

BENEFICIAL EFFECT OF FLAVONOIDS ON SPERM PARAMETERS IN MALES WISTAR RATS EXPOSED TO THE LEAD ACETATE

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ABSTRACT

The present study aims to investigate the beneficial effect of flavonoids (quercetin) to improve the reproductive system efficiency in adult males rats exposed to the lead acetate. Twenty four males Wistar rats about 7 months old with average weight 190 ± 10 gm were divided randomly into four equal groups and treated for 60 days as following. First group (C) was given drinking water only as a control group. Second group (T1) was given quercetin (300 mg/kg/ B .w). Third group (T2) was given lead acetate (10 mg/kg/ B .w). And fourth group was given lead acetate (10mg/kg/B. w) for 30 day then treated by quercetin (300 mg/kg/ B .w) for 30 day. In the end of the experiment all animals were sacrificed and blood samples were collected from the

abdominal vein and serum samples were isolated to measure Interstitial Cell Stimulating Hormone (ICSH) and Testosterone hormone. Samples of caudal epididymis were taken for estimate sperm parameters. The results of study were revealed a significant difference ($p \leq 0.05$) represented by increase in sperm quality, ICSH, Testosterone hormone level, and a significant difference represented by decrease in sperms abnormality in T1 group compared with C group. Also there was a significant difference represented by decrease in sperm quality, Testosterone hormone level, and a significant difference represented by increase in sperm abnormality in T2 group compared with C group, while there were no significant difference in ICSH level in T2 group compared with C group. Also there was a significant difference represented by increase in sperm quality, Testosterone hormone level, a significant difference represented by decrease in sperm abnormality in T3 group compared with T2 group, while there were no significant difference in ICSH level in T3 group compared with T2 group.

KEYWORDS: flavonoids, sperm parameter, wistar rats, lead acetate.

INTRODUCTION

Lead is an ubiquitous element in the environment, it is used in many industrial activities including mining, refining and producing lead – acid batteries.^[1] The alimentary and respiratory tracts are the major routes of lead entry into the body.^[2] Lead generally interferes with a number of body functions such as the central nervous system, the haematopoietic system, liver and kidneys.^[3] The exposure to the lead acetate in male rat causes decrease in spermatids number, epididymis sperms count, Testosterone serum level and effect on prostate function.^[4]

Flavonoids consist of a large group of polyphenolic compounds having a benzo- γ -pyrone structure and are ubiquitously present in plants. They are synthesized by phenylpropanoid pathway. The secondary metabolites of phenolic nature including flavonoids which are responsible for the variety of pharmacological activities like antibacterial, antiviral, antiulcer, anti-inflammatory, antiallergic, antioxidant, and anticancer.^[5] Flavonoids activities and chemical nature dependent on its structure, degree of hydroxylation, degree of polymerization, conjugations and other substitutions.^[6] Functional hydroxyl groups in flavonoids have mediate their antioxidant effect by chelating metal ions or by scavenging free radicals. The chelation of metals could be crucial in the prevention of free radicals generation which damage target biomolecule.^[7] Flavonoids are thought to have health-promoting properties due to their high antioxidant capacity. The arrangement and situation of hydroxyl groups around the nuclear structure determine the antioxidant activity of flavonoids.^[8] One of these flavonoids quercetin (3,5,7,3,4-pentahydroxyflavone), prevents oxidant injury and cell death by several mechanisms, such as scavenging oxygen radicals^[9] protecting against lipid peroxidation^[10] and chelating metal ions, quercetin, a flavonoid found in many plants, is widely distributed in edible fruits and vegetables, quercetin is the major flavonoid in the human diet and its improve the antioxidative defense system by up regulating of antioxidant enzymes.^[11] So the present study aims to investigate the beneficial effect of flavonoids (quercetin) in improving the male reproductive system efficiency that is exposed to by lead acetate.

MATERIALS AND METHODS

Laboratory animals

Used in our experiment twenty four adult males Wistar rats with about 7 months in old, with average weight about (190±10 gm.) obtained from animal house of veterinary medicine college of Al-Qadisiyah university. The animals housed in well ventilated wire-plastic cages and reared under controlled conditions about 12 hour light and 12 hour dark at about 25°. The animals were allowed to acclimatize for 7 days before experimentation.

Biological material

Flavonoid (quercetin 95%) from onion provided by brightol company/ China.

Experiment design

Twenty four adult male Wistar rats divided randomly to the four equal groups and treated for 60 days as following:- Control group(C) given drinking water only .second group (T1): given quercetin orally in dose (300mg/kg/B.w).^[12] The third group (T2):given lead acetate orally in dose (10mg/kg/B.w).^[13] The four group (T3): given lead acetate orally in dose (10mg/kg/B.w) for 30 days then treated by quercetin orally in dose (300mg/kg/B.w) for 30 day.

Sperm parameters

Sperm concentration

Sperm calculated according to the Hinting method (1989).The tail of epididymis was put in (1 ml) of normal saline then dissected to about (200) very small pieces by special microsurgical scissor and keep in 37°C.For examination drop was taken from the mixture by pipette and put on slid and covered by cover slid. The sperm number calculated in 10 microscopic field by using objective lens (40X) of microscope, the sperm concentration (sperm/ml) was calculated from mean of calculated sperm in ten field, then the mean multiply by 1 million.

Abnormal sperm percentage

The abnormal sperm were detected according to siegmund (1979). The smear was prepared by put (50µl)from tail of epididymis and mixed on slide then added drop of eosin-nigrosin stain and mixed to the 30 second, then spread by another slide, after drying the smear examined under objective lens 40X. then calculated (200) sperm, the abnormal sperm detected by the following equation.

$$\text{percentage of abnormal sperm} = \frac{\text{number of abnormal sperm}}{\text{total number of sperm}} \times 100\%$$

Percentage of sperm viability

Taken drop from epididymis content and mix it with drop of eosin-nigrosin stain according to the Barth and Oko method (1994). The smear examined under 40X then calculated (200) sperm, the head of dead sperm were stained, and the head of live sperm don't stained. sperm viability percentage calculated according to the following equation.

$$\text{percentage of live sperm} = \frac{\text{number of live sperm}}{\text{total number of sperm}} \times 100\%$$

Total sperm motility

Maceration the tail of epididymis on warm, dry and clean slide to take the sperm from its content then put a drop of warm normal saline on slide and mix well. Then examined under objective lens (10X) to determination total sperm motility according to the bearden and Fuquay method (1980). The percentage of total sperm motility calculated according to the following equation.

$$\text{percentage of sperm motility} = \frac{\text{number of moving sperms in particular}}{\text{total number of sperm}} \times 100\%$$

Hormonal assays in blood serum

ICSH and Testosterone hormone evaluated by using ELISA technique and done according to the company instruction.

Statistical analysis

A computerized program, the statistical package for social sciences (SPSS) was used to calculate the statistics analysis. The statistical analysis of data had done by.

1. Descriptive statistics :mean± stander error
2. statistical analysis of data was performed on the basis of ANOVA (one way analysis of variance) with least significant difference LSD was detected to compare between groups
3. the confidence limit was accepted at 95% ($p > 0.05$)^[14]

RESULTS

Sperm concentration

Table 1 show there was a significant difference ($p \leq 0.05$) represented by increase in T1 group (78.2 ± 1.28) compared with T2 group (32.8 ± 0.96) and T3 group (60.4 ± 1.63). And there was a significant difference represented by decrease in T2 group (32.8 ± 0.96) compared with

other groups. While there were no significant difference between C group (75.2 ± 1.59) and T1 group (78.2 ± 1.28).

Percentage of abnormal sperm

Table 1 show there was a significant difference ($p \leq 0.05$) represented by increase in T2 group (41.2 ± 0.97) compared with other groups. And there was a significant difference represented by decrease in T1 group (16.2 ± 0.49) compared with other groups. While there were no significant difference between C group (18.6 ± 0.40) and T3 group (19.8 ± 0.67).

Percentage of sperm viability

Table 1 show there was a significant difference ($p \leq 0.05$) represented by increase in T1 group (86.2 ± 0.66) compared with other groups. And there was a significant difference represented by decrease in T2 group (35.8 ± 0.66) compared with other groups. Also there was a significant decrease in T3 group (65.2 ± 1.59) compared with C group (69.2 ± 0.66).

percentage of total sperm motility

Table 1 show there was a significant difference ($p \leq 0.05$) represented by increase in T1 group (64.2 ± 0.67) compared with other groups. And there was a significant difference represented by increase in T3 group (45.2 ± 0.37) compared with C group (42.4 ± 0.75) and T2 group (24.8 ± 0.67). And there was a significant difference represented by decrease in T2 (24.8 ± 0.67) compared with other groups.

Table 1: Effect of quercetin on sperm parameters of adult males wistar rats exposed to the lead acetate.

Groups	C group	T1 group	T2 group	T3 group
Sperm concentration (million / ml)	a 75.2 ± 1.59	a 78.2 ± 1.28	c 32.8 ± 0.96	b 60.4 ± 1.63
percentage of abnormal Sperm (%)	b 18.6 ± 0.40	c 16.2 ± 0.49	a 41.2 ± 0.97	b 19.8 ± 0.67
Percentage of sperm viability (%)	b 69.2 ± 0.66	a 86.2 ± 0.66	d 35.8 ± 0.66	c 65.2 ± 1.59
Percentage of Total sperm motility (%)	c 42.4 ± 0.75	a 64.2 ± 0.67	d 24.8 ± 0.67	b 45.2 ± 0.37

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.

Testosterone hormone

Results in table 2 show there was a significant difference ($p \leq 0.05$) represented by increase in testosterone hormone level in T1 group (10.07 ± 0.11) compared with other groups (C,T2,T3). And there was a significant difference represented by decrease in T2 group (6.78 ± 0.22) compared with other groups. While there were no significant difference between C group (8.2 ± 0.19) and T3 group (8.42 ± 0.29).

Interstitial cell stimulating hormone(ICSH):

Results in table 2 show there was a significant difference ($p \leq 0.05$) represented by increase in ICSH level in T1 group (13.48 ± 1.23) and T3 group (11.88 ± 1.24) compared with C group (7.67 ± 1.14) and T2 group (6.7 ± 0.57). While there were no significant difference between T1 group and T3 group. Also there were no significant difference between T2 and C group.

Table 2: Effect of quercetin on reproductive hormones of adult males wistar rats exposed to the lead acetate.

Groups Hormones	C group	T1 group	T2 group	T3 group
Testosterone hormone (ng/ml)	b 8.2 ± 0.19	a 10.07 ± 0.11	c 6.78 ± 0.22	b 8.42 ± 0.29
ICSH (MIU/ml)	b 7.67 ± 1.14	a 13.48 ± 1.23	b 6.7 ± 0.57	a 11.88 ± 1.24

DISCUSSION

Tables 1,2 in T1 group which is treated with quercetin there is improvement in sperm quality (viability, motility and concentration) due to a stimulatory effect of quercetin.^[15] This result comes with increasing serum testosterone hormone and ICSH concentration due to quercetin have improver effect on plasma gonadotropin concentration especially ICSH.^[16] This results leads to the conclusion that quercetin have an effect on testis by stimulating the

testis and epididymis or hypothalamic-pituitary-testis axis through stimulating Testosterone hormone secretion.^[17] In this field the reproductive hormones have cooperative effect on sperm parameters. ICSH stimulate leydig's cells to produce Testosterone hormone, and then come the role of SSH which stimulate sertoli cells to synthesis androgen binding protein which carries Testosterone to the target sites in the spermatogonia and epididymis for development and maturation of sperm.^[18] Decrease percentage of sperm abnormality may due to ability of quercetin to scavenge endogenous ROS which can damage cell structures like nucleic acids, carbohydrates, lipids, proteins and change their functions.^[19] 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.

In T2 group which is exposed to oxidative stress by lead acetate there is decrease in sperm quality (viability, motility and concentration) due to toxic effect of lead on testis, epididymis, vas deferens, which caused adverse effect on sperm count, sperm motility and retarded the activity of spermatozoa and this results agreed with.^[20] While decreasing number of leydig's cells and Testosterone hormone level consider as indirect reason for this result, lead acetate increases NO and lipid peroxidation in testis and serum, associate with decreasing antioxidant enzymes as SOD and CAT.^[21] Also lead causes shrinkage in seminiferous tubules and loss of the germ cells and apoptosis of Sertoli cells and Leydig's cells, also degenerative changes in mitochondria of testis cells, this leads to decrease number of primary spermatocytes, secondary spermatocytes and spermatids and this results agreed with.^[22] Increase of free radicals due to lead acetate causes changes in spermatozoa membrane which leads to decrease percentage of sperm viability and increase percentage of sperm abnormality.^[23] Lead causes oxidative stress this may be leads to destruction of lining cell for seminiferous tubules (sertoli cell) that is responsible for production of abnormal sperm. The results of the study showed the epididymis duct has few or no sperm and this may due to lead accumulates in all the male reproductive system and drop Testosterone hormone level causes alteration in the epididymis function that is dependent on androgen which leads to higher percentage of abnormal sperm morphology.

In T3 group which is given lead acetate then treated by quercetin there is improvement in sperm quality (viability, motility and concentration) and decrease in sperm abnormality compared with T2 group which given lead acetate only, due to role of quercetin in decreasing of harmful effect of lead acetate by the ability of quercetin to scavenge ROS and

restore the antioxidant systems and this agreed with.^[24] Quercetin have improved effects on gonadotropin and Testosterone hormone which leads to improvement in epididymis function that is dependent on androgen, this leads to decrease sperm abnormality.

CONCLUSION

1. Quercetin has a role in improving the reproductive system efficiency, by increasing ICSH, Testosterone hormone and sperm quality.
2. The use of quercetin at a dose of (300 mg/kg) did not cause any side effects along the period of experiment.

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