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Histopathological study of the effect of glutathione in decreasing pathological changes induced by estrogen in internal organs of albino mice

Ali Salman Dawood
Department of pathology, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq
Corresponding author email: alisloomi555@gmail.com

Hanaa khudair Abaas
Department of pathology, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq
Email: drhanakadiar@gmail.com

***Abstract*---**In recent years, there has been a great deal of attention toward the field of free radical chemistry. Free radicals reactive oxygen species and reactive nitrogen species are generated by our body by various endogenous systems, exposure to different physiochemical conditions or pathological states. A balance between free radicals and antioxidants is necessary for proper physiological function. If free radicals overwhelm the body's ability to regulate them, a condition known as oxidative stress ensues. Free radicals thus adversely alter lipids, proteins, and DNA and trigger a number of human diseases. This study was designed to determine the influence of Glutathione to reduce the pathological changes induced by estrogen. In this study 40 female and male mice are taken at age of 21days with groups of control (10 mouse in each group).The group of experimental animals was treated with estrogen and glutathione diluted with distilled water, as a vehicle, and injected intraperitonially for 12 weeks, once weekly. Each control group was injected with the vehicle of it in the same manner of injection and for the same period. At the end of the experiment period (12weeks) all the animals are euthanasia and the organs include (lung, heart, liver, intestine, spleen and kidney), and the sexual organs of both sexes include (uterus, ovary and testes) were collected for macroscopic and histopathologic examination which preserved in 10% formalin in addition blood sample were taken for biochemical analysis.

***Keywords*---**Histopathological, albino mice, estrogen.

Introduction

Several factors of our modern life style such as excess alcohol consumption, tobacco chewing and smoking habits also exposure to toxic chemical compounds such as estrogen and radiation all add to the free radical production in the body and increase the risk of cancer (Clayson *et al.*, 1994). A balance between free radicals and antioxidants is necessary for proper physiological function. If free radicals overwhelm the body's ability to regulate them, a condition known as oxidative stress ensues. Free radicals thus adversely alter lipids, proteins, and DNA and trigger a number of human diseases. Hence application of external source of antioxidants can assist in coping this oxidative stress (Lobo *et al.,* 2010). Antioxidants are the molecules that prevent cellular damage caused by oxidation of other molecules.Oxidation reactions are known to produce free radicals. Antioxidant reacts with these free radicals and terminates this chain reaction by removing free radical intermediates and inhibits other oxidation reactions by oxidizing themselves (Hamid *et al.*, 2010; Khalil et al., 2013). Glutathione (GSH) is one of the most famous and important antioxidant and plays a major role in the detoxification of endogenous metabolic products, including lipid peroxides, and xenobiotic compounds including pollutants, heavy metals, and drugs (Exner, 2000; Lee, 2008; Alwan and Al-Okialy, 2018). Glutathione is low-molecular weight, water-soluble tripeptide, composed of the amino acids cysteine, glutamic acid, and glycine (Dickinson, 2002).

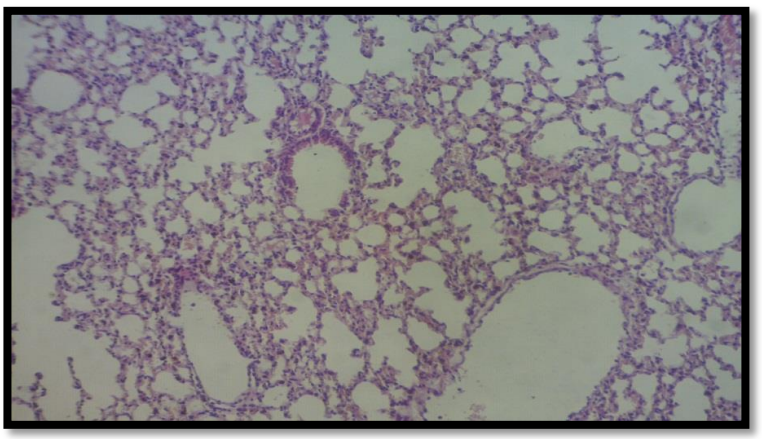
Animal's studies repeatedly demonstrated that Estrogen can induce and promote mammary tumor in rodent (Nilsson *et al.*, 2001). Estrogen also has an important role in pathological processes observed in tissue of reproductive system (Prins and Korach, 2008; Ellem and Risbridger, 2009). Estrogen exerts diverse biological effects in animals and human and many of these results from a direct interaction of the Estrogen with an intracellular receptor that activates the expression of genes encoding proteins with important biological functions (Parker, 1991). One of the most important and notable effect of estrogen is a supper potent mitogenic action in hormone sensitive tissues such as uterus, and breast (Holland and Roy, 1995). Prolonged exposure of target tissue or cells to excessive mitogenic stimulation by natural or synthetic, estrogen has long been considered an important etiological factor for the induction of estrogen- associated cancers in experimental animals (Nandi *et al.*, 1995; Li, 1996) and humans (Grady and Emster, 1996).

Material and method

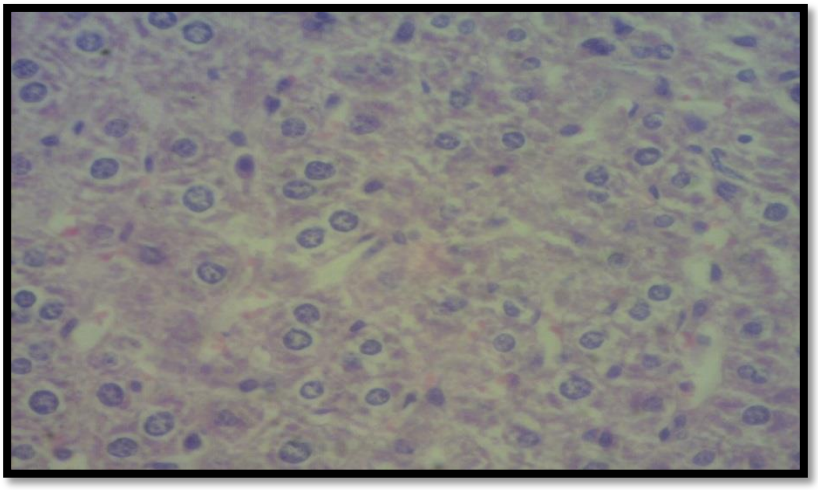
Estrogen and Glutathion**:**-n=40 mice, 20 male and 20 female. This group of animals received estrogen diluted in olive oil .The rout of administration was **(S/C)** at a daily manner along the period of experiment (12 weeks), and Glutathion is diluted in distilled water. The rout of administration was subcutaneous **(S/C) once weekly** along the period of experiment (12 weeks).

Result and Discussion

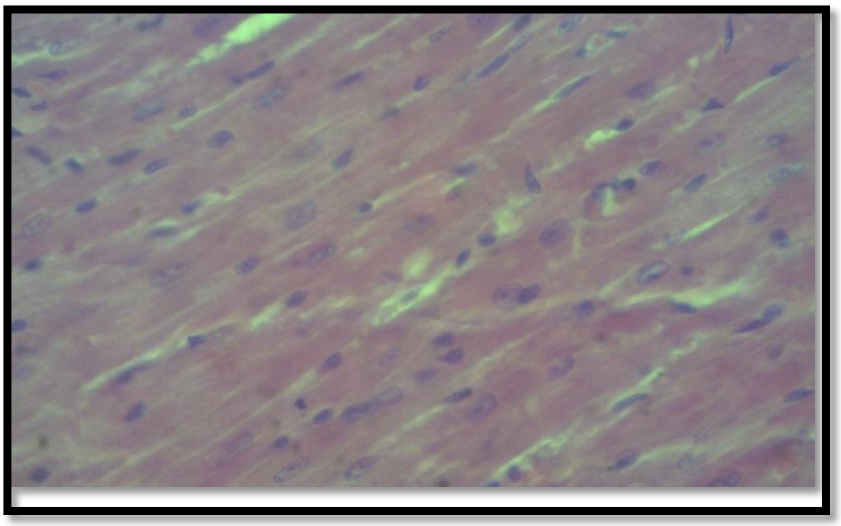
1-Lung
Histopathological section of the pulmonary tissues showed infiltration of interstitial tissues with inflammatory cells (Figure 4-26):.
2-Liver
Histopathological section of liver showed infiltration of inflammatory cells in the hepatic parenchyma and congestion of central vein (Figure 4-27):
3-Heart
Histopathological section of heartmoderat infiltration of inflammatory cells (Figure 4-28):.
4-kidney
Histopathological section of kidney showed infiltration of inflammatory cells in the renal tissues (Figure 4-29):.
5-Intestine
Histopathological section of the showed mild infiltration of inflammatory cells (Figure 4-30):
6-Brain
Histopathological section of brain showed vacculation of astrocyte (Figure 4-31):.
7-spleen
Histopathological section of the spleen showed slight deplesione of lymphoid tissues(4-32)
8-uterus
Histopathological section of the uterus of this group showed distention of the uterine gland and sequamous metaplasia of the epithelial lining cells of this gland (Figure 4-33).
9-ovary
Histopathological section of the ovary showed slight normal follicales
10-Semineferous tubules
Histopathological section of theSemineferous tubules of this group showed slight degeneration of the spermatogonia(Figure 4-34):



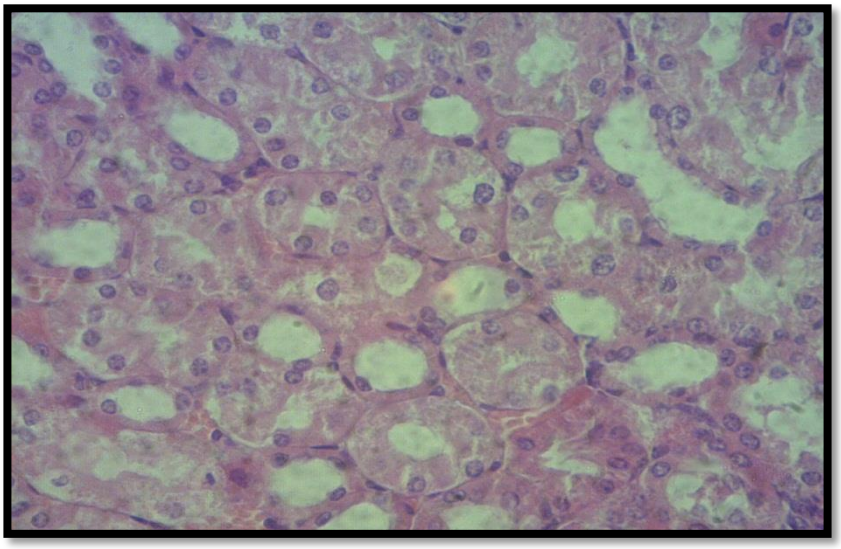
(Figure 4-26): Histopathological section of lung of mouse of (estrogen and Glutathione)treated group showed infiltration of interstitial tissues with inflammatory cells and thickening of the alveolar wallsHandE X100.



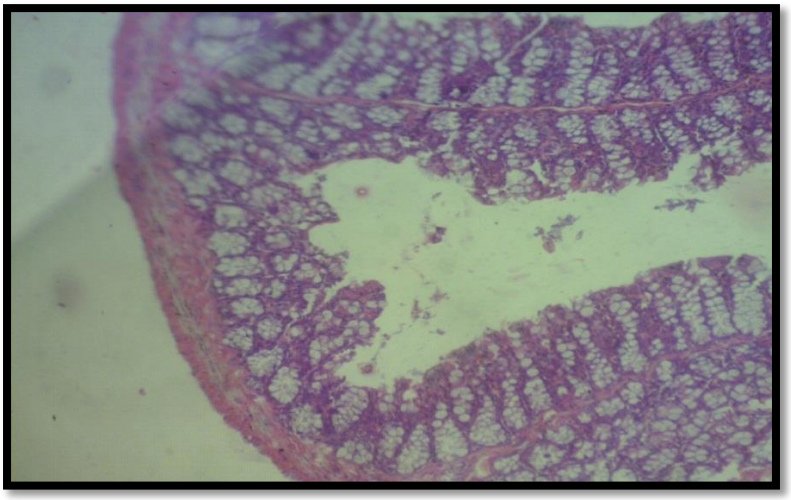
(Figure 4-27): Histopathological section of liver of mouse of (Estrogen and Glutathione treated group showed slight infiltration of inflammatory cells in the hepatic parenchyma HandE X100.



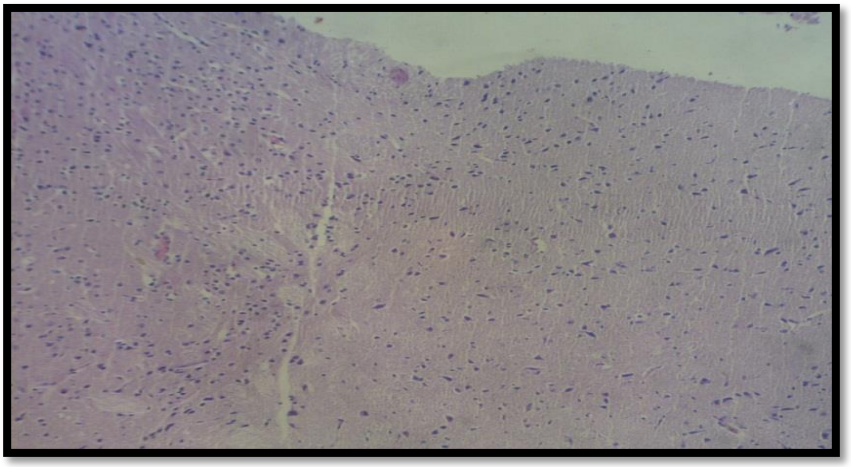
(Figure 4-28): Histopathological section of heartof mouse of (Estrogen and Glutathione) treated group showed mild infiltration of inflammatory cells between myocardial fiberHandE X100.



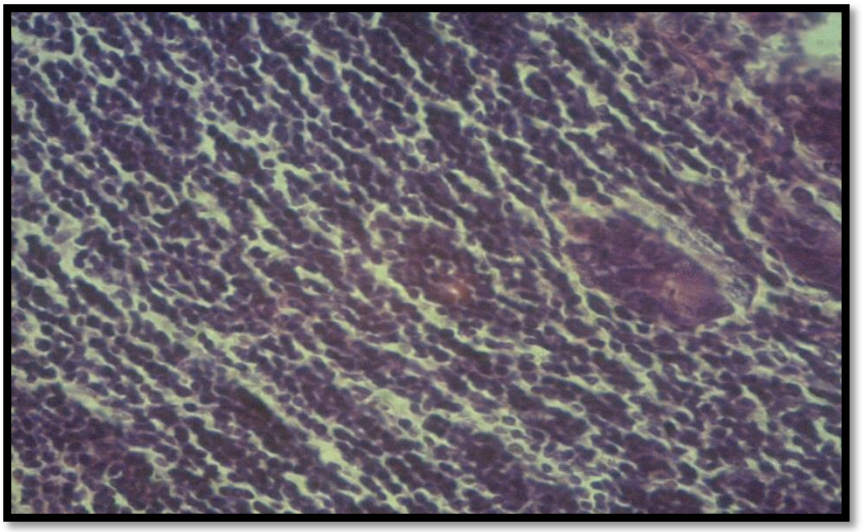
(Figure 4-29): Histopathological section of kidney of mouse of (Estrogen and Glutathion treated group showed degeneration of epithelial cells of the renal tissuesHandE X100.



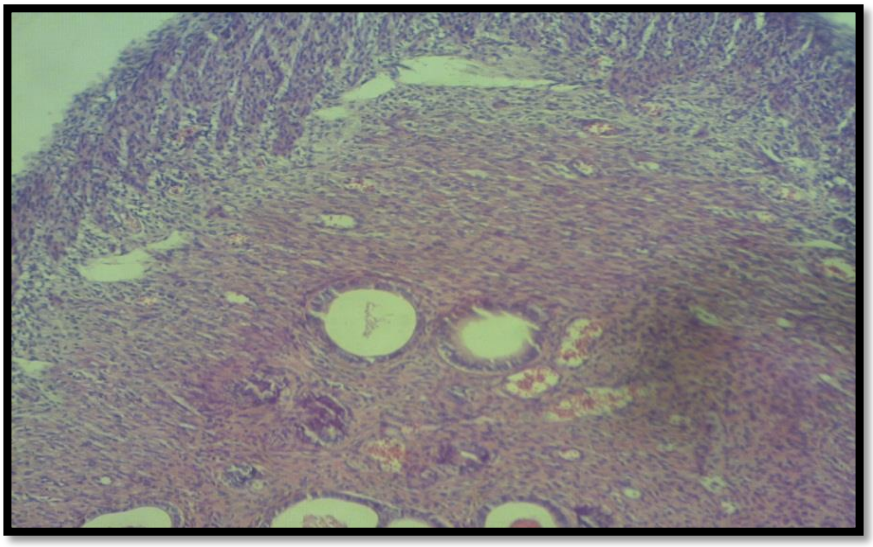
(Figure 4-30): Histopathological section of intestine of mouse of (Estrogen and Glutathione) treated group showed mild infiltration of inflammatory cells in the intestinal layers,HandE X100.



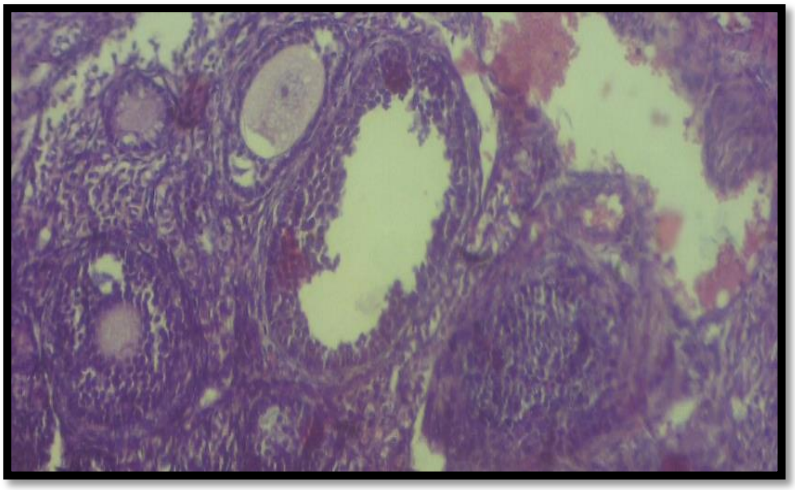
(Figure 4-31): Histopathological section of brain of mouse of (Estrogen and Glutathione)treated group showed infiltration of inflammatory cells of meninges HandE X100.



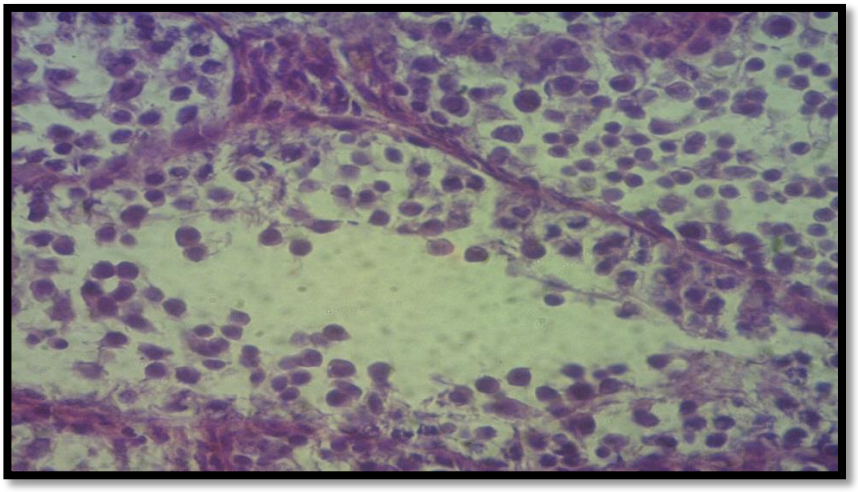
(Fig4-32):-Histopathological section of spleen ofmouse of(Estrogenand Glutathione) treated group showed slightdeplesione of lymphoid tissues,HandE X10.



(Figure 4-33): Histopathological section ofuterus of mouse of (Estrogen and Glutathione) treated group showed infiltration of inflammatory cell in the uterine tissue HandE X100.



(Figure 4-34): Histopathological sectionof the ovary of the mouse of (Estrogen and Glutathione) treated group showed slight normal follicalesHandE X4.



(Figure 4-35): Histopathological section oftestes of mouse of(Estrogen and Glutathione)treated group showed degenerative changes of spermatogoniaHandE X40

Generally most of lesions the second group were disappeared in this group this is returned to the detoxifying ability of Glutathione, (Gamcsik *et al* ., 2012) .Glutathione is one of the most important and potent antioxidants,(Wrotek *et al.*,2020).As it is well known that most of pathological changes result from oxidative stress, due to the excessive production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), (Griendling *et al.*,2016; Andreadou *et al* ., 2020; Gharban and Al-Shaeli, 2021). Glutathione plays a central role as antioxidant defense, as well as in the regulation of pathways involved in cellular homeostasis, not only as a detoxifier of endogenous and exogenous compounds, but also through its participation in processes related to cell proliferation, apoptosis, gene expression, regulation of the immune system, and metabolism of cell compounds improved by(Luc *et al* ., 2019;Zhang *et al.*,2020).Generally the lesions of this group which is treated by Estrogen and Glutathione appeared to be less affected than the first group duo to the antioxidant effect of glutathione and most of them showed moderate lesion ,estrogen effect decreased duo to treatment with glutathione. Estrogen was shown that be able to induced intracellular ROS generation Intracellular ROS have been reported to activate signaling pathways which favor survival and proliferation of cells, both required for malignant transformation (Okoh *et al.*, 2011). ROS may serve as messengers in cellular signaling transduction pathways, and a moderate increase of certain ROS such as superoxide and hydrogen peroxide may promote cellular growth and proliferation and contribute to cancer development (Schimmel and, Bauer,2002). The mechanisms responsible for stimulation of cell proliferation are likely to involve the direct ROS interaction with specific receptors and modulation of the redox states of signaling molecules such as protein kinases and transcription factors (Liu *et al.*, 2000). ROS can affect MAPK signaling pathway which is involved in cell proliferation (Pelicano *et al.*,2004).Glutathione which used in this group as antioxidant have the ability topreventsthe cell damage induced by ROS resulted from estrogen treatment including lipid peroxides,peroxides, free radicals, (Pisoschi and Pop, 2015),others like(Winterbourn, 2016),refer

thatGlutathione can scavenge ROS via non-enzymaticand enzymatic reactions. The non-enzymatic antioxidantactivity is contributed by the free thiol group of glutathione,while(Farhat*et al.*, 2018),pointed to another effect of glutathione to detoxifiesoxidants and electrophiles via enzymatic reactions whichinvolve glutathione reductase, glutathione peroxidase, andglutathione-S transferase. glutathione also involves in metabolismsestrogens, leukotrienes, and prostaglandins and signaltransduction for transcription (Rotar*et al.,* 2014).

The lung of this group showed infiltration of interstitial tissues with inflammatory cells ,but the lesion appeared to be moderate than the first one because of the ability of the glutathione in the regulation of inflammation, this result insisted by (Rodrigues and Percival,,2019).Others like (Droge and Breitkreutz2000),refer that Glutathione (GSH) is required by the immune system to protectshost immune cells through its antioxidant mechanism and provides optimal functioning of lymphocytes and other cells of the immune system .Proliferation of the epithelial lining cells of the bronchiole doesn't observed in this group, associated withglutathione, this result improved by (Sen ,1999),who refer to the ability of glutathione to reduces cell proliferation and increases apoptosis through the activation of several signaling pathways, including calcineurin, NF-_B, protein kinase B, c-Jun N-terminal kinase, apoptosis signal-regulated kinase 1,and mitogen-activated protein kinase.

Other organs like liver showed moderate inflammatory reaction associated with glutathione treatment .In the liver, the role of glutathione (GSH) as an antioxidant is especially relevantbecause it is its main site of synthesis, storage, and export refer by,(Lu, 2009; Lu, 2013).pointed tothe importance of GSHin the liver lies on the central role that this organ has as responsible for the oxidationand elimination of substances such as ethyl alcohol and other toxic products that induceoxidative stress; therefore, the liver requires the presence of antioxidant agents whichprevent or reduce this stress, either by trapping, metabolizing or transforming moleculesinto agents less toxic than ROS,while(Guerra, 2001; Wu et al., 2004) showed that glutathione plays a vital role in the protection against oxidative stress, since it traps ROS ,glutathione may react with different free radicals such as hydroxyl radical, hypochlorousacid,superoxide, peroxynitrite radical, and reduces hydrogen peroxide, thus being the firstcellular defense line against oxygen reactive species (Dröge,2002; Lu,2009) .The second defense line arethe antioxidant enzymes which glutathione uses as cofactor referred by (Mari*et al.*,2010; Pompella*et al.*,2003).glutathione can rapidly improve the ability of anti-oxidative stress and repair the membrane structure of liver cells, which is beneficial to the recovery of liver function and GSH can inhibit thesynthesis of cytokines, reduce aggregation of the activatedeffector cells, prevent the activation of effector cells, anddecrease the cytokine-induced damage to target cells withapromotion effect of cytokines on liver cell inflammationbecause during inflammatory processes, the cells of theimmune system are exposed to large amounts of free radicalsand toxic compounds; hence, these cells need an efficientglutathione system to scavenge the free radicals andtoxic compounds that can otherwise disturb immune functions(Dröge*et al* .,1994 ;Nakamura *et al.*,1997).Moreover, glutathione reduces the activationof nuclear factor κB (NF-κB) and inhibits the synthesisof interleukin 6 (IL-6), IL-8, and tumor necrosis factor α(TNF-α) (Rahman ,2000).

On the other hand**, Heart** demonstrated moderate lesion ,most of these lesions are closely associated with production of ROS and more specifically offree radicals (FR) that damage the structure of organelles and macromolecules (lipids, proteins, carbohydrates and nucleic acids) as a result of estrogen treatment (Cadet ,2014;Pascual*et al.*,2017).ROS produced in these reactionscan alter the intrinsic properties of the membrane such as fluidity, ion transport, lossof enzymatic activity, protein synthesis, DNA damage; which ultimately results in cell death insisted by(Krishnamurthy ,2012;Liu.; 2017).Glutathione plays an important role in the cardiovascular system because it is an importantantioxidant that restores intracellular redox equilibrium and prevents the inactivation ofnitric oxide produced by the endothelium(Kugiyama, *et al.*, 1998; Kugiyama, *et al.*, 2001;Moris,(2017),showed that excessive or sustained increase in ROS generation plays an essential role inendothelial dysfunction.

The kidney of this group showed slight lesion concerned with degenerative changes of the epithelial lining cells of the renal tubules,(Incalza,2018),returned this result to the effect of ROS which may promote endothelial dysfunction ED, the first change seen is endothelial activation, which is characterized byan aberrant pro-inflammatory and pro-thrombotic phenotype of blood vessel endothelialcells. ED is caused by a decrease in NO bioavailability, a decrease in vascular tone, andother phenotypic alterations in the endothelium (Lakshmi,2009)NO is a molecule with vasodilatoryproperties and plays a significant role in vascular homeostasis. High levels of superoxidemay react with NO and generate peroxynitrite, which is a harmful free radical. Onthe other hand, under certain conditions such as low availability of substrate or cofactors, endothelial nitric oxide synthase (eNOS) can produce superoxide instead of NO_ in acondition known as uncoupling. Also, ROS activate the MAPK pathway and inhibit NOSmRNA expression and eNOS activity (Higashi,2014). All these circumstances decrease the amount of NOand cause the loss of endothelial function. Other experimental study in Rate improved by (Zhang, *et al.*, 2017)has been suggested that GSH supplementation may protect cells from immunological cell damage by inhibiting the complement activation cascade and the binding of antibodies to antigens in isolated glomerular mesangial cells from rats,

Intestine of this group which is treated with glutathione appeared less affected by estrogen and demonstrated moderate lesion compared with the first group duo to the effect of Glutathione which is act as a reducing agent and an antioxidant, and involved in the metabolism of xenobioticsand different cell molecules, Glutathione is a free-radical scavengeras it is insisted by (Estrela *et al* .,2006). Glutathione not only plays a main role in intracellular redox balance (Meister, 1988) but is also pivotal in cellular processes such as cell differentiation, proliferation and apoptosis(Traverso*et al.*,2013) .Moreover,(Traverso*et al.*,2013: Meister.1991),pointed that Glutathione was associated with resistance to ionizing radiation and drug-induced cytotoxicity.

The **brain of** this groupshowed moderate lesion concerned with infiltration of inflammatory cell ,and there is no presence of edema compared with estrogen group ,as a result of glutathione supplementation, this result agree with (Hadzic et al.,2005),who is pointed that the immune system is highly affected by the levels of GSH in the body and even small changes in intracellular GSH levels can

affect lymphocyte activity. Hadzic have demonstrated that when depleting GSH from T cells isolated from mice, T cell proliferation and IL-2 production were reduced. More recently by (Zhang *et al* ., 2017), it has been suggested that GSH supplementation may protect cells from immunological cell damage by inhibiting the complement activation cascade and the binding of antibodies to antigens in isolated glomerular mesangial cells from rats, suggesting the possible use of GSH in the treatment of certain immune disorders .

Spleen of this group which is treated with Glutathione appeared slight normal appearances returned to the effect of Glutathione in cell-cycle regulation and microtubular-related mechanisms, which regulates Ca2+ homeostasis, protein function and gene expression, via thiol-disulphide exchange reactions, modulates lymphocyte functions and immune responses, and participates in the mitochondrial mechanisms that link opening of the permeability transition pore complex and activation of cell death (Estrela, 2006)

The male and female genital organs of this this group ,include the **uterus,ovary** and the **testes** showed moderate lesion .Reactive oxygen species(ROS) which is induced by estrogen may produce different pathological processes involving the reproductive tract .ROS can modulate cellular functionsand oxidative stress (OS) can disturb the intracellular milieu, antioxidants act to oppose ROS production, scavenging existing free radicals and promoting repair of ROS -induced damage to cell structures, insisted by (Agarwal and Allamaneni, 2004).The observation of our experimental study showed that the free radicals such as ROS induce lipid damage, inhibition of protein synthesis and ATP depletion (Ray *et al.,* 2004).

On the other hand testes demonstrated moderate lesion ,most of lesion associated with degenerative changes of spermatozoa ,because of the presence of ROS production that damage DNA of spermatozoa leading to defective fertilization (Agarwal *et al.*, 2006).Other scientist like (Lucky*et al.,* 2010) showed that excessivegeneration of ROS induced by estrogen may be lead to produce abnormal morphologically of spermatozoa. Others like (Duru*et al.,* 2000; Misro*et al.,* 2004),refer that when ROS elevated ,viability of the sperm will be affected via depletion of intracellular ATP and the subsequent decrease in axonemal proteins' phosphorylation,for the same reason .High concentrations ofROS induce lipid peroxidation and results in cell death ,insisted by (Agarwal and Prabakaran,2005).

In both these system glutathione play important role to decrease the pathological effect of estrogene,so the glutathione in the view of some scientist like (Hyman,2011),who consider the Glutathione is the greatestof all antioxidants, and the master detoxifier and maestro of the immune system. On the other hand,(Drigen, 2000),consider the glutathione one of the major endogenous antioxidant produced by cells participating directly in the neutralization of free radicals and reactive oxygen species, as well as maintaining exogenous antioxidants such as vitamins C and E in their reduced forms. The lesion of the uterus demonstrated moderate lesion than the first group duo to glutathione treatment influence oocytes, spermatozoa and embryos and their environment (Agarwal and Prabakaran2005). The role of glutathione in this changes as it is refer by (Cavalieri*et al.*,1997;Yager,2000), pointed that there is a series of

enzymes is involved in estrogen synthesisand metabolism, most of those enzymes, catechol-O-methyl-transferase (COMT) and glutathione S-transferases (GSTs) play an important role in the excretion of catecholestrogens (CEs). Catechol estrogens, metabolites of 17b-E2and estrone, are mainly inactivated by COMT. If theconjugation is incomplete, the CEs may be oxidized toCE-semiquinones (CE-SQ) and CE-quinones (CE-Qs).The CE-Qs, in turn, may be conjugated with glutathione,catalyzed by GSTs .Oxidative stress is directly toxic to cells, inducing apoptosis, so Glutathione depletion triggers apoptosis, insisted by (Mari,2009).

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