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Jalal A. Karim

Department of Biology, College of Education, Salahaddin University-Erbil, Erbil-Ira, jalal.karim@student.su.edu.krd

Karim R. Hamad

Department of Biology, College of Education, Salahaddin University-Erbil, Erbil-Iraq

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RESEARCH ARTICLE

Protective Effect of Fenugreek Supplementation against Mercury Toxicity on Sperm Parameters, Serum Testosterone and Testicular Tissue in the Rat

Jalal A. Karim[1], Karim R. Hamad[2]

[1] *Department of Biology, College of Education, Salahaddin University-Erbil, Erbil-Iraq*

[2] *Department of Biology, College of Education, Salahaddin University-Erbil, Erbil-Iraq*

***Corresponding author:**
Jalal A. Karim,
Department of Biology,
College of Education,
Salahaddin University,
Erbil, Kurdistan Region,
Iraq.

E-mail:
jalal.karim@student.su.edu.krd

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ABSTRACT

Background and objectives: The purpose of the current study was to investigate the effects of fenugreek supplementation against toxic effect of mercuric chloride ($HgCl_2$) on Body weight (B.W), testis and epididymis weight, some sperm parameters, serum testosterone, serum MDA, and testis histology in the rat.
Methods: Twenty eight male albino rats weights ranging from 254 to 302 gm and were divided randomly and equally into 4 groups. Group I: control, Group II: Rats received drinking water contained $HgCl_2$ 100 mg/L *ad libitum*, Group III: Rats received drinking water contained $HgCl_2$ 100 mg/L *ad libitum*, and fenugreek (F1) 0.75 mg/kg/day orally by gavage, Group IV: Rats received drinking water contained $HgCl_2$ 100 mg/L *ad libitum* and fenugreek (F2) 1.5 mg/kg/day orally by gavage. The treatments were given for six weeks.
Results: Treatment with F1 caused no substantial changes in body weight, absolute weight, and relative weight of testis and epididymis, sperm count and testosterone. Whereas sperm motility, and normal sperm were increased significantly, and serum MDA was decreased significantly from $HgCl_2$ group. Improvement was observed partially in testis histology. Meanwhile, the protective effect in F2 generally was more than in F1.
Conclusions: the present study revealed that the antioxidant activity of fenugreek supplement reduced toxic effect of mercury. In addition, steroids and/or phytoestrogens in fenugreek possibly compensated the reduced testosterone which resulted at least in part in improvement of sperm parameters and testis histology.
Keywords: Fenugreek; Mercury; Sperm; Testis; MDA; Testosterone

INTRODUCTION

Mercury is a highly toxic metal that causes a wide range of negative neurological, respiratory, immune, renal, dermatological, reproductive, and developmental effect (Risher and Amler, 2005). Investigations showed that male reproductive organs undergo morphological and ultrastructural alteration during exposure to $HgCl_2$. When this metal enters the testis and epididymis disrupts their functions (Penna et al., 2009). It was reported that ingesting $HgCl_2$ orally in the male rats showed the seminiferous tubules lumen with reduced mature sperm clearly visible and appeared dilated. Indeed, the testes clearly showed plasma-rich interstitial flow along with lymphatic vessel and vein dilation. Leydig cells were loosening, and there was a significant decrease and sloughing of germ cells (Boujbiha et al., 2011). In another study, mercury injection i.p. in the rat showed that, seminiferous tubules diameter decreased, indicating decreased spermatogenesis activity. The number of Leydig cells increased, which resulted in reduced interstitial gaps. The decrease in lumen diameter was indicating the discharge of mature spermatids into the lumen (Jahan et al., 2019).

In another study, mercury caused sperm motility defects in mice (Rao and Sharma, 2001). Also the motility of sperm was markedly reduced in the $HgCl_2$ treated rats through drinking water (Boujbiha et al., 2011). In addition, administration of $HgCl_2$ orally caused, a significant decline in sperm motility in the rats (Adelakun et al., 2021).

During treatment the rats with $HgCl_2$ by gavage, revealed the diminish significance of the head and body of epididymis in the sperm count (Heath et al., 2012). Analysis morphology of sperm revealed that prolonged Hg exposure intravenously in rats reduced the proportion of morphologically normal sperm, with sperm head,

banana head, and mostly amorphous morphology defects being the most common abnormalities. In terms of tail morphology, bent tail anomaly predominated (Rizzetti et al., 2017). Besides that, administration of $HgCl_2$ in the rats reduced testicular sperm count and daily sperm production of testis (Abarikwu et al., 2017).

Mercury increases lipid peroxidation which damages the membrane, cause decreased sperm motility, likely due to depletion of intracellular ATP rapidly, and increased sperm morphological problems of subfertile males (Choy et al., 2002). In addition, intoxication by mercury was associated with oxidative stress induction in tissues (Su et al., 2008). Rats were used in an experiment, they were exposed to $HgCl_2$ orally by gavage, liver MDA levels increased (Goudarzi et al., 2017). Administration of $HgCl_2$ i.p. Also, increased MDA level in treated rats (Abarikwu et al., 2017). Furthermore, recent study showed that, $HgCl_2$ in rats, significantly increased MDA product of lipid peroxidation (Adelakun et al., 2021).

It has been demonstrated that, fenugreek seeds have importance for a variety of purposes as a tonic, as a kind of therapy for leg edema and weakness (Yoshikawa et al., 1997). Plasma glucagon, somatostatin, and blood glucose levels are all decreased by the defatted portion of the seeds recipients of fenugreek, also showed decreased levels of glycated hemoglobin (HbA1c) and enhanced sensitivity to insulin (Snehlata and Payal, 2012). In addition, several pharmacological effects of Studies on *Trigonella* including carminative, stomach stimulant, and anti-inflammatory properties, anticarcinogenic, hepatoprotective and antioxidant effect (Yadav and Baquer, 2014).

A non-steroidal chemical called phytoestrogen is obtained from plants, such as fenugreek. (*Trigonella foenum-graecum L.*) (Yusharyahya et al., 2020), and low level of phytoestrogen, affect the testis biologically (Robertson et al., 2002).

There are many studies carried out on the effect of drugs and active ingredients against mercury on the laboratory animals. However in the literature we couldn't find any data concern with the effect of fenugreek seed against mercury on the rat's male reproductive system. Therefore, our research plan established to investigate the impact of fenugreek supplementation (21^{ST} Century Health care Inc. USA) against toxicity of mercury on body weight(B.W.), the weight of testis and epididymis, histology of testis, sperm motility, sperm morphology, sperm count, serum MDA and testosterone in the male rats.

SUBJECTS AND METHODS

Animals

Male albino rats (*Rattus norvegicus*) were obtained from the animal house in department of biology/University of Raparin. Throughout the whole time of acclimation and experiment in department of biology/college of education/Salahaddin University, the rats were housed in customized cages with a steel stainless wire mesh that held standard rat diet (pico Lab. Rodent Diet 20) and water *ad libitum*. The room temperature was fixed at around 24 degrees Celsius, and the light-dark cycle was set to 12/12 hours.

Chemicals

Mercuric chloride ($HgCl_2$) was manufactured by Scharlab S.L. Spain. Fenugreek seeds supplementation was manufactured by 21^{ST} Century Health care Inc. USA.

Design of the Experiment

Twenty-eight male rats weighing (254-302 gm) were randomly divided into four equal groups each of seven rats. The treatments lasted six weeks as the following:

Group I (control): Rats received standard diet and drinking water *ad libitum*, and also received 2 ml distilled water / day by gavage as a vehicle.

Group II (Hg): Rats received standard diet, and drinking water contained ($HgCl_2$)100 mg/l *ad libitum*.

Group III (Hg+F1): Rats received standard diet, drinking water contained ($HgCl_2$) 100 mg/l *ad libitum* and fenugreek seed supplementation 0.75 mg/kg in 2ml distilled water / day by gavage.

Group IV (Hg+F2): Rats received standard diet, drinking water contained ($HgCl_2$) 100 mg/l *ad libitum* and fenugreek seed supplementation 1.5mg/kg in 2 ml distilled water / day by gavage.

Determination of Rats Body Weight

The B.W. of rats was recorded before and after 6 weeks of treatments by digital scale.

Blood Collection

At the end of experiment after fasting for 24 hours, rats were anesthetized with ketamine (100 mg/kg)/xylazine (10 mg/kg) (Flecknell, 2009). Blood samples were collected immediately by 5 ml syringe directly from the heart. The samples were centrifuged using (Sorvall RC-5B Refrigerated Superspeed Centrifuge) for 15 minutes at 3000 rpm. The serum samples were placed in Eppendorf tubes at once without delay the sample were used in determination of testosterone and MDA.

Dissection and Removal of Organs

Animals were dissected after blood samples were taken. The weight of the left testes and epididymides were recorded after they were removed. For fixation, the testes sample were stored in 10% formal saline. The left epididymides were employed for sperm motility, while the right epididymids were employed for sperm counting.

Sperm Count

Each rat's right epididymis was manually homogenized in 5 ml of normal saline. The homogenate was refrigerated

at 4 degrees Celsius for 24 hours to allow sperm to be released from the walls. The samples were then placed in a Neubauer hemocytometer using a light microscope 1µ of the refrigerated homogenate was mixed with 7µl of Eosin 0.2%. The sperm heads were counted in 25 squares (Yucra et al., 2008).

Sperm Morphology

Sperm sample was obtained from the left vas deference. Smearing and drying were performed on the suspensions. After that, the slides were stained with 1% Eosin solution. The slides were washed in running water and dried. Under a light microscope (1000X), sperm morphology such as normal sperm, head defect sperms, and tail defect sperms were identified.

Sperm Motility

Left epididymis was placed into the phosphate buffer 37C°, soon the epididymis cut to release the sperm, and stored in incubator 37C° for 10 minutes. Then one drop of phosphate buffer with sperms was transferred to Neubauer Hemocytometer and read the slide counted the motile sperms and non-motile sperms (Sharma and Singh, 2010).

Histological Sectioning

Serial operations were performed on a preserved testes samples in 10% formal saline. The sample was cooled after being embedded in paraffin wax. A rotary microtome was used to cut the sections. The samples were then stained with haematoxylin (H) and eosin (E) (Bancroft and Gamble, 2008).

Malondialdehyde

The level of malondialdehyde (MDA) in serum was measured spectrophotometrically at 532 nm using an (APEL PD-303 SPECTROPHOTOMETER. APEL CO., LTD. JAPAN). The Thiobarbituric acid reaction (TBAR) method was utilized for this purpose. MDA in µmol/L was used to express lipid peroxidation.

Serum Testosterone

Serum testosterone level was measurement by electrochemiluminescence immunoassay (ECLIA) cobas e411 analyzer.

Statistical analysis

All data in this study were expressed as mean ± S.E. Graphpad Prism Eighteen, version 8.0.2, was used for the statistical analysis. To determine value among groups, data were analyzed using one-way ANOVA and the findings were compared using ANOVA and Tukey's multiple comparisons tests. The difference was considered significant when the P value was less than 0.05.

RESULTS

Effect of Fenugreek Against Mercuric Chloride on Body Weight

In the present study the B.W. of rats in control, Hg, Hg+F1, and Hg+F2 are shown in table 1. The difference in rats B.W was non-significant among all groups before treatment and after treatment as well.

Effect of Fenugreek Against Mercuric Chloride on Organ Weight

The absolute and relative weight of the left testis and epididymis of control, Hg, Hg+F1 and Hg+F2 are shown in table 2 and table 3 respectively. The change in absolute and relative weight of testis and epididymis among all groups were non-significant.

Table 1. Effect of fenugreek supplementation against mercuric chloride on body weight

Groups / Parameters	Control	Hg	Hg+F1	Hg+F2
B.W. before treatment (gm)	274.0± 4.509[a]	275.0± 3.192[a]	276.4± 7.600[a]	281.3± 4.809[a]
B.W. after treatment (gm)	332.1± 10.820[a]	310.1± 6.991[a]	305.3± 9.727[a]	300.4± 10.440[a]

The same letters indicate non-significant differences

Table 2. Effect of fenugreek supplementation against mercuric chloride on absolute weight of testis and epididymis

Groups / Parameters	Control	Hg	Hg+F1	Hg+F2
Left testis (gm)	1.483± 0.049[a]	1.350± 0.048[a]	1.407± 0.049[a]	1.461± 0.045[a]
Left epididymis (gm)	0.261± 0.0122[a]	0.232± 0.0094[a]	0.252± 0.0108[a]	0.270± 0.0237[a]

The same letters indicate non-significant differences

Table 3. Effect of fenugreek supplementation against mercuric chloride on the relative weight of testis and epididymis

Groups Parameters	Control	Hg	Hg+F1	Hg+F2
Left testis %	0.460± 0.014[a]	0.454± 0.019[a]	0.472± 0.021[a]	0.442± 0.060[a]
Left epididymis %	0.086± 0.006[a]	0.081± 0.006[a]	0.084± 0.003[a]	0.141± 0.050[a]

The same letters indicate non-significant differences

Effect of Fenugreek against Mercuric Chloride on Sperm Parameters

Sperm Counting

The sperm count in control, Hg, Hg+F1 and Hg+F2 are shown in figure 1. The sperm count in Hg group was decreased significantly (P<0.001) as compared to the control. It was increased non- significantly in Hg+F1 as compared to Hg group. While, It was significantly reduced (P<0.01) from control. Whereas, in Hg+F2 was considerably higher (P<0.001) as compared to Hg group. Meanwhile, there was a slight decrease in Hg+F2 group as compared to control.

Sperm Morphology

The morphology of the sperm, which includes normal, head-defect, and tail-defect sperm in control, Hg, Hg+F1 and Hg+F2 are shown in figure2, figure3 and figure 4 respectively.

Normal sperm was decreased significantly (P<0.001) in Hg, Hg+F1 and Hg+F2 as compared to control. While, Hg+F1 and Hg+F2 were higher significantly (P<0.001) as compared to Hg. meanwhile, in Hg+F2 was increased significantly (P<0.001) from Hg+F1.

Sperm with head and tail defects increased considerably (P<0.001) in the Hg group compared to the control. While, both defects in Hg+F1 and Hg+F2 were decreased significantly (P<0.01) as compared to Hg group. In both groups (Hg+F1 and Hg+F2) compared to control were significantly (P<0.01) higher. At the time Hg+F2 was decreased significantly (P<0.05) from Hg+F1.

Sperm Motility

The sperm motility in control, Hg, Hg+F1 and Hg+F2 are shown in figure 5. In Hg (P<0. 01) group and Hg+F1 (P<0.001) decreased significantly as compared to control. While, the value in Hg+F2 was not significantly different from control. There is non-significant difference between Hg+F1 and Hg+F2, while both groups increased significantly (P<0.001) form Hg group.

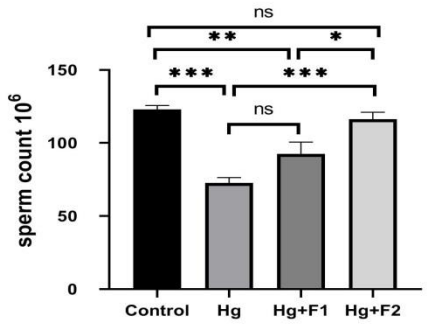

Figure1: Effect of fenugreek supplementation against mercuric chloride on sperm count. Control=122.900±2.734 x10^6/epid, Hg= 72.600±3.780 x10^6/epid., Hg+F1= 92.400±8.232 x10^6/epid., Hg+F2= 116.30±4.714 x10^6/epid. N.S= Non significant differences.
*=P<0.05,**=P<0.01,***=P<0.001

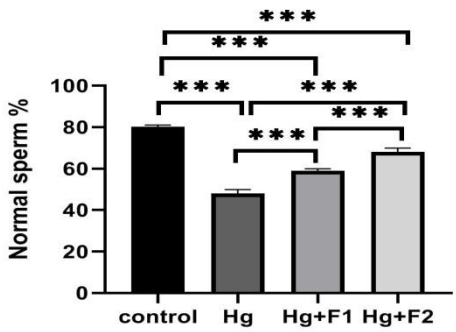

Figure 2: Effect of fenugreek supplementation against mercuric chloride on normal sperm morphology. C= 80.140±0.85 (%), Hg= 48.000±1.83 (%), Hg+F1= 58.860±1.05 (%), Hg+F2=68.140±1.72 (%),
N.S= Non significant differences. ***=P<0.001

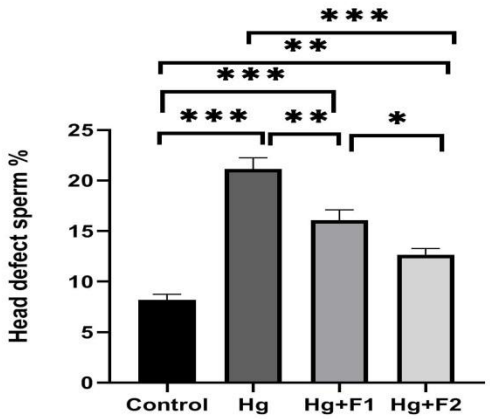

Figure 3: Effect of fenugreek supplementation against mercuric chloride on head defect sperm. C=8.190±0.548 (%), Hg= 21.140±1.10 (%), Hg+F1= 16.080±1.02 (%), Hg+F2= 12.670±0.59 (%), N.S= Non significant differences. *=P<0.05, **=P<0.01, ***=P<0.001

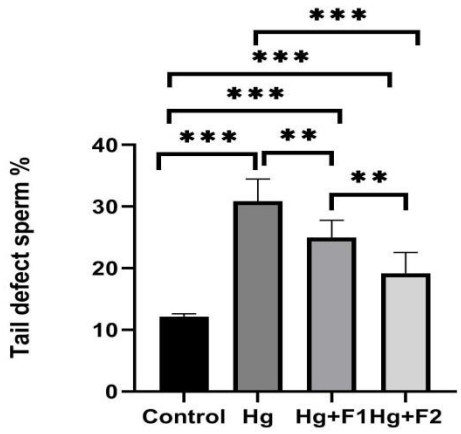

Figure 4: Effect of fenugreek supplementation against mercuric chloride on tail defect sperm. C= 12.100±0.188 (%), Hg= 30.860±1.374 (%), Hg+F1=25.030±1.037(%), Hg+F2= 19.190±1.278 (%).N.S= Non significant differences. **=P<0.01, ***=P<0.001

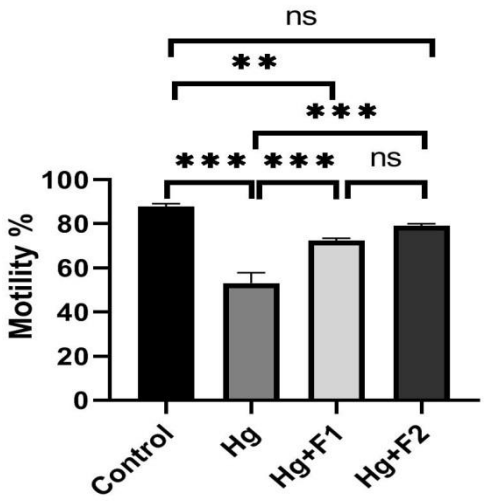

Figure 5: Effect of fenugreek supplementation against mercuric chloride on motility**.** C= 87.86±1.204 (%), Hg= 53.00±4.721 (%), Hg+F1=72.29±1.107 (%), Hg+F2= 79.00±0.975 (%),
N.S= Non significant differences. **=P<0.01, ***=P<0.001

Effect of Fenugreek Against Mercuric Chloride on Serum Malondialdehyde Level

The MDA level in control, Hg, Hg+F1, and Hg+F2 are shown in figure 6. Malondialdehyde in Hg group was increased significantly (p<0.001) as compared to control. In Hg+F1 and Hg+F2 were reduced significantly (p<0.001) as compared to Hg group. While, in Hg+F1 it was higher than control significantly (P<0.05). No significance change found in Hg+F2 in comparison to control.

Effect of Fenugreek Against Mercuric Chloride on Serum Testosterone

The serum testosterone level in control, Hg, Hg+F1 and Hg+F2 are shown in figure 7. Testosterone was diminished significantly (p<0.05) in Hg group in comparison to control. It was also decreased significantly in both groups Hg+F1 (P<0.001) and Hg+F2 (P<0.01) in comparison to control. While, no-significant decrease was found in testosterone level in Hg+F1 and Hg+F2 as compared to Hg group. Meanwhile, its level in Hg+F2 was slightly increased as compared to Hg+F1.

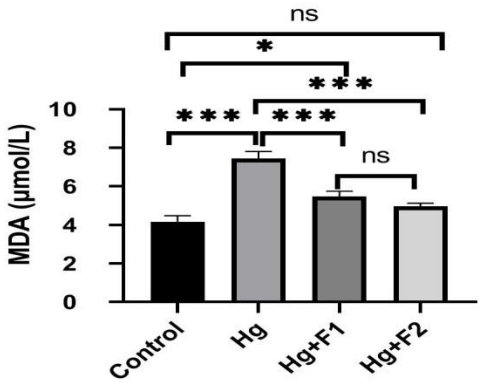

Figure 6: Effect of fenugreek supplementation against mercuric chloride on serum MDA. C= 4.147±0.316 (µ mol/L), Hg= 7.447±0.355 (µ mol/L), Hg+F1= 5.471±0.270 (µ mol/L), Hg+F2= 4.956±0.170 (µ mol/L), N.S= Non significant differences. *=P<0.05, ***=P<0.001

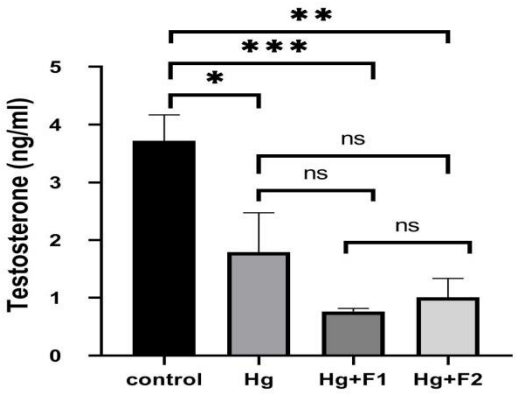

Figure 7: Effect of fenugreek supplementation against mercuric chloride on testosterone. C= 3.721±0.445 (ng/ml), Hg= 1.790±0.683 (ng/ml), Hg+F1= 0.764±0.052 (ng/ml), Hg+F2= 1.008±0.328 (ng/ml), N.S= Non significant differences.*=P<0.05, **=P<0.01, ***=P<0.001

Effect of Fenugreek Against Mercuric Chloride on Testis Histology

The seminiferous tubules in the control testis section display normal architecture with normal characteristics and organization in germ cell layers, normal spermatogenesis activity and Spermatozoa are present in the seminiferous tubules lumen. The section also shows normal interstitial tissue in between (Figure 8).

The histological section of testis in $HgCl_2$ treated rat shows deformities in testis architecture, tissue damage and separation of spermatocytes from basement membrane, spermatid discharge and fragments into the lumen, disorganization in germ cell layer with decreasing spermatogenesis, sloughing of germ cells and few spermatozoa in the lumen of seminiferous tubules, decreased leydig cells, congested blood vessel and edematous condition in interstitial tissues (Figure 9) and (Figure 10).

In certain parts of testis section of rats treated with Hg+F1, seminiferous tubules with slightly improvement, the section shows deformities in testis architecture, degenerative area in the spermatogenic layers, and interstitial tissue (Figure 11). Whereas, other parts show improvement in testicular architecture, organization in germ cells, partially improvement in spermatogenesis activity and in interstitial tissue as well. However, some seminiferous tubules show an increased in lumen diameter indicating decrease in spermatogenesis activity (Figure 12).

The section in Hg+F2 shows improvement in seminiferous tubules better than in Hg+F1. Fenugreek induced improvement in testicular architecture, organization in germ cell layer and restored spermatogenesis activity with spermatozoa in the lumen. Occasionally shows interstitial tissue damage (Figure 13) and (Figure 14).

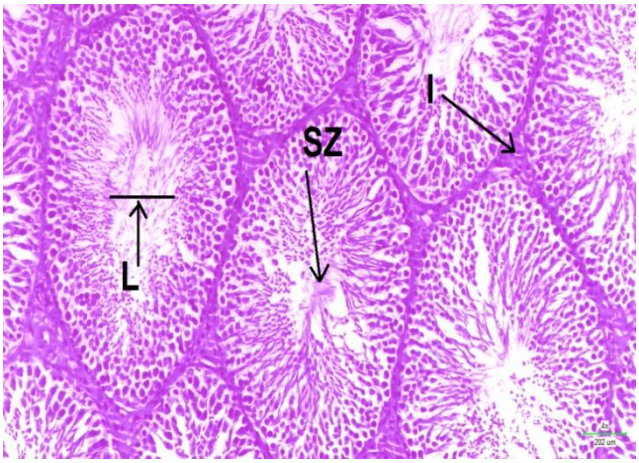

Figure 8: Section from the testis of control rat showing normal architecture of the seminiferous tubules, there are many spermatozoa (SZ) in the lumen (L),also shows normal interstitial tissue (I) (Stain: Haematoxylin and Eosin. 100X).

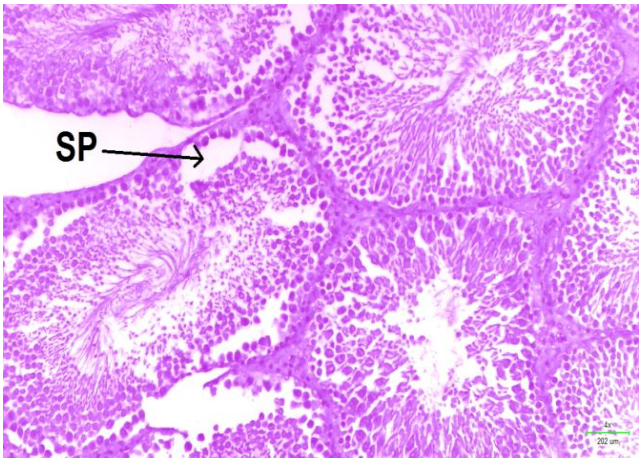

Figure 9: Section from testis of rat treated with $HgCl_2$, displaying abnormalities in the testicle architecture, disorganization in germ cell layer, degenerative area in the spermatogenic layers, and separation of spermatocytes (SP) (Stain: Haematoxylin and Eosin. 100X).

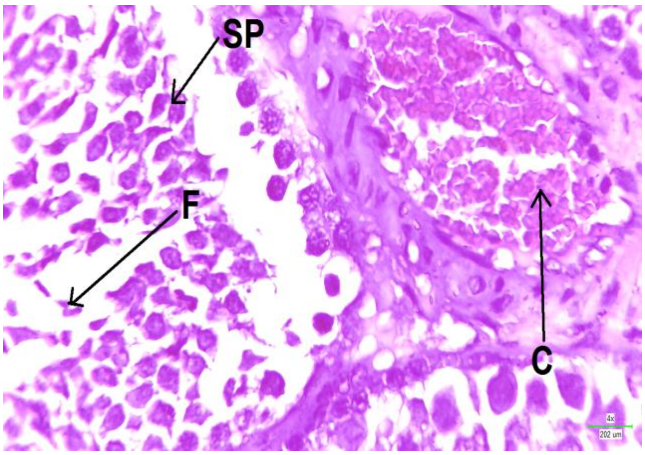

Figure 10: Section from testis of rat treated with $HgCl_2$, display the fragments of degenerated cell (F), separation of spermatocytes (SP) and congested blood vessel (C) in interstitial tissue (Stain: Haematoxylin and Eosin. 400X).

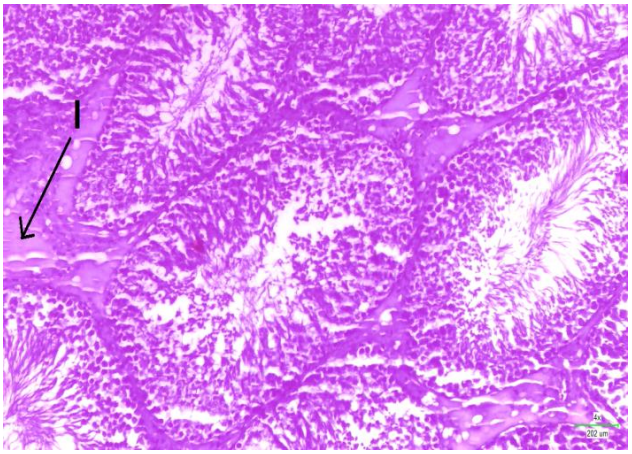

Figure 11: Section from testis of rat of Hg+F1, showing partially improvement, deformities in testis architecture, disorganization in germ cell layer, and tissue damage in interstitial tissue (I) (Stain: Haematoxylin and Eosin. 100X).

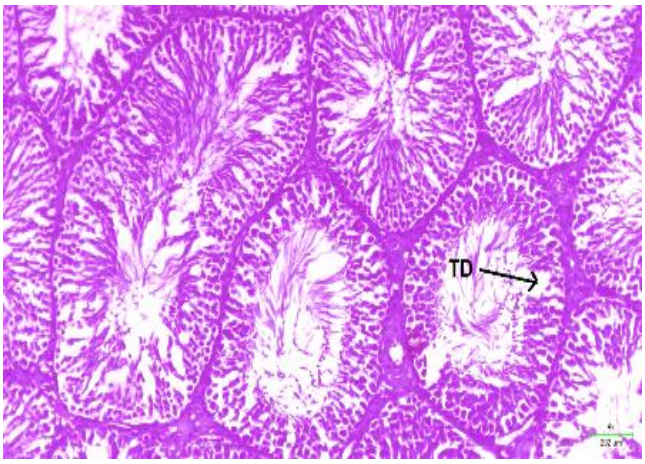

Figure 12: Section from testis of rat of Hg+F1 showing decreased tissue damage (TD), improvement in germ cell layers and interstitial tissue (Stain: Haematoxylin and Eosin. 100X).

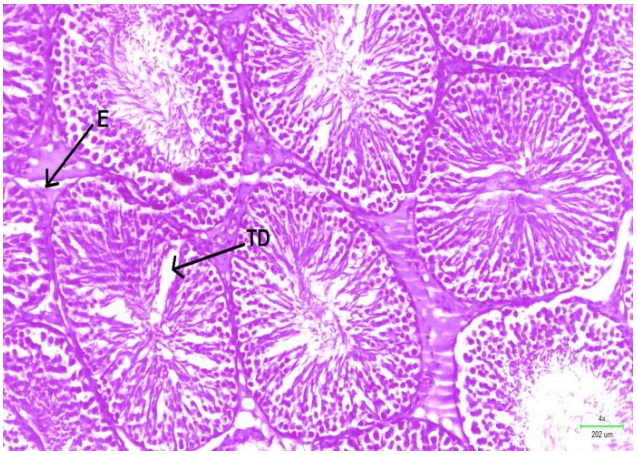

Figure 13: Section from testis of rat of Hg+F2 showing improvement in seminiferous tubules, organization in germ cell layers, decreased tissue damage (TD), increased activity of spermatogenesis, and decreased edema (E) (Stain: Haematoxylin and Eosin. 100X).

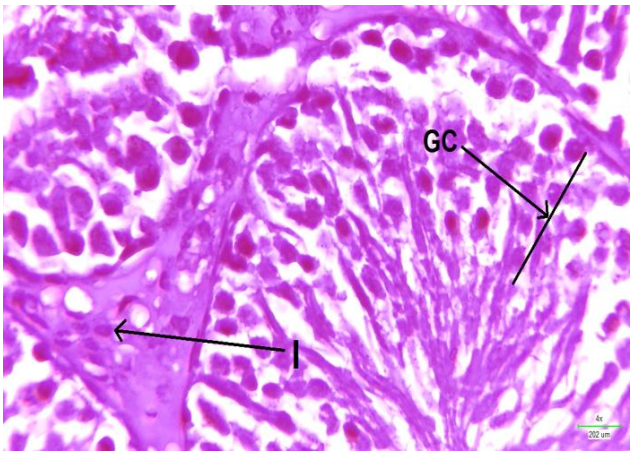

Figure 14: Section from testis of rat of Hg+F2 showing improvement in seminiferous tubules, interstitial tissue and organization in germ cell layer with active spermatogenesis (Stain: Haematoxylin and Eosin. 400X)

DISCUSSION

In the present study $HgCl_2$ caused non-significant change in the B. W. of treated rats, which is supported by (Penna et al., 2009; Rizzetti et al., 2017). First one reported that $HgCl_2$ o.1 µg/ml and 0.01µg/ml for a month had insignificant impact on rats' B.W. The second one treated the rats with $HgCl_2$ 0.07 µg/kg and 4.6 µg/kg daily by i.m for 60 days causing no change in B.W of rats. In contrast $HgCl_2$ 1 µg/kg was administered to rats for 8 weeks decreasing the B.W dramatically (Adelakun et al., 2021). This difference with the present study possibly is dependent on dose, route of administration and experimental period. In both groups (Hg+F1 and Hg+F2) fenugreek supplementation did not alter rats B.W. significantly. In literature although we couldn't find any data concern with the effect of fenugreek against mercury on B.W, however (Hamad, 2021) showed that 5% fenugreek seed powder containing diet and boiled aqueous extract of 5% fenugreek seed containing diet for two weeks caused non-significant change in B.W of female mice, this study more or less, may support the present study.

Mercuric chloride had no appreciable impact on the relative weight and absolute weight of testis and epididymis, which is supported by (Penna et al., 2009; Abarikwu et al., 2017). The first one reported that $HgCl_2$ 0.01 and 0.1 µg/ml for one month in the rat had non-significant effect on testis weight and epididymis weight. And the second one who treated rats with $Hgcl_2$ 5 mg/kg showed that the testis absolute and relative weight was unchanged. In both groups (Hg+F1 and Hg+F2) fenugreek caused non-significant change in absolute and relative weight of testis and epididymis weight. In literature we couldn't find any data concern with the effect of fenugreek against mercury on absolute and

relative weight of testis and epididymis.

Our results demonstrate that sperm count was significantly decreased when the animals treated by $Hgcl_2$, and this is agree with (Boujbiha et al., 2011) who showed considerably decreased sperm count in the rats treated with mercury in drinking water. The significant sperm count reductions were confirmed by (Heath et al., 2012; Rizzetti et al., 2017; Adelakun et al., 2021). In addition, mercury induce endocrine disruptor or generates oxidation in testicular cells (Manfo et al., 2014). Mercury exposure in animals induce disturbance in hypothalamic-pituitary axis. Leads to deficiency in spermatogenesis and steroidogenic activity (Martinez et al., 2014). Indeed in the present study, the significant increase of MDA which is indicating oxidative stress effect of $HgCl_2$, besides the markedly reduction of testosterone by $HgCl_2$, possibly negatively acting on sperm count. Meanwhile such changes in MDA and testosterone of the present investigation are reinforced by investigations of (Manfo et al., 2014; Martinez et al., 2014).

In the present study the significant decrease in normal sperm percentage and significantly increased head defect sperm and tail defect sperm in $HgCl_2$ treated rats are supported by (Rizzetti et al., 2017) who revealed that prolonged exposure to $HgCl_2$ decreased percentage of normal sperms. Whereas, increased sperm head defects and tail defects as well. It was reported that an increase in sperm defects possibly attributed to lipid peroxidation that damages the membrane (Choy et al., 2002). Furthermore, sperm cells membranes contain large amount of polyunsaturated fatty acids, they are vulnerable to oxidative stress of mercury (Kalender et al., 2013).

In the current study, rats treated with $HgCl_2$ significantly reduced sperm motility is in agreement with (Boujbiha et al., 2011) who showed considerably reduced sperm motility in rats receiving treatment with Hg in drinking water. The reduced sperm motility in the treated rats with mercury was confirmed by (Abarikwu et al., 2017; Adelakun et al., 2021). It is well known that, Hg has peroxidate damage (Henkel, 2005). It was reported that lipid peroxidation damages spermatozoa membrane which is linked to sperm motility loss (Henkel, 2005).

The significant increase in MDA in $HgCl_2$ treated rats is supported by (Abarikwu et al., 2017) who reported that MDA level increased 100.8% in Hg treated rats. In addition, the current study is also supported by (Goudarzi et al., 2017; Adelakun et al., 2021). The first one showed that, exposed rats to $HgCl_2$ increased MDA value. The second also showed, increase considerable MDA product of lipid peroxidation in response to Hg toxicity in the rat. In the literature there is no data concern with fenugreek against mercury on serum MDA level. The markedly reduced serum MDA level in both groups Hg+F1 and Hg+F2 against mercury more or less is supported by (Yacoubi et al., 2011) who showed diminished considerably MDA in rats treated with fenugreek powder supplementation and fenugreek seed polyphenol extract. The current study is further may be supported by (Shekha et al., 2014) who demonstrated that fenugreek decreased MDA level, possibly resulting in the oxidants in the rats treated with ethylene glycol consuming antioxidants.

The support for significant decrease in testosterone in $HgCl_2$ treated rats comes from (Heath et al., 2012) who demonstrated reduction testicular testosterone in rats received $HgCl_2$ by gavage. The present study also supported by (Abarikwu et al., 2017; Adelakun et al., 2021) who demonstrated that, rats received $HgCl_2$ markedly reduced plasma testosterone level. The significant decrease of testosterone in both groups Hg+F1 and Hg+F2 from control and non-significantly decreased in both groups from Hg group is supported by (Mansour et al., 2021) who described the significant drops in FSH and LH levels were accompanied by considerable drops in blood testosterone in treated rats with fenugreek seeds. In the present study, since fenugreek changed other studied parameters towards the normal value (particularly sperm parameters and spermatogenesis) except testosterone in Hg+F1 and Hg+F2. Meanwhile, fenugreek seeds contain steroids (Baset et al., 2020) and phytoestrogens(Duke, 2002). So, the reduction in testosterone level, may return to LH inhibition by fenugreek seed steroids and/or phytoestrogens instead of negative feedback effect of testosterone, and the effects of these compounds may compensate testosterone reduction.

In current study, fenugreek supplementation in both groups Hg+F1 and Hg+F2 against mercury markedly improved the above mentioned sperm parameters. However, the Hg+F2 appear more effective than Hg+F1. In our literature there is no information on how fenugreek seeds react with $HgCl_2$. Regardless the source of oxidation, However, antioxidant effect of fenugreek reduced oxidative stress as reported by (Annida and Prince, 2005). More or less, can be depended on to support the present results. So, it would be reasonable to suggest that fenugreek at least in part by its antioxidants improve sperm parameters against Hg. On the other hand, mercury significantly increased sperm abnormalities and associated with reduced testosterone level on the basis of study by (Hamden et al., 2010) who demonstrated restoration of sperm abnormalities by F(steroids) in diabetic rats, so in current study fenugreek steroids at least in part contribute to improving sperm abnormalities against mercury.

Since mercury has oxidative damage effect leading to

decreasing sperm count and motility (Adelakun et al., 2021), and fenugreek seeds aqueous extract has antioxidant potential that helps to reduce oxidative stress leading to elevation of sperm concentration and sperm motility in mice (Kaur and Sadwal, 2020). Therefore, we suggest that improvement in sperm count and motility in the current study is attributed to antioxidant effect of fenugreek supplementation. This is supported by the markedly reduced MDA level in both groups Hg+F1 and Hg+F2. On the other hand, in the current study, the increased sperm count and motility in Hg+F1 and Hg+F2 is supported by (Taha, 2011) demonstrated that fenugreek seeds supplementation caused a significant increase in spermatozoa concentration and motility and significant reduction in dead sperm in Japanese quill. It appears that Hg+F2 improved sperm parameters more than Hg+F1, but there is a limitation to fenugreek effect and higher doses have a negative effect as in an investigation 30% fenugreek in the diet reduced sperm concentration in the rabbit (Kassem et al., 2006).

In the present study, the tissue damage and deformities in seminiferous tubules and interstitial tissue in testis section of mercury treated rat is supported by (Boujbiha et al., 2011) revealed the decreased spermatozoa in the lumen of dilated seminiferous tubules, plasma-rich interstitial flow, a significant decrease and sloughing of germ cells, and the leydig cell loosen. The improvement in testis architecture and histology in Hg+F1 and Hg+F2 against mercury particularly the improvement in Hg+F2 more or less is close to the research conducted by (Sakr et al., 2012) who showed that fenugreek against Adriamycin results improvement in testis architecture, the seminiferous tubules were compact and germ cell layer relatively increased normal cells.

CONCLUSION

This study revealed that both does of fenugreek supplementation (F1 and F2) reduced toxicity of mercury on sperm parameters (sperm count, sperm morphology, and sperm motility) and testis histology, but generally F2 was more effective than F1. Possibly the results are due to the protective effect of fenugreek antioxidants to reduce oxidative effect of mercury, which is supported by changes occurred in serum MDA level. Besides that, steroids and/or phytoestrogens in fenugreek may be through inhibition of LH secretion reduced testosterone secretion and may compensate testosterone functions on sperm parameters and testis histology.

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