



Toxicopathologic Effect of Paraquat (PQ) on Adult Albino Male Testis against Efficiency of olive oil

^aDuha A. Hussien, ^aZainab Jamal Mohammed Jawad and ^aZainab Ismail Ibrahim

^aDepartment of Pathology and Poultry Diseases/ College of Veterinary Medicine /
University of Baghdad - Iraq

*Corresponding Author: ^azainab0jamal@yahoo.com

^azainabalrubaei@yahoo.com

Abstract: This study aimed to investigate the effects of PQ and antioxidant (olive oil) against induced oxidative stress in rats. Male rats (n=48) were randomly divided into 4 groups which administrated orally and daily for six weeks as follows: T1: 3mg /Kg B.wt PQ only, T2: 3mg /Kg B.wt PQ & 2ml /Kg B.wt Olive oil Kg B.wt. T3: 2ml/Kg B.wt Olive oil and T4: distilled water as control group, respectively. After 2, 4 and 6 weeks the blood samples were collected for estimation the testosterone hormone levels and Semen samples for Sperms activity. Three animals from each group sacrificed for the histological study of testis. results showed significant increase of Testosterone level in T3 at 2 and 4 weeks (15.05 ± 0.34) (215.65 ± 3.88) respectively, and there was significant increase of total sperms count (8.5 ± 0.22) and sperms motility (95.52 ± 0.84), decrease of sperm Abnormalities (3.19 ± 0.11) and sperms immotility (37.55 ± 0.44) in T3 as compared with control while a significant decrease of sperms total count (6.37 ± 0.26) and Sperms Motility (85.05 ± 1.21) in T1 and a significant decrease of Sperms total count (6.56 ± 0.22) and Sperms Motility (68.24 ± 1.39) in T2 and also The results showed a significant increase of Sperms Abnormalities (4.12 ± 0.23) and Sperms Immotility (43.82 ± 0.56) in T1 and also The results showed a significant increase of Sperms Abnormalities (5.10 ± 0.11) and Sperms Immotility (57.60 ± 2.33) T2 as compared with control group. The histological sections revealed significant lesions in testes. at (T1) group Testes showed deficiency of spermatogenesis summarized by necrosis of spermatocytes, necrotic debris with infiltration of inflammatory cells in some seminiferous tubules, while the epididymis showed vacuolar degeneration of tubules appear, desquamation of epithelia lining of tubules, lumen filled with debris degenerated cells, sloughed microcilia and



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inflammatory cells infiltration .At vas deference sloughing of the mucosal epithelial lining and stereocilia with congestion of blood vessels and infiltration of inflammatory cells mainly lymphocytes and macrophages were seen .In the other hands, (T2) group notice the testes loss spermatocytes left only with base spermatic cells in the lumen of seminiferous, mild thickening of the testicular capsule due to congestion of blood vessels and edema in the interstitial stroma between tubules, sever destruction of spermatogenesis (germ layer of seminiferous tubules)infiltrated with inflammatory cells, atrophied and shrinkage of tubules other rat show disaggregated and moving away from each other between the tubules, many atrophic necrotic seminiferous tubules replaced with hyalinized eosinophiolic material filled their lumen, few of them appeared calcified (blue-purple in color). germinal cells necrosis, especially in spermatogonia and Leydig cells had an abnormal fibroblast-like appearance It was concluded the paraquat effect on male reproductive system reveled from Sperms activity ,Testosterone concentration, Sperms total account, Sperms Abnormalities, Sperms Motility, Sperms Immotility and histopathological changes of different male reproductive organs including possibilities of having chronic testicular destruction which may lead to male infertility.While, daily consumption of Olive oil can decrease stress oxidative and alteration spermatogenesis in albino rats.

Keywords: Paraquat, olive oil, histopathology, rat.

1. Introduction: Paraquat is a toxic chemical that is commonly used as an herbicide (plant killer), mainly for weed and grass control. In the United States, paraquat is presented primarily as a liquid in several strengths. The US Environmental Defense Agency classifies paraquat as “restricted use.”2014. Exposure to paraquate causes corrosive effects to tissue, including the skin and gastrointestinal tract (Jones and Vale, 2000). Systemic toxicity, counting kidney failure and central nervous system toxicity, is usually related with diquat ingestion. Unlike paraquat, diquat is not selectively concentrated in the lung (Rose and Smith, 1977) and is not known to directly cause pulmonary fibrosis (Vanholder *et al.*, 1981; Jones and Vale, 2000) Essential employments of paraquat incorporate weed control in plantations, estate yields, and woods; weed control before sowing or before trim development; field remodel; preharvest drying up; and amphibian weed control, despite the fact that utilization as a sea-going herbicide in the United States isn't allowed (Parsa, 2018). Paraquat, an operator exceedingly lethal to people and creatures, is a broadly utilized herbicide and furthermore generally utilized by individuals endeavoring suicide in Taiwan. Paraquat is exceptionally lethal and causes hindrance of a



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few indispensable organs, (for example, the liver, kidney, and lung) and all harmed people bite the dust due to multiorgan disappointment, including significant metabolic acidosis, melancholy of myocardial or breath work because of resulting pneumonic fibrosis, and renal or hepatic disappointment (Chen *et al.*, 2018).

Spermatogenesis is a procedure of germ cell multiplication and separation which prompts produce spermatozoa from testis. In addition, the major change spermatozoa are the reduction of DNA material from diploid to haploid via a series of mitotic and meiotic divisions; It is a delicate process and highly sensitive to toxic damages (Mirhoseini *et al.*, 2012; AshokaDeepananda and De Silva, 2013). Moreover, the incitratecontrol and cell associations that happen in the testis give numerous unmistakable focuses by which toxicants can disturb spermatogenesis (Boekelheide *et al.*, 2000). The effects of toxic chemical exposures on the male reproductive system have been demonstrated by using in vivo model testing. A previous study suggested that free radicals who generated by PQ, are highly vulnerable to sperm membrane and mammalian epididymis, subsequently, leading to reduce sperm density (AshokaDeepananda and De Silva, 2013). Therefore, this study was designed to investigate the effects of Paraquat on male reproductive system.

Also we utilized a fluid fat got from olives (the product of Oleaeuropaea; family Oleaceae), a customary tree yield of the Mediterranean Basin. The oil is delivered by squeezing entire olives. It is ordinarily utilized in cooking, regardless of whether for singing or as a serving of mixed greens dressing. It is likewise utilized in beautifying agents, pharmaceuticals, and cleansers, and as a fuel for conventional oil lights, and has extra uses in a few religions. There is restricted confirmation of its conceivable medical advantages. The olive is one of three center nourishment plants in Mediterranean cooking(Howard, 2017).olive oil is a strong antioxidant effect, its Hydrophilic phenols are the most abundant natural antioxidants protecting against damage from free radicals and against the formation of cancer.(Servili *et al.*,2009). This study aims to investigate the Toxicopathology effect of Paraquat administration and olive oil as antioxidant on the adult male reproductive system rats by performed the following parameters:

2-Materials and Methods:



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2.1 Preparation of Paraquat (PQ) doses: Three mg PQ was dissolved in 100ml distilled water to prepare stock solution and prepare other doses. The doses were administered daily to male rats using gastric intubation (Chen *et al.*, 2017).

2.2 Preparation of olive oil doses: The olive oil dosage was calculated by the following equation:

$$\frac{V^1}{W^1} = \frac{V^2}{W^2} = \frac{2 \text{ ml}}{1000 \text{ g}} = \frac{V^2}{100 \text{ g}} = V^2 = \frac{200 \text{ ml/g}}{1000 \text{ g}} = 0.2 \text{ ml injected dose}$$

The doses were administered daily to male rats using gastric intubation (Banihani, 2017).

2.3 Blood collection: blood samples at different intervals of the experiment were collected via cardiac puncture by using disposable medical syringes (5ml). Blood from each rat was kept in disposable tubes which held for not more than four hours before serum isolation. Samples were centrifuged at a speed of 2500 rpm for 15 minutes and then serum samples were stored in freezer at -18°C until used for biochemical test (MDA and Glutathione).

2.4 Experimental design: A total number of forty eight (48) male Albino wistar rats weighting (180-220 g) were used in this experiment. Their ages ranged between (2.5-3.5) months. Experimental animals were housed in plastic cages at (22-25°C) in the animal house of department of Physiology and Pharmacology / College of Medicine -University of Maysan, with controlled lightening and the air of room was changed continuously by using ventilation vacuum. They were left for two weeks for acclimatization with the experimental conditions. Animals had free access to water and standard pellet diet along the experimental period (Hafez, 1970). Forty eight adult male rats were used in this experiment. After acclimatization for two weeks they were divided equally into four groups as follows:

Group one control (C): This group received distilled water daily for 6 weeks

Group two (T1): This group received (3mg & 2ml/Kg B.wt) PQ & Olive oil daily for 6 weeks.

Group three (T2): This group received (2ml/Kg B.wt) Olive oil daily for 6 weeks.

Control Four (T3): This group received (3mg/Kg B.wt) PQ daily for 6 weeks.



The experiment was lasted for 6 weeks. Blood samples were collected after 2, 4, 6 weeks of the experiment for Testosterone concentrations. During of the experiment, three animals from each group were anesthetized and killed for the histological study of testis.

2.5 Statistical analysis: Statistical analysis of data was performed using SAS (Statistical Analysis System - version 9.1). Two ways ANOVA and Least significant differences (LSD) post hoc test were performed to assess significant differences among means. The results were expressed as mean \pm standard errors (SE) and $P < 0.05$ is considered statistically significant (SAS, 2010):.

3. Results:

3.1 Histopathologic examination:

3.1.1 T1 group (paraquat and olive oil): the histopathological lesion of testis at week 2 revealed hyperchromatic germinal layer of spermatogonia, atrophy and necrosis of some seminiferous tubules with congestion of blood vessels, mild infiltration of inflammatory cells and subcapsular odema (figure-1,2), while Some seminiferous tubules showed infiltration of inflammatory cells mainly (macrophage and lymphocytes) with in interstitial stroma. Degeneration and depletion of elongating spermatid hyperchromatic germinal layer of spermatogonia, abnormal space between neighboring sertoli cells (figure-5,6) at week4.results showed week 6, size reduction and shape changes of the seminiferous tubules that affect the smoothness of spermatogenesis with hyalinized necrotic tubule , interstitial inflammatory cells mainly neutrophils and lymphocytes (figure-9) .

3.1.2 T2 (paraquat only): week2 notice the testes infiltration of inflammatory cells, edematous fluid between seminiferous tubules also there was vacuolation of spermatogonia with loss of spermatocytes left only of base spermatic cells in the lumen of seminiferous mild thickening of the testicular capsule due to congestion of blood vessels and edema in the interstitial between tubules, necrosis of tubules. (Figure -12),Notice at week4 there was sever destruction of spermatogenesis (germ layer of seminiferous tubules) necrosis with infiltration of inflammatory cells ,congested blood vessels, atrophied and shrinkage of tubules . Disaggregated and moving away from each other between the tubules (figure-15, 16).many atrophic necrotic seminiferous tubules at week6 replaced with hyalinized eosinophilic material filled their lumen, few of them appeared calcified (blue-purple in color).



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Depletion of germ cells, germinal cells necrosis, especially in spermatogonia and Leydig cells had an abnormal fibroblast-like appearance (figure-20,21).

3.1.3 T3 (olive oil only): Testes showed week 2 Hyperchromatic cells (germ cells) increase spermatocytes in lumen of tubules with congestion of blood vessels (figure-25) while seminiferous tubules at week 4 showed hyperspermatogenesis characterized by increase the sperm amount, hyperchromatic germ cells at the basal of these tubules seen, with interstitial odematous fluid, thickening of fibrous capsule, while subcapsular blood vessels appeared hyperatrophy and filled with odematous fluid (figure -28). week 6 marked thickening of capsule the tubules showed mild to moderate hyperspermatogenesis. with congestion of blood vessels (figure-31).

4. Sperms activity:

4.1 Testosterone concentration: Serum Testosterone concentration in control and treated groups are represented in table (4.4). The concentration of Testosterone shows a significant increase ($P > 0.05$) in T1 and T3 in comparison to control group at 2nd and 6th weeks, while group (T2) shows a significant decrease ($P > 0.05$) in Testosterone concentration comparing with control after sixth weeks of administration of Paraquat and olive oil, Paraquat only and olive oil only. At the meantime, the highest concentration of Testosterone is shown in (T3) after sixth weeks of administration of Olive oil as compared to other groups. Within the time, the concentration of Testosterone reveal no significant difference ($P > 0.05$) between the second, fourth and sixth weeks in T2 group. However, this difference is significant in T1, T3 and control. Also the table showed significant increase ($P > 0.05$) in Testosterone level after the 6th week in comparison to the 2nd and 4th week in T1 and T3.

Table (4.4): Serum Testosterone level (pg/ml) in response to oral administration of T1 Paraquat & olive oil, T2 Paraquat only and T3 olive oil only. (at Paraquat 3 mg/Kg B.wt. and olive oil 2 ml/Kg B.wt. in adult male rats for six weeks.)

Group Week	C Control	T 1	T 2	T 3
2	131.37±1.61	132.94±1.49	121.19±2.09	141.35±1.06



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	C b	C b	A c	C a
4	178.98±4.05 A a	143.60±2.16 B c	120.49±0.86 A d	154.47±1.39 B b
6	148.90±0.63 B c	172.65±4.65 A b	113.70±3.67 A d	215.65±3.88 A a

Mean ± SE (n=12 rats/ group). LSD: 7.5201

Capital letters indicate a significant (P> 0.05) difference within group.

Small letters denote a significant (P> 0.05) difference between groups

4.1.2 Sperms total account: The effect of different doses of Paraquat and olive oil, Paraquat only and olive oil only on mean values of Sperms total account is shown in table (4.5). This table shows a significant decrease (P > 0.05) in Sperms total account in treated groups (T1) and (T2) along the experimental periods comparing with control group. At the meantime, the highest Sperms total account is shown in (T3) as compared to other groups. Within the time, the Sperms total account reveal no significant difference (P> 0.05) between the second, fourth and sixth weeks in control, T1 and T2 groups. However, this difference is significant in T3. The table showed significant increase (P> 0.05) in the Sperms total account after the 6th week in comparison to the 2nd and 4th week in T3.

Table (4.5): Sperms total account in response to oral administration of T1 Paraquat+ Olive oil, T2 Paraquat only and T3 Olive oil only at Paraquat 3 mg/Kg B.wt. and Olive oil 2 ml/Kg B.wt. in adult male rats for six weeks.

Group Week	C Control	T 1	T 2	T 3
2	7.45±0.33 B a	6.37±0.26 A b	7.16±0.07 A a	7.26±0.33 B a



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4	8.05±0.16 AB a	7.03±0.06 A bc	6.56±0.22 A c	7.57±0.23 B ab
6	8.40±0.30 A a	6.84±0.05 A b	6.80±0.22 A b	8.52±0.22 A a

Mean ± SE (n=12 rats/ group). LSD: 0.6672

Capital letters indicate a significant (P> 0.05) difference within group.

Small letters denote a significant (P> 0.05) difference between groups.

4.1.3 Sperms Abnormalities: The effect of different doses of Paraquat and olive oil, Paraquat only and olive oil only on mean values of Sperms Abnormalities is shown in table (4.6). This table shows a significant increase (P > 0.05) in Sperms Abnormalities in treated groups (T1) and (T2) along the experimental periods comparing with control group While group (T3) treated with olive oil only shows a significant decrease (P > 0.05) in Sperms Abnormalities comparing with control group. At the meantime, the highest Sperms Abnormalities is shown in (T2) as compared to other groups.

Within the time, the Sperms Abnormalities reveal no significant difference (P> 0.05) between the second, fourth and sixth weeks in control, T1 and T3 groups. However, this difference is significant in T2. The table showed significant decrease (P> 0.05) in Sperms Abnormalities the 4th week and 6th week in comparison to the 2nd and in T3.

Table (4.6): Sperms Abnormalities in response to oral administration of T1 Paraquat and Olive oil, T2 Paraquat only and T3 Olive oil only (at Paraquat 3 mg/Kg B.wt. and Olive oil 2 ml/Kg B.wt. in adult male rats for six weeks).

Group	C	T 1	T 2	T 3
Control				
Week				



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2	3.57±0.26 A b	3.96±0.10 A a	4.95±0.05 A a	5.09±0.01 A a
4	3.89±0.03 A b	4.12±0.23 A b	5.10±0.11 A a	3.19±0.11 B c
6	4.01±0.10 A b	3.75±0.13 A b	5.09±0.33 A a	3.03±0.09 B c

Mean ± SE (n=12 rats/ group). LSD: 0.4729

Capital letters indicate a significant (P > 0.05) difference within group.

Small letters denote a significant (P > 0.05) difference between groups.

4.1.4 Sperms Motility: The effect of different doses of Paraquat and olive oil, Paraquat only and olive oil only on mean values of Sperms Motility is shown in table (4.7). This table shows a significant decrease (P > 0.05) in Sperms Motility in treated groups (T1) and (T2) along the experimental periods comparing with control. While group (T3) treated with olive oil only shows a significant increase (P > 0.05) in Sperms Motility comparing with control. At the meantime, the highest Sperms Motility is shown in (T3) as compared to other groups.

Within the time, the Sperms Motility reveal no significant difference (P > 0.05) between the second, fourth and sixth weeks in control, T3 groups. However, this difference is significant in T1 and T2.

Table (4.7): Sperms Motility in response to oral administration of T1 Paraquat & Olive oil, T2 Paraquat only and T3 Olive oil only (at Paraquat 3 mg/Kg B.wt. and Olive oil 2 mg/Kg B.wt. in adult male rats for six weeks).

Group	C	T 1	T 2	T 3
Control				
Week				



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2	94.27±1.64 A a	85.05±1.21 A b	74.56±2.97 A c	92.31±1.02 A a
4	91.62±1.31 A a	79.28±2.17 B b	68.38±1.40 B c	95.43±1.60 A a
6	95.68±1.61 A a	78.44±1.14 B b	68.24±1.39 B c	95.52±0.84 A a

Mean ± SE (n=12 rats/ group). LSD: 4.7466

Capital letters indicate a significant ($P > 0.05$) difference within group.

Small letters denote a significant ($P > 0.05$) difference between groups.

4.1.5 Sperms Immotility: The effect of different doses of Paraquat and olive oil, Paraquat only and olive oil only on mean values of Sperms Immotility is shown in table (4.8). This table shows a significant increase ($P > 0.05$) in Sperms Immotility in treated groups (T1) and (T2) along the experimental periods comparing with control While group (T3) treated with olive oil only shows a significant decrease ($P > 0.05$) in Sperms Immotility comparing with control. At the meantime, the highest concentration of Sperm Immotility in (T2) as compared to other groups. Within the time, the Sperms Immotility reveal no significant difference ($P > 0.05$) between the second, fourth and sixth weeks in control, T1 and T3 groups. However, this difference is significant in T2. The table showed significant increase ($P > 0.05$) in Sperms Immotility after the 4th week in comparison to the 2nd and 6th week in T2.

Table (4.8): Sperms Immotility in response to oral administration of T1 Paraquat & Olive oil, T2 Paraquat only and T3 Olive oil only. (at Paraquat 3 mg/Kg B.wt. and Olive oil 2 ml/Kg B.wt. in adult male rats for six weeks).

Group Week	C Control	T 1	T 2	T 3
2	39.18±0.76 A a	41.60±1.88 A a	41.95±0.83 C a	40.35±1.47 A a

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4	37.44±0.78 A c	43.82±0.56 A b	57.60±2.33 A a	39.19±1.01 A c
6	39.74±0.28 A c	42.80±2.21 A b	48.15±1.19 B a	37.55±0.44 A c

Mean ± SE (n=12 rats/ group). LSD: 3.8618

Capital letters indicate a significant ($P > 0.05$) difference within group.

Small letters denote a significant ($P > 0.05$) difference between groups.



Figure- 1: Histopathologic section of rat testes (T1W2), showed subcapsular edema congestion of blood vessels (arrow) with mild degeneration of spermatogenic cells in seminiferous tubules (H&E stain, 200X).

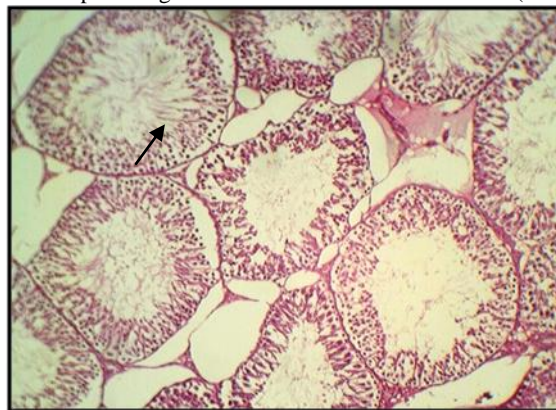


Figure-2: Histopathologic section of rat testes (T1W4), showed Degeneration and depletion of elongating spermatid with empty spaces between tubules with interlobular edema and moderate reduction of Leydig cells (arrow) (H&E stain, 100X).

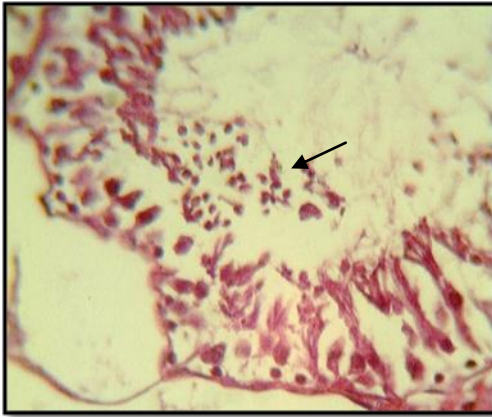


Figure-3: Histopathologic section of rat testes (T1W4) showed complete loss of spermatocytes lining that sloughed in to the lumen with inflammatory cells (arrow) inside the lumen of seminiferous tubules. (H&E stain, 400X).

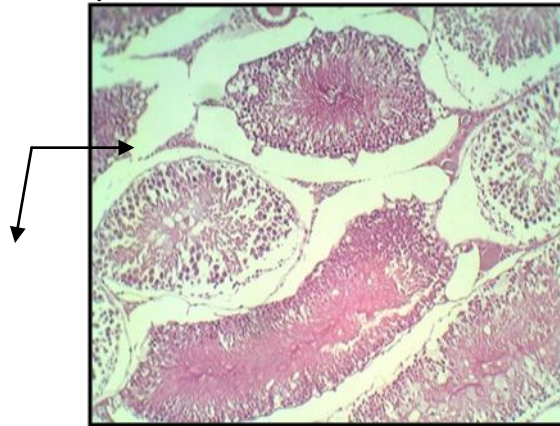
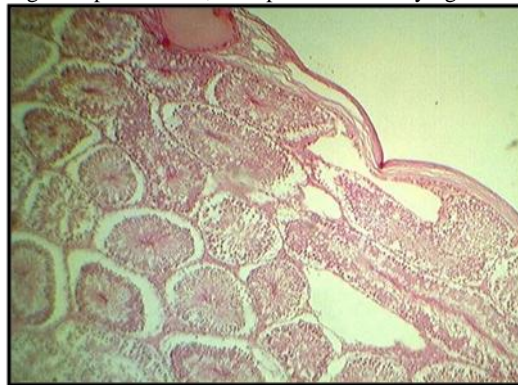


Figure-4: Histopathologic section of rat testes (T1W6) showed tubules at different spermatogenic stage (arrow) with appearance number of elongated spermatozoa, with prominent of leydig cells (H&E stain, 100X).



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Figure-5: Histopathologic section of rat testes (T2W4), showed severe destruction of spermatocytes with infiltration of inflammatory cells and moderate decrease in leydig cells, (H&E stain, 100X).



Figure-6: Histopathologic section of rat testes (T2W4), showed severe necrosis of tubules with infiltration of inflammatory cells, congested blood vessels, disaggregated and moving away from each other between the tubules (arrow), (H&E stain, 100X).

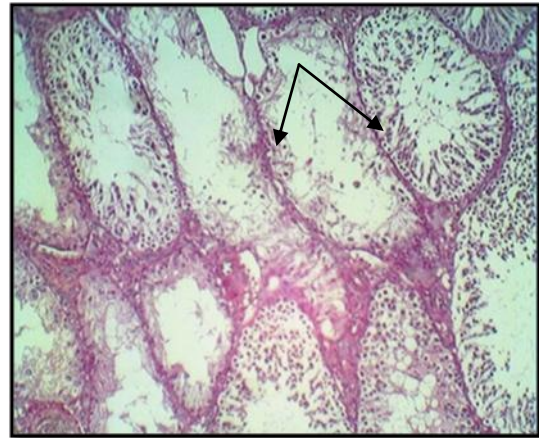


Figure-7: Histopathologic section of rat testes (T2W6), showed sever necrosis of seminiferous tubules epithelia (arrow) with marked reduction of spermatozoa surrounded by slight prominence of leydig cells, (H&E stain, 20X).



Figure-8: Histopathologic section of rat testes (T2W6), showed marked disruption of seminiferous tubules with appearance necrotic calcified mass (arrow) in some tubules (blue-purple in color) and interstitial fibrous connective tissue, (H&E stain, 100X).

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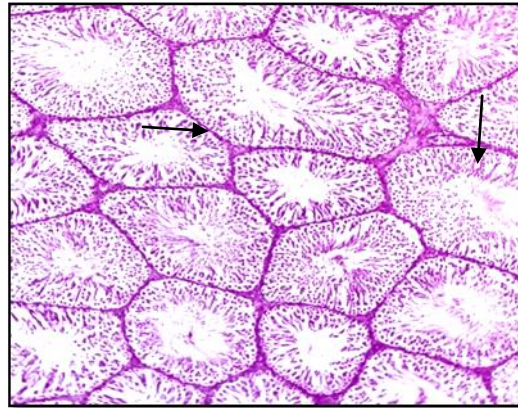


Figure-9: Histopathologic section of rat testes T3 W4, showed congestion of blood vessels, vacuolar degeneration in interstitium (arrow),vacuolar degeneration (H&E 20X).

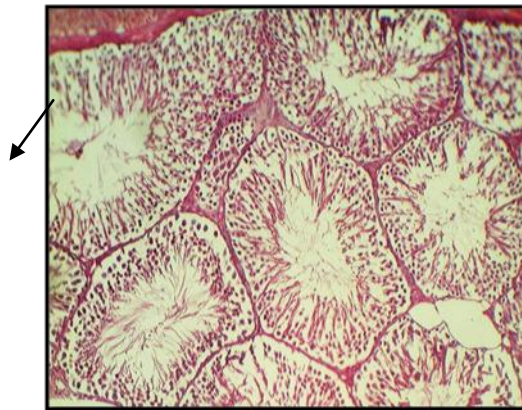


Figure-10: Histopathologic section of rat testes (T3W6), showed thickening of capsule with normal structure of seminiferous tubules normal interstitial leydig cells (arrow),(H&E stain, 100X).

5. Discussion:

5.1: Gross pathology: Gross pathological observation of most of the paraquat treatment group (T1 & T2) rats was showed debilitation and pale carcasses. Lungs and kidneys from all treatment group (T1 & T2) rats showed mild congestion and emphysema. Round flabby heart with pin-point hemorrhages and congestion and pin-point hemorrhages of liver was noticed and Congestion and dark discoloration of spleen in all the treatment group (T1 & T2) rats. These findings were similar to the observations of (Soloukides *et al.*, 2007 and Tamuli *et al.*, 2007) who reported congestion, hemorrhages and emphysema in lungs and spleen.



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5.2 Microscopically changes: T1 group (paraquat and olive oil): the histopathological lesion of testes at week 2 revealed hyperchromatic germinal layer of spermatogonia, atrophy and necrosis of some seminiferous tubules with congestion of blood vessels, mild infiltration of inflammatory cells with subcapsulated odema at week 4, results showed week 6, size reduction and shape changes of the seminiferous tubules that affect the smoothness of spermatogenesis with hyalinized necrotic tubule, interstitial inflammatory cells mainly neutrophils and lymphocytes.

5.3. Testosterone level: The serum level of sexual hormones was known as very useful in evaluation of the fertility of human and animals (Dixon, 1993). In general, a considerable decrease in density of sexual hormones in fertility activities leads to fertility disorders in people who expose to chemical substances (Bjore *et al.*, 1993). In these studies, being exposed to Paraquat causes the anagram of testes structure and considerable changes in Testosterone in rats, some results have been reported in some researches done by (Anuar, 2007) in which the decrease of Testosterone is because of damage in testes. Studies indicate that oxidative stress plays an important role in pathogens of different disease such as cancer, diabetes, vascular and heart disease, Parkinson, Schizophrenia, Atherosclerosis, pulmonary disease and Cataract (Scheffler, 2000). Oxidative stress is because of free radicals and mitochondria are known as the basic production place for free radicals (Cadnes *et al.*, 2000). Experiments with disrupted mitochondria showed that once in the matrix, paraquat reduced by complex I in mammals forms superoxide (Cocheme *et al.*, 2008).

Conclusion: Paraquat had decreased function of male reproductive system from sperms activity, testosterone concentrations; sperm total count, sperms motility and sperms abnormalities. Also the pathological changes illustrated in tissues of testes.

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