International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 23s, 2025
https://www.theaspd.com/ijes.php

Impact Of Estrogen Depletion Induced By Vinylcyclohexene Diepoxide (VCD) On Oxidant /Antioxidant , And Some Cardiac Biomarkers In The Female Rats

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Abstract

Estrogen plays a vital role in womens cardiovascular health before menopause by regulating cholesterol levels ,enhancing blood vessels function ,and reducing inflammation .after menopause ,the decline in estrogen increases the risk of heart disease .

The aim of the current study was to investigate the effects of low level of estrogen by VCD on some cardiac biomarker Troponine-1, Creatine Kinase-MB with oxidant peroxy nitrite and Malondialdehyde (MDA), antioxidant Glutathione (GSH) and nitric oxide (NO). effect of ovarian failure on gene expression α and β on the heart by ovaries failure by vinylcyclohexene (VCD) ,study the effect of the (VCD) on histopathology of heart and ovary. Sixteen female rats (8/group), the first set was the control group, and the second group received (VCD) 80 mg/kg BW/daily for 2 weeks as a VCD group. After collecting samples at the end of the experiment showed a significant increases in serum level of Cardiac Troponin-I,Creatin Kinase-MB, ONOO- , MDA, with a significant decreases in NO and GSH. also there were down regulation in ERα gene expression in heart tissue ,and there were a upregulation in ERβ gene expression compare with control group. The histopathological exam of the heart in VCD group showed shows myocardial degeneration with vaculoation of cardiomyocytes, necrosis and infiltration of neutrophils with inflammatory cell & interstitial hemorrhage, the aorta of VCD group increase thickness of vessels wall due to fibrosis with mononuclear cells infiltration.while in the ovary showed an degeneration of primary follicles with thickness of granulosa with marked vacuolation of interstitial cells with interstitial hemorrhage and sever fibrosis with sever congestion & edema of blood vessels and thickness of endothelium basement membrane with sever eosinophils & fibroblast infiltration .conclusion , In conclusion in the recurrent study revealed that estrogen depletion by VCD caused disturbance in the oxidants /antioxidants damage and Cardiac Troponin and Nitric Oxide , creatinine kinase with positive correlation between estrogen-ERα and negative correlation between estrogen-ERβ

Key words: *VCD, Troponin-1,Creatin Kinase, Female rates, estrogen ,gene expression α β*

INTRODUCTION

The main sex hormones in females, estrogens are involved in both the reproductive and non-reproductive processes. The liver, heart, muscle, bone, and brain are examples of non-reproductive tissues that may synthesise oestrogens. Tissue-specific oestrogen synthesis is consistent with a variety of oestrogen effects (Cui *et al.*, 2013). The placenta, adrenal cortex, and ovaries are where oestrogen is synthesised. Although the placenta takes over as the main location for oestrogen synthesis throughout pregnancy, placental oestrogen production requires input from the foetal and/or maternal adrenal cortex (Kaludjerovic & Ward, 2012).

The effects of estrogen are mediated through its nuclear receptors, ERα and ERβ as well as non-classical G protein-coupled estrogen receptor 1 (GPER1), which differ in tissue distribution and function. Previous studies suggest that the gene expression of these receptors may be influenced by endogenous estrogen levels via feedback mechanisms, reduced estrogen levels could upregulate the expression of one or both receptors

in specific tissues as a compensatory mechanism or suppress them depending on the tissue context (Tang et al .,2019)

Estrogen receptor α (ERα) is predominantly expressed in the uterus and pituitary gland with highest levels in the liver, hypothalamus, bone, mammary gland, cervix, testis, kidney, heart, skeletal muscle, and vagina. Estrogen receptor β (Erβ) expression is high in the prostate and ovary and found exclusively in the granulosa cells , Estrogen receptor α (ERα) activation promotes tumorigenesis in different types of cancer, including breast cancer and the role of ERβ is still unclear , Therefore, inhibition of the ERα has become one of the main strategies for the prevention and treatment of breast cancer (Belachew & Sewasew,2021)

the roles of estrogen receptors and estrogen signaling have been highlighted, with studies reporting their neuroprotective effects on the brain by promoting neurotrophins synthesis and protecting the brain from inflammation and stress(Hwang et al .,2020) E2 has been known to exert its cardioprotective effects by binding to the nuclear receptors ERα and ERβ. However, G protein–coupled receptor GPR30 (G protein–coupled estrogen receptor 1 or GPER) has also gained increased research attention over the past decade. GPR30 is localized in the endoplasmic reticulum and plasma membrane, and is known to be expressed in cardiomyocytes , E2 binds to GPR30 to exert rapid non-genomic events, triggering intracellular signaling cascades that alter gene expression downstream , Such effects are associated with reduced fibrosis, reduced oxidative stress, improved mitochondrial function, attenuation of cardiac hypertrophy, and stimulation of angiogenesis and vasodilation(Aryan et al .,2020)

Oestrogen exerts pleiotropic effects on the cardiovascular system. Its action varies depending on the receptor type and location within the cardiovascular system. In the cardiovascular system, ER-α and ER-β are present in the plasma membrane, cytoplasm, and nucleus , It has been confirmed that oestrogen prevents apoptosis and necrosis of cardiac and endothelial cells, reduces cardiac hypertrophy, and may have significant anti-inflammatory benefits in the ageing body, Oestrogen stimulates the production of nitric oxide (NO), as demonstrated in endothelial cells of the aorta in mice with ER-α receptor knockout , Through membrane oestrogen receptors, oestrogens activate signalling cascades crucial for cell survival and death(Bartkowiak-Wieczorek et al .,2024)

E2 is specifically secreted by the granulosa cells (GC) of developing antral follicles upon follicle-stimulating hormone (FSH) stimulation to strengthen follicular growth and maturation. The increased concentration of E2 contained in the follicular fluid of larger follicles further participates in the selection of a dominant preovulatory follicle, and finally triggers the luteinizing hormone (LH) surge required for ovulation(Chauvin et al .,2022)

For a long time, 4-vinylcyclohexene diepoxide (VCD) has been regarded as a dangerous workplace chemical that encourages ovarian failure. Nevertheless, VCD is also utilised as a study substance to chemically cause premature ovarian insufficiency (POI) in animal models (Cao *et al*., 2020). According to reports, VCD triggers the caspase-mediated apoptosis pathway, which results in the death of granulosa cells and typically results in the loss of ovarian follicles by inducing apoptosis (Sen Halicioglu *et al*., 2021).

In a further investigation, mice were given daily doses of VCD (160 mg/kg/d) or sesame oil for 10 or 20 days in order to eventually cause ovarian failure. On the day of follicle depletion, VCD decreased ($P < 0.05$) the number of primordial (by 93.2%) and primary (by 85.1%) follicles after 10 days of dosing, while nearly all primordial and primary follicles were lost ($P < 0.05$) after 20 days of dosing. The animals' reproductive function was assessed on days 10, 20, and 35 following the start of dosing. The typical duration of ovarian failure, These findings show that prolonged daily dosage can accelerate the rate at which VCD causes ovarian failure (Lohff *et al*., 2006).

Another study examined a mouse model of premature ovarian failure (POF) brought on by 4-vinylcyclohexene diepoxide (VCD), which depletes the follicular reserve by specifically destroying primordial and primary ovarian follicles. Researchers investigated systemic effects outside of the ovaries and optimised VCD timing and dosage (160 mg/kg over 15 days). Emotional disruptions and decreased bone density were among the POF consequences that the model accurately simulated. It also showed a connection between POF and

changes in the makeup of the gut microbiota that are linked to reproductive health. By providing information on systemic effects and directing future studies on ovarian failure and its wider physiological effects, our findings increase the usefulness of VCD-induced POF models. (Cao *et al.*, 2020)

Ovariectomy (OVX), a surgical procedure that involves removing the ovaries from female animals in order to study the effects of ovarian removal and the subsequent reduction in circulating sex hormones, particularly oestrogen, on various physiological and pathological processes, is another experimental method for producing cases of oestrogen deficiency. Numerous problems, including as osteoporosis, cardiometabolic disorders, and inflammatory diseases, have been studied using it (Zhang *et al.*, 2024).

One of the most frequent recognised causes of POF is radiation treatment (Nippita and Baber, 2007). Chemotherapy is less harmful to the ovaries than radiation, Follicle atrophy and a reduction in ovarian follicular reserve can result from direct DNA damage to ovarian follicles caused by ionising radiation. Due to insufficient exposure to oestrogen, this may accelerate the follicles' normal decline, impairing ovarian hormone production and causing uterine malfunctio (Sklar, 2005).

All cancer patients are primarily treated with chemotherapy; numerous studies have already shown that commonly used agents cause premature ovarian insufficiency (POI) by causing the death or rapid loss of primordial follicles and causing damage to blood vessels, stromal cells, and immune components (Reiser *et al.*, 2022). Investigating the effects of low oestrogen levels via VCD on several cardiac biomarkers, such as Troponine-1, Creatine Kinase-MB with oxidants peroxy nitrite and malondialdehyde (MDA), antioxidant glutathione (GSH), and nitric oxide (NO), was the goal of the current study. Examine how the VCD affects the histology of the ovary and heart.

MATERIAL AND METHOD

The animals were kept in plastic cages that were ventilated and had a 12-hour light cycle at 30 ±5°C. They were anaesthetised by inhaling chloroform following a two-week acclimatisation period and an overnight fast. Using sterile syringes, 5 mL of blood was extracted via heart puncture, put in gel tubes devoid of anticoagulant, and centrifuged for 15 minutes at 3000 rpm. Before analysis, the serum was kept at -20°C in Eppendorf tubes (Mahdi *et al.*, 2021).

a16 adult female rats were housed in the veterinary medical college's animal home at Kerbala University. The rats were split into two equal and random groups, with 8 rats in each group serving as the control group (GI) and receiving normal saline (IM). According to the methodology described by Muhammad *et al.* (2009), the animals in the second group were given 80 mg/kg BW of VCD (IM) every day for two weeks. Following the experiment, blood was drawn, serum was extracted, and the serum's parameters were assessed.

Estrogen (pg/ml) estimation of estronen level was measured using a kit manufactured by sunlong Biotech company by the method of (Mousa *et al* .,2009)

cardiac troponin I (pg\ml) estimation of cardiac troponin I (cTnI) was measured using a kit manufactured by sunlong Biotech company (Adams *et al* .,1994)

nitric oxide (NO) µmol\l estimation the method of (chang *et al* .,1998) was used a kit manufactured by sunlong Biotech company

creatine kinase (MB) ng\ml estimation (CK-MB) was used a kit manufactured by sunlong Biotech company by the method of (Penttilä *et al* .,2002)

Glutathione (GSH) ng/L estimation GSH was used a kit manufactured by sunlong biotech company by the method of .(mahdi *et al* .,2021)

malondialdehyde (MDA) ng/ml estimation MDA was used a kit manufactured by sunlong biotech company peroxy nitrate (ONOO-) pg/ml estimation (ONOO-) was used a kit manufactured by sunlong biotech company .

Histological study

Rats are killed at the conclusion of the exposure period, and their ovaries and hearts are removed for histological analysis. Following immediate removal of the heart and ovary, the heart and ovary were

cleansed with a cooled saline solution (0.9%) and the adhering fat and connective tissues were removed. The rat's ovary and heart were removed, immediately fixed with 10% formalin, treated with xylol and ordinary alcohol, imbedded in paraffin, and then cut into sections. The sections were stained with haematoxylin and eosin (H&E) stain. (Suvama et al .,2018)

Statistical analysis

Statistical analysis was done using graphpad prism software 8.0,using the T-test method to measure the differences between the two different groups

Ethical approve

This investigation was conducted in the physiology department ,university of karbala ,collage of veterinary medicine under reference number UOK.VET.PH.2024.098

RESULTS:

The mean values of serum estrogen at the end of the experiment were (17.40 ±0.17, 10.22 ± 0.41) for control group ,VCD group respectively as shown in figure (1),there was a significant decrease in VCD group when compared with control group.and this result agreement with (Brooks *et al* .,2016)

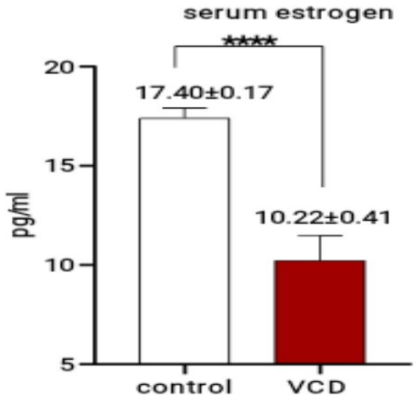

Figure (1) effect of ovarian failure induced 80 mg/kg/daily/2weeks IM injected of VCD in the serum estrogen in rats

A significant decrease in the serum NO and GSH in the VCD group compared with the control group (3.36 ± 0.13,2.69 ±0.18 ; 11.42 ± 0.89 ,7.17 ± 0.97) respectively and this result agreement with (cinthamony et al .,2024)

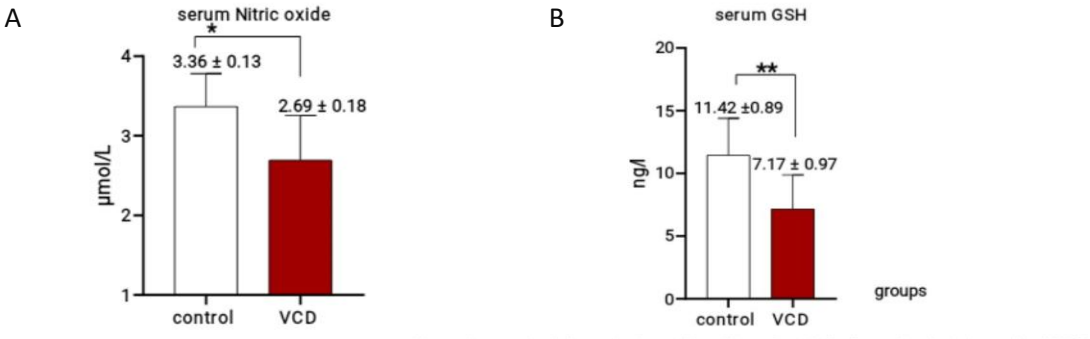

Figure (2) showed a significant decrease in the serum NO and GSH in the VCD group compared with the control group a significant increase in the serum MDA and ONOO- in the VCD group compared with the control group (27.90±0.99, 33.83±0.7; 11.42±0.89,7.17±0.97) respectively and this result agreement with (Behringer,2023)

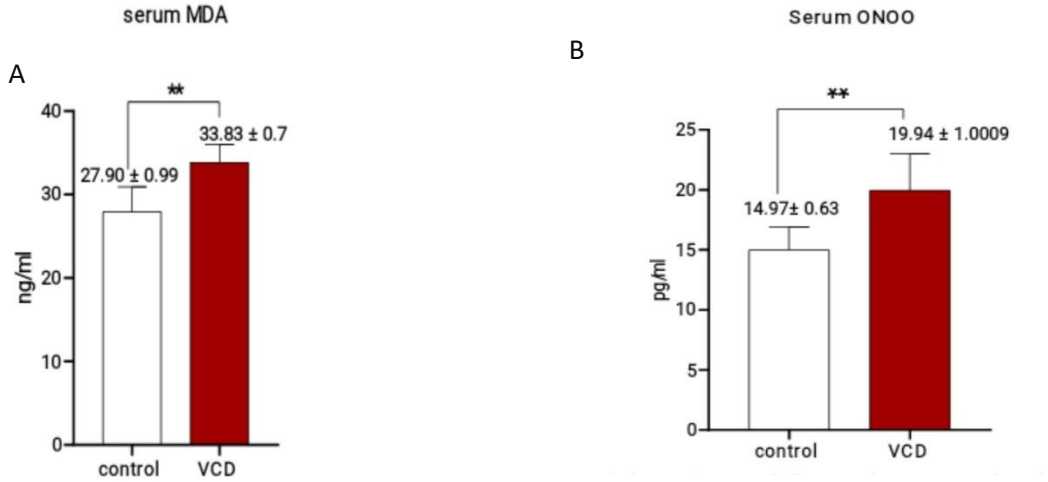

Figure (3) showed a significant increase in the serum MDA and ONOO- in the VCD group compared with the control group a significant increase in the serum Troponine-I and Creatin kinase-MB in the VCD group compared with the control group (24.60±0.5, 30.56±1.44; 1.51±0.07,2.33±0.12) respectively and this result agreement with (Vetter *et al* .,2024)

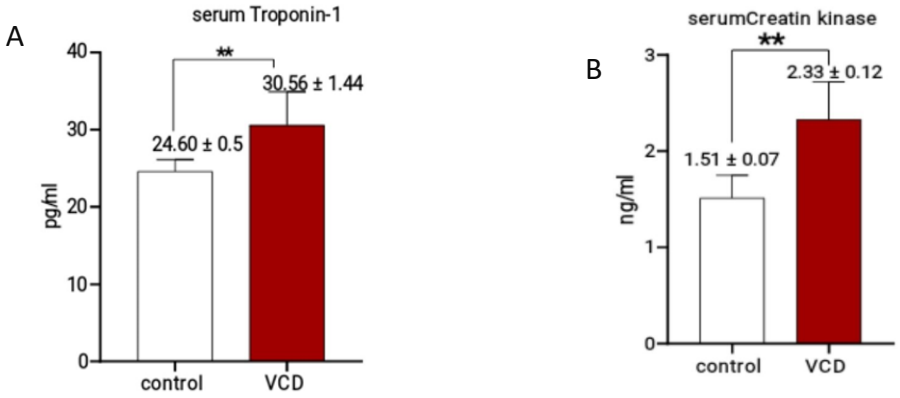

Figure (4) showed a significant increase in the serum Troponine-I and Creatin kinase-MB in the VCD group compared with the control group

In the current study there were a significant decrease in ERα gene expression in heart tissue in the VCD group as compare with the control group as shown in figure (5 and 6)

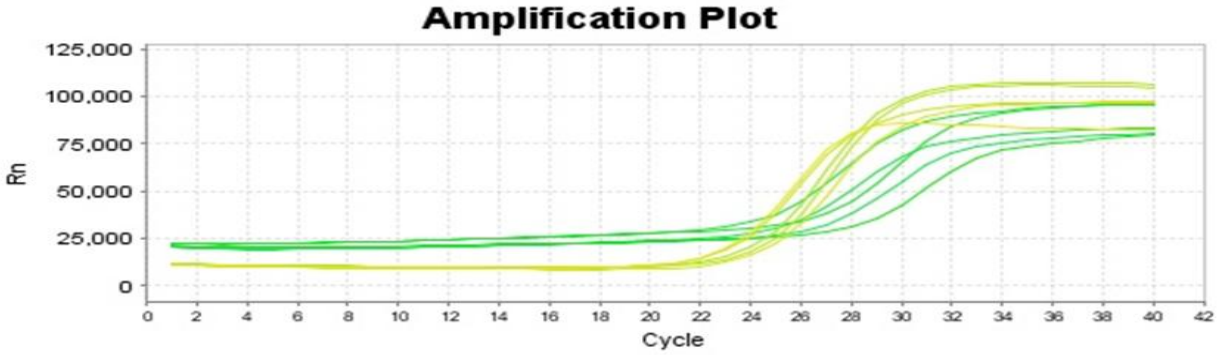

Figure (5): amplification curve of the tested samples represented the ERα gene. This indicate a successful RNA extraction and cDNA synthesis

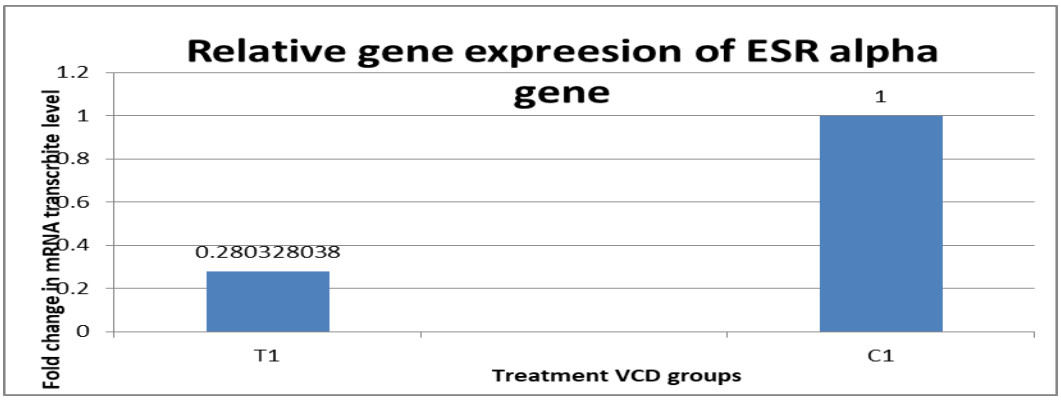

Figure (6): fold change comparison between the group expressed ERα gene . and show a significant decrease in the VCD group as compare with the control group. induced by 80 mg/kg/daily/2 weeks Im injected of VCD

In the curent study there were a significant increase in ERβ gene expression in the VCD group as compare with the control group as shown in figure (7 and 8).

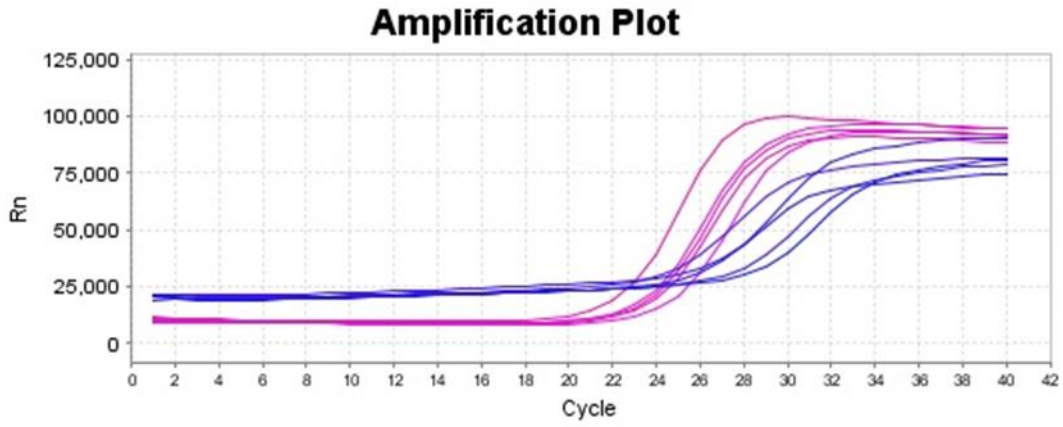

Figure (7): amplification curve of the tested samples represented the ERβ gene. This indicate a successful RNA extraction and cDNA synthesis

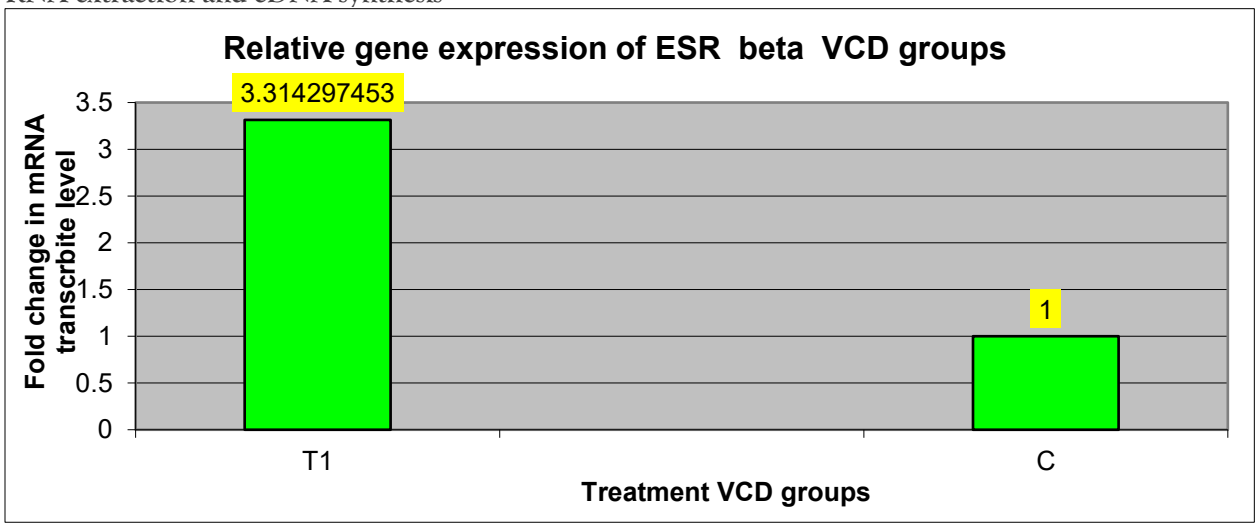

Figure (8): fold change comparison between the group expressed ERβ gene . And show a significant increase in the VCD group as compare with the control group. induced by 80 mg/kg/daily/2 weeks Im injected of VCD

The current study show normal ovary with mature Graafian follicle and well-defined zona pellucida and corona radiata with Liquor folliculi distends the antrum Well developed primary follicles (yellow arrow) with interstitial cells also show a Preantral follicles with Corpus luteum and corpus albicans with of dense irregular connective tissue.

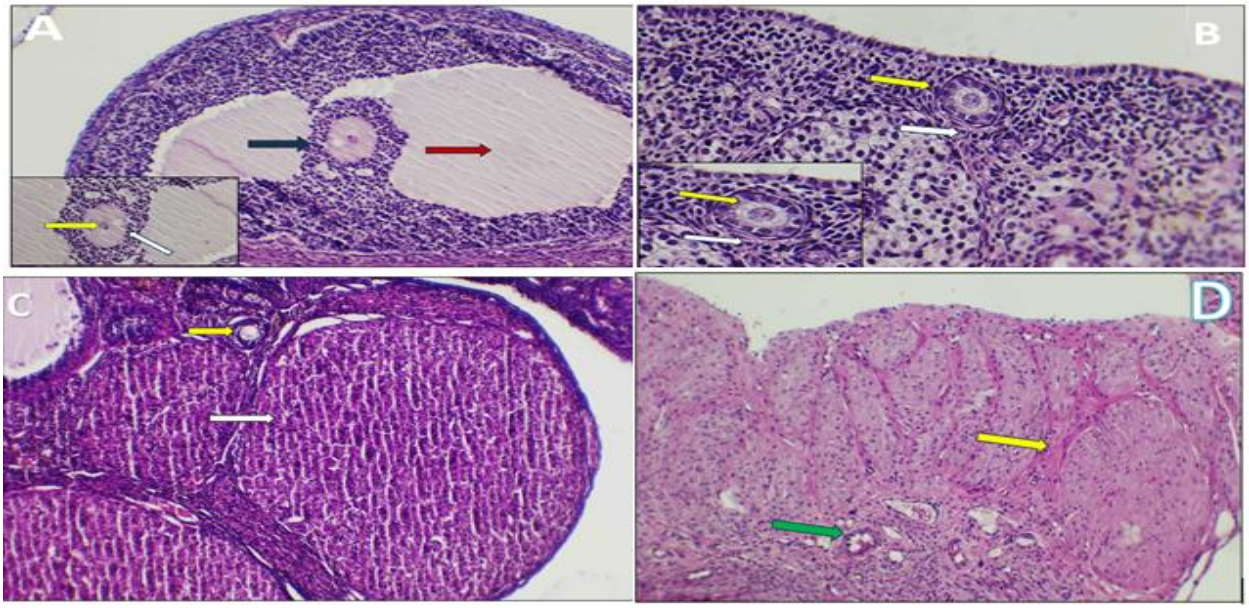

Figure (9A): histological section in the ovary of Control group at 14 days showed mature Graafian follicle has oocyte at a peripheral position (yellow arrow) & well-defined zona pellucida (white arrow) and corona radiata (black arrow) with Liquor folliculi distends the antrum red arrow. (9B) Well developed primary follicles (yellow arrow) with interstitial cells (white arrow Figure (9C) showed Preantral follicles (yellow arrow) with Corpus luteum (white arrow) (9 D) showed corpus albicans with of dense irregular connective tissue (yellow arrow) and some blood vessels (green arrow) (H&E stain X10&X40)

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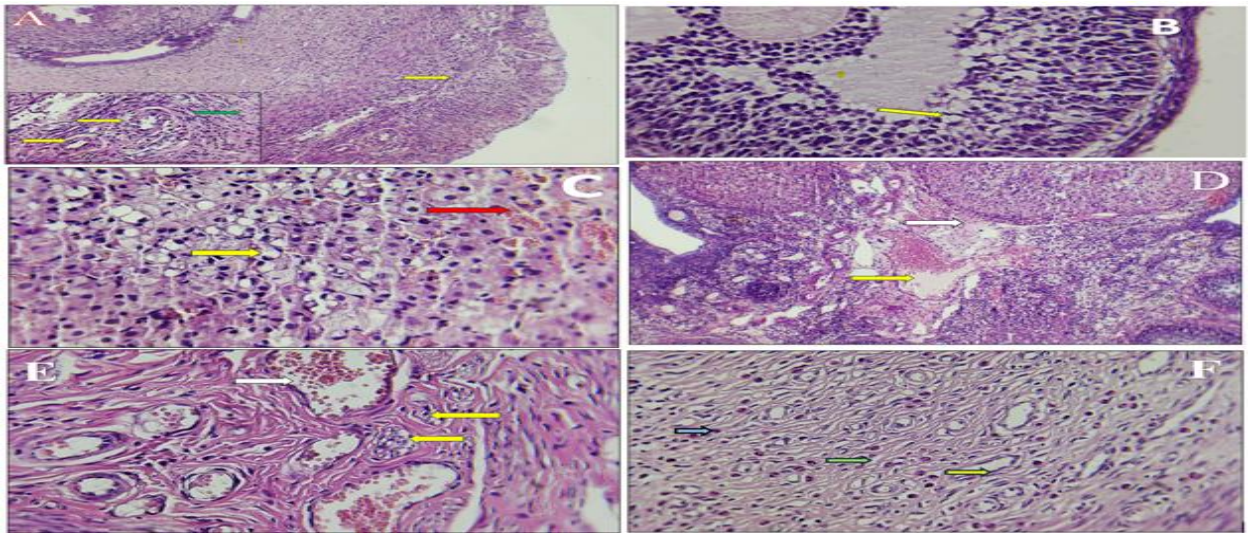

Figure (10A) histopathological section in the ovary of VCD group at 14 days showed degeneration of primary follicles (yellow arrow) (H&E stain X10+40) (10B) showing the thickness of granulosa with Marked vacuolation of zona pellucida (yellow arrow)(H&E stain X40) (10C) vacuolation of interstitial cells (yellow arrow) with interstitial hemorrhage(black arrow)(H&E stain X40) (10D) showed sever fibrosis (white arrow) with sever congestion & edema of blood vessels(yellow arrow) (H&E stain X10) (10E) showed degeneration

of growing follicles as well as primordial follicles collagen fibers (yellow arrow) surround congested blood vessels (white arrow) (H&E stain X40),(10F) showed thickness of endothelium basement membrane(yellow arrow) with sever eosinophils(green arrow) & fibroblast (blue arrow) infiltration (H&E stain X40).
In the current study the control group show normal histological myocardium cells and the Nucleus of the myocytes is represented, on the other hand the VCD group shows myocardial degeneration with vacuolation of cardiomyocytes, necrosis and infiltration of neutrophils with inflammatory cell & interstitial hemorrhage.

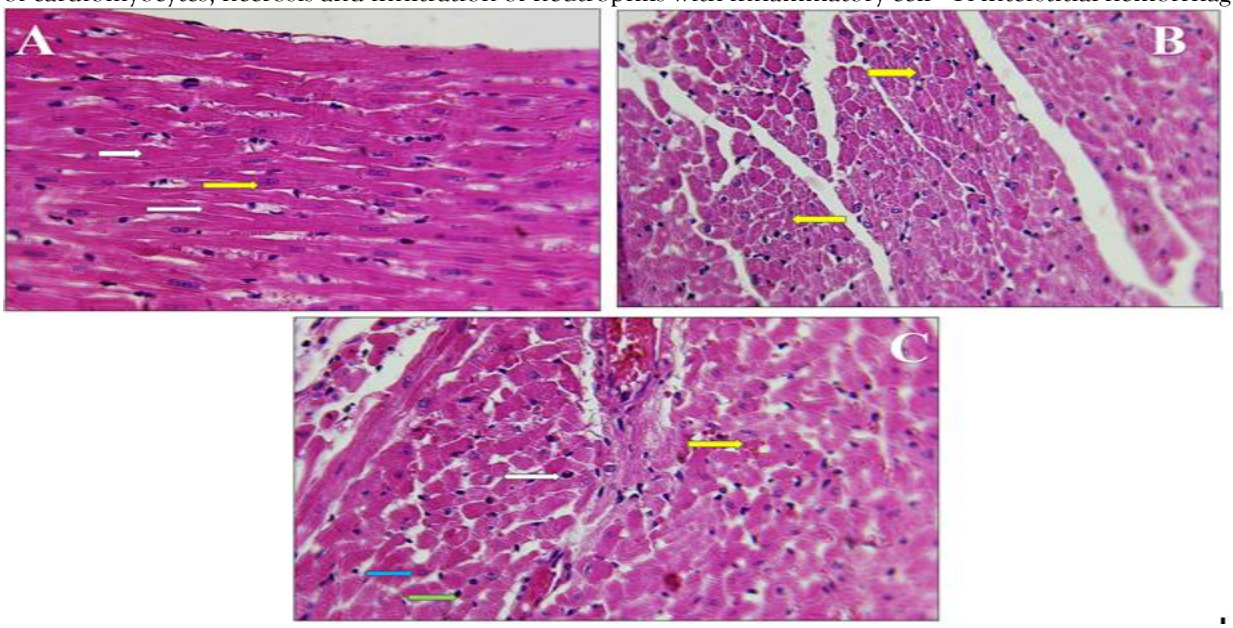

Figure (11A): histopathological section in the myocardium of control group at 14 days showed Nucleus of the myocytes is represented (yellow arrow) and muscle fiber is also indicated (white arrow) with intercalated discs (white arrow) (H&E stain X40). (11B): histopathological section in the myocardium of VCD group at 14 days showed myocardial degeneration with vacuolation of cardiomyocytes yellow arrow (H&E stain X4) . (11C): histopathological section in the myocardium of VCD group at 14 days showed necrosis (blue arrow) & infiltration of neutrophils (white arrow)with MNCs inflammatory cell (green arrow) & interstitial hemorrhage (yellow arrow)(H&E stain X40)

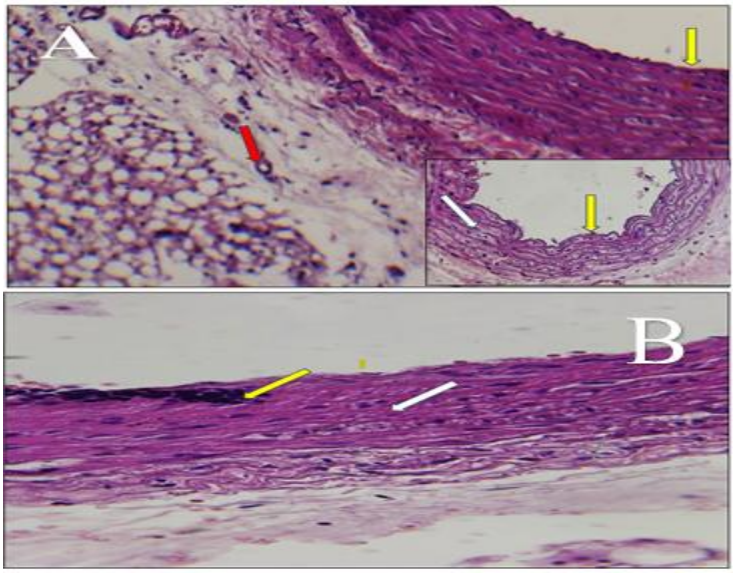

Figure (13A): histopathological section in the aorta of control group at 14 days showed Tunica intima (yellow arrow) & Tunica media that large number of fenestrated elastic lamella with smooth muscle fibers (white arrow) and Tunica adventitia dense irregular C.T. with small B.V.(black arrow) *(H&E stain* X10+40) F)(13B): histopathological section in the aorta of VCD group at 14 days showed increase thickness of vessels wall due to fibrosis (white arrow) with mononuclear cells infiltration (yellow arrow) *(H&E stain X10+40*

DISCUSSION:

In the current study there was a decrease in the level of estrogen in the serum in the VCD group compared to the control group as a result of ovarian failure**.**

The ova hazardous characteristics of VCD, an industrial chemical metabolite, are well known. According to studies, VCD only damages primordial and primary follicles, which are tiny pre-antral follicles, while leaving bigger, more mature follicles unaffected (Muhammed *et al*., 2009). These early-stage follicles have rapid atresia as a result of the bioactivation of VCD within the ovary. Ovarian failure results from this targeted follicular destruction, which also damages the ovarian reserve (Kappeler & Hoyer, 2012).

The depletion of primordial and primary follicles directly impacts estrogen synthesis, as these follicles are primary sites for estrogen production in the ovary. With the loss of these follicles, there is a marked reduction in circulating estrogen levels. This decline mirrors the hormonal changes observed during natural menopause, where diminished follicular activity leads to decreased estrogen production.(Mark-Kappeler *et al* .,2011)

Reactive epoxides are produced as a result of VCD's bioactivation in ovarian tissue. Larger, more mature follicles are unaffected by these reactive metabolites, but they specifically harm tiny pre-antral follicles, such as primordial and primary follicles. The process causes the granulosa cells of these follicles to undergo apoptosis, or programmed cell death, which accelerates their atresia and depletion. (Muhammad et al., 2009).

Granulosa cells found in primordial and primary follicles are in charge of aromatising androgens to produce oestrogen (Gervásio *et al*., 2014). The number of granulosa cells drastically drops when VCD depletes these follicles, which lowers aromatase activity and, in turn, lowers the generation of oestradiol. The ovary's capacity to make oestrogen is directly impacted by the loss of these early-stage follicles, which causes a noticeable drop in the amount of oestrogen in the blood. (Mayer *et al*., 2004).

Because it regulates NO, oestrogen is essential for preserving vascular homeostasis. When oestrogen binds to endothelial cells' ERα and ERβ receptors, it triggers signalling cascades such the PI3K/Akt pathway, which phosphorylates and activates eNOS. By converting L-arginine to NO, this enzyme lowers inflammation, inhibits platelet aggregation, and promotes vasodilation. (Robert, 2023 and Chakrabarti *et al*.,2008)

Additionally, oestrogen maintains NO bioavailability by upregulating antioxidant defences and suppressing reactive oxygen species (ROS). Accordingly, endothelial dysfunction is closely associated with oestrogen deprivation (Strehlow *et al.*, 2003).

Reduced eNOS phosphorylation results from ERα/ERβ's inability to activate the PI3K/Akt pathway in the absence of oestrogen binding. Research indicates that mice with ER deletion had reduced baseline NO levels and compromised vasodilation. (Khalil, 2013)

The transcription of the eNOS gene is upregulated by oestrogen. Long-term oestrogen loss in VCD-treated mice reduces endothelial cell levels of eNOS mRNA and protein, which restricts the ability to produce NO. Lee and Knowlton (2012).

L-arginine is a necessary substrate for NO production. Through cationic amino acid transporters (CAT-1), oestrogen increases the absorption of L-arginine. Decreased CAT-1 expression restricts substrate availability in hypoestrogenism, which affects eNOS activity. (Arnal *et al*, 2010)

In conditions when oestrogen levels are low, ADMA, an endogenous eNOS inhibitor, is increased. Dimethylarginine dimethylaminohydrolase (DDAH), which degrades ADMA, is often enhanced by oestrogen. Reduced DDAH activity after VCD permits ADMA to build up, further inhibiting the generation of NO. (Xia *et al.*,, 2014).

The oestrogen shortage brought on by VCD is considerably exacerbated by oxidative stress. Through GST-mediated conjugation, the electrophilic diepoxide groups in VCD deplete glutathione (GSH), the primary antioxidant in cells, impairing granulosa cells' capacity to combat the accumulation of reactive oxygen species (ROS) (Song *et al.*,2023). ROS formation, including superoxide ($O_2 \bullet^-$) and hydrogen peroxide (H_2O_2), is further increased in these cells by mitochondrial dysfunction, which is characterised by disturbances in electron transport chains (ETC) (Cozzolino,2023).

When ROS generation exceeds cellular antioxidant capability, oxidative stress results. As the primary redox regulator, glutathione (GSH), a γ-glutamyl-cysteinyl-glycine tripeptide, directly neutralises ROS and supports antioxidant enzymes including glutathione reductase (GR) and glutathione peroxidase (GPx) (Sies & Jones 2020). Research indicates that exposure to VCD disrupts redox equilibrium by reducing ovarian granulosa cell GSH by 40–50% in a 24-hour period (Devine *et al.*, 2001).

MDA, on the other hand, is a sign of oxidative membrane damage and is generated during lipid peroxidation. Chronic diseases such as ovarian dysfunction are associated with elevated MDA levels, which are correlated with reduced cell integrity (Shi *et al.*, 2023).

These ROS inhibit CYP19A1 and the steroidogenic acute regulatory (StAR) protein, which are essential for the movement of cholesterol and the activity of aromatase. Lipid peroxidation produces malondialdehyde (MDA), which combines with aromatase to generate inactive adducts that exacerbate damage. Studies conducted on human granulosa cells (KGN cells) treated with VCD showed a 40% decrease in aromatase activity in addition to increased MDA, establishing a clear connection between oxidative stress and impaired oestrogen production (Zhou *et al.*,2023).

Reactive intermediates produced by the metabolism of VCD trigger NADPH oxidase to generate $H2O_2$. In Fenton reactions, excess H_2O_2 combines with Fe^{2+} to produce hydroxyl radicals ($\bullet OH$), which remove hydrogen atoms from polyunsaturated fatty acids (PUFAs) and start lipid peroxidation (Kwiecień *et al.* 2020).

Additionally, VCD causes granulosa cells' mitochondrial electron transport chains (ETC) to malfunction, which increases electron leakage and the generation of superoxide ($O_2 \bullet^-$). Lipid peroxidation is fuelled by mitochondrial ROS (mtROS), especially in ovarian follicles that are rich in mitochondria (Abolaji *et al.*, 2016). Follicle susceptibility to ROS-induced apoptosis is increased when GSH levels are low because it disrupts the thioredoxin and peroxiredoxin systems. Two important indicators of VCD-induced follicular degeneration are cytochrome c release and caspase-3 activation (Zhang *et al.*, 2022).

Because oestrogen plays a crucial role in regulating antioxidant defences, peroxynitrite (ONOO), a reactive nitrogen species associated with oxidative stress, rises as oestrogen levels fall. By directly neutralising free radicals like superoxide (O_2^-) and nitric oxide (NO), as well as by upregulating enzymes like glutathione

peroxidase and superoxide dismutase (SOD), oestrogen improves cellular resilience (Olufunmilayo & Holsinger, 2023).

O_2^- and NO may build up as oestrogen levels fall because the body's antioxidant ability declines. Peroxynitrite ($O_2^- + NO \rightarrow ONOO^-$), a strong oxidant that damages lipids, proteins, and DNA and exacerbates tissue failure, is created when these molecules quickly combine. Studies demonstrating increased oxidative stress indicators in low-estrogen situations indicate the association between elevated peroxynitrite levels and illnesses such as chronic inflammation, dementia, and cardiovascular disease (**Schieber & Chandel.**, 2014).

Women's cardiovascular health is maintained in large part by oestrogen, and the reduction of oestrogen that comes with menopause can raise the risk of cardiovascular disease. In addition to being direct (rapid vasodilation) and indirect (involving changes in the genetic expression of proteins that regulate vascular tone and the response to injury), estrogen's cardioprotective effects are mediated by ER-α and ER-β (Mendelsohn, 2002).

This vasodilatory action helps to keep blood flow at its best and lessens cardiac strain Additionally, it has been demonstrated that oestrogen has anti-inflammatory properties, lowers oxidative stress, and positively affects lipid profiles by raising HDL and lowering LDL levels. (Fan and others, 2021).

Blood arteries stay dilated and blood flow is maintained under ideal circumstances because oestrogen can stimulate the creation of nitric oxide. (KE Kypreos and others, 2014) Vasoconstriction and increased arterial stiffness result from endothelial function deteriorating due to oestrogen shortage. These alterations may result in an increased heart workload and increase the risk of ischaemic damage to myocardial tissue. Even at a subclinical level, occasional ischaemia of the heart muscle causes cTnI to be released into the bloodstream. (Hillegass *et al.*, 2010)

The antioxidant activity of oestrogen is another important component of its cardioprotective actions. In the cardiovascular system, oestrogen lessens oxidative stress and counteracts free radicals. (Siow and others, 2007) The heart is more susceptible to oxidative injury when there is insufficient oestrogen present. In addition to affecting myocardial function, this rise in oxidative stress can trigger inflammatory pathways that lead to the degeneration of cardiac cells (Wassmann *et al.*, 2001). As a result of the accumulated stress and damage to the heart, injured cardiac cells produce cTnI. (Weil *et al.*, 2018)

Myocardial tissue is better able to withstand the stresses of an increased workload or mild injuries when oestrogen is present. (Persky *et al.*, 2000) On the other hand, maladaptive remodelling of the heart muscle results from the disruption of this adaptive process caused by oestrogen shortage. This may eventually lead to decreased contractility, fibrosis, and an increased susceptibility to cardiac damage. (Patel, 2015)

In order to ensure effective cardiac contraction, oestrogen affects the control of calcium channels and intracellular calcium homeostasis. Dysregulation of calcium homeostasis may arise in estrogen-deficient situations, affecting myocyte function and possibly resulting in cellular damage. The resulting stress and injury to the cardiomyocytes can cause cTnI to be released, indicating that the heart muscle's integrity is being jeopardised. (Dworatzek & Mahmoodzadeh, 2019).

Increases in CK-MB were only supposed to be a sign of heart damage, not myocardial infarction. Creatine kinase MB (CK-MB) has been widely employed as a diagnostic marker for skeletal muscle damage and myocardial infarction. Serum CPK and serum CK-MB activity fluctuation is influenced by a number of variables, including physical exercise (Schneider *et al.*, 1995). When comparing the VCD group to the control group, it was found that the amount of creatine kinase MB rose. This suggests that a drop in oestrogen levels damages the cardiac cells, which is in line with (Majumdar and Nirwane ,2016)

The antioxidant enzymes glutathione peroxidase and superoxide dismutase (SOD), which counteract reactive oxygen species (ROS) and stop oxidative damage to muscle cells, are enhanced by oestrogen. (Massafra *et al.*, 2000)

By blocking nuclear factor-kappa B (NF-κB) signalling and suppressing pro-inflammatory cytokines including interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF-α), oestrogen lessens inflammation-induced muscle deterioration. (Ghisletti and colleagues., 2005)

By enhancing sarcoplasmic reticulum (SR) activity, guaranteeing effective calcium reuptake through SR calcium ATPase (SERCA), and avoiding cytoplasmic calcium excess, oestrogen controls calcium handling in muscle cells (Romero-Martínez *et al.*, 2023).

The observation of a significant decrease in estrogen receptor alpha (ERα) gene expression in the cardiac tissue of the 4-vinylcyclohexene diepoxide (VCD)-treated group, compared to controls, provides critical insights into the molecular mechanisms underlying the cardiovascular alterations previously reported in this experiment (increased heart rate, reduced P/QRS amplitudes). ERα, a nuclear receptor that mediates the genomic effects of estrogen, plays a central role in maintaining cardiovascular homeostasis by regulating cardiac contractility, ion channel function, and protection against hypertrophy, fibrosis, and oxidative stress(Puglisi et al .,2019). This finding suggests that VCD-induced ovarian failure and estrogen depletion disrupt not only hormonal signaling but also the heart's intrinsic capacity to respond to estrogen, potentially exacerbating cardiac dysfunction.

ERα expression is modulated by ligand-dependent and ligand-independent mechanisms. Estrogen binding stabilizes ERα and enhances its transcriptional activity, while estrogen deficiency can lead to receptor downregulation(Mal et al ., 2020).the VCD group ovarian follicular depletion causes systemic estrogen loss, which likely drives cardiac ERα downregulation. This creates a feedforward loop that can lower estrogen reduces ERα expression, further blunting the heart's ability to respond to residual estrogen, amplifying dysfunction (Menazza & Murphy., 2016).

Also, VCD generates reactive oxygen species (ROS) in ovarian tissue, and systemic oxidative stress may extend to the heart. ROS can decrease ERα via decrease DNA Methylation which Oxidative stress promotes hypermethylation of the ESR1 promoter, silencing transcription(Mahalingaiah et al ., 2015).Also study found that ROS can inhibit histone acetyltransferases (HATs), reducing chromatin accessibility at ESR1 loci (Dumasia et al .,2017).In addition, ROS-induced inflammation upregulates NF-κB, which represses ERα transcription by competing for transcriptional coactivators (Nakajima & Kitamura.,2013).

ERβ's regulation of potassium channels might stabilize repolarization, potentially explaining why T wave parameters remained unchanged despite ERα reduction (Yang et al ., 2012).Study found that ERβ enhances mitochondrial biogenesis and oxidative phosphorylation improving energy efficiency in cardiomyocytes(Mahmoodzadeh & Dworatzek., 2019). Also, Rodent studies report increased cardiac ERβ expression post-ovariectomy, coinciding with ERα downregulation(Naaz et al ., 2002).Although, the ERβ upregulated in ischemic hearts, where it attenuates apoptosis and inflammation (Wang et al .,2009).

In the curent study the VCD group show a degeneration of primary follicles with thickness of granulosa with Marked vacuolation of zona pellucida also vacuolation of interstitial cells with interstitial hemorrhage and sever fibrosis with sever congestion & edema of blood vessels and thickness of endothelium basement membrane with sever eosinophils & fibroblast infiltration

The VCD group's primary follicle degeneration exhibits notable histological alterations, underscoring the detrimental impact of chemical exposure on ovarian function. Increased follicular atresia and apoptosis, which severely impair folliculogenesis, are among the study's major findings (Cao *et al.*, 2020).

A continuous degenerative process is suggested by the thickening of granulosa cells, cytoplasmic vacuolation, and loss of intercellular cohesiveness. Follicle disintegration, which eventually results in decreased reproductive function, is greatly influenced by this structural compromise (Li *et al.*, 2024).

Significant vacuolation, fragmentation, and weakening of the zona pellucida also occur, which impairs oocyte viability in addition to its protective role. Interstitial cell vacuolation exacerbates ovarian insufficiency by indicating metabolic dysfunction and decreased steroidogenic activity (Sen *et al.*, 2021).

Interstitial haemorrhage, which is characterised by widespread blood extravasation and capillary rupture, is another important histological characteristic seen. Haemorrhage is a key component of ovarian toxicity

because of the accelerated follicular degeneration caused by the increased vulnerability to oxidative stress and inflammation (Özel *et al*., 2020).

Serious eosinophil and fibroblast infiltration characterises the inflammatory response in VCD-induced ovarian damage. According to (Cao *et al*.,2020), fibroblast proliferation leads to excessive collagen deposition, which causes fibrosis, whereas eosinophilic infiltration indicates a continuous inflammatory process fuelled by oxidative stress and cellular damage.

This protracted inflammatory condition is a major factor in the development of ovarian failure and worsens tissue damage. Overall, the histological results in ovarian tissue exposed to VCD show a complicated interaction between inflammation, vascular anomalies, fibrosis, and degenerative alterations (Qin *et al*., 2024).

Oestrogen insufficiency in VCD patients may have an impact on the heart. By encouraging vasodilation through the generation of nitric oxide, oestrogen improves endothelial function. This action is lessened by low oestrogen, which might limit blood flow and perhaps cause ischaemic damage to heart tissue, which can result in degeneration (Reckelhoff, 2006).

In the present study the control group show normal histological section of aorta with normal Tunica intima & Tunica media that large number of fenestrated elastic lamella with smooth muscle fibers. The aorta of VCD group increase thickness of vessels wall due to fibrosis with mononuclear cells infiltration

Additionally, because oestrogen has anti-inflammatory and antioxidant qualities, a lack of it may raise inflammatory cytokines and oxidative stress, which can lead to cardiomyocyte damage and fibrosis (Crescioli, 2021).

Furthermore, oestrogen maintains the integrity of the mitochondria by restricting the opening of the mitochondrial permeability transition pore (mPTP), which stops the release of cytochrome c and the start of apoptosis. Furthermore, oestrogen reduces oxidative stress by suppressing ROS generation by blocking NADPH oxidase and strengthening antioxidant defences through Nrf2 activation (Simpkins & Dykens, 2008). Further opposing pro-fibrotic pathways, ERβ reduces collagen deposition and cardiac stiffness, whereas oestrogen enhances contractility via modifying calcium-handling proteins. Low oestrogen throws these defences off balance, leading to oxidative damage, fibrosis, mitochondrial malfunction, and apoptosis. As indicators of cardiac degeneration, this cascade encourages the loss of cardiomyocytes, structural remodelling, and compromised contractile performance (Simpkins & Dykens, 2008; Klinge, 2017).

Conclusion:

In conclusion. VCD induced estrogen depletion, which impacts the serum Oxidant /Antioxidant balance with positive correlation between estrogen-ERα and negative correlation between estrogen-ERβ, damaging the heart, leading to a risk heart disease

Acknowledgments: In acknowledging the animal house of karbala University that provided the animals, the authors express their gratitude

Novelty Statement: This study provides a new insight into the relationship between ovarian failure and its result in the administration of VCD, which leads to a decrease in the percentage of serum estrogen. Thus, there was a decrease in estrogen alpha receptors in the heart, as well as an increase in estrogen beta receptors in the heart. This reveals the effect of estrogen deficiency on the heart muscle and its health in females. Unlike other studies that dealt with only one topic of estrogen deficiency, this study focused on all aspects from the aspect of antioxidant deficiency, high oxidation indicators, as well as high levels of cardiac enzymes in the blood as a result of ovarian damage and low levels of estrogen in the blood. The genetic aspects were also taken into account in the study.

Author Contributions: Dr. Wefak Jbori Albazi and Dr. Khawla Ibrahim Adel conceptualized and supervised the study, contributed to the experimental design, and provided scientific guidance throughout the research process.

Dr. Haider Al-Karawi conducted the laboratory experiments including examination of gene expiration of alpha and beta estrogen receptors in the heart .analysis of the serum estrogen, antioxidant and heart parameters.

Dr. Wefak Jbori Albazi and Dr. Khawla Ibrahim Adel analyzed the experimental data, reviewed related literature, and prepared the initial draft of the manuscript.

All authors contributed to the interpretation of results, revised the manuscript critically, and approved the final version for submission.

Conflict of interest: The authors have no conflict of interest to declare.

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