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# Effect of Pyrethroid on Fertility of Adult Rats

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**ABSTRACT**

**Sixty three Adult female albino rats were randomly divided into seven groups as fellow group (1) normal control, Inductive group (1) in this group animals received low dose 12 mg/kg) from permethrin (perm), Inductive group (2),in this group animals received high dose ( 75mg/kg.) from permethrin, group 4: treated group, animals received vitamin (Vit) E. (500 mg/ mg/Kg.) plus low dose of permethrin (12 mg/k.) group 5: treated group, animals received Vit E. (500 mg/ mg/Kg.) plus high dose of permethrin (75 mg/k.) ,Group 6: treated group, animals received Vit E. (250 mg/ mg/Kg) plus low dose of permethrin (12 mg/k. ) and group 7: treated group, animals received Vit E. (250 mg/ mg/Kg.) plus low dose of permethrin (75 mg/k. ) every doses received daily. At the end of the experimental period Blood samples were withdrawn,we determine triglyceride (TG), total cholesterol (TC), blood urea and creatinine. Also Estradiol and progesterone, total $T_3$, $T_4$ and TSH levels in plasma of female were measured. The excised ovary in each group used to measure MDA, as a marker for lipid peroxidation (LPO) and GSH content in ovary tissue. In the same time the ovarian tissue which were removed after sacrifice, it was used for histological examination for the evaluation and assessment of the d fibrous formation, in injuries tissue. Permethrin with different doses (12 and 75 mg/kg respectively) significantly ($p \leq 0.05$) increased the MDA and significantly decreased ($p < 0.05$) in GSH content in ovary tissue versus control animal. Though treatment with vitamin E after permethrin with different dose (250 and 500 mg/kg respectively) significantly deceased the MDA content versus permethrin group (12 and 75 mg/kg respectively) and Vit E treatment increased GSH content in a group treated with (permethrin 75 mg/kg b.w). The level of both serum estrogen (E2) and progesterone were significantly ($p<0.05$) decreased in a permethrin group in both**

**doses (12 and 75 mg/kg) when compared to control group. While, treatment with Vit E ameliorate the level of E2 and progesterone. Treating rats with permethrin led to a significant decrease in triiodothyronine (T3) and thyroxine (T4) as well as a significant increase of thyroid-stimulating hormone (TSH) when compared to control group. Administration of Vitamin E and permethrin caused an improvement in the levels ofT3 and T4 and TSH.This study was designed to elucidate premethrin effects at doses (12and75mg\kgw b) for 30 days, causing reproductive toxicity through several biotransformation metabolites processes. Focusing on ovarian injures and reproductive efficacy. treated ovarian tissue showed histological alterations represented by reduction number of different follicles, with absent mature follicles in high dose premethrin, or follicles without oocytes and zone granulose residual with vacuolar change finally necrotic areas. Special stain for connective tissue fibersMasson'strichrome, showed accumulation of fibrous proportional to the dose. while the Immuno- reactive of TNF-ɑ assayment many necrotic area evidence of premethrin toxicity, also its dwindling effect on proliferating cell nuclear antigen (PCNA)which affected the efficiency of DNA, and consequently on the cell proliferative activity. In contrast, evaluate the effective therapeutic role of Vitamin E at doses (250 and 500 mg \kg bw),as an antioxidant clarified, which is explained by the histological in the results. This study reveals that chronic exposure to premerthin even low dose alters ovarian cycle activity . Hence, Premethrin exposure threat fertility of humans and animals.it is speculate that the positive effect of the Vitamin E by its antioxidant activity able to protect ovarian tissues the premerthin toxicicity**

## INTRODUCTION

Pesticides have increased with the increasing awareness of their utility in agriculture, animal husbandry, post-harvest technology and in public health (Milne, 2000). Pesticides may cause reproductive toxicity through several mechanisms; direct damage to structure of cells, interference with biochemical processes necessary for normal cell function and biotransformation resulting in toxic metabolites ( McLachlan, 2001; Anju and Bechan, 2010). Pyrethroids as one of these categories, is a group of synthetic chemical analogs of the naturally occurring toxin pyrethrin . It is derived from the dried flowers of Chrysanthemum cinerari-aefolium. They are very stable in sunlight and generally effective against most agricultural insect pests when used at very low doses (Anudurga and Jayanthi 2018). Pyrethroids are considerably less toxic to mammals than other classes of insecticides like organochlorines, organophosphates, and carbamates. Because of their low human toxicity, pyrethroids are widely used to control insects in agriculture and in the home (Anju and Bechan 2010). In this study, we used permethrin. Permethrin is an example of the pyrethroid insecticides, which are used to control a wide varietyof insects in agricultural, veterinary and domestic uses. It is used in sprays, pet flea shampoos, lice shampoos, municipal mosquito abatement products, and aerosol bombs. It is also used on agricultural crops, particularly fruits and vegetables. Permethrin and other pyrethroid insecticides have also been measured in high proportion of household dust samples (Meeker *et al*., 2009). The European Union classifies chemicals and labeled permethrin as hazardous to the environment (http://echa.europa.eu). Many studies have reported undeniable links between insecticides and serious health consequences including endocrine disruption, fertility problems (Colborn*et al*., 1993; George *et al*., 2002 ), cancers (Bassil*et al*., 2007). There are some data, albeit limited, regarding altered reproduction or endocrine function due to pyrethroid

insecticides in animal and in vitro studies that showed that some pyrethroid insecticides and their metabolites may cause endocrine disruption and have adverse effects on semen quality (Meeker *et al*., 2009). Also, some reports have shown destructive effects on hormones of the endocrine system.

Vitamin E (α-tocopherol)) is a fat-soluble antioxidant in cells and protect cellular membranes and lipoproteins from peroxidation. In addition, vitamin E has an effective role in inhibiting the free radical formation and so reduce lipid peroxidation in biological systems (Xiaoyuan and Peter, 2000). The previous study had shown that vitamin E deficiency can lead to infertility due to its polyphenol components rapidly generates free radicals and protects activity of testis (Jalili *et al*., 2014). Furthermore, antioxidants have a substantial role in the female reproductive (Mulla *et al*., 2018).

The present study has been planned to investigate the biochemical and histopathological effects of permethrin, on the proliferation of ovarian follicles in adult rats and evaluate the protective role of vitamin E in rat ovarian tissue.

## MATERIALS AND METHOD

### Materials

Vitamin E (α-tocopherol acetate) was purchased from Sigma and Permethrin from NODCAR

### Animals

Sixty (60) Adult female albino rats (180 ± 20 g) obtained from the National Organization for Drug Control and Research (NODCAR), Giza, Egypt, was used in this study. The rats were housed in wire mesh fence cages for two weeks to become acclimated to the environment. During the entire research, the rats were kept under standard conditions (temperature 25 ± 2oC, 12 hours. light and, 12 hours. darkness cycles). Animals were fed a pellet standard rat diet and water *ad libitum*. Maintenance of the animals was approved by the animal ethics committee in accordance with the guide for the care and use of animals by NODCAR.

### Experimental design

The rats were randomly divided into seven groups (6 rats each) and treated as follow:
Group 1: Normal control, animals received distilled water (0.5 ml /kg. b. w. p. o. daily).
Group 2: Inductive group (1), in this group animals received low dose (1/125 LD50 12 mg/kg. b.w. p. o, daily) from permethrin (Cantalamessa 1993).
Group 3: Inductive group (2),in this group animals received high dose (1/20 $LD_{50}$ 75mg/kg. b.w. p. o, daily) from permethrin (Cantalamessa 1993).
Group 4: treated group, animals received low dose of permethrin (12 mg/k. b.w.p.o daily) Vit E. and then received (500 mg/ mg/Kg. b.w.p.o) after half hour.
Group 5: treated group, animals received high dose of permethrin (75 mg/k. b.w.p.odaily) and then received Vit E. (500 mg/ mg/Kg. b.w.p.o) after half hour.
Group 6: treated group, animals received low dose of permethrin (12 mg/k. b.w.p.o daily) and then received Vit E. (250 mg/ mg/Kg. b.w.p.o) after half hour.
Group 7: treated group, animals received high dose of permethrin (75 mg/k. b.w.p.odaily) and then received Vit E. (250 mg/ mg/Kg. b.w.p.o) after half hour.

The experiment maintained for 30 days. At the end of the experiment, serum will collect under anesthesia (50 mg sodium pentobarbital i.p) using orbital sinus technique and tissues (ovary and uterine) take after humanity decapitation of rats.

**Blood samples and tissue collection**

At the end of the experimental period, all animals are fasted overnight. Blood samples were withdrawn from the retro-orbital plexus vein. Serum was separated by centrifugation at 3000 rpm at 4$^{o}$C and stored at -20 °C until further biochemical analysis.

The excised ovary in each group was fixed with 10% formaldehyde for histopathological examination, and the remnants of the ovary tissues were stored at -80°C for later analysis.

**Measurement of serum biomarkers**

The collected sera were used to determine triglyceride (TG), total cholesterol (TC), blood urea and creatinine. All the previous biochemical tests were done using commercial kits of Human, Biosystem, Spectrum, and Biomed diagnostic Inc.

Estradiol and progesterone levels in plasma heparinized tube of female rats were analyzed by using enzyme immune assay test kit (Catalog Number 10000) of Estradiol (Tsang *et al*., 1980) and assay Kit (Catalog Number: BC-113) of progesterone (Radwanska *et al*., 1978) were used for assessment process. Determination of total $T_3$ (Klee 1996), $T_4$ (Nelson and Wilcox 1996 and TSH (Hood *et al*., 1999) levels by Radioimmunoassay (RIA) via using commercial kits.

**Preparation of ovarian homogenate**

The ovaries were excised and washed in ice cold isotonic saline solution containing 1 mM EDTA. Homogenization for 1 ovary from each animal was performed in 8 mL of cold buffer (50 mM potassium phosphate buffer, pH 7.4, containing 1 mM EDTA) using a Potter-Elvejham homogenizer at 4°C. The crude tissue homogenate was then centrifuged at 8000 rpm for 15 minutes at 4°C and the supernatant was removed and kept at -20°C for estimation of malondialdehyde (MDA) and glutathione content. The other ovary was used for histological preparation.

**Ovarian oxidative stress**

MDA, as a marker for lipid peroxidation (LPO) was measured calorimetrically in ovarian homogenate according to the method of Ohkawa*et al*. (1979). GSH content in ovary tissue was determined according to (Ellman 1959).

**Histological investigation**

The ovarian tissue were immediately removed after sacrifice, And it was prepared for histological examination using technique with hematoxylin and eosin (H-E), according to Bancroft and Gamble(2002).Special histochmicallystain for connective tissue fibrosis Masson's trichrome technique is a three-colors taining protocol used in histology, necessary for the evaluation and assessment of the d fibrous formation, in injuries tissue, according to Richard. 2009.

### Immunohistochemical investigation

Immune-reaction is the process of localizing proteins in tissues by exploiting the principle of antibodies binding specifically to antigens. The visualization of theantibody is commonly accomplished by conjugating an enzyme to the antibody. This can produce a color changing reaction given protein located.The Tumor Necrotic Factor- ά(TNF-ά)can be detected by Immuno-reaction technique for necrotic effect of permethrin toxicity according to (Mclaughlin,et.al.,1990,). Expression of proliferating cell nuclear antigen were detected with PCNA immunohistochemistry was performed as described by (Picut et al., 2008) and PCNA-positive cells demonstrated brown nuclear deposits.

### Software testing

From immunehistochemically, selected stained slides, percentage of positively stained necrotic area detected by using soft ware web application(Image J)program, at website (https://youtu.be/wsXCbaYB6-M)for image areas analysis ,and To evaluate and measured TNF-a immune staining necrotic are and the mean percentage were representation graphically.

### Statistical analysis

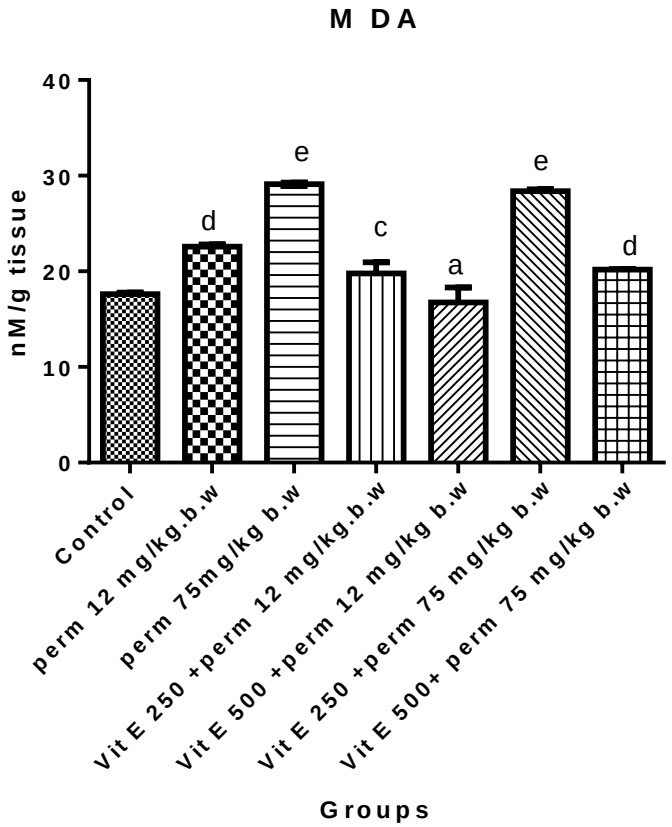
Data are expressed as mean ± SE. Comparisons between different groups were done using one way analysis of variance (ANOVA) followed by multiple comparisons test using the software Graph PadIn Stat and Statistical Package for the Social Sciences program (SPSS version 20.0, Chicago, USA). A probability level of less than 0.05 was accepted as statistically significant.

## RESULTS

### Effect on oxidative stress

Regarding the oxidative stress marker, permethrin with different doses (12 and 75 mg/kg b.w respectively) significantly ($p \leq 0.05$) increased the MDA content in ovary tissue versus control animal. Though treatment with vitamin E after permethrin with different dose (250 and 500 mg/kg.bw respectively) significantly deceased the MDA content versus permethrin group (12 and 75 mg/kg b.w respectively) according to concentration dependent manner.Also, permethrin with different dose caused significantly decreased($p < 0.05$) in GSH content in ovary tissue when compared with control group. However, the Vit E treatment increased GSH content in a group treated with (permethrin 75 mg/kg b.w). (Fig 1 B).

A




B

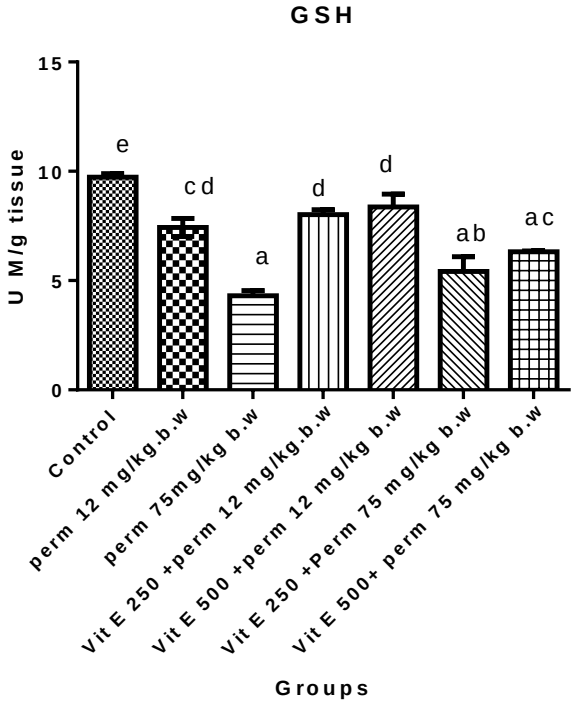



**Figure (1) Effect of permethrin and vitamin E on (MDA & GSH) in ovarian tissue**

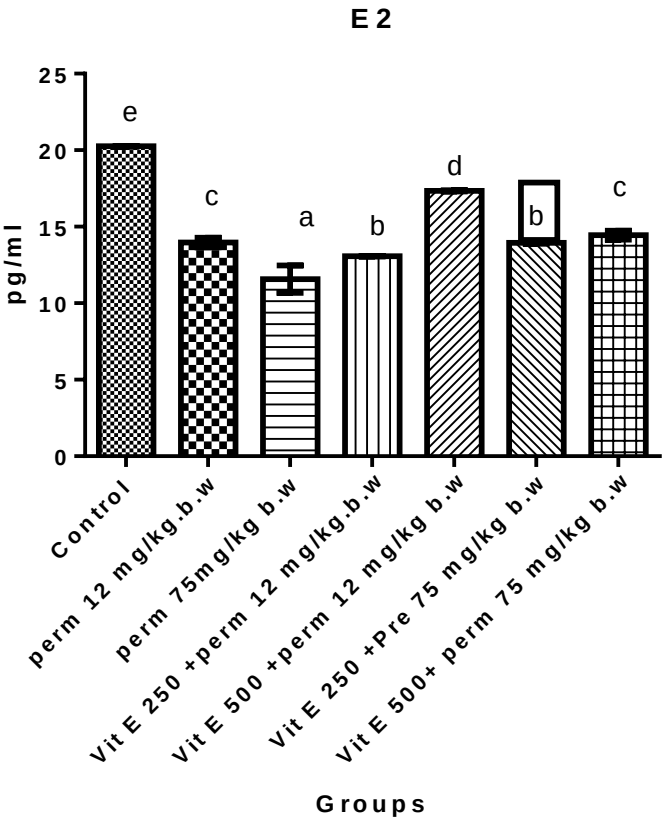
Data are expressed as Mean ± SE (n = 6 rats), Means with different superscript letters are significant different at $p < 0.05$. Data are analyzed with oneway ANOVA followed by DuncanTest at $p < 0.05$.

**Effect on ovarian hormone**

The level of both plasma estrogen (E2) and progesterone were significantly ($p<0.05$) decreased in a permethrin group in both doses (12 and 75 mg/kg b.w) when compared to control group.

While, treatment with Vit E ameliorate the level of E2 and progesterone according to the concentration dependent manner.

A



B

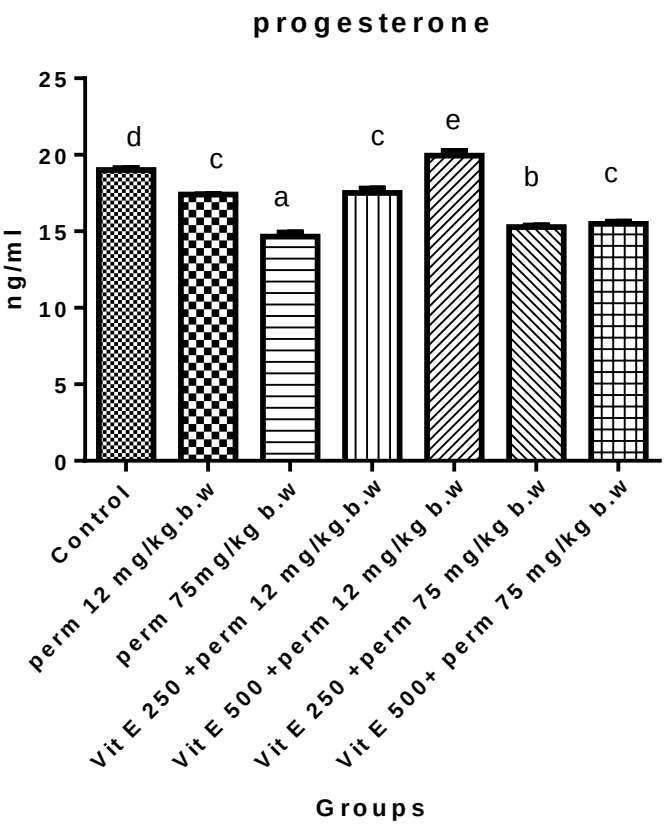


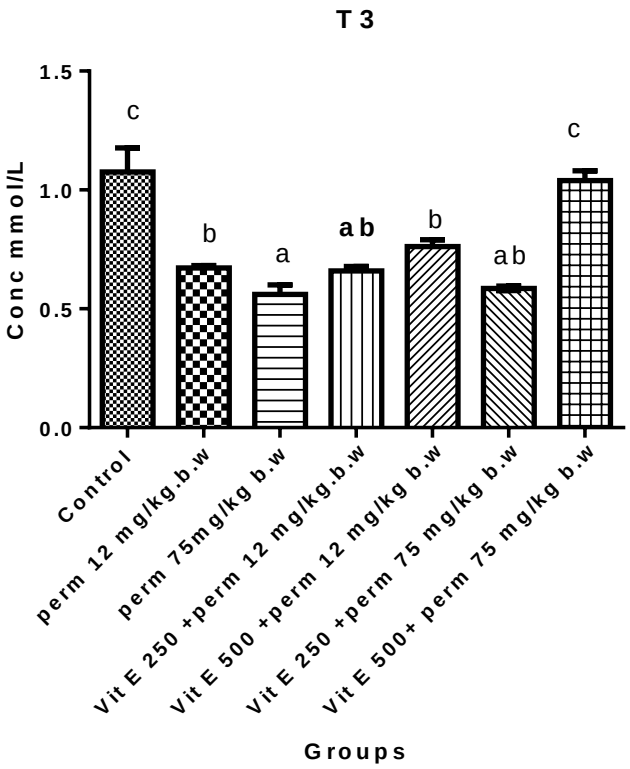

**Figure (2): Effect permethrin and vitamin E on (Estrogen and progesterone)**

Data are expressed as Mean ± SE (n = 6 rats), Means with different superscript letters are significant different at $p < 0.05$. Data are analyzed with one way ANOVA followed by DuncanTest at $p < 0.05$.

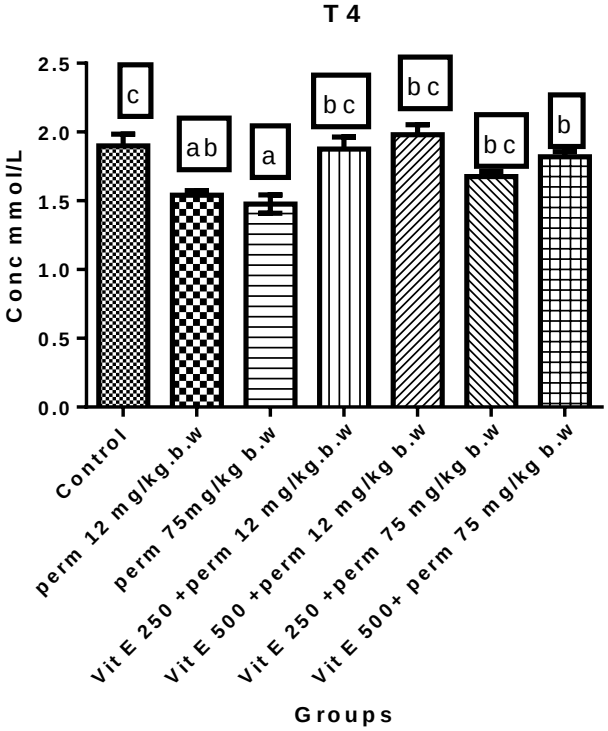
## Effect on thyroid function

Treating rats with permethrin led to a significant decrease in triiodothyronine (T3) and thyroxine (T4) as well as a significant increase of thyroid-stimulating hormone (TSH) when compared to control group. Administration of Vitamin E and permethrin caused an improvement in the levels ofT3 and T4 and TSH according to the concentration dependent manner as shown in (Figure 3).

A


T 3
Conc mmol/L
1.5
1.0
0.5
0.0
c
b
a
a b
b
a b
c
Control
perm 12 mg/kg.b.w
perm 75mg/kg b.w
Vit E 250 +perm 12 mg/kg.b.w
Vit E 500 +perm 12 mg/kg b.w
Vit E 250 +perm 75 mg/kg b.w
Vit E 500+ perm 75 mg/kg b.w
Groups

B


T 4
Conc mmol/L
2.5
2.0
1.5
1.0
0.5
0.0
c
a b
a
b c
b c
b c
b
Control
perm 12 mg/kg.b.w
perm 75mg/kg b.w
Vit E 250 +perm 12 mg/kg.b.w
Vit E 500 +perm 12 mg/kg b.w
Vit E 250 +perm 75 mg/kg b.w
Vit E 500+ perm 75 mg/kg b.w
Groups

C

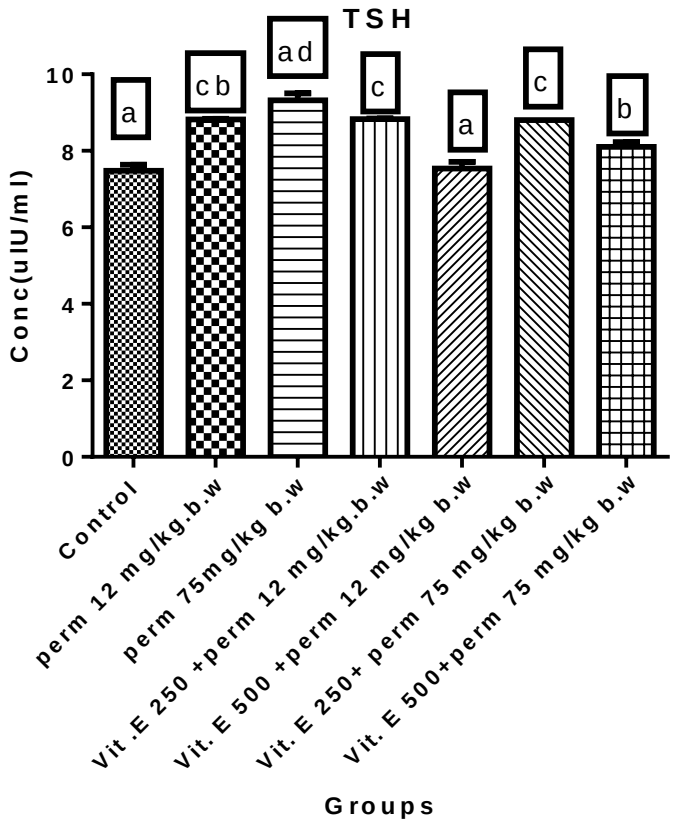


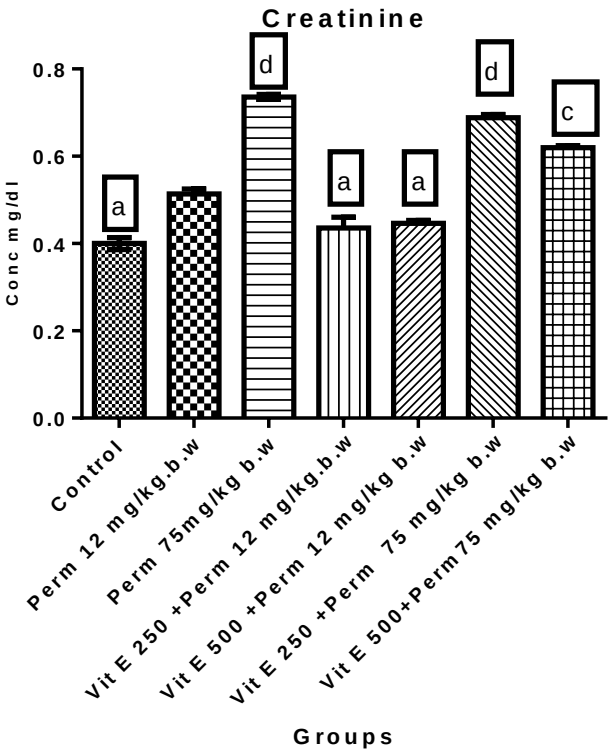

**Figure (3): Effect of permethrin and vitamin E on plasma (T3, T4 7 &TSH)**

Data are expressed as Mean ± SE (n = 6 rats), Means with different superscript letters are significant different at $p < 0.05$. Data are analyzed with one way ANOVA followed by Duncan Test at $p < 0.05$.

## Effect on renal biomarker

As shown in Fig (4) treated rats with permethrin led to significant increase in the level of blood creatinine with different doses (12 and 75 mg/kg.b.w respectively) and with high dose in urea when compared with normal control ($p < 0.05$ ). Administration of Vit E with different concentration after permethrin led to ameliorate the level of blood urea and creatinine.

A




B

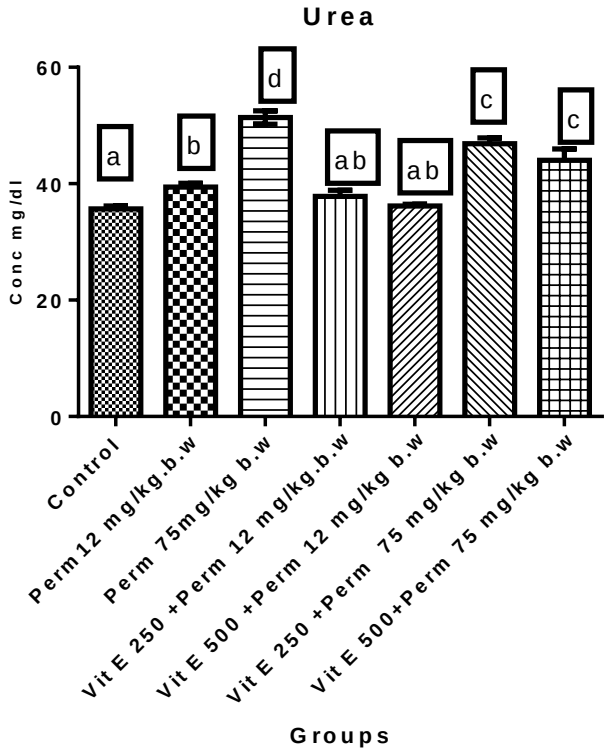


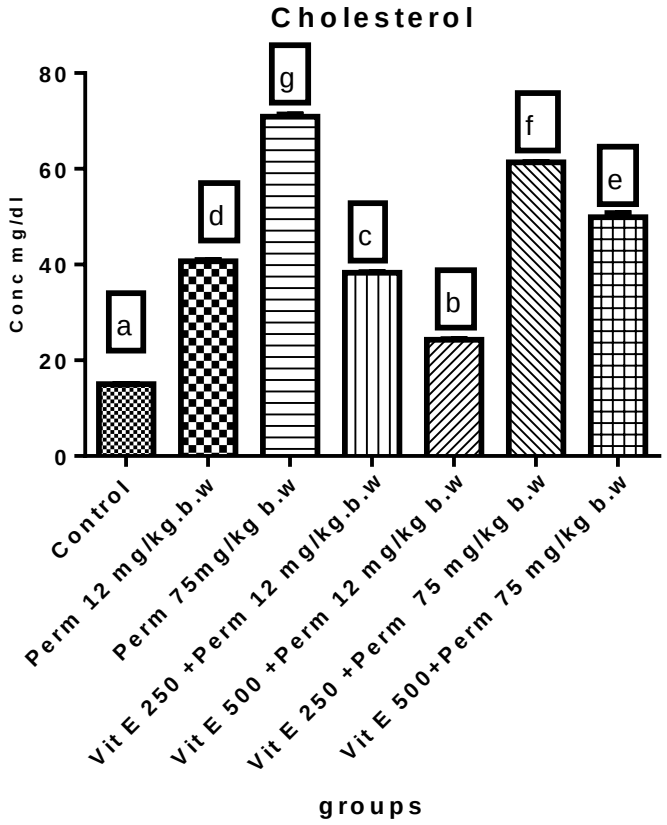

**Figure (4):Effect of permethrin and vitamin E on creatinine and urea evels**

Data are expressed as Mean ± SE (n = 6 rats), Means with different superscript letters are significant different at $p< 0.05$. Data are analyzed with one way ANOVA followed by DuncanTest at $p< 0.05$.

## Effect on cholesterol and triglycerides

Fig (4): illustrate the effect of different dose of permethrin on the level of Total cholesterol and triglycerides. It is clearly that different dose of permethrin caused a significantly increased in the level of cholesterol. While, high dose of Permethrin caused a significantly increase in triglycerides level when compared to control group. According to dependent concentration manner Vit E ameliorate these changes.

A



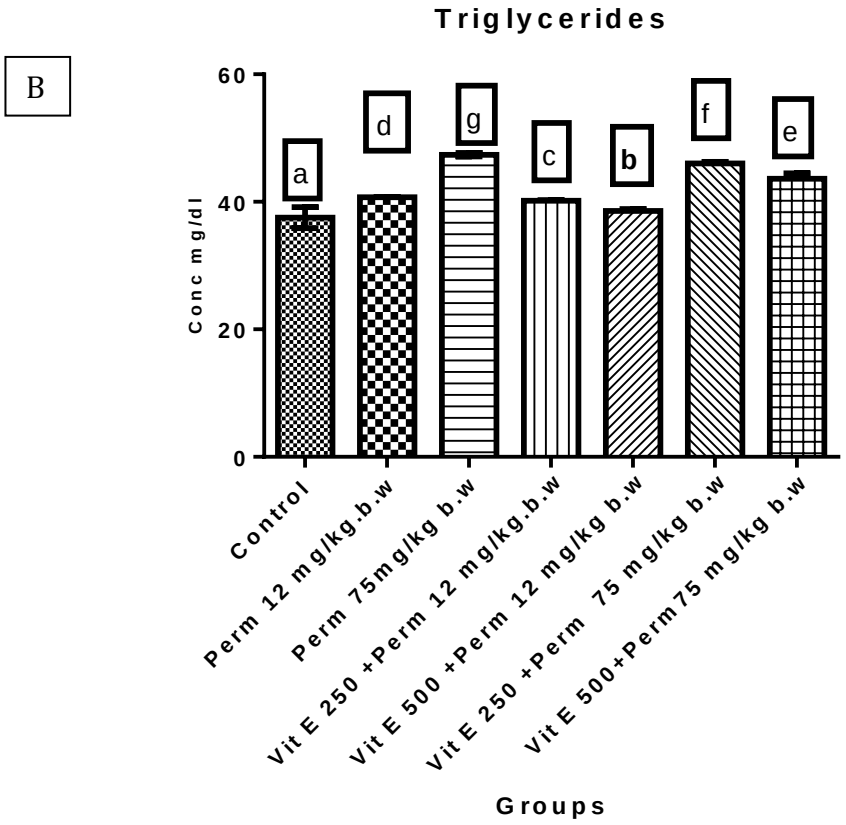



**Figure (5): Effect of permethrin and vitamin E on cholesterol and triglyceride**

Data are expressed as Mean ± SE (n = 6 rats), Means with different superscript letters are significant different at $p< 0.05$. Data are analyzed with one way ANOVA followed by Duncan Test at $p< 0.05$.

## Histological and immunological investigations

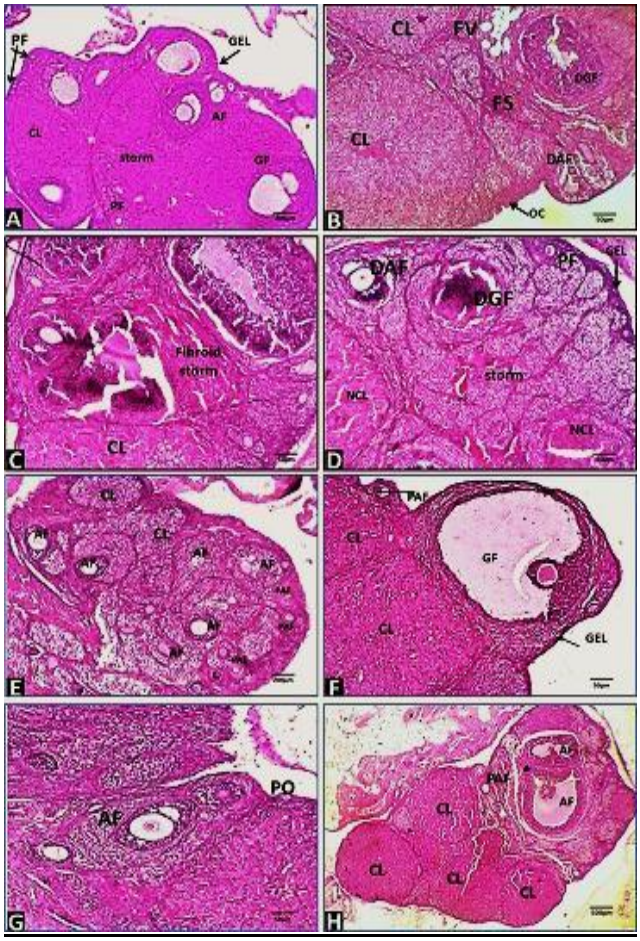



**Figure (6): Sections from ovarian tissue in HE stain in (A)Normal structure ovarian tissue showed germinal epithelial layer(GEL),promodial F.(PF),anterial F(AF), graffian F(GF), corpus latium(CL)and stromal tissue(X100) (B)Ovary of (perm 12) group illusterate degenerated antrial( DAF) and graffian follicle (DGF), thickening ovarian capsule (OC),necrotic corpus latium(CL) fibrosis sheeth (FS) extended in stromal tissue and fatvacuoles( FV) (x400), (C)and (D)Ovary of (perm 75) group showing membrane granulosa collapsed of grafficfollicle, cellular hydropic change, cellular deprees, necroticcorpouslatium (NCL),antralfollicle fully degeneration with hyaline material and ill deformed oocyte (DAF-AGF) and stromal tissue inflammation invaded by fibrous bands andgerminalepithelialayer(GEL) (x400) (E)and (F)Ovary of (V 250 + perm12 and V500) group noticedregenerated ovarian graffian F (GF) ,antral (AF ), Pre-antral F (PAF)CorpusLuteum (CL) and (GEL)(x100X400).(G)and (H) Ovary of (Vit 250 + perm 75 and Vit 500) group Showed the Partially improved overian tissue development antral follicles(AF)and persistence of post-ovulatory corpora lutea(CL) pycnotic ovum nucleus (PO) and atrophy follicles (AF)**

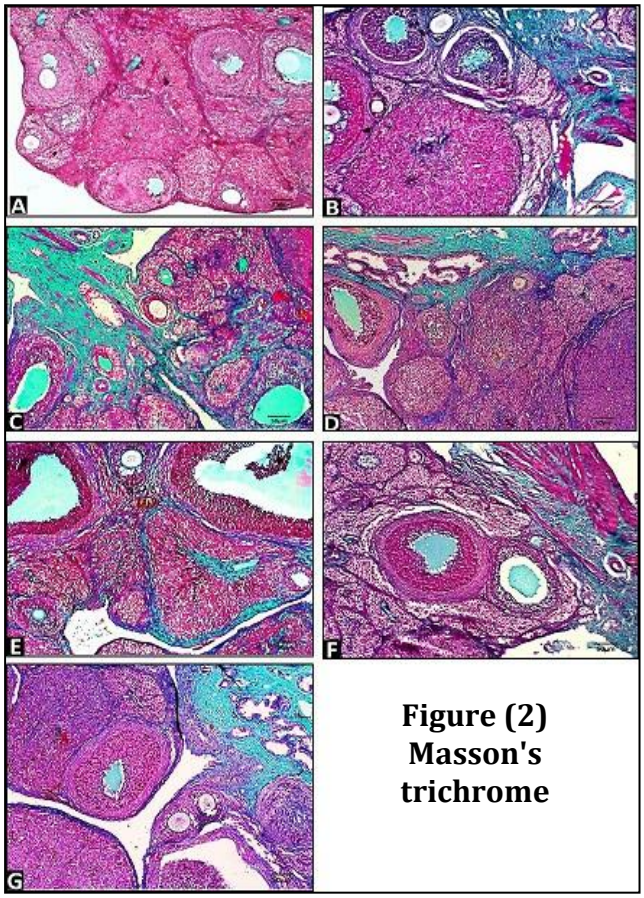



**Figure (7): Sections from ovarian tissue inMasson's trichrome staining for fibrous tissue (A)Control group showing very little fibers,(MassonX100) (B)perm. 12 group showinga few fibrous tissue . (C)perm 75 group showing massively Increase fibrosis inovarian medulla (D)prem 12 + Vit 250 and (E)perm 12+ Vit 500 groups showing remarkable decrease fibroticarea ( F)perm 75 + Vit 250 and (G)perm 75 + Vit 500 showing slightly decrease fibrous area ,(MassonX400**

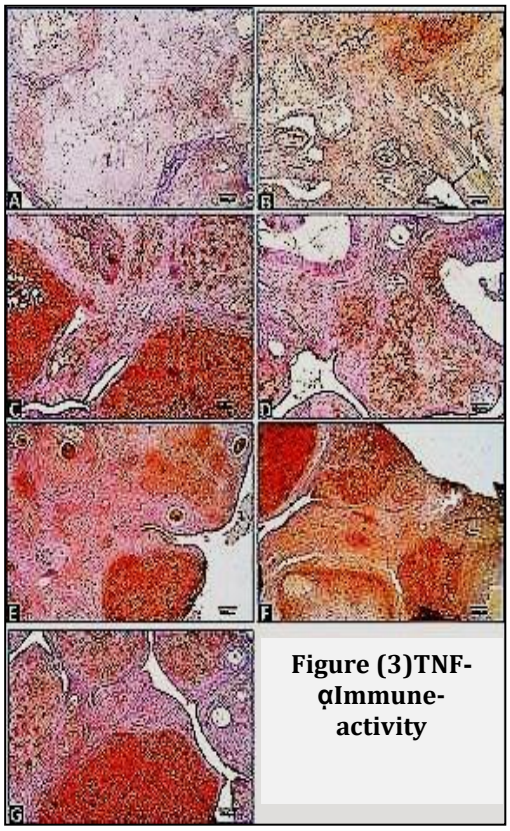



**Figure (3)TNF-ąImmune-activity**

**Figure (8): Sections from ovarian tissue in Immunohistochemical staining forTNF-ą. (A)Control group showing no evidence of TNF-ąreaction. (B)perm 12 group showing a few TNF-ą reaction.(C)perm 75 group showing massively positive reaction to TNF-ą (brown chromogenic sites compared to control.(D) Vit 250 + perm.12 and (E). Vit 500 + perm.12 group showing remarkable decrease TNF-ą -positive reaction area (F) Vit 250 + prem.75 and(G)perm 75 + Vit 500 showing slightly decrease TNF-ą -positive reaction area.( TNF-ą X100) .**

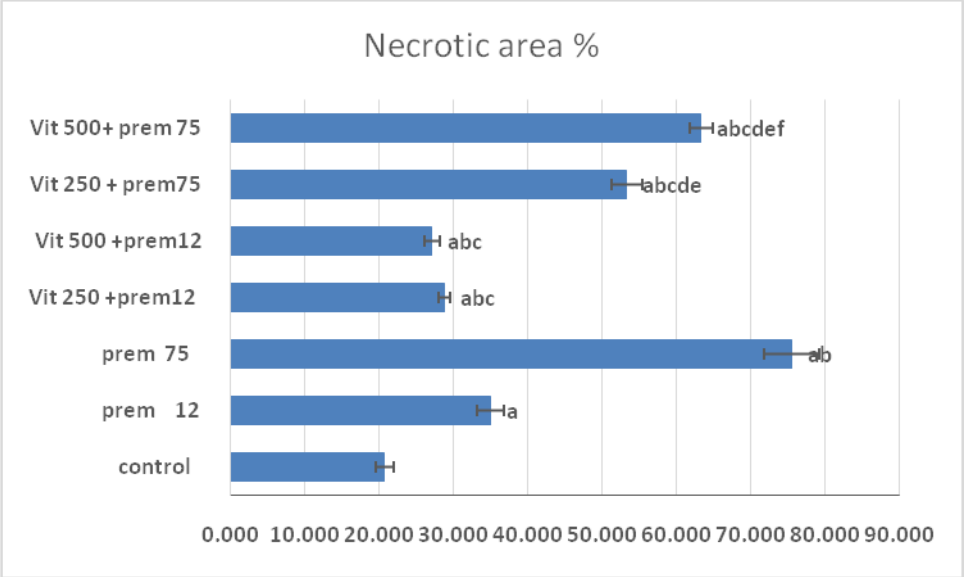



**Figure (9): Chart illustrating the percentage of TNF-ά immune reaction in ovarian tissue sections of all groups. The values are presented as mean ± standard error and significance ($p < 0.05$) compared to (a) control group, (b) perm 12 groups, and (c) perm 75 group. (d) perm 12+Vit 250 group, (e) perm 12+ Vit 500 group. and (f) perm 75+ Vit 250 group.**

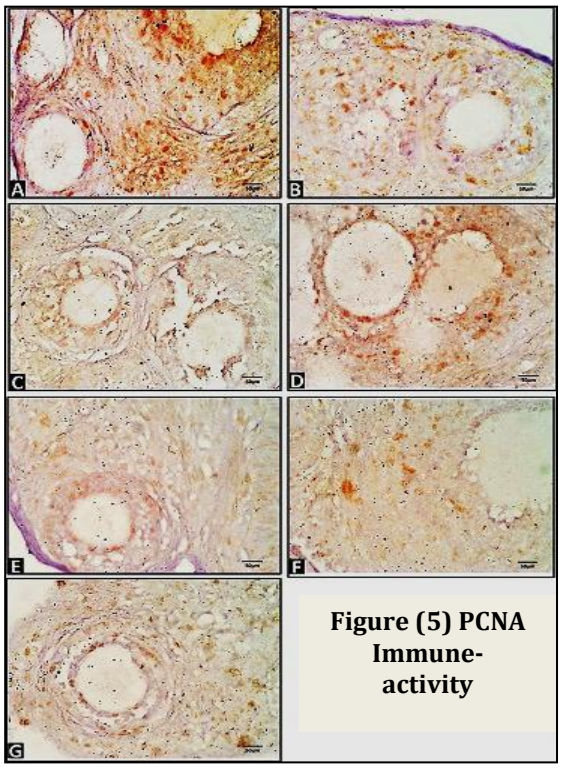



**Figure (5) PCNA Immune-activity**

**Figure (10) Sections from ovarian tissue in Immunstaining for PCNA.(A)Control group showing normal distribution PCNA reaction. (B)perm 12 group showing a few PCNA reaction. (C)perm 75 group showing negative reaction to PCNA brown chromogenic sites compared to control. (D)prem. 12 + Vit 250 and(E) Vit 500 + perm.12 group showing remarkable PCNA –positive**

**reaction area.(F) Vit 250 + perm 75 And (G) Vit 500 + perm.75 showing slightly increase PCNA positive area ,( PCNA X400)**

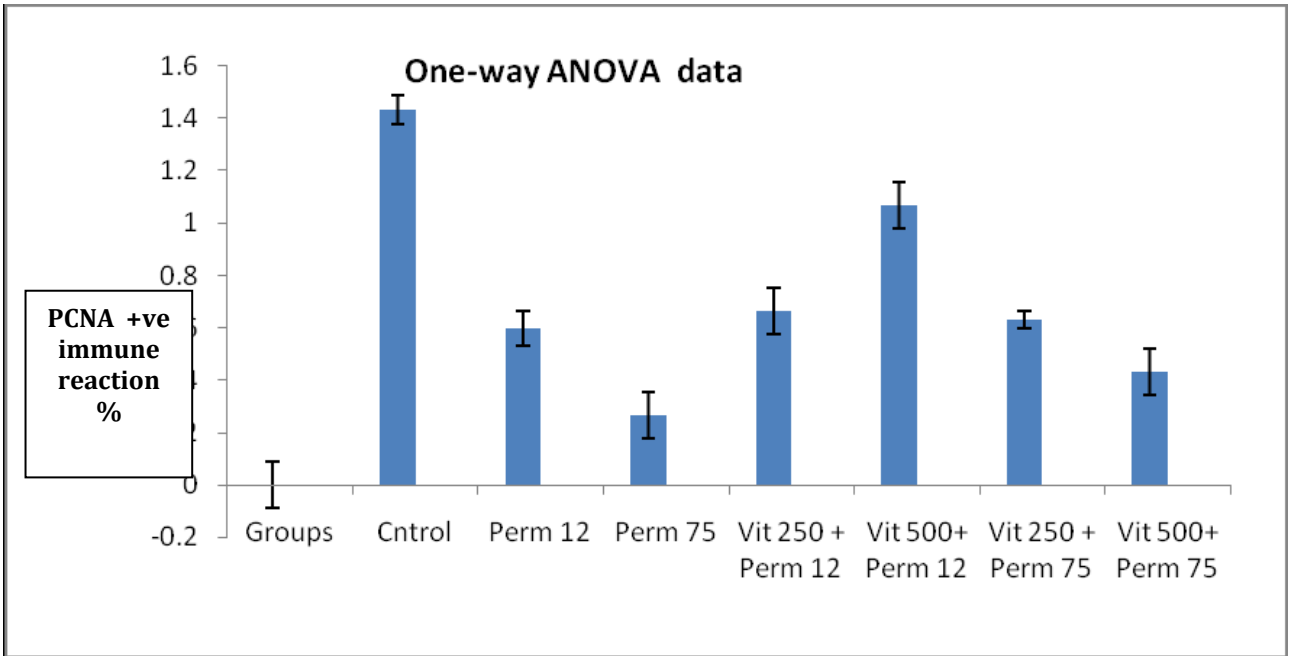



**Figure (11):Chart illustrating the percentage of PCNA immune reaction in ovarian tissue. The values are presented as mean ± standard error and significance ($p < 0.05$) compared to control group**

## DISCUSSION

It is confirmed that pyrethroids can induce toxicity due to the induction of oxidative stress in humans and animals (Changwon *et al*., 2020). Toxicants that interfere with ovarian function can directly affect the ovary or indirectly by acting at the level of hypothalamus orpituitary gland or both. (Guney*et al*., 2007a, b) In the present study, the protective effect of Vitamin E on permethrin-induced oxidative stress was evaluated in rats.

The widespread application of pyrethroids has led to contamination of various environmental compartments with a high lipophilic nature facilitates their bioaccumulation in membranes leading to the generation of reactive oxygen species (ROS) that elicits oxidative damage in animals [ Aydin 2011, Asari*et al*., 2010 , Mossa, *et al*., 2013].

In the present study, permethrin administration to rats resulted in a marked dose-dependent increase in the lipid peroxidation (LPO) as indicated by the increase in the level of malondialdehyde (MDA) in ovaries of permethrin and reduced GSH content compared with the control group. These results are consistent with the previous studies as reported for otherInsecticide ino vary**,** such as malathion (pyrethroid) (Somayyeh*et al*., 2018**),** diazinon (Zinat*et al*., 2014)**,** methomyl (Haneia*et al*., 2013)**,** Endosulfan and Malathion (Koc *et al*., 2009), and in other organs as Cypermethrin (pyrethroid) in liver and kidney **(**Olusegun *et al*., 2019),Cypermethrininliver, kidney, brain, spleen testis and Erythrocytes (Murat *et al*., 2016) and Fipronil in liver and kidney (Mohamed and Ahmed 2018).Pyrethroids induced cellular oxidative damage and accumulation of peroxidation products have been reported in literature [ Mossa*et al*., 2013, Sankar *et al*., 2010, Sankar *et*

*al*., 2012]. These oxidative products have been extensively used as markers of oxidative stress. In the present study, permethrin exposure generated oxidative stress in ovary of the female rat as evidenced by significant elevation of the lipid peroxidation and protein oxidation products, namely MDA. The increase in the levels of MDA in these organ is an indication of permethrin-induced lipid peroxidation leading to tissue injury.
In an extensive review (Wang*et al*., 2016) reported that, the generation of reactive oxygen species (ROS) or reactive nitrogen species (RNS) might play critical roles in oxidative stress and the related toxicities induced by permethrin. The oxidative stress induced by permethrin might be dose- and tissue-dependent. In addition, it was reported that, PER could decrease the antioxidant defence system resulting in damage to cellular macromolecules, such as DNA, lipids, and proteins. Following oxidative stress, cell death can occur via apoptotic or necrotic mechanisms. During this process, DNA damage, enhanced lipid peroxidation and protein damage may occur **(**Weidingerand Kozlov, 2015).

Uner*et al*., 2001 reported that pretreatment of rats with Vitamin- provided significant protection against the elevation of MDA concentrations in cerebral and hepatic tissues, induced by pyrethroids. Cells have several ways to reduce the effects of oxidative stress, through repairing damage (damaged nucleotides and lipid peroxidation created by products) or reducing oxidative damage by enzymatic and non-enzymatic antioxidants [Ogutcu*et al*., 2006]. Vitamin E, a non-enzymatic antioxidant, is a lipid-soluble vitamin present in biological membrane [Kumar *et al*., 2004, Uzunhisarcikli and Kalender, 2011]. Vitamin E allows free radicals to remove a hydrogen atom from the antioxidant molecule rather than from polyunsaturated fatty acids; thus, it breaks the chain of free radical reactions; the resulting radical is relatively inactive [ Pascoe *et al*., 1987]. Minerals and vitamins are essential for the normal functioning of the reproductive system [ Mohammadi*et al*., 2013].In the present study, the groups those received vitamin E plus perm (Perm12+250E,perm12+ 500 E, Perm75+250E and Perm75+500E. mg/kg) significantly decreased the level of MDA compared to perm treated rats and increased the GSH content compared to perm treated rats. Ehab and Mostafa, 2017 reported that vitamin E prevents the oxidative stress of permethrin to a significantly higher level). It is possible that pyrethroids can be transported through blood to the liver for metabolism and may produce cellular damage in erythrocytes (Gabbianelli*et al*., 2013).

Kalender *et al* 2004 observed that endosulfan administration increases MDA level in rats and vitamin E protects cells and cellular structures from oxidative damage by reducing the MD A level. . Vit E is a lipid-soluble vitamin that is incorporated in the lipid bilayer of the cellmembrane. It has a hydroxyl group that acts as a hydrogen atom donor for OH•, yielding a non-radical product, and prevents FPN-induced lipid peroxidation, as indicated by a significant decrease in MDA levels (Niki, 2013; Nordberg and Arnér 2001; Small *et al*., 2012). Since it has been reported that OH• reacts with Vit E and GSH at the same constant rate (Simic et al., 1992), supplementation with Vit E may increase GSH in the cytoplasm, which is required in ROS scavenging and GPx recycling. The results of thyroid hormones analysis in this study showed that treating animals with permethrin induced a decrease in T3 (triiodothyronine) and T4( thyroxine) and increase in thyroid-stimulating hormone TSH. This result agree with the result of ( Shui-Yuan *et al*., 2018 ) who found that deltamethrin decreased T4 and T3 in the female.

Also PM (permethrin) induced a dose dependent decrease in the serum levels of T4, and T3 and increase in the serum level of TSH (Suqing *et al*., 2002).The biochemical results of the pyrethroid Lambda-cyhalothrin (LCT) treated group revealed a decrease in T3, T4 and increase in TSH (Wael 2018). A similar result was obtained by [ Abdul-Hamid and Salah 2013], who investigated the toxicity of Deltamethrin on thyroid function. It is assumed that the reduction in thyroid hormones may be the result of increased hepatic enzyme activity.

In the present study vitamin E increase T3, T4 in the groups which treated by vitamin E + permethrin. This result agree with Naeem (2016) who reported that The supplementation with Iron, Zinc, Vitamin E or Vitamin C significantly increased the level of thyroid hormones (FT4 and FT3), moreover, the level of TSH was ameliorate , might be due to their antioxidant properties, also this result parallel with (Heba *et al*., 2020) who found that the administration of vitamin E with CYPermethrin improved all parameters studied (T3, T4 and TSH).

In this study, the hormone assays showed a significant reduction in the levels of progesterone (Pg) and estradiol (E2) in the blood of the perm-treated rats, in agreement with previous reports of (Samira *et al*., 2017, Ventura *et al*., 2016 and Das *et al*., 2014). This effect has been attributed to oxidative damage in the ovaries, caused by the pesticides. In the same time the groups which treated with vitamin E amelioarted the levels of E2 and Pg2, this result agree with (Saviour et al., 2018)who observed that vitamins C and E attenuated the effect of crude oil which produced a significant ($p<0.05$) decrease in serum estradiol and progesterone levels in female rats, compared to control by producing a significant increase in the hormonal levels of rats exposed to crude oil (Bonny light crude petroleum oil). And also the result of this study agree with (Uboh *et al*., 2011) who reported that Vitamin E is more effective than A and C in ameliorating the adverse effect associated with exposure to gasoline vapour (GV) on the female serum sex hormonal (FSH, LH, estradiol and progesterone) levels in rats.

In the present study treatment of permethrin by the low and high dose resulted in significant increase in the cholesterol and with high dose in triglyceride, Xiao *et al*., 2015 reported that Serum parameters further permethrin treatment (0.5 mg/kg/day) elevated glucose, triglycerides, free fatty acid, and cholesterol levels in mice fed low-fat diet, also Dhivya *et al*., 2011 found that permethrin treatment (1/50 LD(50), from 6th to 21st day of life showed higher levels of cholesterol these results parallel with the present study, in the same time deltamethrin significantly ($P<0.05$) increased the levels of plasma total lipid (TL), cholesterol, triglyceride (TG) (Mokhtar *et al* (2006)), and It was observed that a higher concentration of bifenthrin raised level of cholesterol in all the treated groups (Ikram *et al* 2016). These results agree with the result of the present study. Administration of vitamin E with permethrin in the present study ameliorate cholesterol and triglyceride, this result agree with ( Wilson *et al*., 2016).

In the present study permethrin increase urea and creatinine in the tow uses dosed , Solignum® is a brand of insecticide and wood preservative commonly used by carpenters, furniture makers and roof builders in general for wood preservation. Its main active constituent is permethrin, Kingsley and Iniobong (2014) illustrated that biochemical analysis results showed a significant increase in marker enzymes namely alanine aminotransferase, aspartate aminotransferase and alkaline phos-phatase activity at the end of the fourth week.Similarly, total bilirubin, serum urea, creatinine and electrolytes in a dose

dependent manner in treated rats by Solignum when compared with untreated control group this result agree with the present study, also (Atef 2010) showed that the activities of serum glutamic oxaloacetic acid transaminase (GOT), glutamic pyruvic acid transaminase (GPT), alkaline phosphatase (ALP), and acid phosphatase (ACP), and the values of creatinine, urea, and uric acid were statistically increased 2 were orally given malathion at a dose level of 100 mg/kg body weight (BW) for a period of one month., this agree with the present study. Administration of vitamin E with permethrin in the present study ameliorate urea and creatinine this result agree with ( Wilson *et al*., 2016),

The ovary contains a finite number of germ cells (oocytes) within primordial follicles. Primordial follicles give rise to all mature oocytes and are thus required to sustain the female fertile lifespan (Wallace and Kelsey 2010). Permethrin metabolism yields phenoxybenzyl alcohol from a metabolic pathway involving cytochrome P450 (Kaneko.2010)**,** that indicated tissue damaged and highly apoptotic rate. Permethrin administration causes degeneration of ovarian follicles and oocytes in a dose-dependent manner, such as loss of cell junctions in granulosa cell layer, pyknotic cell morphology with condensed chromatin, vacuolizationcytoplasm**(**Kotil and Yon 2015),which agree with current observation .Oxidative stress is another potential permethrin mechanism induced toxicity due to reactive oxygen species(ROS) generation ,which affected cell membrane. Also, activities of antioxidant enzymes such as CAT, glutathione peroxidase (GPx), and reduced glutathione (GSH) reduction in the in dose- and time-dependent manner , that are agreed with (Sharma et al., 2018a) and explained our findings as vacuolar and necrotic changes .A decrease in the antioxidant defense system and an increase in ROS, leading tocellular macromolecules damage such as lipids، proteins, and DNA.All these deteriorative changes evidence of apoptosis and necrotic change (Wang *et al* 2016)**, a**lso increases caspase-3 reactivity in ovarian follicles elevate apoptotic and necrotic rate (Kotil and Yon 2015)**.**The Permethrin tissue injury, can be responded by fibrosis adaptive feature. Under normal physiological conditions, affected tissues are remodeled and tissues regenerated without permanent damage. However, if the balance between the synthesis and degradation of the components of the extracellular matrix (ECM) is altered, fibrosis or excess accumulation of the ECM - can disrupt tissue structure, function, and fiber accumulation (Shawn, *et al*., 2016)This corresponds to the current examination of ovarian tissue and establishes fibrosis as a hallmark of tissue acclimatization also indication to slight improvement in vitamin intake group.

The cytokine tumor necrosis factor (TNF-α) is a major mediator of inflammation,with actions directed towards both tissue destruction and recovery from damage [Beutler (1999).The secretion of TNF-α is one of the immune escape mechanisms of the tumor,deviating the immune response toward a T helper 2 (Th2) profile (Balkwill *et.al***.,** 2005).OtherStudiessuggest that TNF-α leads to chronic inflammation, angiogenesis, and other processes, which promote the initiation, growth, and metastasis (Lebrec, *et al*., 2015).

The current investigation illustrates increasing the level of necrotic tissue examined byTNF-αimmune activity according to the permethrin dose compared to the control group, which was in line with(Greenfeld ,*et al*., 2007)who explains that TNF-is a protein with countless functions and is expressed in the ovary to regulate germ cell cyst lysis (GCN), which explains the association of TNF-activity with the damaging effect of permethrin. Proliferating cell nuclear antigen **PCNA** is important for both DNA synthesis and DNA repair (Essers *et al* 2005).It is a DNA clamp that acts DNA polymerase processivityand replication essential , also

acts as a scaffold to recruit proteins involved in DNA replication, repair, and chromatin remodeling. (Moldovan *et al*., 2007). It is expressed in the phases G1 and S of the cell cycle and controls the equilibrium among cell apoptosis and proliferation.(Zhou *et al*., 2014 and. Schönenberger*et al*., 2015) .Therefore it can be used as a marker for cell proliferation in ovarian follicles damaged , which lacking PCNA-positive cells in permethrin dose tissue which means slightly expression of PCNA comparing with untreated group .Vitamin E protective role in the present study, regard improved of PCNA reaction in ovarian tissue indicated to cell proliferating in experimental groups. **(**Carlson and Ehrich, 2008)

The obtained results proved that antioxidants such as **vitamin E** improve the cell defense system and protect cells from oxidative stress. This indicates an improvement in cell proliferation resulting from the toxicity of pesticides and the resulting free radicals that cause oxidative stress to damage DNA. This stops the cell cycle, causing aging or apoptosis (Carlson and Ehrich 2008). Collectively, these findings show that permethrin is a critical impacting on female fertility and reproductive longevity.(Seng *et al*., 2017).

## CONCLUSIONS

Finally, the study reveals that chronic exposure to premerthin even low dose alters ovarian cycle activity. Hence, Premethrin exposure threat fertility of humans and animals.it is speculate that the positive effect of the Vitamin E by its antioxidant activity able to protect ovarian tissues the premerthin toxicity.

and lacking PCNA positive cellsin different follicles ,membrane granulose and pri-antrial follicle (fig.10B –C ).Whileexaminationofvitamin E administration showed amelioration of various degrees in the treated groups as showed,(Fig.10- D and G) compared with control and represented graphically ( Fig 6 ) .

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