Ameliorative effect of Quercetin and Hesperidin on Antioxidant and Histological Changes in the Testis of Etoposide-Induced Adult Male Rats

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Abstract :

Aim of study investigate the protective potential of Quercetin and/or Hesperidin against Etoposide (ETO)-induced testicular toxicity in male rats.

Material and method : Twenty five adult male rats were divided into five groups. Control group (C) : was given orally Distilled water, The first group (G1): was treated with (20) mg / kg bw of Etoposide , Second group (G2) : received oral gavage (20) mg / kg of Etoposide, then given 20 mg / kg of Quercetin, Three group (G3): was given with (20) mg/ kg Etoposide with Hesperidin at dose 25 mg / kg bw, Four treatment group (G4): was given Etoposide with Quercetin and Hesperidin at same doses previous .

Result : Etoposide treatment caused significant a decrease ($P<0.05$) in body weight , testis weight and epididymis and also a decrease ($P<0.05$) enzymatic (SOD, CAT, GSH) and increased MDA induced by ETO, The histological changes resulted there was necrosis and degeneration in seminiferous tubule , presence of congestion in blood vessels between seminiferous tubules , the vacuolation and disintegration of Spermatogonia , a significant reduction in seminiferous tubule diameter and height of Epithelial layer , thickness of epithelial layer and diameter of tubules in epididymis , also in sperms stored in the epididymis with impaired spermatogenesis, as well as decrease in number of Leydig cells and Sertoli cells . In contrast, QE and/ or HES treatments significantly attenuated the harmful effects induced by ETO .

Conclusion :This suggests that Quercetin and/or Hesperidin may be a potential therapeutic against ETO induced testicular toxicity by restoring normal spermatogenesis, testicular and epididymal structural , antioxidant levels and inhibition of Lipid peroxidase (MDA) .

Introduction :

Oxidation is a chemical reaction that removals electrons from a substance to the oxidizing agent, and cause cells damage by produce free radicals , The important mechanisms of free radicals by restrict the cellular functions by lipid peroxidation which in turn causes damage to proteins, lipids and nucleic acids leading to the cell death (1) . Lipid peroxidation levels were used as marker for oxidative damage in cells and tissues , Lipid peroxidation is analyzed in biological samples by measuring the quantity of Malondialdehyde, To prevent damage from ROS, cells possess different antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GSH-PX) and catalase (CAT) (2) . The alterations in the testis, kidney and liver functions induced by anticancer drugs are closely associated with formation of reactive oxygen species (ROS) in the tissues

(3) ,(4). Etoposide (ETO) is one of the most commonly used agents in cancer chemotherapy for the treatment of leukaemia and many solid tumor's including testicular and ovarian cancers, which are common in children and young adults in their reproductive years (5). ETO is a chemotherapeutic agent that interacts with topoisomerase II, a nuclear enzyme that is essential for DNA replication, transcription, recombination and chromosomal segregation. ETO induces cell death by blocking top II catalytic function leading to high levels of DNA strand breaks (5). Although ETO is effective in the treatment of cancer, it does not act solely on cancer cells; it also causes the death of normal proliferating cells, including the male germ cells. In the testis, However, it can also act on premeiotic DNA synthesis and kills primary spermatocytes (6).Other studies have demonstrated that the administration of etoposide during the prepubertal phase causes the reduction of several testicular morphometric parameters (7), (8) and Sertoli cell alterations (9).

The use of herbal medicines has recently gained popularity in Europe and the United States. Citrus fruits, such as oranges and vegetables such as onion , these plants contains compounds called phytochemicals that can be included flavonoids are polyphenolic secondary metabolites that are abundantly present in vegetables, fruits and beverages (10). In particular, flavonoids are widely recognized as naturally occurring antioxidants that inhibit lipid peroxidation in biological membranes (11). Also flavonoids have been found to exhibit anti-carcinogenic and anti-inflammatory properties (12) . Hesperidin is a flavanone glycoside, abundantly found in lemon and oranges, HES is also known as hesperidin 7-rhamnoglucoside, its exhibits various biological and pharmacological properties such as anti-inflammatory, analgesic, anti-fungal, anti-viral and anti-cancer properties and protect against ischemia-reperfusion tissue damage (13). HES has also been found to inhibit cell cycle progression in human pancreatic cells and to contribute to the intracellular anti-oxidant defense system by acting as an agent against superoxide, singlet oxygen and hydroxyl radicals (14) . Quercetin bio-pharmacological properties may offer promising options for the development of more effective chemo-preventive and chemotherapeutic strategies because of its powerful antioxidant and free-radical scavenging properties (15) It has been shown that quercetin has highly potent antioxidant and cyto-protective effects in preventing apoptosis and oxidative damage induced by cadmium in both in vivo and in vitro studies.Quercetin's antioxidant and physicochemical properties enable it to counteract the damaging effects of oxidation caused by ROS or other types of free radicals in many types of tissues and cells ,which contributes to the genesis of atherosclerosis, diabetes, ischemic heart disease, heart failure, and hypertension (16) Quercetin has exhibited anticancer potential against a wide range of cancers , also been shown to enhance the anti-cancer effects of several chemotherapeutic drugs (17).

The aim of the current study is to evaluate the effect of QR and/or HES on the reproductive system, an potential of flavonoids in the improvement of testis , epididymis architecture and antioxidant level impairment associated with Etoposide -induced Oxidative stress in male rats.

1. Material and Method :

2.1 Animals

Adult male rats (185-200 g) , these animals were obtained from Veterinary College , Al-Qadisiyah University , Iraq . All animals were kept under uniform and controlled conditions of temperature and light/dark (12/12 h) cycles, fed with standard rodent diet and water ad libitum. The animals were allowed to acclimatize to the laboratory condition for one week before commencement of the experiment.

2.2 Chemicals

Quercetin (QE) and Hesperidin (HES) powder were purchased from (Sigma–Aldrich Chemical Co., USA) , Etoposide (ETO) was purchased from NIPON KAY AKU CO . LTD (TOKYO 100-0005. JAPAN). The doses of QE , HES and ETO used in the present study were selected based on our preliminary experiments and in accordance with previous reports (9 , 13, 18)

2.3 Experimental Design

Twenty-five sexually mature male Wistar rats (185-200 g b. wt) were randomly divided into four groups (n=5). Rats of the **Control group** (**C: D.W**) :received (1ml) of the distiller water and kept as normal control, **First group (G1: ETO)**: was treated with single orally of ETO at a dose of 20 mg/kg per day for 60 days. In addition, the **^{2}nd group (G2:ETO + QE)** was given ETO (20 mg/kg), then QE (20 mg/kg.B.W) for 60 days, while the **^{3}rd group (G3: ETO+HES)** was given orally ETO and HES at doses of (20 , 25 mg/kg.B.W) for 60 days , while **^{4}th group (G4: ETO+QE+HES)** received ETO , QE , HES at same doses at same doses previous for 60 days. Twenty-five hours after last dose, the rats were weighed and sacrificed under light ether anesthesia.

2.4 Assessments of antioxidant and peroxidation

2.4.1 Assessment of SOD concentration

were assessed by using the modified photochemical Nitrobluetetrazolium (NBT) method in utilizing sodium cyanide as peroxidase inhibitor (19).

2.4.2 Assessment of Glutathione concentration

by the method of Carlberg and Mannervik (20).

2.4.3 Assessment of CAT activity

Was measured according to Aebi (21) .

2.4.4 Assessment of lipid peroxidation

The level of peroxidation product; Malondialdehyde (MDA) was measured according to Guidet and Shah, (22) .

2.4.5 Histomorphometricl measurements

Numbers of primary , secondary spermatocytes , leydig's and sertoli cells calculated according to the Al-wachi *et al.*, (23)

2.4.5 Histopathological examination:

Testes and Epididymis were fixed in 10% solution formalin, embedded in paraffin wax and sectioned at measure 3 µm . Sections were then stained with Hematoxylin and Eosin (H&E) stain and set in slides for light microscopic examinations .

1. Result

3.1 Body weight

In Table (1) showed a significant decrease ($P < 0.05$) in the body weight gain rate in the group of animals treated with Etoposide (T1) at dose (20 mg / kg bw) as compared with the control group and other treated group , While QR or / and Hesperidin treated groups (T2, T3, T4) showed a significant increase ($P < 0.05$) in the weight gain as compared with Etoposide group.

3.2 Weights of Reproductive organ

Table (1) shows that ETO (T1) caused significant decrease in reproductive organ when compared with the control and treated groups others , Administration of QE and HES / or their combination prevented testicular and epididymis weights decrease as observed in rats treated with ETO alone .

3.3 Assessments of antioxidant and peroxidation

3.3.1 MDA concentration

MDA concentration was significantly ($P < 0.05$) increased in the serum of rats treated with Etoposide at dose (20 mg / kg bw). In the rats treated with Quercetin , hesperidin and/ or their mixture on MDA level was significantly ($p < 0.05$) decreased compared with animals administered Etoposide alone .

3.3.2 Glutathione Level :

Etoposide treatment caused a significant decrease in the serum Glutathione level in the Etoposide group (ETO) compared with the control and other groups , QE and HES / or their Synergism

produced a potential increase in Glutathione level in experimental groups compared with Etoposide groups .

3.3.3 SOD and catalase activities :

ETO also induced significant reduction in SOD and catalase activities when compared to control group and other groups, Rats pre-treated with QE and HES / or their Synergism led to a significant increase in SOD and catalase levels when compared to the ETO-treated rats .

3.3.4 Measurement histological :

Led administration of Etoposide caused significant reduction in diameter of seminiferous tubules , Epithelial height of seminiferous tubules , diameter of seminiferous tubules in epididymis and thick of epithelial layer in epididymis when compared with the control and others treated rats . Pre-treated with QE and HES / or their Synergism protected against ETO-induced reduced in parameters mentioned previously and reached mean treated (**ETO + QE + HES)** a significant to control group.

3.4 Number of Spermatogenic cell , Leydig cells and sertoli cells

The volume density of spermatogonia, spermatocytes , spermatids , Leydig cells and sertoli cells decreased significantly ($p<0.05$) in rats Etoposide treated as compared with control group and other groups , While led administration of QE and HES / or their combination improved significant observed in (of spermatogonia, spermatocytes , spermatids , Leydig cells and sertoli cells) , where treated group of combination (**ETO + QE + HES)** to reach the normal value obtained from control group when comparing with Etoposide- treated alone ,

3.5 Histological alterations

The histopathological examination showed satisfactory changes in the testicular and epididymis of rat treated with (20mg/kg BW) of Etoposide for 60 days, which showed complete inhibition of the spermatogenesis, As well as obvious presence necrosis and degeneration in the seminiferous tubules , and presence of intercellular debris in the lumen and decrease or none of sperm in seminiferous tubules , smallness diameter of the seminiferous tubules , and decrease number of spermatogonia , spermatocyte , Leydig cells , presence of congestion in blood vessels between seminiferous tubules , the vacuolation and disintegration of Spermatogonia (Figure 3,4) when comparing to normal section of testis in control group (Figure 1,2) . In sections of epididymis rat treated Etoposide showed epididymis tubules is empty from sperm , atrophy and degeneration with decrease of high Epithelial lining of tubules epididymis , desmoplasia proliferations in connective tissue (Figure 13,14) , when comparted to section of epididymis was taken from control group (Figure 11,12) .

Administration of QE (20 mg/kg B.W) and HES (25 mg/kg B.W) / or their combinations at same dose concurrently with (20 mg/kg BW) of Etoposide protection of testicular and epididymis tissues of the oxidative damage caused by the toxicity of the Etoposide treated, where caused regression of pathological lesions and improved of the histological structure of testis with regeneration of testicular and Epididymis tissues, in testis was observed improve of spermatogenesis and full seminiferous tubule by sperm , proliferation of leydig's cell (Figure 5,6,7,8) and approached group Synergistic (**ETO + QE + HES)** (Figure 9,10) in their structural with testicular sections taken from control group . In sections of Epididymis , showed the lumen of epididymis tubule is full of sperm, with megakaryocyte giant cells (Figure 15,16) inner lumen of epididymis tubule a few in other some and Desmoplasia proliferations (Figure 17,18)and observed an increase in high of Epithelial cells lining of the tubules , With presence Stereocilia (Figure 19,20), approached section group (**ETO + QE + HES)** of Epididymis taken from control group (Figure 11,12) **.**

Table (1): Shows the effect of Etoposide , Quercetin and Hesperidin on the body weight and reproductive organs in male rats.

Groups	***Body weight (gm)***	***Testis weight (mg)***	***Epididymis (mg)***
Control (C)	**48.38 ± 0.31 c**	**583.6 ± 0.74 c**	**231.9 ± 1.04 c**
G1: ETO	**19.73 ± 0.42 f**	**395.1 ± 1.23 f**	**167.4 ± 0.78 f**
G2: ETO + QE	**43.89 ± 0.55 de**	**571.3 ± 0.44 d**	**218.5 ± 0.87 d**
G3: ETO + HES	**44.04 ± 0.42 e**	**568.2 ± 0.44 d**	**215.3 ± 1.44 d**
G4: ETO + QE + HES	**46.53 ± 0.69 cd**	**580.3 ± 0.98 c**	**228.9 ± 1.02 c**
LSD	**2.47**	**3.29**	**3.35**

Values are expressed as Mean ± SE ; n= 5 / group.

Different small letters denote between groups differences, P< 0.05 Vs. control.

Table (2): Shows the effect of Etoposide , Quercetin and Hesperidin on the Antioxidant and lipid peroxidation in male rats.

Group	*SOD activity (U/ml)*	*Glutathione (µmol/L)*	*Catalase (U/mL)*	*MDA (µmol/L)*
Control (C)	**1.96 ± 0.01 C**	**3.53 ± 0.03 c**	**0.81 ± 0.009 c**	**1.27 ± 0.011 d**
G1: ETO	**0.79 ± 0.01 f**	**1.68 ± 0.011 f**	**0.31 ± 0.005 e**	**2.33 ± 0.011 a**
G2: ETO + QE	**1.75 ± 0.008 e**	**3.18 ± 0.008 e**	**0.71 ± 0.005 d**	**1.41 ± 0.011 c**
G3: ETO + HES	**1.79 ± 0.006 e**	**3.25 ± 0.007 e**	**0.69 ± 0.005 d**	**1.48 ± 0.011 b**
G4: ETO + QE + HES	**1.91 ± 0.005 d**	**3.43 ± 0.008 d**	**0.79 ± 0.009 c**	**1.30 ± 0.011 d**
LSD	**0.046**	**0.045**	**0.027**	**0.032**

Values are expressed as Mean ± SE ; n= 5 / group.

Different small letters denote between groups differences, P< 0.05 Vs. control.

Table (3): shows Effect of Etoposide , Quercetin and Hesperidin on diameter and thickness of seminiferous tubules in Testis , diameter and thickness of tubules in Epididymis in adult male rats .

Group	*Diameter of Seminiferous tubules in testes*	*Thickness of Epithelial layer in Seminiferous tubules*	*Diameter of Seminiferous tubules in Epididymis*	*Thickness of epithelial layer in epididymis*
		(µm)		
Control (C)	12.56 ± 0.22 b	3.46 ± 0.05 c	10.60 ± 0.13 b	1.09 ± 0.03 c
G1: ETO	8.1 ± 0.17 d	2.76 ± 0.03 f	7.66 ± 0.26 c	0.76 ± 0.05 d
G2: ETO + QE	11.46 ± 0.16 c	3.17 ± 0.03 e	10.04 ± 0.16 b	1.00 ± 0.03 c
G3: ETO + HES	11.28 ±0.26 c	3.14 ± 0.01 e	10.01 ± 0.35 b	0.98 ± 0.03 c
G4: ETO + QE + HES	12.04 ± 0.22 bc	3.27 ± 0.03 d	10.40 ± 0.29 b	1.02 ± 0.02 c
LSD	**0.762**	**0.138**	**0.823**	**0.130**

Values are expressed as Mean ± SE ; n= 5 / group.

Different small letters denote between groups differences, P< 0.05 Vs. control.

Table (4): Shows the effect of Etoposide , Quercetin and Hesperidin on the spermatogenic cells count , Sertoli cell and Leydig cell in male rats .

Groups	*Spermatogonia*	*Spermatocyte*	*Spermatids*	*Sertoli cells*	*Leydig cells*
Control (C)	75.6±0.58 C	94.4±1.02 c	106.2±1.02 c	23.2±0.58 c	16.6±0.74 c
G1: ETO	43.4±1.02 E	40.4±1.50 e	52.8±1.24 g	11.8±0.66 e	8.8±0.38 e
G2: ETO + QE	66.6±0.50 d	87.6±0.86 d	91.2±0.66 f	18.2±0.66 d	14.4±0.50 b
G3: ETO + HES	65.4±0.74 d	85.2±0.80 d	88.6±0.81 e	17.8±0.58 d	14.2±0.40 b
G4: ETO + QE + HES	73.8±0.86 c	92.2±1.15 c	97.4±0.92 d	21.4±0.74 c	15.8±0.37 c
LSD	2.30	3.85	2.90	2.16	1.82

Values are expressed as Mean ± SE ; n= 5 / group.

Different small leers denote between groups differences, P< 0.05 Vs. control.

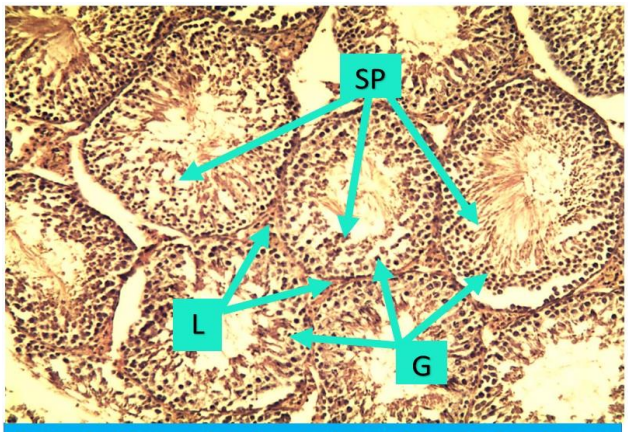

Figure –1 : Cross section from testis control rat showing there are normal structure tissue characterized by complete spermatogenesis and presence high number spermatogonia (G) , primary and secondary Spermatocyte (SP) in the lumen , there is presence of leydig's (L) (H&E stain, X10) .

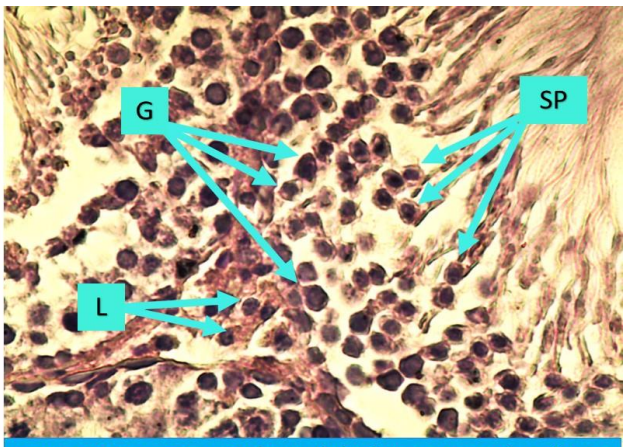

Figure –2 : Cross section seminiferous tubules from testis control rat showing there are normal structure tissue characterized by complete spermatogenesis and presence high number spermatogonia (G) , primary and secondary Spermatocyte (SP) in the lumen , there is presence of leydig's (L) (H&E stain, X40) .

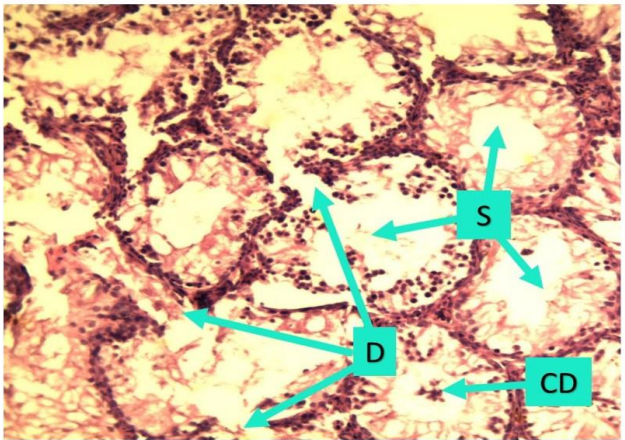

Figure – 3 : Cross section seminiferous tubules from testis Etoposide group rat showing there are spermatogenesis stops , lumen is empty from sperm (S) , With degeneration (D) of with seminiferous tubules , intracellular cell debris (CD) (H&E stain, X10).

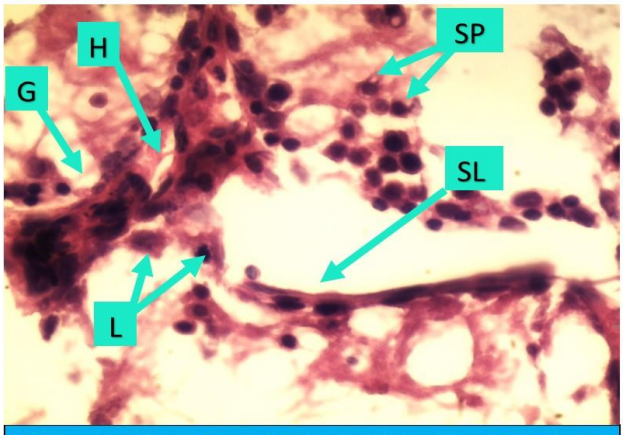

Figure – 4 : Cross section seminiferous tubules from testis Etoposide groups rat showing there are illustrate disintegration of Sprmatogonia (G) , and few spermatocyte (SP) , disintegration and necrosis of Liydig cells and very few (L) , blood congestion within the tubules (C) , With basal membrane dissociation (SL) (H&E stain, X40) .

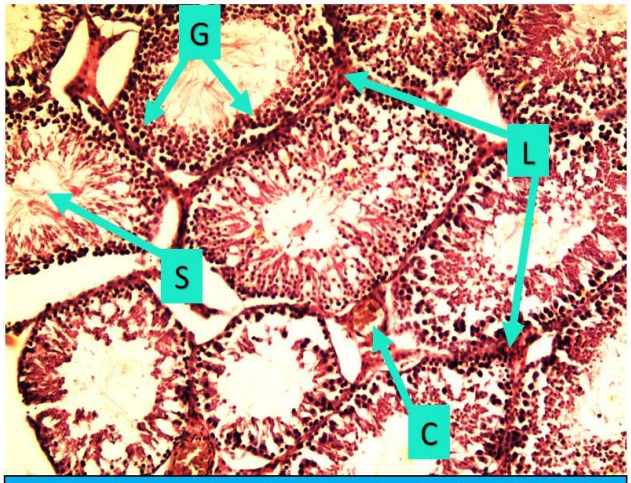

Figure –5 : Cross section from Seminiferous tubule of testis (ETO + QE) group rat showing there are improved in structural tissue through full Seminiferous tubule lumen by sperm (S) , improved semi-complete spermatogenesis and presence spermatogonia (G), presence of leydig's (L) , with simple blood congestion (C) (H&E stain, X10) .

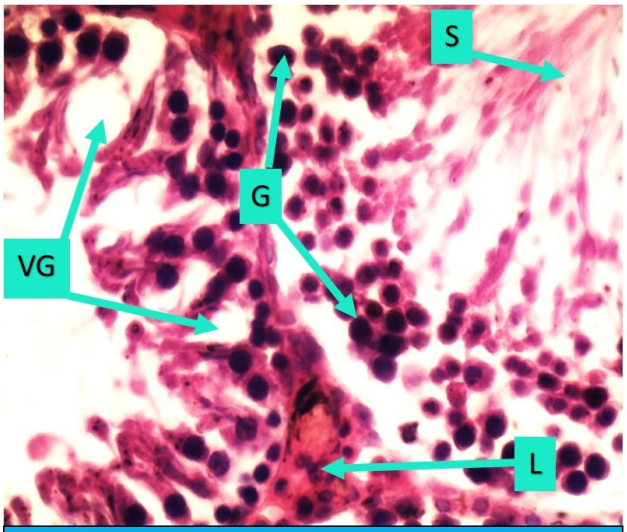

Figure –6 : Cross section from Seminiferous tubule of testis (ETO + QE) group rat showing there are full lumen by sperm (S) , abundance of spermatogonia (G) with few disintegration in it (VG) , presence of leydig's (L) , with simple blood congestion (C) (H&E stain, X40).

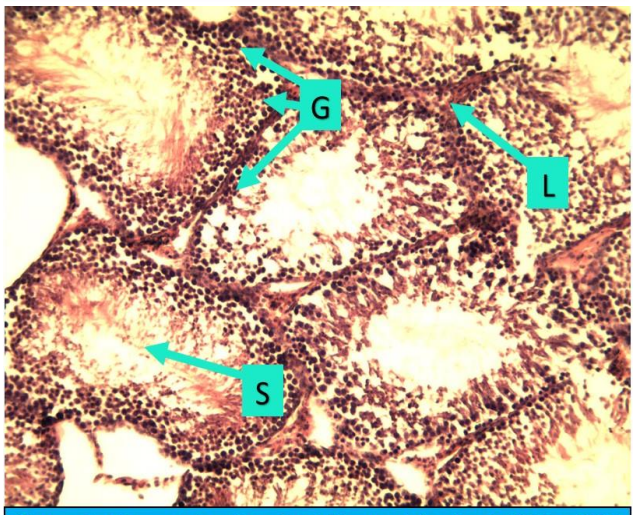

Figure –7 : Cross section from Seminiferous tubule of testis (ETO + HES) group rat showing there are improved in histological changes , and fullness lumen with sperm (S) , abundance of spermatogonia (G), increase of leydig's cell (L) , (H&E stain, X10).

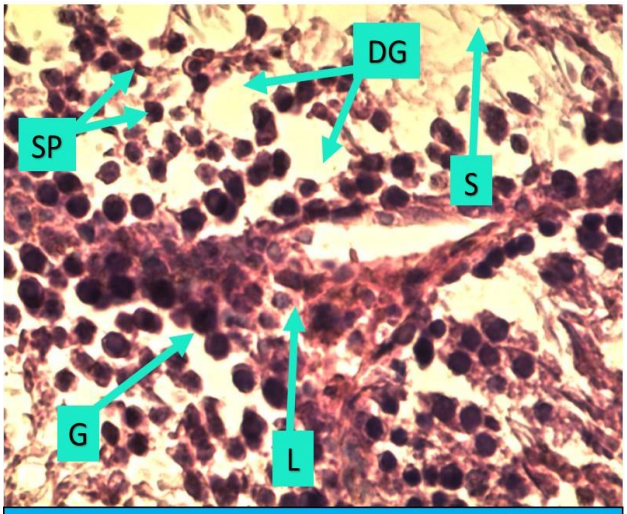

Figure –8 : Cross section from Seminiferous tubule of testis (ETO + HES) group rat showing there are full lumen by sperm (S) , rise of spermatogonia (G) and spermatocyte (SP) . with few disintegration in it (DG) , great number of leydig's cell (L) , (H&E stain, X40).

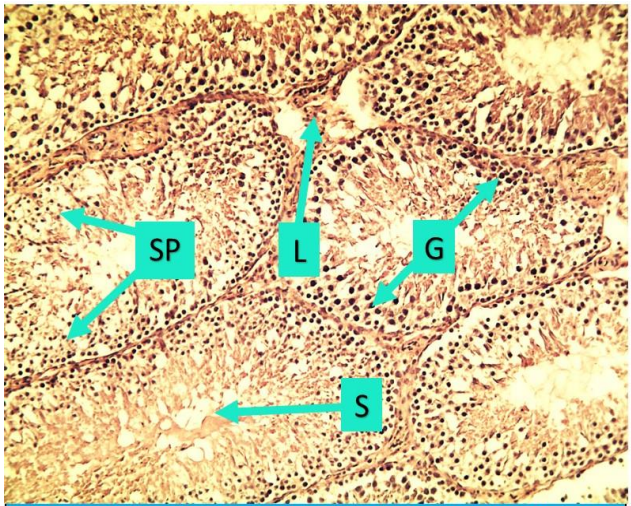

Figure – 9 : Cross section from Seminiferous tubule of testis (ETO + QE + HES) group rat showing there are normal structural of seminiferous tubule , full lumen by sperm (S) , rise of spermatogonia (G), abundance of leydig's cell (L) , (H&E stain, X40).

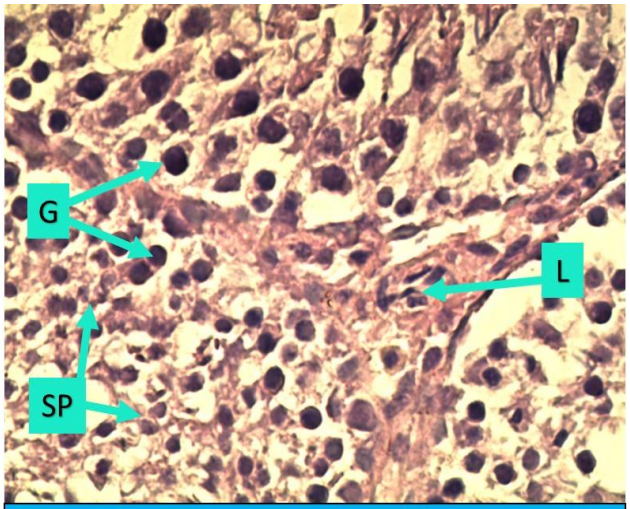

Figure – 10 : Cross section from Seminiferous tubule of testis (ETO + QE + HES) group rat showing there are full lumen by sperm (S) , spread of number spermatogonia (G) and Spermatocyte (SP) , proliferation of leydig's cell (L) , (H&E stain, X40).

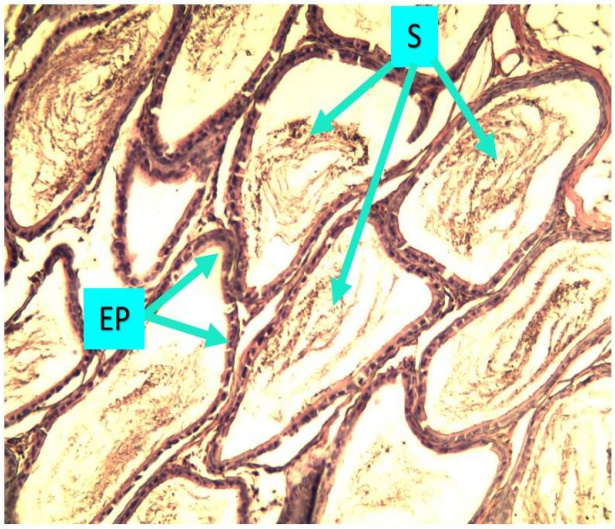

Figure –11 : Cross section from Epididymis control group rat showing normal epididymal structure with presence of large numbers of sperms in the lumen (S) , with normal Epithelial cells (H&E stain, X10) .

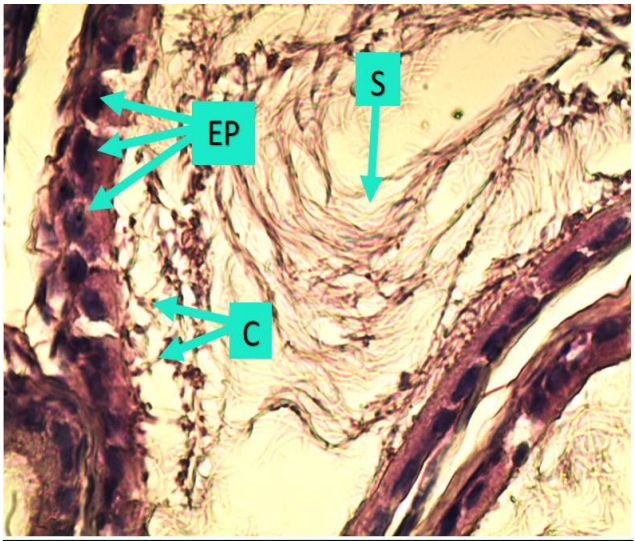

Figure –12 : Cross section from Epididymis control group rat showing fullness epididymal tubule of sperms (S) , with normal Epithelial cells (EP) , presence of Cilia (C) (H&E stain, X40) .

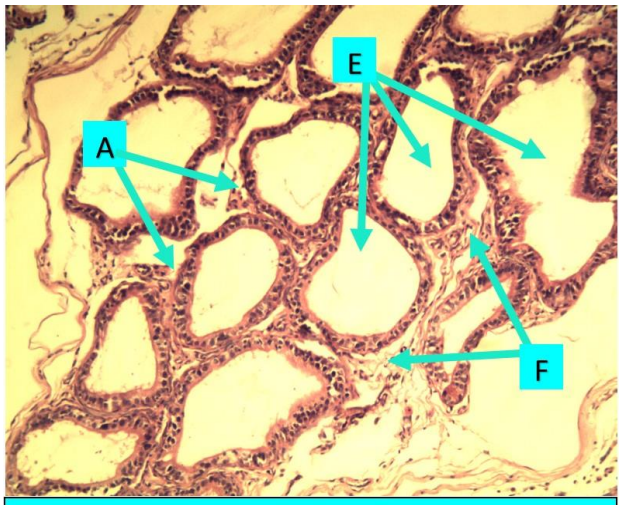

Figure –13 : Cross section from Epididymis Etoposide group rat showing epididymal tubule is empty of sperms (E) , with atrophy tubules (A) , Desmoplasia proliferations (F) (H&E stain, X10) .

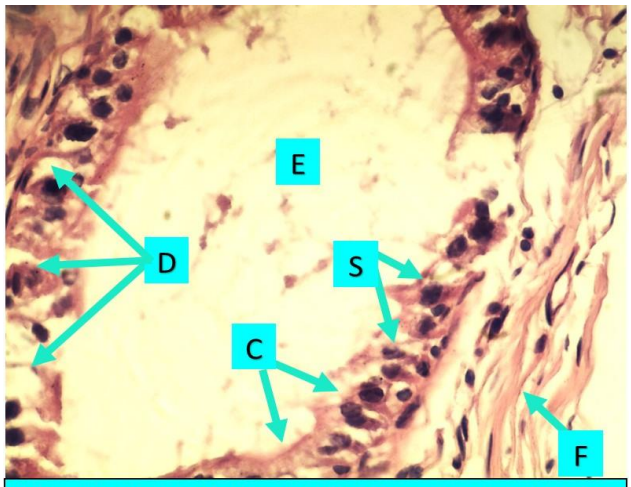

Figure –14 : Cross section from Epididymis Etoposide group rat showing epididymal tubule is empty of sperms (E) , hyperplasia (H) with atrophy and Degenration of Epithlial cell lining of Epididymal tubule (D) , Desmoplasia proliferations (F) (H&E stain, X40) .

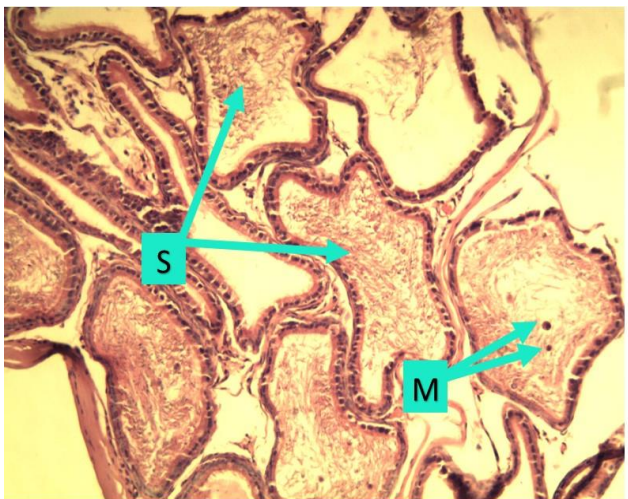

Figure –15 : Cross section from Epididymis ETO + QE treated group rat shows that epidermis tubule is full of sperm (S), with megakaryocyte giant cells (M) inner lumen of tubule epidermis . (H&E stain, X10) .

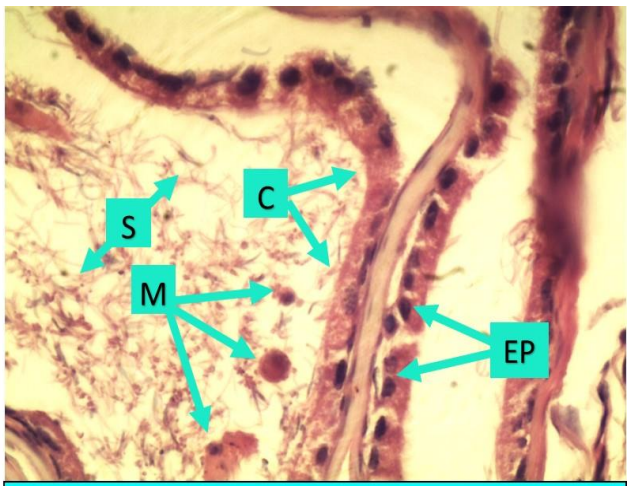

Figure –16 : Cross section from Epididymis ETO + QE treated group rat shows epidermis is full of sperm (S) , tubule is lined with normal Epithelial cells (EP) , cilia (C) , with giant megakaryocyte cells (M) inside lumen of tubule epidermis . (H&E stain, X40).

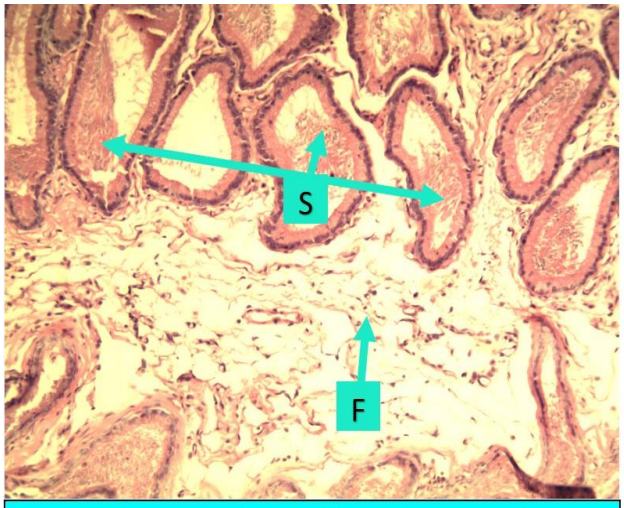

Figure –17 : Cross section from Epididymis ETO + HES treated group rat shows some that epidermis tubule is almost full of sperm (S) , a few in other some and Desmoplasia proliferations (F). (H&E stain, X10) .

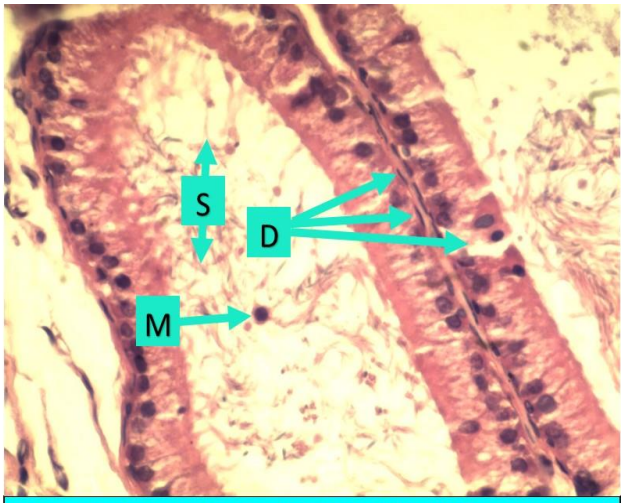

Figure –18 : Cross section from Epididymis ETO + HES treated group rat shows that the epidermal germs contain medium numbers of sperm (S) and simple degeneration (D), with megakaryocyte giant cells (M) within lumen of tubule epidermis . (H&E stain, X40).

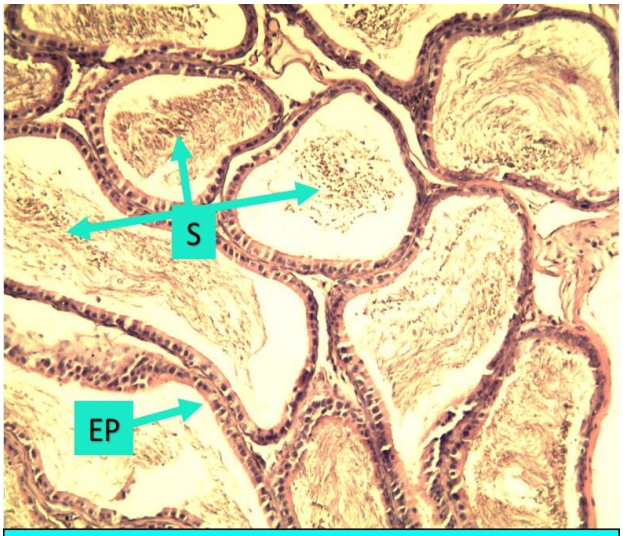

Figure –19 : Cross section from Epididymis ETO + QE + HES treated group rat shows : The epididymal tubule is full of sperms (S) , with normal Epithelial cells (EP) . . (H&E stain, X10).

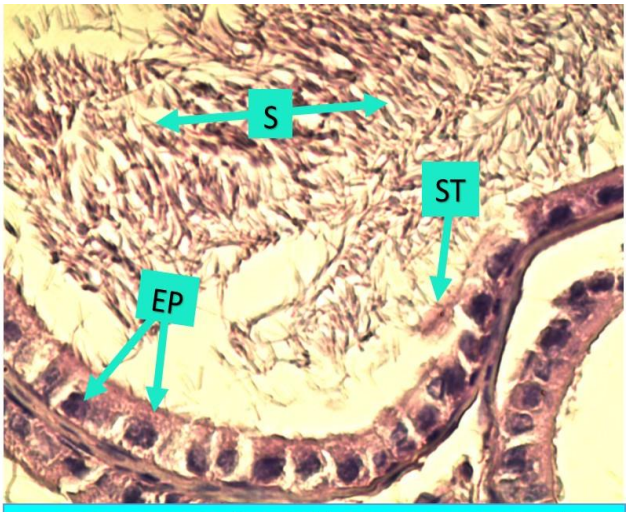

Figure –20 : Cross section from Epididymis ETO + QE + HES treated group rat shows : that epididymis is full of sperm (S), and observed an increase in high of Epithelial cells lining of the tubules (EP) , With presence Stereocilia (ST) .(H&E stain, X40) .

4. Dissections

The alterations in the testis functions induced by anticancer drugs are closely associated with formation of reactive oxygen species (ROS) in the tissues. Since oxidative status is known to affect several physiological processes in tissues (3,4). Led administration of ETO induced lipid peroxidation and decreased the activities of SOD , GSH and CAT in rats . Lipid peroxidation, an indicator of oxidative stress in tissues cause peroxidative damage of cellular lipid content . The reduced activities of SOD and CAT have resulted in the accumulation of these highly reactive free radicals and eventually generate reactive oxygen species (ROS) that lead to harmful effects in tissues. This damage occurs due to an imbalance between the generation of reactive oxygen species and antioxidants .

Our study showed of reduction in the body weight and of the reproductive organs is due to effect of Etoposide on the Spermatogenesis . Therefore, few Spermatogonia cells in the testis and sperm store in the epididymis, as indicated by the results, may have a role in decrease organs weight (9) , whereas indicate (6) cause decrease in the weight of testis and epididymis to the toxic effects of Etoposide, which caused degeneration, Necrosis, which led a significant reduction in the diameter of the semifinal tubules of testis and epididymis, this result agreed with the results of a previous study (4,25) . Our results indicate that drug Etoposide therapy led to destruction of seminiferous epithelium and epididymis tubule resulting in tubular degeneration , necrosis , atrophy and decrease in spermatogenic cells , as shown in earlier studies that etoposide can damage DNA synthesis (7,10), and is a very potent inducer of programmed cell death in adult male rat germ cells (28). Previous work suggested that differentiating spermatogonia , postmeiotic germ cells and protein synthesis in the testis are more sensitive to chemotherapeutic agents, but mitotic cells are also vulnerable (10, 25). Etoposide was found to increase the tissue MDA levels,In general cellular damage in the testis is the result of an improper balance between ROS generation and scavenging activities. Excessive ROS production that exceeds critical levels can overwhelm all antioxidants defense strategies of spermatozoa and seminal plasma causing oxidative stress (4).

Testis are highly susceptible to damage by excessive concentrations of ROS due to the high content of polyunsaturated fatty acids within their plasma membrane. The lipid peroxidation destroys the structure of lipid matrix in the membranes of spermatozoa, and it is associated with loss of motility and impairment of spermatogenesis. All the above reports clearly suggest the role of oxidative stress in Etoposide induced reproductive abnormalities (24,26). Chronic treatment with the single agent cyclophosphamide resulted in a decrease in the expression of the stress response genes in pachytene spermatocytes and round spermatids (27) , showed current study decrease of Leydig cells, which is responsible On the completion of histological of the testis , epididymis and spermatogenesis , as well as reduction of Sertoli cells , which led to depletion in its' function of secreting of androgen

binding protein (ABP) which enhance accumulation of testosterone and dihydrotestosterone in high concentrations within the seminiferous tubules and the interstetium of the testis (8). These find out led to adverse effects on the structure of the testis and spermatogenesis that might due to reduction of serum testosterone levels in male rats, agreement with reported previous study (28,29). Other dose-dependent histological alterations, such as moderate and accentuated depletion of the seminiferous epithelium in adult etoposide-treated rats have also been reported (8,30) . The results of this study showed a significant increase in serum MDA level and decrease CAT , SOD , GSH in the Etoposide treated group , this result is in agreement with several reports demonstrated that ETO could cause oxidative damage and increase in serum MDA level (3, 4) . Previous studies have shown various actions of etoposide reporting that the drug can oxidize GSH and protein SH groups in HL-60 cells, also etoposide treatment cannot amplify the loss of essential antioxidants, but increases lipid hydroperoxide concentration in serum (31,32). Etoposide activates oxidation by the interaction of phenoxyl root with thiols (-SH) group in the glutathione molecule, which inhibits the action of glutathione as an anti-oxidative stress agent, or by stimulating many of the enzymes or inflammatory agents such as monooxygenases, CYP 450 and prostaglandin synthetase , which lead to cellular toxicity (31).

Flavonoids are considered as antioxidant which remove the free radicals produced after Etoposide treated , Received QE and HES / or their combinations, may increase testicular weights and epididymis with increasing diameter of seminiferous tubules and high epithelial cells , because of QE and HES have antiperoxidative effect, and its oral administration was given protection to the male reproductive organs and cells by decreasing the lipid peroxidation (33) . The increases in body weight and reproductive organ after treatment with QE , HE alone or in combination may be attributed to flavonoids , which have antioxidant effective protect of tissue induced-oxidation . The apparent increase in weight body and reproductive organ were observed in treatments with HES and QE and its approach to the control group rate is due to the strength of the synergistic effect of flavonoids were stimulates cell performance, improves overall body health and protects against diseases that are affected by oxidative stress (16,34). Flavonoids are known to improve erectile function by their antioxidant and anti-inflammatory properties (2,34). These results are in agreement with these reported by Khaki *et al.,* (16) by significantly improved body weight, epididymal , testis weight and decrease MDA and enhanced the serum SOD , CAT , GSH levels , In additional, improved spermatogenesis in STZ-induced diabetic rats . indicted study a significant improvement in body weight and feed/gain ratio was recorded after hesperidin incorporation into broiler diets at the level of 20 mg/kg . Our result confirmed the findings of Izawa et al., (15) and Taepongsorat *et al.,* (35) . Izawa *et al.,* (15) evidenced that quercetin can inhibit the testicular damage induced by DEPs in mice , Taepongsorat *et al.,* (42) showed

that the tubular area of seminiferous tubules increased with Quercetin treatment in a time- and dose-dependent manner. On the other hand, Led treatment with QE and HES, showed an increase leydig's cells and Sertoli cells produce androgen binding protein (ABP) which transport of 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 , and may lead to increase lumen and diameter of seminferous tubules (28,36). These increase may be accompanied with an increasing LH concentration and gene expression for LHr gene as well as Cyp11a1 , its responsible for testosterone synthesis, Quercetin have improver effect on plasma gonadotropin concentration especially LH (28,44) . Studies on the effects of QE on oxidative damage in cultured chicken spermatogonial cells showed that it had no deleterious effects at doses of 1 and 10 μ g/Ml, QE at (1) μg/mL increased the numbers of spermatogonial cells and reduced Aroclor-induced oxidative damage in the testes (37,38).

Moreover, Showed histological changes improved by treatment with Quercetin and Hesperidin and regeneration structural to normal , these flavonoids are known to protect the cell membrane against damage induced by free oxygen radicals (16,39), through their attaches to the Metals ion , preventing it from binding to hydrogen peroxide, whereas etoposide has been shown to cause free radical production (40). Flavonoids can also increase the activities of antioxidant enzymes with a concomitant decrease in lipid peroxidation. This is synonymous with decreased oxidative stress (41). Quercetin acts an increase that Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) , a compound that regulates the expression of antioxidant proteins that protect against oxidative damage , Quercetin also inhibit xanthine oxidase activity, thereby resulting in decreased oxidative injury (42) . Accordingly, Izawa *et al.*, (15) demonstrated that quercetin can inhibit the testicular damage in mice.

The mechanisms contributing to its effectiveness involve the quenching of free radicals, increasing the antioxidants status and metal-chelating activities of quercetin. Thus HES has been shown to reduce oxidative stress in various in-vivo and in-vitro studies , in treatment with rats, HES decreased MDA level signifying attenuation in lipid peroxidation thereby proving its stabilizing power on membranes (11). Enough evidence has been garnered for HDN proving to be effective antioxidant in Cisplatin mediated oxidative stress (10). Reported study of Ilankeswaran *et al.*, (12) indicate that Hesperidin offers protection to the liver by decreasing the levels of lipid peroxides and maintaining the levels and activities of non-enzymatic and enzymatic antioxidants in STZ-induced diabetic rats. This could be due to its free radical scavenging, antioxidant as well as membrane-stabilizing property of Hesperidin . These finding is consistent with previous reports which indicated that QE and HES increased the antioxidant activities of SOD and CAT and improved histological testis and epididymis structural and spermatogenesis (4,43) .

Led treatment flavonoids causes to increase their effectiveness , In combination (QE +HES) in our study showed synergistic potential , Many explanations have been recorded action of mechanism of this Flavonoids, due to have the ability to form non-covalent bonds within the lipid layers close to the cell's plasma membrane, which greatly enhances its antioxidant activity in this combination and regeneration of endogenous antioxidants level (43,44). Numerous studies have indicated the synergistic and protective role of flavonoids against many diseases and pathological changes generated by exposure to various chemicals and drugs in vitro or in vivo of animals experimental , Reported by Lien *et al.,* (45) a dietary supplementation by the flavonoids Hesperitin and Naringenin at the same time, an increase SOD and total antioxidant activity level and scavenging superoxide ability were enhanced, and serum TBARS level was decreased in laying hens , In a study by Hozayen *et al.,* (38), found that pretreatment with Hesperidin and/ or Rutin (combination) may improvement of testicular dysfunction caused via doxorubicin – induced, by amelioration in the levels of (testosterone, LH and FSH) and antioxidant (CAT , GSH , SOD) and decrease MDA level in male rats .

Conclusion

We conclude that Quercetin and/or Hesperidin the investigated increases the improved of rat reproductive tissues and antioxidant level to Etoposide-induced oxidative damage , As these antioxidant flavonoids are known to decrease the risk of degenerative diseases.

Acknowledgement :

We would express our sincere thanks to all members of Teachers in the Dept. of Biology , College of Education., Univ of Al-Qadisiyah .

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RJPT Research Journal of Pharmacy and Technology
ISSN: 0974-360X(Online) 0974-3618(Print)

Research Journal of Pharmacy and Technology
Scopus coverage years: 1997, 2005, from 2011 to Present
Publisher: A and V Publication
ISSN: 0974-3618 E-ISSN: 0974-360X

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Acceptance Letter

23 October 2017

Dear Author(s)

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Dr./Mr. Hussein K. AL-Mayali / University of Al-Qadisiyah, IRAQ

Greetings, with reference (JPT 36) to your article entitled:

Ameliorative effect of Quercetin and Hesperidin on Antioxidant and Histological Changes in the Testis of Etoposide-Induced Adult Male Rats

We wish to bring to your kind notice the following

√ **We acknowledge the receipt of the above mentioned article.**

√ **The above mentioned article(s) has been sent to the reviewer of expert comments**

√ **The above mentioned article(s) have been accepted for publication in the** (Research Journal of Pharmacy and Technology). The probable date of publication is; **Vol:10 (No:11-12): 29 December-:2017**