present study. Oral administration of quercetin did not cause any signs of toxicity in treated rats compared with untreated controls.

Body Weight

Table 1: Effect of quercetin supplementation on body weight of experimental rats.

Parameters	Experimental Groups			
	QR (0)	QR (10 mg/kg)	QR (20 mg/kg)	QR (40 mg/kg)
Initial weight	52.43±5.3	56.32±6.2	58.14±6.1	51.62±5.9
1st week	79.74±7.8	86.91±8.9	84.87±9.1	76.42±8.1
2nd week	101.64±10.3	107.57±10.8	108.84±11.3	99.79±10.1
3rd week	130.12±12.9	136.85±14.6	134.45±14.5	129.82±13.2
Final weight	155.86±16.2	159.53±17.1	156.49±16.1	145.94±15.2
Body weight gain	103.43±10.6	103.21±10.9	98.35±10.2	94.32±10.2

The data are presented as mean ± S.D.

*: significantly different from control (P< 0.05)

Body weight gain = Final weight - Initial weight

The effects of quercetin on the body weights were shown in Table 1. In all dietary groups, animals did not continuously gain body mass during the feeding period.

The administration of QR at the doses 10 and 20 mg/kg caused an insignificant increase in body weights. While, QR at the dose 40 mg/kg caused an insignificant decrease in body weights as compared with the control group.

Organ Weights and Relative Organ Weights

Organ weights and relative organ weights of control and experimental male rats are presented in Table 2. Organ weights were found to be similar to control animals. There were no statistical differences between the experimental groups and the control groups at any concentration of quercetin.

Table 2: Effect of quercetin supplementation on organ weight of experimental rats.

Parameters	Experimental Groups			
	QR (0)	QR (10 mg/kg)	QR (20 mg/kg)	QR (40 mg/kg)
Organ weights (g)				
Testes	2.73± 0.26	2.71± 0.26	2.81± 0.31	2.17± 0.27
Epididymis	1.13±0.11	1.12±0.17	1.15±0.11	1.04±0.11
Relative organ weight				
Testes	1.752± 0.130	1.767± 0.120	1.800± 0.110	1.487± 0.099
Epididymis	0.725±0.080	0.736±0.080	0.759±0.068	0.713±0.045

The data are presented as mean ± S.D.

*: significantly different from control (P< 0.05)

Relative organ weight = Organ weight /Body weight x 100

Biochemical Parameters

The effects of quercetin supplementation on serum MDA, SOD and CAT levels

Biochemical results related to antioxidant status were given in Table 3. The MDA levels, the end product of lipid peroxidation, were insignificantly decreased at the doses of 10 and 20 mg/kg and significantly increased at the high dose of quercetin (40 mg/kg) treated groups compared to control group.

The CAT and SOD activities were significantly increased in 20 mg/kg and significantly decreased in 40 mg/kg compared to control group at the end of the 28 days.

The effects of quercetin supplementation on serum total cholesterol, triglycerides, HDL, LDL, VLDL and Total lipids levels

The results presented in Table 4 demonstrate the effect of quercetin on the lipid profile.

The result showed that the quercetin treated groups were significantly different (p<0.05) for all parameters including lipid profile (total cholesterol, TG, HDL, LDL, VLDL and total lipids) compared to controls. Quercetin treatment significantly decreased T.Ch, TG, LDL, VLDL and T.lipids.

On the other hand, HDL in QR groups (20 and 40 mg/kg) was increased in all treated groups compared to control.

While, QR at a dose of 10 mg/kg caused insignificant alterations in the HDL.

Table 3: The effects of quercetin supplementation on serum MDA, SOD and CAT levels

Parameters	Experimental Groups			
	QR (0)	QR (10 mg/kg)	QR (20 mg/kg)	QR (40 mg/kg)
MDA (nmol/ml)	214.23±22.6	203.54±22.6	200.74±22.8	321.52±30.5*
SOD (U/mg)	11.48±1.98	12.41±1.65	24.98±2.51*	8.12±1.69*
CAT (U/mg)	11.05±1.22	11.82±1.55	14.51±1.65*	7.52±1.07*

The data are presented as mean ± S.D.

*: significantly different from control (P< 0.05)

Table 4: Effect of quercetin on total cholesterol, triglycerides, HDL, LDL, T.Lipid levels of experimental rats

Parameters	Experimental Groups			
	QR (0)	QR (10 mg/kg)	QR (20 mg/kg)	QR (40 mg/kg)
T. CH (mg/dl)	95.32±9.85	90.46±10.13*	87.15±8.10*	70.12±6.98*
TG (mg/dl)	77.15±8.71	61.55±7.11*	56.75±6.11*	40.65±4.25*
HDL (mg/dl)	25.78±2.36	29.88±2.36*	33.92±3.52*	41.24±5.65*
LDL (mg/dl)	54.11±6.11	50.27±5.98*	41.88±4.25*	20.75±2.98*
VLDL (mg/dl)	15.43±1.85	12.31±1.32*	11.35±1.65*	8.13±0.98*
T.Lipid (mg/dl)	292.79±32.53	273.47±26.81*	238.05±24.32*	205.89±20.80*

The data are presented as mean ± S.D.

*: significantly different from control (P< 0.05)

Total serum testosterone, LH and FSH levels

As shown in Table 5 serum sex hormones monitored in male rats showed a statistically significant difference. Serum testosterone and LH levels were significantly increased in QR at the doses of 10 and 20 mg/kg compared with the control group. Treatment of QR (40 mg/kg) caused significant decrease in testosterone level compared to other treated groups and the control.

With respect to LH levels, there were significant decrease in its main value in high dose of QR (40 mg/kg) compared to controls. However, there was no significant change in FSH compared to control group.

Table 5: The effect of Quercetin on serum total testosterone, FSH and LH levels of experimental rats

Parameters	Experimental Groups			
	QR (0)	QR (10 mg/kg)	QR (20 mg/kg)	QR (40 mg/kg)
Testosterone (ng/ml)	2.90±0.25	4.10±0.51*	4.22±0.45*	0.95±0.10*
FSH (mIU/ml)	3.24±0.36	3.28±0.41	3.30±0.365	3.19±0.3
LH (mIU/ml)	2.90±0.28	3.21±0.52*	3.11±0.29*	1.34±0.11*

The data are presented as mean ± S.D.

*: significantly different from control. (P< 0.05)

Effects of quercetin on Sperm quality

The effects of quercetin on epididymal sperm concentration, sperm motility and abnormal sperm number are presented in Table 6. There is a significant increase in sperm count, motility, viability and increase in normal sperm number at doses 10 and 20 mg/kg of QR compared to control group. The administration of QR (40 mg/kg) caused a significant (P<0.05) decrease in count of spermatozoa. Also, QR (40 mg/kg) significantly decreased the epididymal sperm motility and increased the total abnormal sperm number compared with the control group.

Table 6: Effects of quercetin on sperm quality of experimental rats

Parameters	Experimental Groups			
	QR (0)	QR (10 mg/kg)	QR (20 mg/kg)	QR (40 mg/kg)
Sperm count (×10⁶)	78.3 ± 8.21	89.2 ± 9.85*	96.1 ± 10.1*	40.4 ± 4.21*
Sperm motility (%)	52.8 ± 6.2	69.5 ± 7.65*	75.1 ± 7.58*	35.6 ± 3.98*
Normal sperm (%)	68.7 ± 7.25	86.4 ± 8.98 *	92.4 ± 9.85*	45.3 ± 4.65*
Viability (%)	73.13 ± 8.2	88.5 ± 9.21*	96.45 ± 9.87*	46.5 ± 5.1*

The data are presented as mean ± S.D.

*: significantly different from control (P< 0.05)

Histopathological changes in the Testis tissues

Cross sections of the control testes showed normal architecture of all seminiferous tubules (Figure 1C). The tubules are lined with spermatogenic epithelium. The germ cells include differentiated spermatogenic cells could be easily differentiated from each other. The spermatogonia located on or near the basal lamina. The primary spermatocytes are rounded cells with large spherical nuclei. The secondary spermatocytes cannot be easily seen because they pass this stage readily. The late spermatids appear normal. The central Lumina could easily be delineated in almost all the tubules and the majority of them is occupied by spermatozoa.

The spermatogenic and Sertoli cells that rest on the basal lamina in the seminiferous tubules of QR (10 mg/kg) treated group were observed normal structure and clearly identified with characteristic oval and obvious nucleoli. Leydig cells were found in the interstitial connective tissue between the seminiferous tubules, and the tubules appeared to be normal size and shape (Figure 1D).

Histological examination of the testis of QR (20 mg/kg) showed nearly normal histological architecture of the seminiferous tubules with spermatogenic stages where the connection between the stages are strong as shown in the control sections.

QR (10 and 20 mg/kg) groups, showed the lumen of seminiferous tubules filled with spermatozoa higher than those in QR (0) and QR (40 mg/kg) (Figure 1E). However,

histological examination of the testis of QR (40 mg/kg) revealed a remarkable wide degenerative interstitial tissue with liquid infiltration and disorganization of the seminiferous tubules. The Sertoli cells and Leydig cells are abnormal with necrosis of Leydig cells in the degenerated interstitial tissue, depletion of germ cells, remarkable decrease in the number of spermatozoa and impaired spermatogenesis (Figure 1F).
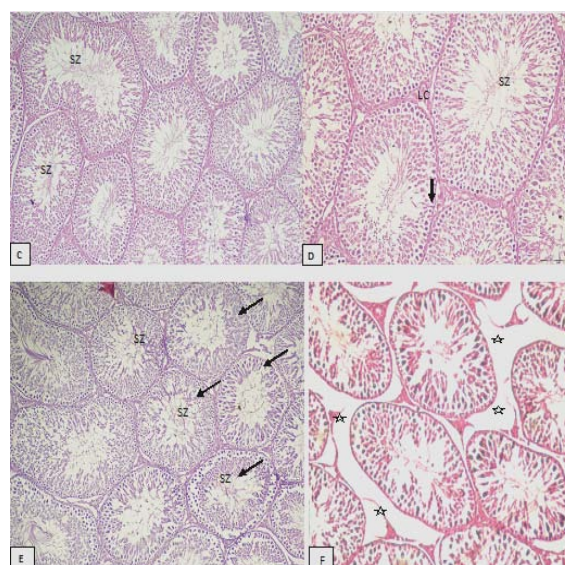

Figure 1: Photograph of testicular section of **(C)** control rat shows the normal structure with regular seminiferous tubule and the lumen filled with spermatozoa (SZ), **(D)** 10 mg/kg quercetin showing normal configuration with spermatogenic stages, Sertoli cell (arrow) and Leydig cells (LC) in the interstitial tissue, **(E)** 20 mg/kg showing normal architecture with the successive stages of spermatogenesis including spermatogonia and the lumen filled with spermatozoa, **(F)** 40 mg/kg quercetin showing a remarkable wide degenerative interstitial tissue (stars) with liquid infiltration (arrow) and disorganization of the seminiferous tubules (H&E, 400X).

DISCUSSION

Natural compounds and dietary components as antioxidants against diseases and injures attracted a growing number of scientific searchers. Quercetin an important flavonoid provides beneficial effects on health due to its antioxidant function. In the present study, quercetin administration had no signs of toxicity on rats at different doses (10, 20, 40 mg/kg) during the experimental period. This result was in agreement with Ruiz[27] who stated that the daily oral administration of QR (30, 300, 3000 mg/kg for 28 days) did not cause mortality in both sexes of Swiss mice. Aydin[28] showed that quercetin, either alone or in combination with methotrexate, showed no signs of morbidity or mortality during the study. Also, Selvakumar[29] stated that no mortality was observed in the quercetin experimental group.

Oral administration of quercetin had no effect on body weight and relative organ weights. Furthermore,

quercetin did not disturb the body weight gain in male rats compared with control rats QR (0). These findings were similar to a number of studies addressed the effects of quercetin on body weight. Ruiz[27] found that quercetin had no significant effects on food intake, water consumption and body weight gain in the male or female Swiss mice. Taepongsorat[30] showed that there were no significant differences of rat body weights between the control and quercetin (30, 90 and 270 mg/kg) after 3, 7 and 14 days. Verma[31] stated that the body weight of quercetin-treated rats remained comparable to the control group rats throughout the experimental period. Also, Esmaeilzadeh[32] stated that there were no significant changes in body weight, the daily intakes of energy and macronutrients between groups and dietary intake of quercetin.

It was obvious that quercetin has dose dependent manner on the malondialdehyde levels. Where, QR at low doses (10 and 20 mg/kg) induced insignificant decrease in MDA levels. These results were in agreement with Atef[33] who observed that daily oral administration of 20 mg/kg of quercetin for seven consecutive days significantly decreases the MDA level in diabetic rats.

Another study indicated that the administration of quercetin (15 mg/kg) for four weeks decreases the serum MDA level in streptozotocin-induced diabetic rats[10]. Meanwhile, a significant increase in the MDA levels was observed in QR (40 mg/kg). This result came accordance with Mi[34] who stated that quercetin alone at dose of 75 mg/kg led to a significant increase of MDA.

The present study revealed that quercetin plays a positive antioxidative role as indicated by the increment in SOD and CAT activities at QR (10 and 20 mg/kg). This finding is consistent with previous reports which indicated that quercetin increased the antioxidant activities of SOD and CAT[35]. Also, Annapurna[36] showed that quercetin increases serum antioxidant enzymes. Karampour[37] stated that quercetin treatment (259 mg/kg, PO and 100 mg/kg, IP) improved heart conditions and resulted in a significant reduction in MDA levels and elevation in GPx activity. Meanwhile, high dose of QR (40 mg/kg) causes a significant decrease in SOD activities. Mi[34] found that quercetin administration (75 mg/kg) caused a significant reduction in the SOD activity.

Indeed, the ability of quercetin at low doses to protect against oxidative stress induced cellular damage is associated with free radical scavenging as well as chelatory properties[38]. In addition to the direct scavenging of free radicals, other antioxidant mechanisms contribute to the potent action of quercetin against oxidative stress, such as the chelation of iron and calcium and the inhibition of lipid-peroxidation (MDA), as well as other biochemical mechanisms, such as the inhibition of enzymes, including xanthine oxidase and nitric oxide synthase[39]. Also, quercetin can distribute on the surface of the lipid bilayers as well as in the aqueous phase and scavenge free radicals[40].

The hypolepidemic effect of quercetin may be due to that quercetin reduces *de novo* synthesis of fatty acids and consequently cholesterol biosynthesis and lipoproteins formation[41]. Supports to our results came from several previous studies where quercetin presented the largest percentage reduction of cholesterol in the triton-induced hyperlipidemic rats[42]. Lee[43] had reported that quercetin-rich supplementation reduces serum concentrations of total cholesterol and increases serum concentrations of HDL-cholesterol. Also, quercetin was found to restore the lipid profiles to normal[44].

The present results clearly indicate that quercetin induced fertility at the doses 10 and 20 mg/kg; as indicated by a marked increase in the testosterone and LH levels. Similarly, Khaki[45] found that quercetin increased the serum testosterone levels and had beneficial effects on sperm parameters in streptozotocin-induced diabetic male rats. Also, Ma[46] reptorted that quercetin can increase serum testosterone levels in male rats. While, quercetin at the high dose caused a significant decrease in testosterone level, this result agreed with the results of a previous study by Aravindakshan[47] who stated that the treatment with high dose of quercetin (300 mg/kg bw, two injections) reduced the fertility rate of male rats. In the present study quercetin has no significant difference in serum FSH, while, QR (40 mg/kg) caused a significant decrease in testosterone and LH levels.

The positive effect of quercetin (10 and 20 mg/kg) on sperm characteristics was observed. Besides, it decreased the total abnormal sperm number in experimental groups as compared with the control group. Similarly, Taepongsorat[30] determined that quercetin improved the sperm motility, concentration and viability in rats. On the other hand, QR (40 mg/kg) had contrary effect on the sperm motility, viability and count. Khanduja[48] showed that the incubation of human semen with quercetin induced an irreversible and dose-dependent fall in sperm motility and sperm viability. It was previously reported that quercetin can act dose-dependently as either an agonist of endogenous steroids at low doses or an antagonist at high doses[49].

In addition, Robaszkiewicz[50] showed that quercetin decreased production of reactive oxygen species in chicken testicular germ cells. Also, Chandel[51] found that quercetin had no deleterious effects on oxidative damage in cultured chicken spermatogonial cells at doses of 1 and 10 µg/ml. Mi and Zhang[52] showed that quercetin displayed protective effects on embryonic chicken spermatogonial cells from Aroclor 1254-induced oxidative damage through increasing intracellular antioxidant levels and decreasing lipid peroxidation.

The consequences of the antioxidant efficacy of quercetin that were confirmed by histological examinations showed that administration of quercetin caused highly regular seminiferous tubules with normal interstitial tissue with high number of spermatozoa in the lumen of the

seminiferous tubule at low doses of QR (10 & 20 mg/kg). Accordingly, Taepongsorat[30] showed that the tubular area of seminiferous tubules increased with quercetin treatment in a time- and dose-dependent manner. Also, Izawa[53] demonstrated that quercetin can inhibit the testicular damage in mice. Quercetin has protective effect in the testicular tissue of rats. The mechanisms contributing to its effectiveness involve the quenching of free radicals, increasing the antioxidants status and metal-chelating activities of quercetin.

On the other hand, QR (40 mg/kg) has a degenerative effect on the interstitial tissue; the germ and the Leydig cells where they had an abnormal appearance with necrosis. Testosterone is produced by Leydig cells in the testes and decreased number of Leydig cells and their nuclear area diminished the production of testosterone[54] which might have affected the fertility. Reduction in number of Sertoli cells resulted in decreased number of spermatogonia, as Sertoli cells play a critical role in spermatogenesis by providing the physical support, nutrients and hormonal signals, necessary for successful spermatogenesis[55]. Izawa[53] confirmed that, the high-dose quercetin group, sperm production (DSP) and Sertoli cell were decreased, but the incidence of morphologically abnormal sperm was increased. Elevated LPO biomarker like MDA was demonstrated to have fundamental implications on testicular physiology and sperm function[56].

The bioactivity of quercetin is attributed to its specific molecular structure, in which the oxygen active group, phenolic hydroxyls and 2,3-unsaturated double bond give quercetin strong antioxidant ability not only from accepting oxygen free radicals, but also by forming metal chelation compounds[57]. Furthermore, quercetin was an effective antioxidant because of its ability to interact with and penetrate lipid bilayers. Its higher diffusion rate into the membranes allowing it to scavenge oxyradicals at several sites through out the lipid bilayer[58].

CONCLUSION

Therefore, in this study, we evaluated the safe effects of quercetin, an antioxidant agent, on reproductive system at low doses in neonatal male rats. These results indicated that antioxidants from foods, such as quercetin, could improve sperm health parameters and testicular androgenesis at low doses.

An increased consumption of antioxidants in the diet of individuals is strongly recommended, however, further studies are needed before antioxidants can be used safely as food additives and supplements.

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