

EFFECT OF FLAVONOIDS ON THICKNESS AND DIAMETER OF TESTICULAR SEMINIFEROUS TUBULES IN ADULT MALES WISTAR RATES EXPOSED TO OXIDATIVE STRESS BY LEAD ACETATE

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Abstract

The current study aims to determination the effect of flavonoids on thickness and diameter of testicular seminiferous tubules in adult males wistar rats exposed to lead acetate which cause oxidative stress. 40 male wistar rats, each 200±10g on average, were randomly divided to four equal groups and received the following treated for 60 days: First group (C) received nothing except water as a control. Quercetin at dose (300 mg/kg/B.w.) was administered to the second group (T1). Lead acetate at dose (10 mg/kg/B.w.) was administered to the third group (T2). The fourth group received quercetin (300 mg/kg/B.w) for 30 days after receiving lead acetate (10mg/kg/B.w) for 30 days. All of the animals were sacrificed at the end of experiment. Samples of testis, epididymis and vas deference were taken for histopathological study. A significant difference ($p \leq 0.05$) in the seminiferous tubule thickness and leydig cell count was reported in a histological examination. sertoli cells, primary and secondary spermatocytes in T1 group in compared with C group. Whereas there were no significant difference in seminiferous tubules diameter in T1 group in compared with the C group. Additionally, there was a significant difference ($p \leq 0.05$) between the T2 group and the C group in terms of the thickness of the seminiferous tubules, leydig's cells and sertoli cells number, primary and secondary spermatocytes, and the diameter of the seminiferous tubules. Additionally, there was a significant difference between the T3 group and the T2 group as evidenced by an increase in seminiferous tubule thickness, leydig's cells, sertoli cells, primary and secondary spermatocytes, and a decrease in seminiferous tubule diameter. Also histological sections in control group revealed a complete spermatogenesis, normal testicular tissue architecture with normal seminiferous tubules, epididymis and vas deference tissues. In T1 group there was complete spermatogenesis, the epididymis duct filled with the sperm and extended, vas deference has normal smooth muscles fibers and sperm filled the lumen of it. In T2 group there was vaculation of spermatogonia and few number of sperms, sertoli and leydige cells, the epididymis duct empty with degeneration and destruction of stereocilia, the vas deference empty from sperm and there was mild destruction in the stereocilia and sloughing the epithelial layer of it. In T3 there was large number of spermatogonia, primary and secondary spermatocytes with spermatids, the epididymial duct filled with the sperm .Vas deference has normal smooth muscles and the sperm filled the lumen of it.

Keywords: Flavonoids, Oxidative Stress, Wistar Rats, Lead Acetate, Testicular Histopathology.

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Introduction

The chemical process of oxidation involves the transfer of electrons from a material to an oxidizing agent. Free radicals can be produced during oxidation processes, and they can trigger cell-damaging chain reactions (Hamid, et al., 2010). Due to negative alterations that can occur during spermatogenesis, epididymal maturation, and sperm capacitation, oxidative stress is a significant factor in male infertility (O'Flaherty, 2020). Very important cause of male factor infertility is oxidative stress. Its evaluation offers crucial data that can direct treatment plans targeted at enhancing male reproductive potential (Dutta, et al., 2019). Lipid peroxidation appears to be one of the most significant mechanisms and the series of events through which free radicals interfere with cellular functioning, ultimately leading to cell death. (Lakhanpal and Rai, 2007). The more comprehensive free radical concept of aging serves as the foundation for the free radical theory of infertility. It claims that the malfunction of sperm cells is caused by free radicals. It is as hotly contested as the free radical theory of aging itself. Free radicals and oxidative stress are also anticipated to be connected to a number of pathogenic diseases that affect male fertility. Heavy metal ions, such as lead, can induce generation of reactive radicals and cause cellular damage.

Lead is a common element in the environment and is utilized in a variety of industrial processes, such as lead mining, lead refining, and the production of lead-acid batteries. (Flora, et al. 2004). The two main ways that lead enters the body are through the digestive and respiratory tracts (Fischbein, 1999). Lead often affects a variety of bodily processes, including those of the central nervous system, the hemopoietic system, the liver, and the kidneys. (Kalia and Flora, 2005). According to reports, lead generates reactive oxygen species (ROS) like super-oxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl (OH) radicals, and lipid peroxides, which cause oxidative stress. (El-Nekeety, et al., 2009). The exposure to the lead acetate in male rat causes decrease in spermatids number, epididymis sperm count, testosterone serum level and effect on prostate function (poma, et al., 2003).

Antioxidants are compounds found in large quantities in fruits and vegetables that may shield cells from damage brought on by free radicals which are unstable molecules. They interact with free radicals, stabilize them, and possibly even prevent some of the harm that caused by free radicals (Hamid, et al., 2010).

Natural substances called flavonoids, which are typically found in plants as secondary metabolites, provide significant health benefits. These benefits include antioxidant, antibacterial, antiviral, anti-allergic, anticancer, anti-inflammatory, and antiulcer actions (Middleton, 1998). The placement of functional hydroxyl groups around the nuclear structure, which has a significant impact on numerous of antioxidant mechanisms activity as radical scavenging and chelation ability of metal ion, determines the antioxidant activity of flavonoids (Pandey, et al., 2012). One of the most important dietary flavonoids, quercetin is a pigment found in many different plants, especially berries, apples, onions, tea, and broccoli. (Williamson and Manach, 2008). It is importance antioxidant in pharmacology (Dajas, 2012). Quercetin improves the antioxidant defense system in the body by up regulating of antioxidant enzymes (Sangai and verma, 2012).

So this work is aimed at investigating the role of quercetin in attenuating oxidative stress and testicular-histopathology induced by lead acetate.

Materials and methods

Laboratory animals:

forty adult male Wistar rats, each about six months old and weighing about (200±10 gm) received from the Al-Qadisiyah University's Veterinary Medicine College's animal home . The animals were raised in controlled environments in well-ventilated wire-plastic cages. Prior to the experiment, the animals were given 10 days to acclimate.

Biological material:

Flavonoids (Quercetin 95%) from onion provided by Brightol Company/ China

Experiment design:

Forty adult male Wistar rats were randomly separated into four equal groups, and each group received the following treatment for 60 days: Control group (C) received nothing but water. Giving oral doses of quercetin to the second group (T1) (300mg/kg/B.w) (Taepongsorat, 2008). The 3rd group (T2): given lead acetate orally in dose (10mg/kg/B.w) (Makhlouf, et al., 2008). The 4th group (T3): given lead acetate orally in dose (10mg/kg/B.w) for 30 days and then treated by giving quercetin orally at dose (300mg/kg/B.w) for 30 day.

Histological Studies:

After the animals were killed, five samples of each group's testis, epididymis, and vas deferens were collected, fixed with 10% formalin in a solution that had to be 10:1 the size of the specimen, and then left to dry for 72 hours (Luna, 1968). Following fixation, the specimens were transferred, the tissue was removed, and the formalin solution was rinsed out with tap water for three to four hours. to the following steps: Dehydration of specimen, clearing by using alcohol for dehydration, embedding Paraffin is considered to be either soft (melting point 48-50C°) or hard (melting point 56-60C°) (Luna, 1968), Blocking left for 24 hours in refrigerator to give the paraffin time to solidity, Cutting by using the rotary microtome with thickness is about (5-8mm). Staining with the following stains: Alum Haematoxylin and Eosin. This stain for showing general histological components. It is a routine stain in histological work and consists of: a- Haematoxylin crystals (5gm). b- Alcohol 100 (50ml). c- Ammonium or potassium alum (100gm). d- Distilled water 1000 ml. e- Mercuric oxide (red) (2.5gm.). And Periodic acid Schiff (PAS) for carbohydrate, muco-protein, glycoprotein and basement membrane. It composed of A- Schiff reagent solution which is composed of 1- Basic fuchsine (1gm). 2- Distilled water (200ml). 3-Normal hydrochloric acid (20 ml). 4- Anhydrous sodium bisulphate (19gm). B- 1% periodic acid solution C- Light green stock (10ml) and D.W. (50ml). D- Harris hematoxylin solution. These stain according to (Luna, 1968).

Method of histological measurement count by using ocular micrometer:

The ocular micrometer (OM) and stage micrometer (SM) were used for histological measurement. OM is special circular glass graduated horizontal and vertical (0-100) unit, while the stage micrometer is glass slide contain 2 millimeter horizontal line in mid and divided into 200 unit which equal 10 micron or 0.01 millimeter and used as following.

- 1-putting the OM in mid of shift between lower and upper ocular lens.
- 2-putting SM on microscope stage and moved it until appearance line of horizontal graduated in the field.
- 3-moving of ocular lens which contain O.M. with moving of slide until matching of SM zero with M.O.
- 4-recording of other tow number which depend on the type of microscope.
- 5-Value of each unit of OM for choice lens by:
$$\frac{\text{number of maching line SM} \times 10 \text{ micron}}{\text{number of mached line of OM}}$$
- 6-Removed of SM and putting examined slide instead of it.
- 7-the result divided (1.4) micron which is constant factor (CF).

Statistical analysis:

The statistical set for social sciences (SPSS), a computer application, was utilized to calculate the statistics analysis. The data had been statistically analyzed by

- 1.Descriptive statistics :mean± stander error.
- 2.One-way analysis of variance (ANOVA) was used to statistically examine the data, and LSD was used to compare the groups. The confidence limit was accepted at 95% (p>0.05) (Joda, 2008).

Results:**Effect of quercetin on thickness and diameter of testicular seminiferous tubules in adult males wistar rats exposed to oxidative stress by lead acetate:****Thickness of seminiferous tubules epithelium (μm):**

The results displayed in (Table 1) shown a significant difference ($p \leq 0.05$) characterized by increase in seminiferous tubules thickness in T1 group (10.6 ± 0.45) and T3 group (10.38 ± 0.18) in compared with other groups, and there was a significant difference represented by decrease in T2 (4.7 ± 0.19) compared with other groups, and there were no significant difference between T1 group and T3 group.

Diameter of seminiferous tubules (μm):

The findings in (Table 2) indicated a significant difference ($p \leq 0.05$) represented by increase in seminiferous tubules diameter in T2 group (17.46 ± 0.16) compared with other groups, and there was a significant difference represented by decrease in T3 group (9.54 ± 0.16) compared with control group (10.6 ± 0.24). While there were no significant difference between T1 group (10 ± 0.31) compared with C (10.6 ± 0.24) group and T3 group (9.54 ± 0.16).

Table 1 Effect of quercetin on thickness and diameter of testicular seminiferous tubules of adult males wistar rats exposed to oxidative stress by lead acetate

Groups	C group	T1 group	T2 group	T3 group
Parameters				
Thickness of Seminiferous Tubules (micrometer)	b 8.28 ± 0.57	a 10.6 ± 0.45	c 4.7 ± 0.19	a 10.38 ± 0.18
Seminiferous Tubules diameter (micrometer)	b 10.6 ± 0.24	bc 10 ± 0.31	a 17.46 ± 0.16	c 9.54 ± 0.16

Number= mean \pm S.E.

Different litters= Significant differences ($p < 0.05$).

C group = control group.

T1 group = Orally gavage quercetin (300mg /kg/B.W once daily, dissolved in 1 ml drinking water) for 60 days.

T2 group = Orally gavage lead acetate (10mg/kg/ B.W once daily ,dissolved in 1 ml drinking water) for 60 days.

T3 group = Orally gavage lead acetate (10mg/kg/B.W once daily ,dissolved in 1 ml drinking water) for 30 days then given quercetin (300mg/kg/b. w) for 30 days.

Effect of quercetin on number of testicular cells in adult males wistar rates exposed to oxidative stress by lead acetate:

Primary Spermatocytes:-

Table 2 revealed there was a significant difference ($p \leq 0.05$) represented by increase in number of primary spermatocytes in T1 group (170.8 ± 0.37) compared with other groups, and there was a significant difference represented by increase in T3 group (163.6 ± 0.98) compared with C group (159.6 ± 1.29) and T2 group (103 ± 2.00). Also there was a significant decrease ($p \leq 0.05$) in T2 group (103 ± 2.00) compared with other groups.

Secondary Spermatocytes:

Table 2 revealed there was a significant difference ($p \leq 0.05$) represented by increase in number of secondary spermatocytes in T3 group ($321.6 \pm .92$) compared with other groups, and there was a significant difference represented by increase in T1 group (301.8 ± 1.11) compared with C group (261.8 ± 0.97) and T2 group (172 ± 1.04). Also there was a significant difference represented by decrease in T2 group (172 ± 1.04) compared with other groups.

Sertoli Cells:

Table 2 revealed there was a significant difference ($p \leq 0.05$) represented by increase in number of sertoli cells in T1 group and T3 group (51 ± 0.54), (50.4 ± 0.50) respectively compared with other groups, and there was a significant difference represented by decrease in T2 group (23.2 ± 0.70) compared with other groups. While there were no significant difference between T1 group and T3 group.

Leydig's Cells:

Table 2 show there was a significant difference ($p \leq 0.05$) represented by increase in numbers of leydig's cells in T1 group (43.4 ± 1.02) compared with other groups, and there was a significant difference represented by decrease in T2 group (20.8 ± 1.31) compared with other groups. While there were no significant difference between T3 group and C group.

Table 2 effect of quercetin on number of testicular cells in adult males wistar rats exposed to oxidative stress by lead acetate:

Groups parameters	C group	T1 group	T2 group	T3 group
Primary spermatocytes	c 159.6±1.29	a 170.8±0.37	d 103±2.00	b 163.6± 0.98
Secondary spermatocyte	c 261.8±0.97	b 301.8±1.11	d 172± 1.04	a 321.6± .92
Sertoli cells	b 44.4±0.4	a 51±0.5	c 23.2 ±0.70	a 50.4 ± 0.50
Leydig's cells	b 33.8± 2	a 43.4±1.02	c 20.8 ± 1.31	b 34.8± 0.37

Histopathological study:

Testis histopathological:

Rats in the control group had normal seminiferous tubules with a large amount of spermatogonia, primary, secondary spermatocytes, sertoli cells, and spermatozoa, according to testicular sections., there was presence of leydig's in the interstitial tissue of testes (fig1).

Testicular sections of rats in T1 group which given quercetin show there was complete spermatogenesis character's by presence of spermatogonia and high number of primary and secondary spermatocytes with presence of spermatozoa and spermatid in the lumen of seminiferous tubules (fig 2) .

Testicular sections of rats in T2 group which given lead acetate show there was vaculation of spermatogonia and few number of primary and secondary spermatocytes, few sperms production and the lumen of seminiferous tubules wide, few numbers of sertoli cells and presence of spermatid multinucleated giant cells within the seminiferous tubules (fig 3)

Testicular sections of rats in T3 group which given lead acetate then treated with quercetin show there was complete spermatogenesis characters' by presence of spermatogonia ,primary and secondary spermatocytes with spermatid also there was spermatozoa in the lumen of seminiferous tubules, and prominent sertoli cells which were support spermatogonia (fig 4).

Epididymis histopathology:

Epididymis sections of rats in control group show normal epididymial duct filled with the sperms, normal epithelium lining epididymis duct and presence of long stereocilia (fig 5)

Epididymis sections of rats in T1 group which given quercetin show the epididymis duct extended, dilated and complete filled with sperms, there was normal epithelium of epididymis duct and presence of long stereocilia (fig 6).

Epididymis sections of rats in T2 group which given lead acetate show the epididymis duct empty with degeneration and destruction of stereocilia and low epithelial cells lining the epididymial duct (fig 7).

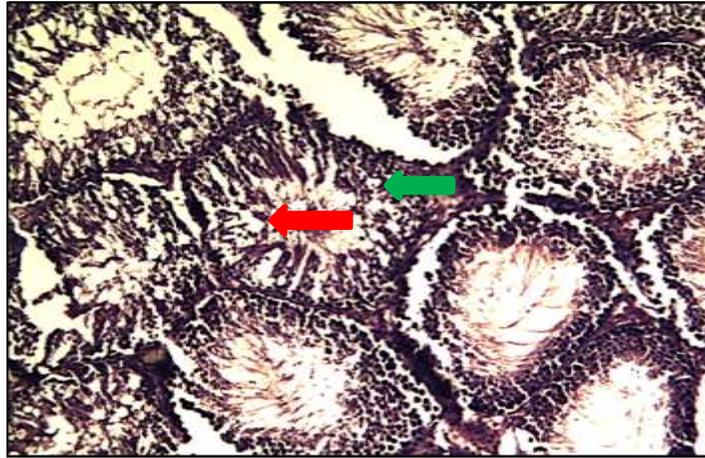
Epididymis sections of rats in T3groupwhich given lead acetate then treated with quercetin show the epididymial duct has normal and high epithelial cells and stereocilia. The epididymial duct wide with large amount of sperms in the lumen of it (fig 8).

Vas deference histopathology:

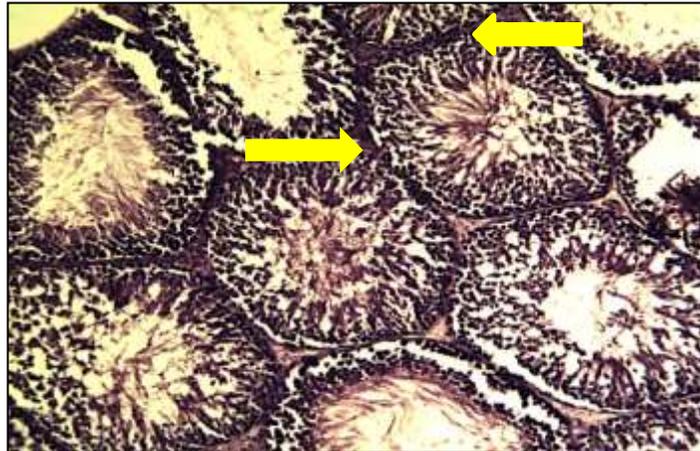
Vas deference sections of rats in control group show normal vas deference wall characters by normal columnar epithelium with stereocilia, normal smooth muscles fiber and normal serosa, the sperms filled the lumen of vas deference (fig 9). Vas deference sections of rats in T1 group which given quercetin show normal vas deference tissue characters by normal epithelium lining it ,normal smooth muscles fibers and sperms filled the lumen of vas deference (fig 10)

Vas deference sections of rats in T2 group which given lead acetate show the conductive tube of vas deference was empty from sperms and there was mild destruction in the stereocilia and sloughing the epithelial layer of vas deference, (fig11).

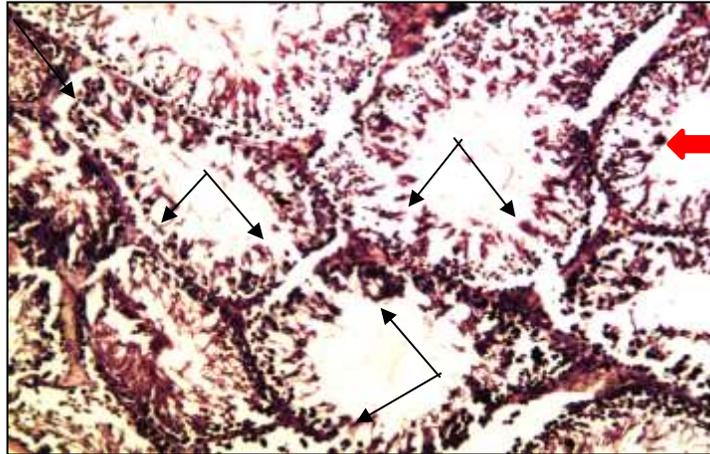
Vas deference sections in T3 group which given lead acetate then treated with quercetin show there was high columnar epithelial with long and extended stereocilia and thick vas deference wall due to normal smooth muscles fiber (fig 12).



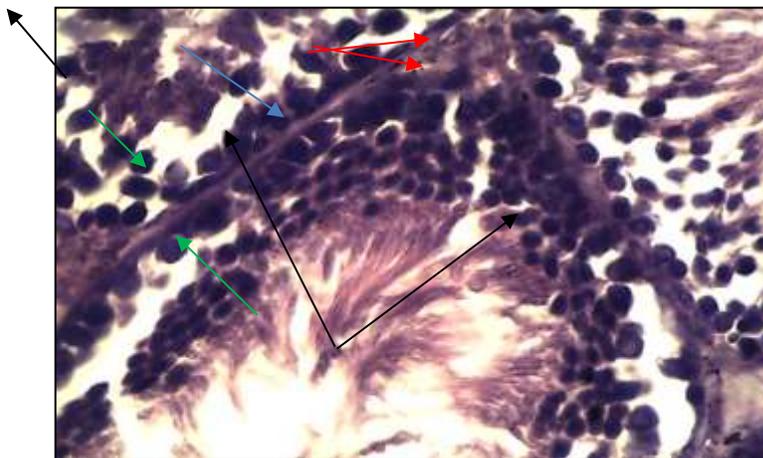
(fig-1) cross section of seminiferous tubules in control group. Primary and spermatocytes, with spermatozoa (red arrow), there is presence of leydig's in the interstitial tissue of testes (green arrow) 100X H&E.



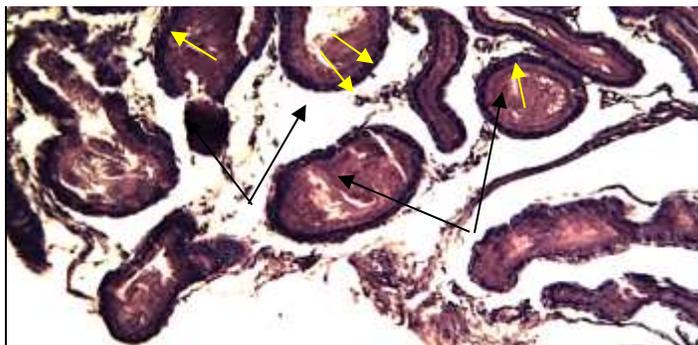
(fig-2) cross section of rat seminiferous tubules in T1 group (red arrow) 100X H&E.



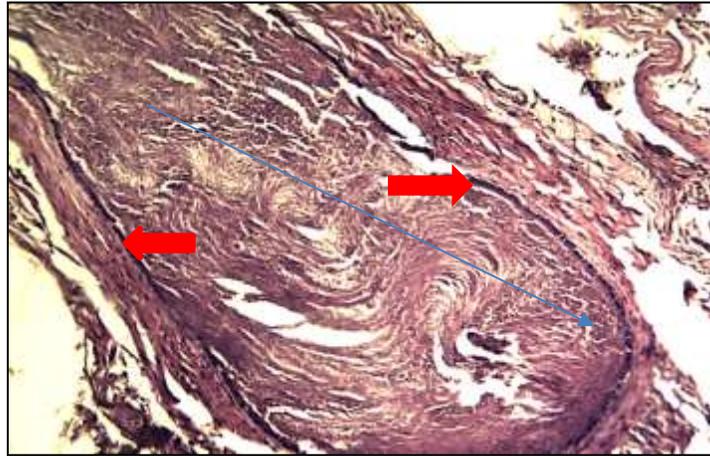
(fig-3) cross section of rat seminiferous tubules in T2 group. Spermatogonia (thin arrows) presence of spermatid with in seminiferous tubule (red arrow) 100X H&E.



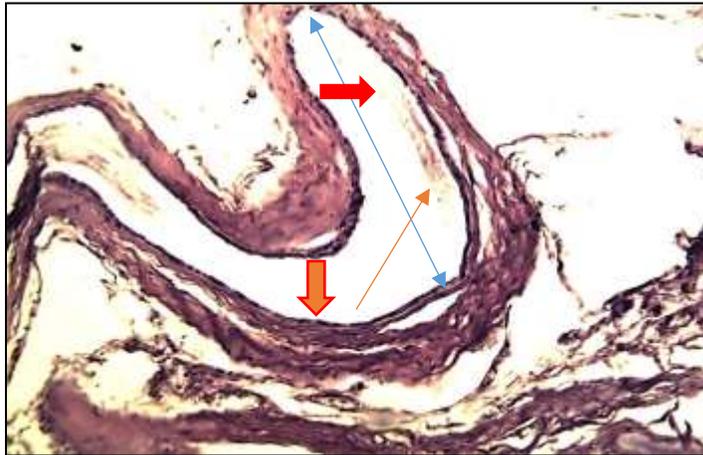
(fig-4) cross section of rat seminiferous tubules in T3 group. Primary and spermatocytes, with spermatid (two head arrows) presence of lidigs cells in the interstitial tissues spermatid with in seminiferous tubule (red arrow), sertoli cells (thin green arrow), and basement membrane (blue arrow) 400X H&E



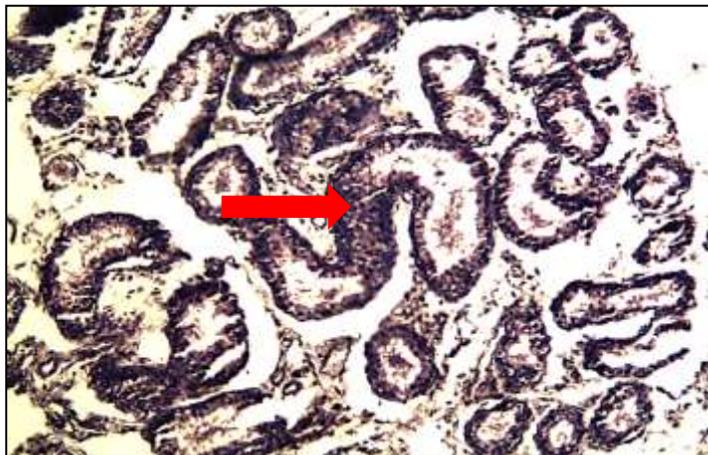
(fig-5) cross section of rat epididymis in control group. Normal duct filled with sperm (thin black arrows) Normal epithelium lining epididymis tubules (thin yellow arrows) 100X H&E.



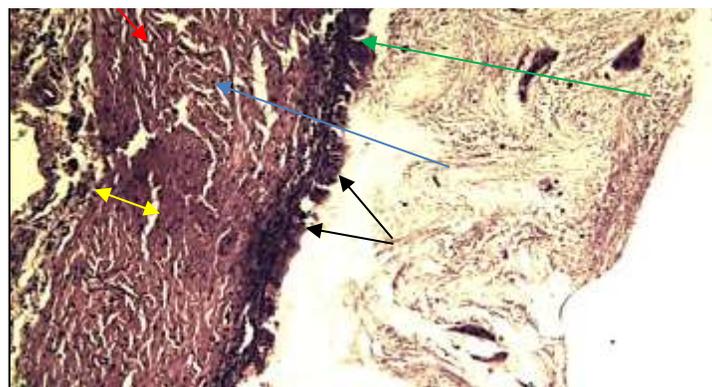
(fig-6) cross section of rat epididymis in T1 group. Epididymis duct (two head blue arrow), lining with normal epithelium (green arrows) 100X H&E



(fig-7) cross section of rat epididymis in T2 group. Epididymis duct empty (two head blue arrow), absence of sertocelia in the epididymis duct (red arrows) 400X H&E.



(fig-8) cross section of rat epididymis in T3 group. Epididymis duct is wide and filled with sperm (red arrow), 400X H&E



(fig 9) Vas deference sections of rats in control group show normal vas deference wall (blue arrow), normal columnar epithelium (black arrow) smooth muscles fiber (red arrow), normal serosa (yellow arrow), and the sperms filled the lumen of vas deference (two head green arrow) 100X H&E.

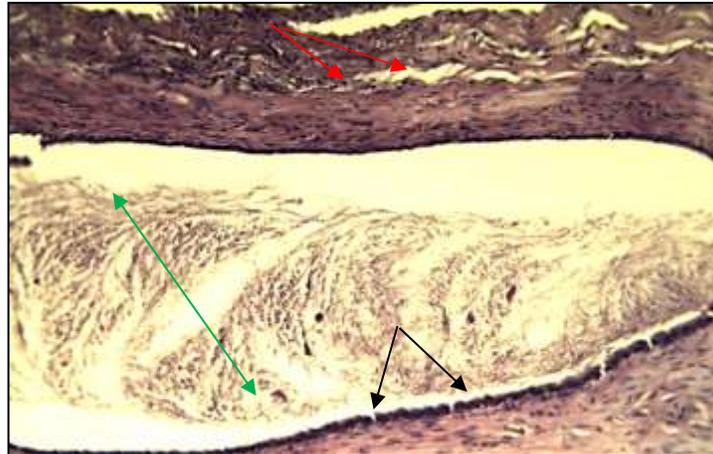
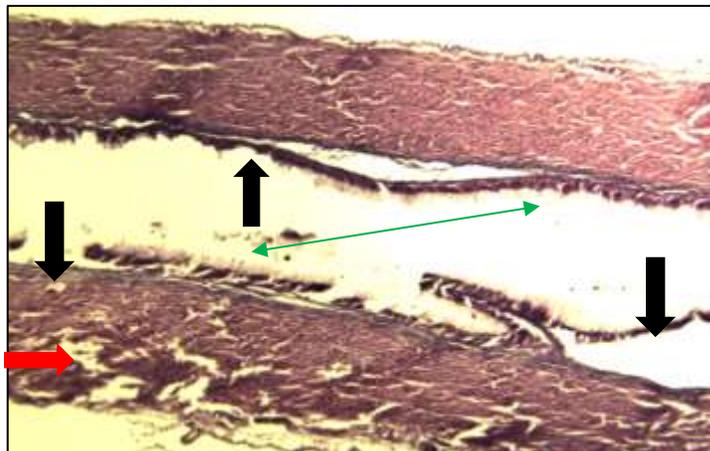
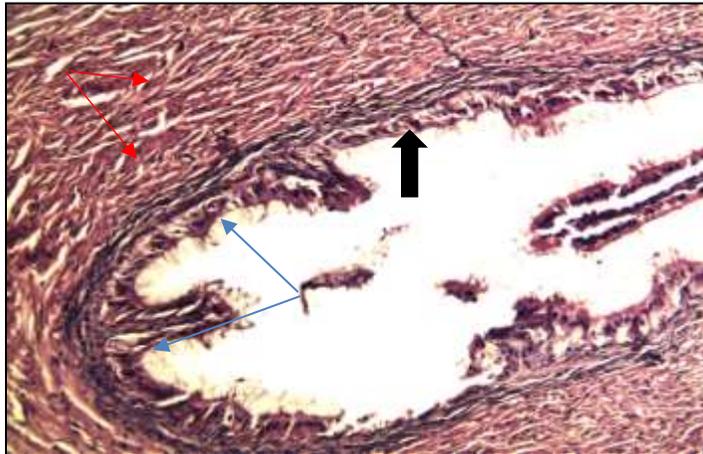


fig 10) Vas deference sections of rats in T1 group, vas deference (black arrow), normal smooth muscles fibers (red arrow) and sperms filled the lumen of vas deference (green arrow) 100X H&E.



(fig11) Vas deference sections of rats in T2 group the, conductive tube of vas deference was empty from sperms (green arrow) and there was mild destruction in the stereocilia and sloughing the epithelial layer of vas deference (black arrow) and smooth muscle (red arrow) 400X H&E.



(fig 12) Vas deference sections in T3 group there is high columnar epithelial (black arrow) with long and extended stereocilia (blue arrow) and thick vas deference (red arrow) 400X H&E .

Discussion:

Table 1 in T1 group which is received quercetin there is a significant increase in seminiferous tubules thickness compared with T2 and control group this increase due to quercetin stimulate testosterone synthesis in testis through increase the activity of enzymes which are responsible for synthesis testosterone hormone which is essential for normal growth of testis which result to increase the primary and secondary spermatocyte and spermatid, therefore increasing seminiferous tubules thickness and decrease seminiferous tubules diameter (Pineda and Dooly, 2003). In T2 group which is received lead acetate there is a significant reduction in the thickness of seminiferous tubules in compared with T1 group, T3 group and C group, and a significant increase in seminiferous tubules diameter due to the lead acetate exposure stimulated production of free radicals , causing oxidative deterioration of lipids, proteins , DNA and starting many pathological conditions causing shrinkage in seminiferous tubules, apoptosis and loss of the germs cells, spermatocytes, sertoli and leydig's cells (Makhlouf, etal., 2008). Free radicals may leads to defect in mechanism of sperm production which leads to decreasing number of germ cells (Holstein, etal., 2003). thus we can consider that the thickness of tubule related to sperms production of the some tubules .When

number of germ cells are reduce this leads to decrease seminiferous tubules thickness and increase seminiferous tubules diameter.

In T3 group which is given lead acetate and then treated with quercetin, show significant increase in seminiferous tubules thickness compared with T2 group and there is a significant decrease in seminiferous tubules diameter compared with T2 group this may be due to the role of quercetin in decreasing of harmful effect of lead acetate which linked with ability of quercetin to chelation of lead ions, and inhibition of the action of oxidases and restore the antioxidants systems (Rice-Evans, et al., 1997).

Table 2 in T1 group which is given quercetin, the results revealed there is a significant increase in sertoli and leydig's cells numbers compared with control group this increase is may be quercetin have improver effect on plasma gonadotropin concentration especially ICSH (Farhomand and Sadeghi, 2015).In this field ICSH increases number of leydig's cells and may the SSH increases number of sertoli cells .Leydig's cells produce testosterone hormone that act on primary spermatocytes to convert to spermatids .sertoli cells produce androgen binding protein which carrying testosterone hormone to the target sit also act as nurse cells to the spermatogonia and play important role in differentiation of primary spermatocytes to secondary spermatocytes this leads to increase spermatogenesis (Pineda and Dooly, 2003).

In T2 group which is given lead acetate ,the results show there is a significant decrease in sertoli and leydig's cells number ,primary and secondary spermatocytes compared with control group ,due to lead acetate administration inhibit spermatogenesis by decreasing stages length related to spermiation and beginning of mitosis (Leiva, et al., 2011). Free radicals leads to defect in mechanism of sperms production this results agreed with, also lead exposure induce generation ROS which causes loss and apoptosis of germ cells , sertoli and leydig's cells, this results in decrease number of primary and secondary spermatocytes (Makhlouf, et al., 2008).

In T3 group which is given lead acetate and then treated with quercetin the results show there is increase in numbers of sertoli ,leydig's cells ,primary and secondary spermatocytes compared with T2 group, due to quercetin may alleviate the damage induced by lead to some extent ,by the ability of quercetin to scavenge ROS (Leiva, et al., 2011). Quercetin may affect indirectly on sperms numbers by the stimulation of the sex organs (Taepongsora, et al., 2008).furthermore the antioxidant activity of quercetin is very important for mitotic divisions for spermatogonia and then produce normal sperm without abnormalities in the seminiferous tubules, while the role of epididymis is complementary to provide sperms requirements to still normal and life.

The histological sections of rats in group T1 which given quercetin (300 mg/kg/B.W) revealed a complete spermatogenesis, normal testicular tissue architecture with normal seminiferous tubules, epididymis and vas deference tissues. This result leads to conclusion that the quercetin antioxidant activities given protection to the testis tissues from oxidative stress (Choi, et al., 2005). Additionally, quercetin affects the testicles via other sex organs via activating the testicles and epididymis or the hypothalamic-pituitary-testicles axis by promoting the release of the hormone testosterone (Ma, et al., 2004). In this area, sperms and testis cells are cooperatively affected by reproductive hormones. The goal of SSH is to encourage sertoli cells to synthesize androgen binding protein, which carries testosterone to the target region in the spermatogonia and epididymis for formation and maturation of sperms. ICSH stimulates leydig's cells to create testosterone hormone (Pineda and Dooly, 2003).

The histological sections of rats in T2 group which is given lead acetate (10 mg/Kg/B.W) show there is sever deteriorate of spermatogenesis and drop in testicular function, might

contaminate the environment with excessive levels of this possible reproductive toxin, which could result in physiologic, biological, and histological abnormalities, this results agreed with (Jegede, et al., 2013). Lead exposure induced generation ROS that caused shrinkage in seminiferous tubules and loss and apoptosis of the germ cells, Sertoli cells, Leydig's cells, as well as deteriorating alterations in testis cell mitochondria, this leads to decreasing number of spermatocytes (Boots, et al., 2008). Also lead acetate causes decrease testicular functions and alteration in the histological pattern in the testis, this results agreed with (-Elgawisha and Abdelrazekb, 2014).

The testicular functions of the rats in the T3 group that were given lead acetate and afterwards treated with quercetin have been restored, according to histological sections. Because of its capacity to remove ROS and replenish the antioxidant systems, quercetin may have a function in reducing the adverse effects of lead acetate. (Pineda a Taepongsorat nd Dooly, 2003) Additionally, quercetin has positive effects and raises gonadotropin hormone levels, which promotes the growth and development of germ cells. (Farhomand and Sadeghi, 2018). Additionally, antioxidants like quercetin increase epithelial cell function and reduce free radical production. (Taepongsorat, et al., 2008).

CONCLUSION:

1. Quercetin have role in improvement the reproductive system efficiency, by increase of sperm quality.
2. That use of the quercetin at a dose of (300 mg/kg) did not causes any side effects along period of experiment.

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