Alexandria Journal of Veterinary Sciences

www.alexjvs.com

AJVS. Vol. 54 (1): 79-96. Jul. 2017

DOI: 10.5455/ajvs.269380

Protecting Role of Selenium on the Cytotoxic Effect of Profenofos on Rabbit Fertility

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Key words:
Profenofos – Histopathology- Caspase- 3 –Biochemical- Hormones- Antioxidants- Reproductive toxicity – Selenium- Liver - kidneys - Rabbits.

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Abstract

Profenofos is an Organophosphorous compound and acaricide used in Egypt with cumulative toxic effects causing reproductive dysfunction and increase in reactive oxygen species production. Protective and anti-oxidant effects of selenium can improve the activity of radical scavenger. So, the aim of this study was to elucidate the protective effects of Selenium against reproductive toxicity induced by Profenofos in male and female New Zealand rabbits (n=18). Three experimental groups (3 males and 3 females per each group) receiving a combination of Profenofos (70 mg/ kg b. w.) and/or selenium in drinking water (25 mg/ kg b. w.) for 90-day was divided as follows: non treated (control), Profenofos alone and Profenofos + selenium group. Profenofos caused a decrease in body and organs weights compared to control group. It had a fetotoxic effect resulting in a significant reduction in pregnancy rate and the number of viable feti in treated animals. Also, Profenofos caused a significant elevation in ALT, AST, MDA, urea and creatinine, while a significant decrease was noticed in SOD, GSH, testosterone, estrogen and progesterone in all treated rabbits compared to control group. Concomitant administration of selenium with Profenofos alleviated the induced alterations. In addition to, histopathological results revealed that testes, epididymis, ovary and uterus alterations represented by degenerative and inflammatory changes as a result of Profenofos exposure. Profenofos had a carcinogenic effect on uterus as leiomyoma with rhabdomyosarcoma which gave positive immunoreactivity with Desmin and Smooth muscle actin. Also, Profenofos caused histopathological and histochemical effects in liver and kidney tissues. There was intensive positive immunoreactivity for caspase- 3 (dark brown granules) was observed in the cytoplasm of apoptotic testicular, ovarian and uterine cells that indicating the severity of Profenofos toxicity. On the other hand, examined tissues of Profenofos in combination with selenium treated group showed apparent reduction of caspase-3 immunoreactivity to be more or less similar to the control group. In conclusion, selenium is a valuable cytoprotective which reduced the oxidative stress toxicity induced by Profenofos in the reproductive system of male and female as well as liver and kidney albino rabbits.

1. INTRODUCTION:

Organophosphorous compounds (OPIs) are synthetic pesticides that developed in the last 50 years and are commonly used in agriculture (Salem and Kobeasy, 2006*)* .Due to their wide use, they contaminate food, water and air and consequently adverse health effects in humans, animals and fish (Sharma *et al.,* 2005). The use of pesticides not only increases the crop yields but also helps in controlling ectoparasites on livestock. Residues of the grain protecting pesticides may also come in feeds and causes severe acute and chronic poisoning in farm animals and human as a result of its harmful commutative effect (Memon *et al.,* 2014).

Pesticides in the environment may play an important role in contributing the causes of fertility problems in

dairy livestock as a result of endocrine imbalance (Campagna *et al*. 2009). The exposure of males to pesticides can adversely effect on its fertilization capacity through a direct genetic or epigenetic effect of their residues on the male germ cells either during spermatogenesis or sperm maturation as well as by the direct exposure of oocyte during fertilization to the pesticide residues in the seminal plasma (Ghuman *et al.*, 2013). In females, the pesticide exposure induced alterations which include poor reproductive behavior, infertility, pregnancy loss, growth retardation, intra-uterine fetal demise and ovarian failure (Uzumcu and Zachow, 2007). In addition, they can cross through the placenta and enter the fetal blood stream in which may accumulate and affect its development or function (Barr *et al*., 2007). Also, they cause adverse effects on liver (Akhgari *et al*., 2003) and urinary system (Rodrigo *et al*., 2001).

Profenofos [0-4-bromo-2-chlorophenyl-0-ethyl S-propyi phosphorothioate] is a broad spectrum OPIs and acaricide used in Egypt. It is a pale yellow liquid under trade name: Selecron 72% EC (Farrag and Shalby, 2007). Its main physiological effect is the inhibition of cholinesterase (ChE) activity enzyme in blood and nervous system, resulting in the accumulation of acetylcholine (a major transmitter) which may lead to death (Khalil *et al*., 2014). In spite of the extensive use of Profenofos, information related to its effects on health with particular reference to reproductive toxicity is scarcely (El-Kashoury, 2009).

Profenofos can induce oxidative stress by generating free radicals and altering antioxidant levels of the free radical scavenging enzymes (Nashwa *et al*., 2012). This oxidative stress is a consequence of imbalance between the body antioxidant system and pro-oxidant state. Endogenous enzymatic and non-enzymatic antioxidants are essential for the conversion of reactive oxygen species (ROS) to harmless metabolites as well as to protect and restore normal cellular metabolism and functions (Bebe *et al*., 2003).

Profenofos toxicity disrupt the endocrine system so that it called Endocrine Disrupting Chemical (EDC) (Memon *et al*., 2014) which may induce atrophy of the male reproductive organs during sexual differentiation leading to disruption of the pituitary testicular feedback mechanisms and subsequently, Leydig cells hyperplasia (Ostby *et al*., 1999). Zidan *et al.* (2009) assessed the toxicity of Profenofos on rat by significant changes in some blood biochemical parameters such as AST, ALT, creatinin and urea as well as a significant reduction in serum testosterone levels.

Apoptosis is an active and regulated process of cell death through a series of biochemical and morphological changes, such as caspase family activation, DNA fragmentation, cell volume loss, and chromatin condensation (Vaskivuo *et al*., 2000). Overproduction of ROS by Pesticides in both intra and extra cellular spaces, resulting in massive cellular damage and death has been associated with lipid peroxidation and alterations of protein in living organisms (El-Kashoury, 2009).

In the recent years, many research works has been focused on the use of natural materials as antioxidants against the toxic oxidative materials to ameliorate their toxic and cell damaging effects. Protective and anti-oxidant effects of selenium have also been described by Zeng *et al*. (2013), who reported that, it can improve the activity of radical scavenger through epithelium proliferation and may improve endothelial protein efficiency.

So, the aim of this study was to investigate:

1- Conception and pregnancy rate.

2 -The reproductive biology function, hormonal assay, in rabbit that had been exposed to Profenofos.

3- The histopathological alterations and immunohistochemical state of rabbit tissues (tests, epididymis, ovary, uterus, liver and kidney) under Profenofos effect.

4- Additionally, to investigate an ameliorating effect of selenium against Profenofos administration on the biochemical, histopathological and immunohistochemical changes.

2. MATERIALS AND METHODS:

2.1. Animals:

Eighteen healthy adult New Zealand white rabbits (9 male and 9 female) of the same age, with a weight range of 2–2.5 kg were used for this study. They were housed in a well-ventilated animal house and each group caged separately, at a temperature of 29-32°C. The animals exposed to 10–12 h of daylight under proper hygienic conditions and received food and water ad libitum. All animals were acclimatized for one week before being dosed. The present study was carried out in National Research Center.

2.2. Chemicals:

2.2.1. *****Profenofos: produced by Syngenta multi-national comp. under trade name: Selecron 72% EC.

2.2.2.*****Sodium Selenite (Na_2SeO_3): it was obtained from Sigma Aldrich Chemicals, St. Louis, MO, USA.

2.3. Animal groups and dose administration:

It was applied according to Memon *et al.* (2014). The rabbits were divided into three equal groups of six rabbits in each (3males+ 3femals) and they gave different treatment daily in drinking water for 90-day.

1 - Control group (GP I): Animals were given water without any treatment.

2 - Profenofos group (GP II): Animals were provided 70 mg Profenofos /kg b.w.

3 - Profenofos + Selenium group (GP III): Animals were provided 70 mg Profenofos /kg b.w. + Sodium Selenite 0.50 mg/kg b.w. (Said *et al*., 2012). At the end of experiment, animals were sacrificed and tissues (testis, epididymis, uterus, ovary, liver and kidney) were taken for histopathological, histochemical and immunopathological examinations.

2.4. Evaluation of pregnancy and viability rate:

It was performed according to Morgan and Abd El-Aty (2008). The female rabbits were mated with male rabbits in the same group and kept until fertilization is proved.

2.5. Body and organ weights:

The weight of rabbit's body, testes, epididymis, liver and kidney were recorded after scarification.

2.6. Biochemical assay:

Blood samples: was collected directly from the ear vein every two weeks then kept frozen till assayed at -20^{o}c and serum samples were prepared by centrifugation at 3000 rpm for 10 min.

2.6.1. Serum samples were analyzed for rabbit's serum transaminases including aspartate aminotransaminase (AST) and alanine aminotransaminase (ALT) according to Retiman and Frankel (1957).

2.6.2. Urea and creatinine (Young 2000).

2.6.3. MDA content was measured according to the earlier method reported (Zhang, 1992).

2.6.4. Total antioxidant capacity level (TAC) was measured according to Cortassa *et al.* (2004).

2.6.5. SOD activity was determined according to the previous report (Rai *et al*., 2006).

2.6.6. Reduced Glutathione was assayed according to the previous reports (Mohandas *et al.*, 1984).

2.6.7. Part of each serum sample are analysed for testosterone, estrogen and progesterone hormone determination according to methods of **Maxey *et al.* (1992)**. Serum was also assayed for testosterone, estrogen and progesterone hormone; it was assayed by direct enzyme linked immunoassay (ELISA) according to Maxey *et al*. (1992).

2.7. Pathological studies:

2.7.1. Histopathological examination: Testes, epididymes, uteris, ovaries, livers and kidneys were dissected and preserved in 10% neutral formalin solution for fixation, then dehydrated through ascending grades of alcohol, cleared in xylene, embedded and blocked in paraffin. Sections of 3–5-µm thickness were taken and stained with hematoxylin and eosin as described by Suvarna *et al*. (2013) then examined by light microscope.

2.7.2. Immunohistochemical evaluation:

* Caspase-3: Deparaffinized tissue sections were used for detection of apoptosis in cells according to Ozmen and Mor (2012). All steps for immunohistochemical evaluation were carried out using image analysis software (Image J, 1.46a, NIH, USA).

* Desmin and Smooth muscle actin (SMA): They were applied according to Gruchala *et al.* (1997) for recognizing of tumors of myogenic origin.

2.7.3. Histochemical staining: Masson's Trichrome Technique to define the connective tissue elements and Periodic Acid- Schiff method (PAS) to demonstrate collagen fibers as well as bromophenol blue stain to demonstrate protein content was performed. All these procedures were applied according to Suvarna *et al*. (2013).

2.8. Statistical analysis:

Data were subjected to analysis of variance according to Snedecor and Cochran (1982. Values were expressed as mean ± SE. Statistical comparisons between the means of different experimental groups were made with completely randomized one way ANOVA ″Student_ Newman_ Keuls test ″ by COSTAT program version one. A probability ″P″ value of <0.05 was assumed for statistical significance.

The mean ± S.D. Values were calculated for each group to determine the significance of intergroup difference. Each parameter was analyzed separately using two-way ANOVA analysis of variance. P values <0.05 were considered to be significant (Snedecor, and Cochran, 1982).

3. RESULTS AND DISCUSSION:

OPIs occupy a prime position in pest management, due to their high insecticidal activity, rapid metabolism and rapid decomposition in soil and water. Also, it was reported that they are widely used in home and industry as pesticides, plasticizes, flame retardants and lubricants (Hammam and Abd el Mottaleb, 2007).

Profenofos danger contributes to its ROS production. The mammalian tissues contain several enzymes scavenging ROS such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-PX) and glutathione S-transferase (GST) to control ROS level and protect cells under stress conditions. Some compounds as Selenium (Se) contribute to detoxification process from ROS and occurs as selenoproteins in enzymes. Also, it is very important in animal nutrition as an anti-oxidant which may act directly by neutralizing free radicals or by reducing peroxide concentrations and repairing oxidized membranes as well as through quenching iron to decrease ROS production (Flora *et al*., 2009) in addition to, its anticarcinogenic properties (Zeng *et al.*, 2013).

3.1. Effects on health and mortality:

Test group animals administrated Profenofos only showed clinical symptoms like temporarily stopped eating food, fatigue, nasal dripping, tremor, convulsion, occasionally diarrhea and dragging their hind limbs were noted. Mortality was occurred in one male rabbit during the experimental period. On the other side, none of these symptoms appeared on GP I and III rabbits. These symptoms were mimic to that of Memon *et al*. (2014) in rabbits and Khan *et al*. (2009) in male goats. Hendawy *et al*. (2012) reported that no deaths were recorded throughout the experimental rats exposed to Profenofos.

3.2. Effects on body weight and organs weights:

Changes in the body and organ weights after insecticide dosing have been used as a valuable index of insecticide-related organ damage (Mossa *et al*., 2011).In the present study, animals exposed to Profenofos; their body weight was significantly $p<0.05$ decreased as compared to control animals as shown in Table (1). While GP III animals showed no difference in body weight compared to control animals.

The present results were similar to those reported by Ambali *et al.* (2007) as a result of feed consumption reduction; while Ahmed *et al*. (2000) reported that the body weight was significantly increased. On the other hand, Shaker *et al*. (1988) reported, no effect on body weight gain as a result of pesticides administration. Moreover, Salem and Kobeasy (2006) aimed it to metabolic process interference by Profenofos due to gastrointestinal tract pathological lesions, which was reflected in mal-absorption of nutrients.

Also, group II were showed significant decrease in their testes and epididymis weight and GP III animals showed no difference as compared to control group (Table 1).

Profenofos exerted toxic effects on testicular tissues and disrupting the testicular function in treated animals and this associated with significant reduction in testes weight (Memon *et al*., 2014). The decrease in testicular and epididymal weight in treated rats may be due to reduced tubule size, spermatogenic arrest and inhibition of steroid biosynthesis of leydig cells as mentioned previously by El-Kashoury (2009).

Data in Table (1) indicated that, rabbits treated with 70 mg Profenofos showed increase in liver and both kidneis weights which were in agreement with that obtained by Salem and Kobeasy (2006). While disagree with those stated by Undeger *et al*. (2000) due to Dimethoated and Methyl Parathion administration in male rats.

Table (1): Effect of Profenofos and Se on the body, testes, epididymis, liver and kidneys weight (Mean ± SE):

parameters	Group I (Control) (n=6)	Group II (Profenofos) (n=6)	Group III Profenofos+ Se (n=6)
Body wt. (Kg)	2.4 ±13.5 [a]	2.0 ±14.2[b]	2.3 ±.26.2 [a]
Testes wt. (g)	4.22±0.22[a]	2.71±0.13[b]	4.02±1.20[a]
Epididymis wt. (g)	0.36± 0.02 [a]	0.25 ± 0.01 [b]	0.32± 1.02 [a]
Liver wt. (g)	66.2 ±20.6[a]	70.23 ±11.1[b]	65.8 ±21.5[a]
Kidneys wt. (g)	134.2 ±30.0[a]	144.7 ±11.5[b]	132.0 ±10.2[a]

Means within the same row are bearing different letter superscripts (a & b) differ significantly ($P \leq 0.05$).

3.3. Conception and pregnancy rate:

The present study showed that the long-term exposure of female rabbits to Profenofos had reduction effect in pregnancy rate and number of viable feti in treated group compared to the control group. Two females in group II gave birth and the 3rd one didn't pregnant till the end of experiment. All the feti were weak and dead within 10days so the pregnancy rate represented 67% with zero % feti survived. Those results nearly approach to results of Mlynarczuk *et al.* (2010). Protective effect of Se may improve pregnancy rates and reached to 100%. These studies have confirmed a

link between antioxidant-poor diet and pregnancy loss (Said *et al*., 2012).

3.4. Biochemical studies:

Organophosphates are well absorbed after up take via the oral, dermal or inhalation (Leng *et al*., 1997). There were some biochemical parameters as well as some enzymes activities and hormones were investigated in the present study.

3.4.1. Effects of Profenofos on liver function tests:

The present results demonstrated that there was significant increase in Aspartate transaminase (AST) and Alinine transaminase (ALT) values in GP II comparing with the control group under the influence of daily administration of Profenofos as shown in Table (2) and Fig. (A). This was ascribed to its damaging effect on the liver which liberating their interacellular enzymes into the blood stream. The obtained results coincide with those obtained by Abdel-Megeed *et al*. (2001), who found the marked increase in serum AST and ALT activity in response to pesticides manifested their potential hepatotoxic actions as hepatic necrosis in accompanied by abnormal increase in serum level of transaminase. Also, our results agreed with Rezg *et al*. (2008).

3.4.2. Effect of Profenofos on kidney function tests:

GP II animals showed a significant increase in serum urea level comparing with the control group as shown in Table (2) and Fig (B). Any factor that reduce glomerular filtration rate (GFR) will cause an increase in serum urea nitrogen (Anderson and Cockayne, 1989). It was suggested that uremia was due to increase catabolism of body proteins, decreased renal blood flow as a result of general circulatory distress or renal damage from the pesticide. These results agreed with Hanafy *et al*. (1989).

Table (2) and Fig (B) showed that a significant increase in creatinine level in GP II comparing with the control group. Creatinin is a non protein nitrogenous substance formed during muscle metabolism of creatinine and phosphocreatine and filtered by renal glomeruli. Accumulation of creatinine in the blood is used as significant marker for toxicity in kidneys (Finco, 1997). These may be due to renal tissues damages that will explained by the histopathology. Our results were coincided with Zidan *et al*. (1998) and Rahman and Siddiqui (2006).

3.4.3. Effect of Profenofos on antioxidants activity:

3.4.3.1. Malondialdehyde (MDA):

Serum MDA was significantly elevated in response to Profenofos treatment for 90 days compared with normal control group. On the other hand, Se induced a non-significant change compared with control group table (2) and Fig (C). Antioxidant parameters represented as biomarkers, levels of MDA, a major oxidation product of poly-unsaturated fatly acids, have been considered to be the most significant indicator of membrane lipid peroxidation arising from the interaction of reactive oxygen types with cellular membranes .So the increase of MDA levels could be earlier diagnostic index in Profenofos toxicity (Nagy *et al*., 2006). Increasing MDA level in our study went hand in hand with the results of Ogunro *et al*. (2005).

3.4.3.2. Glutathione Peroxidase:

It was apparent from Table (2) and Fig (C) that GPII afforded a significant decrease ($P<0.05$) in serum glutathione peroxidase when compared with GPI. Whereas, Se induced a non-significant change in serum Glutathione peroxidase compared with control group.

The long-term treatment with OP causes a gradual depletion of GPx, and GST (Song *et al*., 2006). Our results were compatible also with Fang *et al*., (2002). They reported that a considerable decline in GSH content in the tissue may be due to its utilization to challenge the prevailing oxidative stress under the influence of ROS generated. Meanwhile, the administration of Profenofos caused a significant decrease in the levels of glutathione peroxidase (GPx) and reduced glutathione (GSH), and an increase in the lipid peroxidation (LPO) level (Gamal *et al*., 2006).

Se is a crucial component of several major metabolic pathways, including antioxidant defense function, thyroid hormone metabolism, and immune system. Se, an important trace element, its deficiency is involved in heart disease and increased cancer risk and it is a cofactor of glutathione peroxidase which confers protection to cell from free radicals (Burk *et al*., 2003). Se is a component of the unusual amino acids selenomethionine and selenocysteine. Se plays a key role in the physiological functioning of the thyroid gland which produces thyroid hormone (TH) and in every cell that uses TH, by participating as a cofactor for tri-iodothyronine deiodinases (Pieczy and Grajeta, 2015).

3.4.3.3. Total Antioxidants Capacity (TAC):

It was apparent from Table (2) and Fig (D) that treatment of rabbits with Profenofos afforded a significant decrease ($P<0.05$) in TAC after the end of the study when compared with normal control group

while Se induced a non significant change in TAC content of the liver compared with control group.

The decreased level of serum TAC reflects a lower total antioxidant capacity which is considered as indicator to all antioxidants (Nagy *et al*., 2006). This is probably due to the depletion of the antioxidant molecules consumed in the process of protecting cells against ROS generated by Profenofos (Ogunro *et al*., 2005).

3.4.3.4. Super oxide Dismutase (SOD):

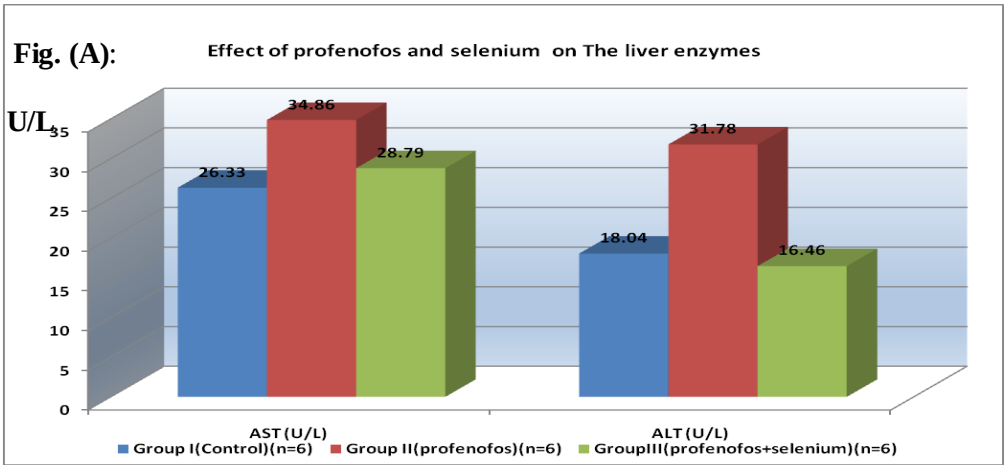
The results of this study revealed that GP II elicited a significant decrease ($P<0.05$) in serum SOD level after the end of the study when compared with control group. Supplemented of normal rabbits with Se for 90 days elicited non significant changes in serum SOD activity compared with normal control group after the end of the study (Table 2 and Fig. E).

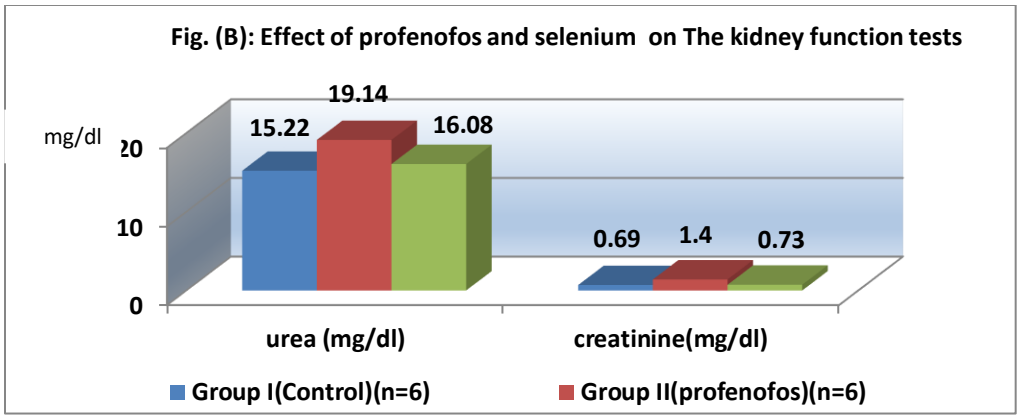
The enzymes that provide the first line of defense include superoxide dismutase (SOD). Our results are reinforced by (Verma and Srivastava, 2003). Changes in blood SOD activities have been determined to develop due to the generation of free radicals which causes red blood cell damage, damage occurs in tissues, primarily in the liver. Pesticides are reported to inhibit the enzymatic defense also in rat tissues (Ojha *et al*., 2011).

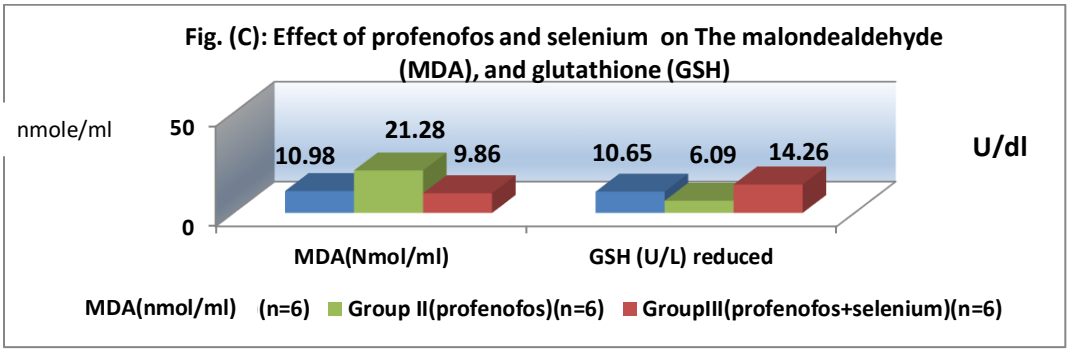
Table (2): Effect of Profenofos and Se on the biochemical parameters (Mean ± SE):

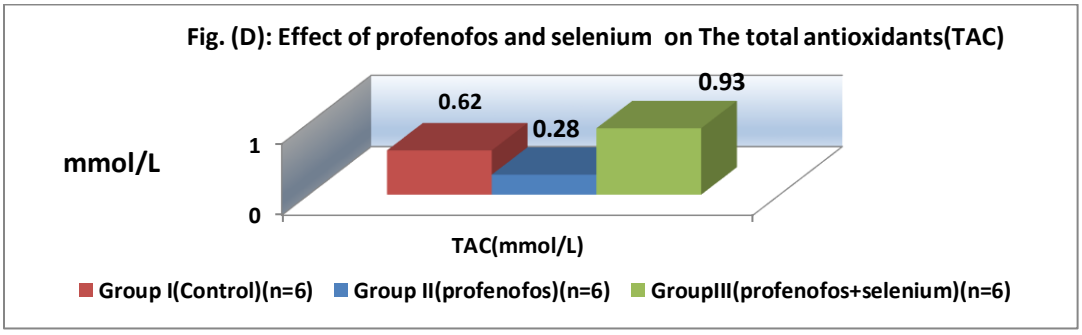
parameters	Group I (Control) (n=6)	Group II (Profenofos) (n=6)	Group III Profenofos+ Se (n=6)
AST (U/L)	26.33 ± 0.55[a]	34.86 ± 1.07 [b]	28.79 ± 0.04 [a]
ALT (U/L)	18.04± 0.34[a]	31.78 ± 1.06[b]	16.46 ± 0.55 a
urea (mg/dl)	15.22± 0.01[a]	19.14± 0.68[b]	16.08± 0.11[a]
creatinine(mg/dl)	0.69 a ±0.03 [a]	1.4±0.01[b]	0.73±0.03[a]
MDA (Nmol/ml)	10.98 ±0.37[a]	21.28±0.25 [b]	09.86±0.15 [a]
TAC (mmol/L)	0.62±0.24 [a]	0.28±0.02 [b]	0.93±0.02 [a]
Superoxide dismutase(SOD/ml blood)	262.81 ± 13.89[a]	115.43 ± 7.27[b]	209.78 ± 10.53[a]
GSH (U/L)	10.65 ± 0.51[a]	6.09 ± 0.47[b]	14.26 ± 0.76[a]

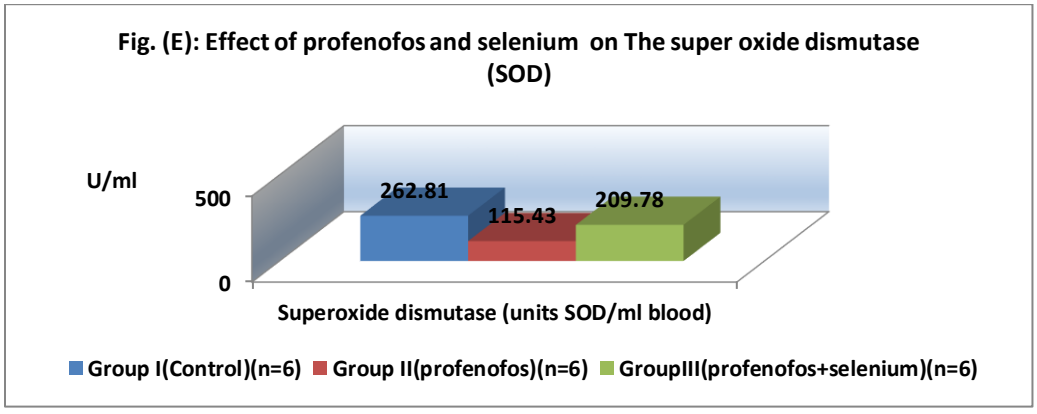
Means within the same row are bearing different letter superscripts (a & b) differ significantly ($P\leq0.05$).











Superoxide dismutase (U/ml)

3.4.4. Effect of Profenofos on hormonal profile:

3.4.4. 1.Testesterone hormone:

The data in Table (3) and Fig (F) revealed significantly decreased in serum testosterone hormone in GP II against GP I. But revealed non-significant changes in GP III in compared with GP I

The present results confirm the previous reports of (El-kashoury and El-far, 2004) who mentioned that administration of rats with Profenofos induced a significant decrease in thyroid hormone levels which is an essential to the normal development of testes in the neonate (El- Kashoury 2009).

Se is an essential trace nutrient for humans and animals which required for normal testicular development and spermatogenesis in rats (Behne *et al*., 1996). The selinodeiodinase enzymes control the metabolism of thyroid hormone, which is essential for the normal development (Defrance *et al*., 1995) and function (Latchoumycandane *et al*., 1997) of testes in rats. In support of these findings, earlier results (El-Kashoury and El-Far, 2004) revealed that treatment of rats with Profenofos at the same dose and time interval decreased markedly (T_3) level in plasma in comparison with the control group.

Se is important for normal spermatogenesis and largely as a component of seleno-proteins phospholipid hydroperoxide glutathione peroxidase (PHGPx/GPX4) and Seleno-protein. Most of the selenium found in the testis is associated with PHGPx/GPX4. It serves as a powerful antioxidant protecting cells from oxidative stress. PHGPx also appears to be involved as a structural protein to provide normal sperm motility (Yugal *et al*., 2013). It has also been shown that a variant to this protein is necessary for normal chromatin condensation and subsequent normal spermatozoa head formation (Wiltbank *et al*., 2007).

3.4.4.2. Estrogen hormone:

Table (3) and Fig. (F) showed significantly decreased in estrogen hormone in group II against the control group and Non-significant changes in selenium group.

Overview of the various ways in which pesticides may disrupt the hormonal function of the female reproductive system and in particular the ovarian cycle. Disruption can occur in all stages of hormonal regulation and induce ovarian dysfunction (Farr *et al*., 2004). Pesticides may cause reproductive toxicity through several different mechanisms: direct damage to the structure of cells, interference with biochemical processes necessary for normal cell function, and biotransformation resulting in toxic metabolites. Reproductive effects that have been associated with pesticide exposure in women are decreased fertility, spontaneous abortions, stillbirth, premature birth, low birth weight, developmental abnormalities, ovarian disorders, and disruption of the hormonal function (Schettler *et al*., 2003).

3.4.4.3. Progesterone hormone:

Also, table (3) and Fig. (F) showed significantly decreased in progesterone in GP II against the control group. While non-significant changes were observed in selenium group.

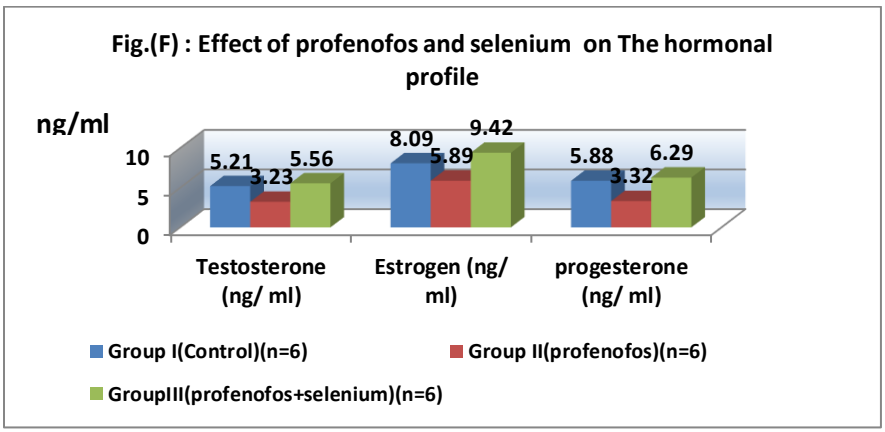
Pesticides inhibit various enzymes which inhibits progesterone synthesis (Reini *et al*., 2008). Then added that hormonal balance, i.e. a proper level of sexual hormones, is important to preserve female reproduction and maintain fertility. This balance can be disturbed by changing levels of estrogen or progesterone. Estrogen levels may be decreased by several pesticides. Treatment of rats with the insecticide heptachlor suppressed estrogen concentrations in blood and reduced the production of estrogen by ovarian cells of treated rats. Exposure to pesticides revealed reduction in body weight, induce many other health problems in animals and these might be reproductive disorders (Aly *et al*., 2009).

Se was proved as an indispensable nutrient necessary for both normal growth and reproduction in animals. Selenium deficiency, female reproduction, pregnancy and lactation: Among the major outcomes resulting from Se deficiency in females are infertility, abortion and retained placenta. In addition to, the offspring born from Se-deficient mother suffer from muscular weakness. During the early stage of pregnancy, low concentration of Se in serum, has been associated with low birth weight of child at birth Pieczy and Grajeta (2015).

Table (3): Effect of Profenofos and selenium on the hormonal profile (Mean ± SE):

parameters	Group I (Control) (n=6)	Group II (Profenofos) (n=6)	Group III Profenofos+ Se (n=6)
Testosterone(ng/ ml)	5.21 ± 0.3 [a]	3.23 ± 0.1 [b]	5.56 ± 0.6 [a]
Estrogen (ng/ ml)	8.09 ± 0.3 [a]	5.89 ± 0.4 [b]	9.42 ±0.5[a]
progesterone (ng/ ml)	5.88 ± 0.2 [a]	3.32 ± 0.2 [b]	6.29 ±0.3[a]

Means within the same row are bearing different letter superscripts (a & b) differ significantly (P≤0.05).



3.5. Pathological examination:

The results of histopathological examination of tests, epididymis, ovary, uterus, liver and kidney revealed that Profenofos exhibited pathological changes in these organs and considered as toxic substance.

3.5.1. The histopathological results:

3.5.1.1. Effect of Profenofos on male:

OPIs can cause various histopathological changes in the male reproductive system of mammals as they have the ability to cross the blood-testis barrier inducing oxidative stress and lipid peroxidation that damage the biological membranes in the testes result in testicular dysfunction and male infertility (Muralidhara, 2007).

- Testis:

The above mentioned biochemical results were supported histopathologically as testicular changes were observed in rabbits. In this study, the gross appearance of testicles was prominently atrophied in GP II males. While other animals showed normal appearance testis. Histological findings of testes of control group (GP I) revealed that normal seminiferous tubules with complete series of spermatogenesis containing small groups of Leydig cells and high spermatozoa concentration in the lumen. While Profenofos GP II animals, in correlation with the control one revealed prominent vacuolations absence of all internal layers except scattered cells along the basement membrane, leading to complete cessation of spermatogenesis as well as presence of giant spermatids in the lumen of some tubules were found in the seminiferous tubules. Some tubules showed atrophy and necrosis of the spermatogenic cells**.** Most of the tubules showed thickening and hyalinization of their basement membranes. Interstitial space showed clearly Leydig cells elimination or hypertrophy and vacuolations Infiltration of mononuclear inflammatory cells was noticed. Prominent interstitial edema, congestion and hemorrhages were also observed (Fig. 1). On the other side, of GP III animals showed signs of regeneration and Se could protect testis against toxic effect of Profenofos (Fig. 2).

All these lesions confirmed our opinion that Profenofos had testicular toxic effect by its direct effect on the testis tissues. The previous results were supported by the results of Memon *et al.* (2014) in OPI intoxicated animals. In this respect, Dessouki *et al.*(2012) stated that the spermatogenesis and the sperms themselves may also be damaged by oxidative effects of OPI. The presence of pesticide residues in the fluids surrounding spermatozoa have negative influence on sperm quality (Ghuman *et al.* ,2013) as well as alter spermatozoa chromatin and DNA structure and fertilization competence (Salazar-Arredondo *et al.* ,2008). These pesticides caused spermatozoa dysfunctions due to their cumulative

effect or their chronic exposure (Selvaraju *et al*., 2011).

Exposure to OPI may cause endocrine changes both directly (as hormone agonists or antagonists) and indirectly (altering circulating levels of hormones by influencing rates of hormone synthesis or metabolism), which can severely affect steroid hormone actions (Moustafa *et al*., 2007). Hypertrophy in Leydig cells results, disrupt the functioning of the testes to release testosterone hormone for normal spermatogenesis that creates fertility problems (Thomas *et al*., 2008).

There is evidence that some OPI may have in-vivo genetic effects, suggesting a possible link with cancer with long term or repeated heavy exposure (Hatjian *et al*., 2000) which inducing chromosome damage so they could be a mutagenic or carcinogenic effect (Hammam and Abd el Mottaleb , 2007).

In this work, Se used as an antioxidant which came in accordance with Uzun *et al*. (2009) and Shittu *et al.* (2013) who observed that vitamins C and E ameliorate Malathion and Chlorpyrifos testicular toxicity, respectively.

- Epididymis:

Pathological changes of epididymis (GP II) characterized by severe oedema and congestion between epididymis tubes and lacking of sperm. Also, epithelial vacuolations and focal inflammatory cell infiltrations were observed. The tubules were deshaped with loss of cilia of the epithelial lining and possessed little or no spermatozoa (Fig. 3). Lesser histological changes were noted in group I and III. These changes were noted previously by Hendawy *et al.* (2012).

3.5.1.2. Effect of Profenofos on female:

- Ovary :

The macroscopic examination of the ovary in this work was apparently normal except some congestion. Histopathologically, GP I ovaries showed normal structure with different stages of Graffian follicles. While GP II revealed ovarian necrosis, atrophy of follicular cells with interstitial edema, congestion, severe hemorrhage and decreasing the number of growing follicles. Also, one case showed vacuolar degeneration in granulosa cells of matured Graffian follicle accompanied by mononuclear cell infiltrations in the interstitial tissue. On the other hand, GPIII showed no abnormal alterations as compared to the control group; however, these tissues showed more follicular maturation.

Similar findings observed by Sangha *et al*. (2013) in rats and Ghuman *et al*. (2013) in buffaloes. Also, Ranjana and Mishra (2015) reported adhesion of primary follicle, cytoplasmic clumping and increased atretic oocyte in H. fossils ovary treated with Malathion.

These lesions could be due to a lack of available proteins necessary for oogenesis or else inhibition of the hypothalamus or pituitary that induced by pesticide (Pocar *et al*. 2005). Faundez *et al.* (1996) added that the endocrine imbalance leading to a reduction in circulating estrogen and progesterone and this came in accordance with our work. In this respect, Agarwal *et al*. (2012) stated that OPIs may cause ovaries toxicity as it affect multiple physiological processes from oocyte maturation to fertilization, embryo development and pregnancy as a result of ROS which produced by multiple cells like macrophages and parenchymal steroidogenic cells in ovaries (Halliwell and Gutteridge ,1988).

The ovarian exposure to pesticides can have temporary or permanent impact on fertility depending upon the developmental stage at which damage occurs. When its impact on growing or antral follicles, they have temporary influence on reproductive functions as these follicles are replaced by recruitment of other follicles. But they extensively destroy oocyte contained in the primordial and primary follicles that have a permanent effect on reproduction (Uzumcu and Zachow, 2007). Also, the pesticide exposure during neonatal period causes damage to germ cells in ovaries thus producing sterility in the adult dairy animals (Pocar *et al*., 2005).

In addition to, this work discussed the role of Se which reduced Profenofos cytotoxicity and blocked generation of ROS that showed previously by Said *et al.* (2012) and Madhavi and Kumar (2010) when used Se and curcumin against radiation and OPIs, respectively.

- Uterus:

In the present investigation, the macroscopic examinations of uteri showed edema, congestion and haemorrhage in Profenofos treated animals. The histopathological examination of uteri in control group (GP I) revealed that normal structure. While GP II rabbits showed focal areas of chronic metritis with focal and diffuse subepithelial mononuclear cell infiltrations as well as erosion, desquamation of the uterine epithelium and fibrosis. Uterine glands showed necrosis, periglandular fibrosis and inflammatory infiltrations with cystic dilatation. There

were pronounced congestion and other parts revealed edema and haemorrhage. Sub-serosal inflammatory cells infiltrations were observed (Fig. 4& 5)**.**On the other side, administration of Se preserved the glandular and lining epithelium in the uterine structure.

The same histopathological changes described by Ahmad (2012) who stated Pyrethroids induced early embryonic death. More over Ghuman *et al*. (2013) mentioned that the endocrine disrupting effects of pesticide residues could be through their binding to estrogen receptors, which ultimately influences the hypothalamic–pituitary–gonadal axis. Moreover, the observed chronic metritis might be a possible reason for fetal losses in which endometrial fibrosis interrupted blood supply to the fetus, resulting in low body weight gain or increased death rate in the feti (Ullah *et al*., 2006). Also, Sallam *et al*. (2015) observed that the exposure of bovine to cypermethrin decreased the progesterone concentrations which support our findings.

One case in Profenofos treated GPII showed leiomyoma with rhabdomyosarcoma which grossly appeared as a single large grayish white fleshy mass with multiple foci of necrosis, bulging and trabeculated on cut surface. Microscopically, leiomyoma is a benign smooth muscle tumor appeared as proliferated of smooth muscle cells and separated by well vascularized connective tissue .Smooth muscle cells are elongated with eosinophilic or occasional fibrillar cytoplasm and distinct cell membranes. Areas of degenerations associated with lymphocytes and mast cells were seen. In addition to, this case showed rhabdomyosarcoma which appeared as large, multinucleated, eosinophilic elongated tumor cells with eccentric eosinophilic granular cytoplasm and Prominent cross striations (Fig. 6). Similar findings previously described by Crum *et al.* (2011) in human. Also, Sharma *et al*. (2005) reported that certain OPIs are associated with carcinogenesis as a result of DNA damage.

3.5.1.3. Effect of Profenofos on liver tissues:

The macroscopic appearance of Profenofos treated liver showed hepatomegaly, congestion and haemorrhage. The liver of GP I showed the normal histological structure. While GP II revealed massive, diffuse vacuolar degenerative changes and disorganization. Prominent periportal hepatocytes necrosis with dilated and congested blood vessels was seen. Focal and diffuse mononuclear inflammatory cells infiltrations were detected in the portal area as well as Kupffer cells proliferation .Oedema with evident interlobular and subcapsular haemorrhage were also noticed. In addition to, dilated and hyperplasic bile ducts and newly formed bile ductules as well as portal fibrosis were pronounced (Fig. 7). On the other side, Se could ameliorate the toxic effect of Profenofos in GP III that gave the normal hepatic microscopical appearance.

The adverse effect of Profenofos was similar results were obtained by Abu Aita *et al*. (2012). Therefore, this study suggested Se had ameliorating effects which was equally shown the protection of vitamins as antioxidant in hepatic oxidative damage (Mossa *et al.*, 2011). Liver enzymes are more sensitive measure of hepatotoxicity and their disturbing levels was supported by histopathological changes in this study. Therefore, the increase in these enzymes may be due to liver dysfunction and disturbance in the biosynthesis of them with alteration in the permeability of liver membrane takes place (Khalil *et al.*, 2014).

3.5.1.4. Effect of Profenofos on kidney tissues:

The control rabbits showed normal structure kidneys. On the other side, the kidney of GP II exhibited inflammatory cell infiltrations in the interstitial spaces associated with degenerative change in the epithelial cells lining the renal tubules which had casts in their lumen. Some tubules showed epithelial desquamation and others revealed necrotic epithelium as well as shrinked glomeruli. Severely congested blood vessels with focal hemorrhagic areas and oedema were noted. The renal corpuscles showed congestion and hypercellularity and wide urinary space. There was thickening in the Bowman's corpuscle (Fig. 8). Moreover, GP III kidneys showed nearly normal structure except somewhat congestion. These changes confirmed the above mentioned biochemical results of kidney function and that was in accordance with those obtained by Salem and Kobeasy (2005) and Shalby (2006). Selenium was significantly more effective at recovering activities of SOD, CAT and GPx in liver and kidney of Malathion treated rats then was tocopherol (Al-Othman *et al.*, 2011).

3.5.2. Immunohistochemical evaluation:

- Caspase-3 immunoreactivity:

Oxidative stress occurs when the production of ROS exceeds the body’s natural antioxidant defense mechanisms, causing DNA, proteins and lipids damage that initiate apoptosis (Bartsch and Nair, 2000). Disruption of the outer mitochondrial membrane by apoptotic stimuli results in the release

of cytochrome c into the cytoplasm where it initiates a cascade of caspase activation and results in apoptosis (Anuradha *et al*., 2001). Caspases are a family of cysteine proteases as inactive proenzymes and become activated when apoptosis (programmed cell death) is initiated (Llopis *et al*., 2003). Organochlorine insecticides are also capable of inducing apoptosis by multifunctional pathways when cells exposed to oxidative stress, they often die by apoptosis or necrosis (Ozmen and Mor, 2012). Failure of apoptosis is one of the main contributions to tumor development (Dias *et al*., 2000). To date, little information exists on the effect of Profenofos on caspase-3 expression as well as the effect of Se in correction of the Profenofos toxicity.

In this study, testis**,** ovary and uterus of control group revealed that negative and/or low caspase- 3 immunohistochemical reactivity. However, few scattered cells exhibited faint light brown granules. On the other hand, intensive positive immunoreactivity for caspase- 3 (dark brown) was observed in the cytoplasm of germinal cells of GP II compared with the control. The apoptotic positive cells were seen to be prominent in germinal epithelium, Sertoli cells and Leydig cells. Slight caspase-positive reaction was also seen in spermatids (Fig. 9). Also, marked increase in caspase- 3 expressions in ovarian granulosa and theca interstitial cells as well as uterine epithelial cells (Fig 10). While animals of GP III showed apparent reduction of caspase-3 immunoreactivity in their tissues including testis (Fig 11), ovary (Fig 12) and uterus (Fig 13) to be more or less similar to the control group.

These findings agreed with that of Elgawish and Abdelrazekb (2014) who explore caspase-3 in mice testes to lead acetate. This study supported the findings of Ozmen and Mor (2012) with apoptotic activity in Sertoli and Leydig cells. The caspase positive reaction in Leydig cells correlated with decreased serum testosterone level. Meanwhile, treatment of Profenofos with Se showed noticeable alleviation in histopathological and biochemical changes. Consequently, antioxidants could reduce cell death (Said *et al*., 2012).

- Desmin and Actin immunohistochemistry:

Desmin is a class-III intermediate filament protein expressed on smooth, skeletal and cardiac muscle cells and is useful in diagnosis of tumor with myoid origin. Also, the antibody recognizes actin isotypes alpha of smooth muscle and those cells with myofibroblast differentiation. It labels smooth muscular cells, myofibroblasts and myoepithelial cells that are a useful marker for the identification D'Angelo *et al.* (2009).Leiomyoma with rhabdomyosarcoma case showed positive immunoreactivity for Desmin (Fig 14) and SMA (Fig 15). Variation in immunohistochemical reactivity to Desmin and SMA might be a diagnostic feature of genital leiomyosarcomas of domestic animals; where there is diffuse and uniform immunoreactivity to Desmin and SMA in leiomyomas, but irregular or diffuse immunostaining in genital leiomyosarcomas. Variations in the immunoreactivity of smooth muscle tumors to SMA and Desmin might be related to the degree of differentiation of each tumor (Cooper and Valentine, 2002). A variety of exogenous and endogenous hormones can influence the mitotic index and the development of tumor cell necrosis (Asakawa *et al.*, 2008).

3.5.2. Histochemical Evaluation:

In the present work, periglandular or perivascular fibrosis which demonstrated with Masson-trichrome stain in the uterine tissues (Fig. 16). Profenofos treated group showed decrease in the polysaccharides content of testis (Fig. 17), hepatocytes (Fig. 18) and renal cells (Fig. 19) as well as the basement membranes of the proximal and distal convoluted tubules appear thick as compared to control. On the other side, PAS stained tissues of GP III appeared more or less as control (Fig. 20&21). These results came in accordance with that of Rao (2006) who reported that the glycogen depletion in the tissues is an indication of typical stress response in fish challenged with pesticides. Shalaby (2006) mentioned that the reduction in carbohydrate components could be due to the release of hydrolytic enzymes from the ruptured lysosomes under the pesticide effect. Also, staining hepatic and renal tissues with bromophenol blue stain revealed reduction in the protein content in Profenofos-treated rabbits (Fig .22&23). While, Se caused a marked preservative effect on protein content of these cells (Fig. 24).The observed changes mimic to that of El-Khayat *et al.* (2010).

4. CONCLOSION:

1-The studied criteria included the changes in body and organs weight under the effect of drinking polluted water by 70 mg Profenofos for 90 days were detected as a result of ROS generation.

2-Profenofos has one of serious problems that threatened mammal's life and toxic effects as represented by increased levels of liver enzymes and

kidney function test and decrease testosterone progesterone and estrogen hormone levels.

3-Varies degrees of harmful changes in the histological structure of testis, epididymis, ovary, uterus, liver and kidney were observed.

4-Se not only eliminates the toxic effects of Profenofos but also acts as a suitable antioxidant and free radical scavenging activities normalizing the toxic effect induced by Profenofos.

5-For all that, the adverse health effects are clearly minimized by selecting the right pesticide at proper time of application and using the right method. It is therefore, necessary to follow the international recommendations and enforce national legislation.

6-The exposed workers living in the agriculture zones should be periodically examined to estimate any mutagenic effect which results from their contact with pesticides.

7-In grains and cereals the level of selenium is generally low but much higher levels can be found in products from the seleniferous areas.

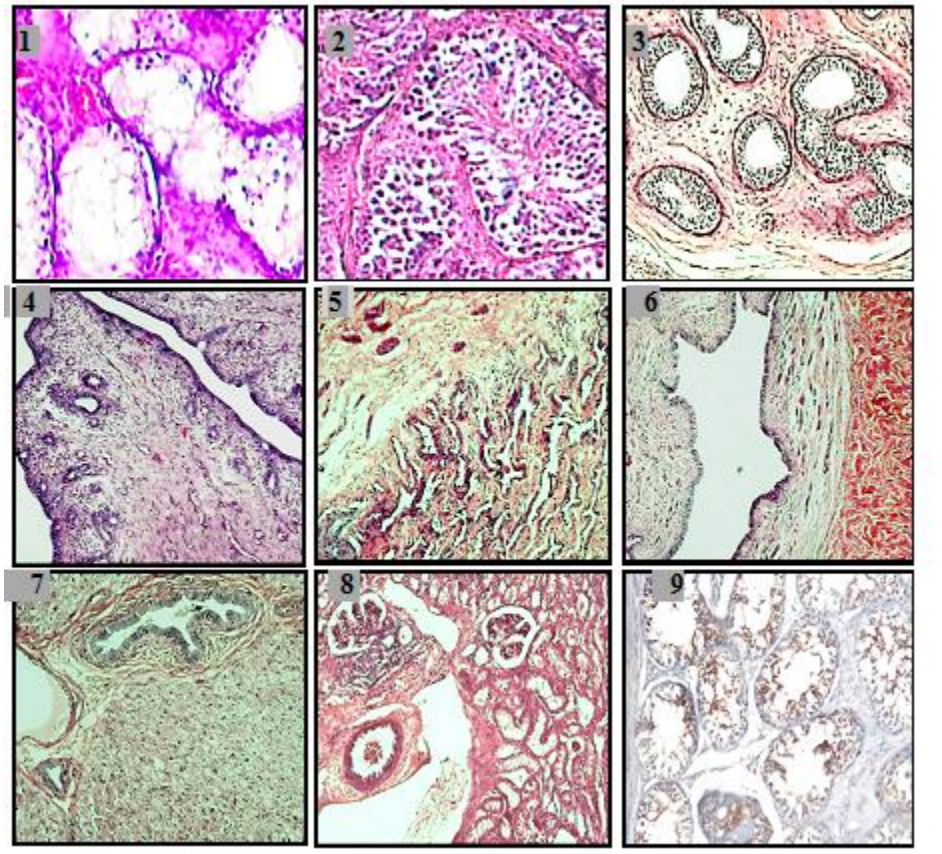


Fig.1: Rabbit testis (G II) showing epithelial vaculations and disorganization of seminiferous tubules ,thickening of basement membranes ,no spermatocytes ,Leydig cells hypertrophy, Prominent interstitial edema, congestion and inflammatory cells infiltration (H&E stain, x400).

Fig.2: Rabbit testis of (GP III) animals showed signs of regeneration with normal tubules and interstitial tissue (H&E stain, x400).

Fig.3: Rabbit epididymis (G II) showing prominent epithelial vaculations, interstitial edema, complete absence of sperm, loss of cilia of the epithelial lining and focal inflammatory cell infiltrations (H&E stain, x100).

Fig. 4: Rabbit uterus (G II) showing chronic metritis with focal and diffuse subepithelial mononuclear cell infiltrations, periglandular fibrosis and necrosis, cystic dilatation and congestion (H&E stain, x100).

Fig.5: Rabbit uterus (G II) showing chronic metritis with periglandular fibrosis and glandular necrosis, cystic dilatation, glandular hyperplasia and congestion (H&E stain, x100).

Fig.6: Rabbit uterus (G II) leiomyoma with rhabdosarcoma (H&E stain, x100).

Fig.7: Rabbit liver (G II) showing dilated and hyperplasic bile ducts, newly formed bile ductules, portal fibrosis, vacuolar degeneration, inflammatory cells infiltrations in the portal area (H&E stain, x100).

Fig.8: Rabbit kidney (G II) showing interstitial inflammatory cell infiltrations, epithelial degeneration, shrinked glomeruli, congested blood vessels and oedema (H&E stain, x100).

Fig. 9: Rabbit testis of (G II) showing positive apoptotic cells prominent in germinal epithelium, Sertoli cells and Leydig cells (Caspase-3 immunostain, X 100).

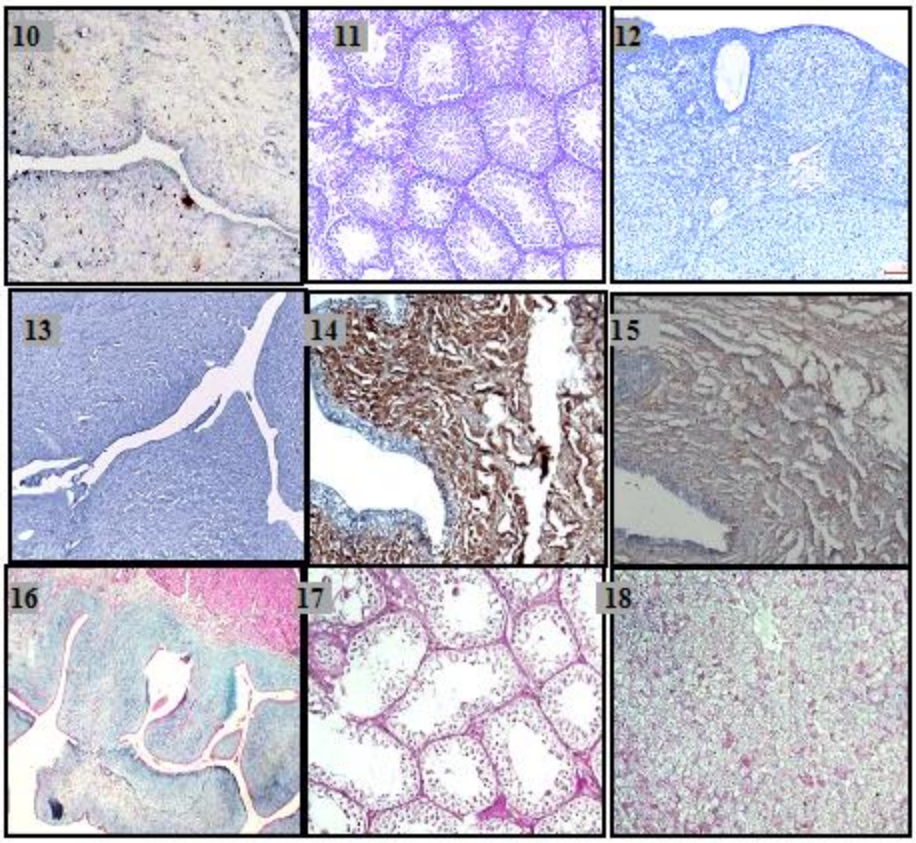


Fig.10: Rabbit uterus (G II) showing marked positive apoptotic in caspase- 3 expressions in uterine epithelial cells (Caspase-3 immunostain, X 40).
Fig.11: Rabbit testis (G III) showing apparent reduction of caspase-3 immunoreactivity (Caspase-3 immunostain, X 40).
Fig.12: Rabbit ovary (G III) showing decreasing of caspase-3 immunoreactivity (Caspase-3 immunostain, X 40).
Fig.13: Rabbit uterus (G III) showing apparent reduction of apoptotic caspase-3 immunoreactivity (Caspase-3 immunostain, X 40).
Fig.14: Rabbit uterus of (G II) showing positive immunoreactivity for smooth muscle markers desmin (Desmin immunostain, X 100).
Fig.15: Rabbit uterus of (G II) showing positive immunoreactive to actin (SMA immunostain, X 100).
Fig.16: Rabbit uterus (G II) showing marked proliferation of fibrous connective tissue (Masson's Trichrome stain, X40).
Fig.17: Rabbit testis (G II) showing severe decrease in the polysaccharides content of the seminiferous tubular with thickening basement membrane (PAS stain, x100). Fig.18: Rabbit liver (G II) showing decrease in the polysaccharides content of the hepatocytes (PAS stain, x100).

6. REFERENCES

Abdel-Megeed M.I.,Radwan U.M.,Hindy A.Z. and Zrook A.2001.Liver functions under stress of certain common pesticides residue used on fruits and vegetables orally administrated. Annals Agric. Sci., Ain Shams Univ., Cairo, Egypt, 46(1): 383-404.

Abu Aita, N. A.; Hashesh, M. A. and Mohamed, A. H. 2012. Clinicopathological and cytogenetic studies on the ameliorative effect of Propolis against Profenofos toxicity in rats. Global Veterinaria; 9 (6): 669-682.

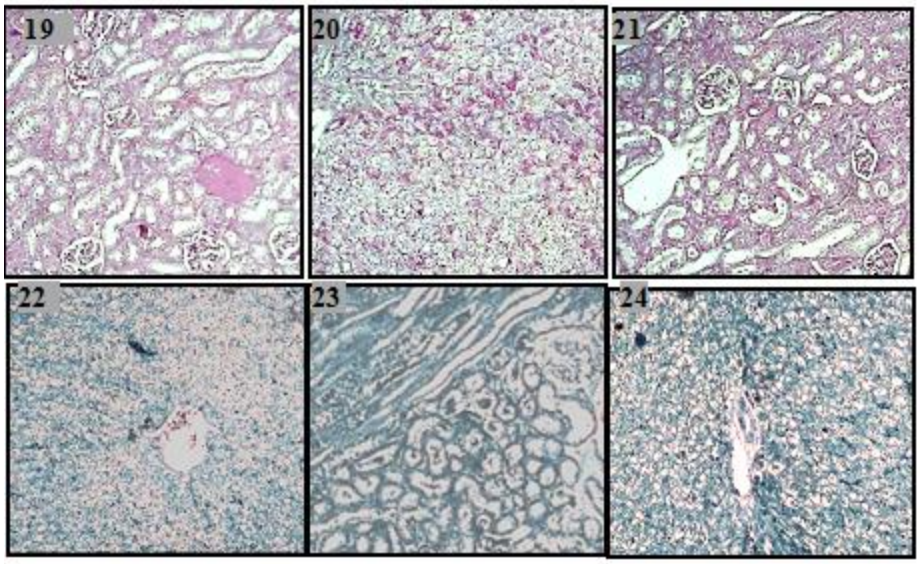
Agarwal, A., Aponte-Mellado, A., Premkumar, B.J., Shaman, A. and Gupta, S. 2012. The effects of oxidative stress on female reproduction: a review. Reproductive Biology and Endocrinology; 10: 49.

Ahmad, L.; Khan, A. and Khan, M. Z. .2012.Pyrethroid-Induced Reproductive Toxico-Pathology in Non-Target Species Pak Vet J.; 32(1): 1-9.

Ahmed, M.M., Sahar, L..,Nasir, F. and Minhas, R. 2000. Pesticide induced changes in secretaryactivity of the thyroid gland in rats. Pakistan. J. Zoo, 32(4): 333-336.

Akhgari, M., Abdollahi, M., Kebryaeezadeh, A., Hosseini, R. and Sabzevari, O. 2003.Biochemical evidence for free radical induced lipid peroxidation as a mechanism for subchronic toxicity of Malathion in blood and liver of rats. Hum Exp Toxicol. 22: 205-11.

Al-Othman, A. M. , Al-Numair , K. S. , El-Desoky , G. E.,Yusuf , K. Z.; Al Othman , A., Aboul-Soud, M. A. M. and Giesy, J. P. 2011. Protection of -tocopherol and selenium against acute effects of malathion on liver and kidney of rats. African Journal of Pharmacy and Pharmacology;5(8):1054-1062.



(Fig.19): Rabbit kidney (G II) showing severe decrease in the polysaccharides content of the tubular epithelium (PAS stain, x100).
(Fig. 20): Rabbit liver of (G III) showing restored polysaccharides content of the hepatocytes (PAS stain, x100).
(Fig. 21): Rabbit kidney of (GIII) showing restored polysaccharides content of tubular epithelium (PAS stain, x100).
(Fig. 22): Rabbit liver of (G II) showing severe decrease in the protein content of the hepatocytes (Bromophenol blue stain, x100).
(Fig. 23): Rabbit kidney of (G II) showing decrease in the protein content of tubular epithelium (Bromophenol blue stain, x100).
(Fig. 24): Rabbit liver of (G III) showing restored protein content of the hepatocytes (Bromophenol blue stain, x100).

Aly, H., O. Domenech, B. Ashraf, N. Abdel Naim. 2009. 1254 impairs spermatogenesis and induces oxidative stress in rat testicular mitochondria Food Chem. Toxicol., 47 (8): 1733–1738.

Ambali, S., Akanbi, D. , Igbokwe, N., Shittu, M. and Kawu, M. 2007. Evaluation of subchronic chlorpyrifos poisoning on hematological and serum biochemical changes in mice and protective effect of vitamin C. J. Toxicol. Sci., Vol.32 pages: 111-120.

Anderson, R. and Cockayne, D. 1989. "Clinical chemistry concepts and applications.", Allston. Ann. Corale. Editor, Philadelphia, London, PP:367-383.

Anuradha, C.D.; Kanno, S. and Hirano, S. 2001. Oxidative damage to mitochondria is a preliminary step to caspase-3 activation in fluoride-induced apoptosis in HL-60 cells. Free Radic. Biol. Med.; (31): 367–373.

Asakawa, M. G., Goldschmidt M. Une, H. Y. and Nomura.Y. The Immunohistochemical Evaluation of Estrogen Receptor-{alpha} and Progesterone Receptors of Normal, Hyperplastic, and Neoplastic Endometrium in 88 Pet Rabbits. Vet Pathol March 2008 , 45: 2: 217-225 .

Barr, D.; Bishop, A. and Needham, L. 2007. Concentrations of xenobiotic chemicals in the maternal-fetal unit. Reproductive Toxicology;23(3):260-266.

Bartsch, H. and Nair, J. 2000.Ultrasensitive and specific detection methods for exocytic DNA adducts: markers for lipid peroxidation and oxidative stress, Toxicology; 153:105–114.

Bebe, F.N. and Panemangalore, M. 2003. Exposure to low doses of endosulfan and chlorpyrifos modifies endogenous antioxidants in tissue of rats. J. Environ. Sci. Health, 38(3): 349-363.

Behne, D.; Weiler, H. and Kyriakopoulos A. 1996. Effects of selenium deficiency on testicular morphology and function in rats. J. Repord Fertile. 106: 291-297.

Burk RF, Hill KE, MotleyAK. 2003. Seleno protein metabolism and function evidence for more than one function for selenoprotein P.J Nutr 13315175-20S.

Campagna, C.; Guillemette, C.; Ayotte, P. and Bailey, J. L. 2009. Effects of an environmentally relevant organochlorine mixture and a metabolized extract of this mixture on porcine sperm parameters in vitro. Journal of Andrology 30: 317–24.

Cooper, B.J.; Valentine, B.A. Tumors Of Muscle. In: Meuten, D.J. (Ed.). Tumors in domestic animals. 4.ed. Ames: Iowa State University, 2002. p.319-363.

Cortossa, S., Aon, M.A., Waston, R.L., ´Rourke, B. 2004. A mitochondrial oscillator dependent on reactive oxygen species. Biopysic. J, 87: 2060-2073.

Crum, C. P., Lee, K. R. & Nucci, M. R. 2011. Diagnostic Gynecologic and Obstetric Pathology. Saunders. Publisher – Google Scholar

D'angeloe, E. & Prat, J. 2009. "Uterine Sarcomas: A Review," Gynecologic Oncology.Publisher – Google Scholar

Defrance, L.R., Hess, R.A., Cooke, P.S. and Russell, L.D. 1995. Neonatal hypothyroidism causes delayed sertoli cell maturation in rats treated with propylthiouracin: Evidence that sertoli cell controls testis growth. Anat. Rec. 242: 57-69.

Dessouki, A., Hassan, A., Ali, S. and Loutfy, N. 2012. Alterations in p53 gene, testicular and hepatic tissues of albino rats due to profenofos administration: a possible protective effect of vitamin c. J. Plant Prot. and Path., Mansoura Univ., Vol. 3 (5): 415 – 433.

Dias, S., Hattori, K.; Zhu, Z.; Heissig, B., Choy, M., Lane, W.; Wu, Y.; Chadburn, A.; Hyjek, E.; Gill, M., Hicklin, D.J., Witte, L., Moore, M.A . and Rafii, S. 2000. Autocrine stimulation of VEGFR-2 activates human leukemic cell growth and migration. Journal of Clinical Investigation. 106:511-521.

Elgawish,R. A. R. and Abdelrazek,H. M .A. 2014.Effects of lead acetate on testicular function and caspase-3 expression with respect to the protective effect of cinnamon in albino ratsToxicology Reports. ; 1:795–801.

El-Kashoury, A.A. and El-Far, F.A. 2004.Effect of two products of profenofos on thyroid gland, lipid profile and plasma APO-1/FAS in adult male albino rats, Egypt. J. Basic and Appl. Physiol., 3: 213-226.

El-Kashoury, A. A. 2009. Influence of Subchronic Exposure of Profenofos on Biochemical Markers and Microelements in Testicular Tissue of Rats. Nature and Science,;7(2).

El-Khayat, Z. , Rasheed, W. , Ramzy, T., Hussein, J. ,Agaiby, M., Morsy, S., Morsy, F. and Shaffie, N. 2010. Protective effect of garlic oil against liver injury in experimental animals. Journal of Medicinal Plants Research ;4(22):2359-2369.

Fang, Y.Z., Yang, S., and Wu, G., 2002. Free radicals, antioxidants, and nutrition, Nutrition.; 18:872–879.

Farrag A.H. and Shalby E.M. 2007. Comparative Histopathological and Histochemical Studies on IGR, Lufenuron and Profenofos Insecticide Albino Rats. Journal of Applied Sciences Research, 3(5): 377-386.

Farr SL, Cooper GS, Cai J, Savitz DA, Sandler DP .2004.Pesticide useand menstrual cycle characteristics among premenopausalWomen in the Agricultural Health Study. Am J Epidemiol 160:1194-1204.

Faundez, R., E. ,Sitarska, W. K. and Duszewska,A.M. 1996. The effect of persistent chlorinated hydrocarbons on the secretion of estradiol and progesterone by bovine granulose cells in vitro. Zentralbl. Veterinarmed. A, 43: 317-323.

Finco, D. R. 1997."Kidney function in : Clinical Biochemistry of Domestic Animals." 5th Ed., Jiro, J. Kaneko, Academic Press PP: 441-484. Egypt J. Comp. Path. Clin. Patho, 2 (2): 251-256.

Flora, S. J. S., 2009. Structural, chemical and biological aspects of antioxidants for strategies against metal and metalloid exposure. Oxidative Medicine and Cellular Longevity. 2: 191–206.

Gamal, H. A., Farrag A, H., El Sharkawy S, L.., and Wafaa E. A.. 2006.Effects of Profenofos on Antioxidant Enzymes Activities and Gastric Mucosa in Rats. JASMR.; 1(2):125134l.

Ghuman, S. Si.,Gill and, J.P.S. and Jasbir, S. B. 2013.Impact of pesticide residues on fertility of dairy animals: A review Article in The Indian journal of animal sciences · December .

Gruchala A, N. Wasilewska A, Sikora A. k, Rys J, Szklarski W, Jaszcz A, Lackowska B, Herman K. 1997. Rhabdomyosarcoma. Morphologic, immunohistochemical, and DNA study. Gen Diagn Pathol. 1997 Feb;142(3-4):175-184.

Halliwell B, Gutteridge JM. 1988. Free radicals and antioxidant protection: mechanisms and significance in toxicology and disease. Human Toxicology 7, 7–13.

Hammam, F. M. and Abd el Mottaleb, E. M. 2007. Studies of the Genotoxic and Histopathological effects of the Organophosphorous insecticide 'Profenofos' on white rats. The Egyptian Journal of Hospital Medicine ; 29: 685– 706 .

Hanafy, N. S. M., Arbid, M. S. and Afify, M. M. H. 1989. "Biochemical and histopathological effects of the organophosphorous insecticide (Tamaron) in rats." Egypt J. Comp. Path. Clin. Patho, 2 (2): 251-256.

Hatjian, B. A.; Mutch, E.; Williams, F. M.; Blain, P .G. and Edwards, J .W. 2000. Cytogenetic response without changes in peripheral cholinesterase enzymes following exposure to a sheep dip containing diazinon in- vivo and in- vitro. Mutation Res , 472(1-2): 85-92.

Hendawy, A. A. , Mansour, H. Z., El-Sayed, A. A.; Abd El-Aziz ,A. D. and Hamza, R. Z.. 2012. Ameliorative Role and Antioxidant Effect of Propolis and Ginseng against Reproductive Toxicity of Chlorpyrifos and Profenofos in Male Rats. Life Science Journal;9(3) .

Khalil, S. M.,Taha, K. M., Farid, H. E. and Atia, A. F. 2014. Antioxidant role of olive "Olea europeavar picual" leaves extract on Profenofos induced oxidative stress in male rats. International Journal of Advanced Research; 2(7): 635-650.

Khan, A., Faridi, H.A.M., Ali, M., Khan, M.Z., Siddique, M., Hussain, I. and Ahmad, M. 2009.Effects of cypermethrin on some clinicohematobiochemical and pathological parameters in male dwarf goats (Capra hircus). Exp. Toxicol. Pathol., 61: 151–160.

Latchoumycandane, C., Gupta, S.K. and Mathur, P.P. 1997. Inhibitory effects of hypothyrodism on the testicular functions of postnatal rats. Biomed. Lett., 56 : 171-177.

Leng, G., Kuhn, K. H. and Idel, H. 1997. "Biological monitoring of pyrethroids in blood and pyrethroid metabolits in urine. Applications and limitations." Sci. Total Environ., 199: 173-181.

Llopis, S.P. , Ferrando, M.D. and Pena, J.B. 2003. Fish tolerance to organophosphate induced oxidative stress is dependent on the glutathione metabolism and enhanced by N-acetylcysteine, Aquat. Toxicol. ;65(4) :337–360.

Madhavi K. and Kumar R. 2010. In Vivo Toxicological Evaluation Of Organophosphate Pesticide On Female Albino Mice: Therapeutic Effects Of CurcuminIJPSR; 1 (9):86-92.

Maxey, K.M., Maddipati, K.R., and Birkmeier, 1992. J. Interference in enzyme immunoassay.J. Clin. Immunoassay 15, 116-120.

Memon, S. A.; Shaikh, S. A. and Memon, N. 2014.Effects Of Profenofos An Endocrine Disrupting Chemical On Leydig's Cells In Rabbits. The Journal of Animal and Plant Sciences, 24(1):167-171.

Mlynarczuk J, Wrobel M H and Kotwica J. 2010. Effect of environmental pollutants on oxytocin synthesis and secretion from corpus luteum and on contractions of uterus from pregnantcows. Toxicology and Applied Pharmacology 247: 243–49.

Mohandas, J, Marshall, JJ., Duggin, GG, Horvath, JS and Tiller, D 1984. Differential distribution of glutathione and glutathione related enzymes in rabbit kidney: possible interactions in analgesic neuropathy. Cancer Re., 44: 50865091.

Morgan A M. and Abd El-Aty A. M 2008.Reproductive Toxicity Evaluation of Pestban Insecticide Exposure in Male and Female Rats Toxicol. Res. Vol. 24, No. 2, pp. 137-150

Mossa, A. H., Refaie, A. A. and Ramadan, A. 2011. Effect of Exposure to Mixture of Four Organophosphate Insecticides at No Observed Adverse Effect Level Dose on Rat Liver: The Protective Role of Vitamin C. Research Journal of Environmental Toxicology, 5: 323-335.

Moustafa, G.G., Ibrahim, Z.S. Hashimoto, Y. Alkelch, A.M. Sakamoto, K.Q. Ishizuka, M. and Fujita, S.2007. Testicular toxicity of profenofos in matured male rats. Arch. Toxicol.; 81(12):875-881.

Muralidhara, S.B. 2007. Early oxidative stress in testis and epididymal sperm in streptozotocin-induced diabetic mice: its progression and genotoxic consequences, Rep. Toxicol. 23 :578–587.

Nagy, G. J. Ward, D.D. Mosser, A. Koncz, P. Jr.Gergely, C. Stancato, Y. Qian, D. Fernandez, B. Niland, C.E. Grossman, T. Telarico, K., Banki A. . 2006.Regulation of CD4 expression via recycling by HRES-1/RAB4 controls Susceptibility to HIV Infection. J. Biol. Chem., 281(45): 34574-34591.

Nashwa A. A, Mahitab A. H. and Amira H. M. 2012.Clinicopathological and Cytogenetic Studies on the Ameliorative Effect of Propolis Against Profenofos Toxicity in Rats Global Veterinaria 9 (6): 669-682.

Ogunro, P.S., Ogungbamigbe,T.O. Ajala M.O. and Egbewale,B.G. 2005.Total antioxidant status and lipid peroxidation in HIV-1 infected patients in a area of south western Nigeria. Afr. J. Med. Sci., (24): 221-225.

Ojha, A., Yaduvanshi, S.K., and Srivastava. N. 2011. Effect of combined exposure of commonly used organophosphate pesticides on lipid peroxidation and antioxidant enzymes in rat tissues. Pestic. Biochem. Physiol.; 99: 148-156

Ostby, J., Monosson, E., Kelce, W. R., WOLF, C. J., Lambright, C. and Gray, L. E. 1999. The fungicide procymidone alter sexual differentiation of the male rat by acting as an androgen-receptor antagonist in vivo and in vitro. Toxicology and Industrial Health;15(1-2): 80-93.

Ozmen, O. and Mor, F. 2012. Apoptosis in adult rabbit testes during subacute endosulfan toxicity. Pesticide Biochemistry and Physiology; 102:129–133.

Pieczy,A. J. and Grajeta G. 2015. The role of selenium in human conception and pregnancy.

Pocar, P., Fischer, B., Klonisch, T. and Hombach-Klonisch, S. 2005. Molecular interactions of the aryl hydrocarbon receptor and its biological and toxicological relevance for reproduction. Reproduction 129,379–389.

Rahman, M. F.; Siddiqui, M. K. 2003. "Biochemical enzyme activity in different tissues of rats exposed to a noval phosphorothionate (RPR.V)." J. Environ. Sci. Health B, 38 (1): 59-71.

Rai, S., Wahile, A., Mukherjee, K., Saha, B.P. and Mukherjee, P.K. 2006.Antioxidant activity of Nelumbo nucifera [sacred lotus] seeds. J. Ethnopharmacol., 104: 32227.

Ranjana, A. and Mishra, A.P. 2015. Histopathological alterations of profenofos on the ovary of the fresh water air-breathing fish Channa gachua. The Asian Journal Of Animal Science; 10(1):8-13.

Reini W B., Chris MG, Thomas, P T and Scheepers J.2008.Egypt. J. Comp. Path. & Clinic. Path. Vol. 21 No. 4 (December) 2008; 75 – 9275.

Rao, J.V. 2006. Toxic effects of novel organophosphorus insecticide (RPR-V) on certain biochemical parameters of euryhaline fish, Oreochromis mossambicus. Pesticide Biochemistry and Physiology, 86: 78–84.

Reitman, S. and Frankel, S. 1957. "A colorimetric method of pyruvic transaminases." Am. J. Clin. Pathol., 28: 5763.

Rezg, R., Mornagui, B., ElFazza, S., Gharbi, N. 2008. "Biochemical evaluation of hepatic damage in subchronic exposure to malathion in rats: effect on superoxide dismutase and catalase activities using native PAGE", CR Biol., 331 (9): 655-562.

Rodrigo, L., Hernandez, AF., Lopez-Caballero, JJ., Gil, F. and Pla, A. 2001. Immunohistochemical evidence for the expression and induction of paraoxonase in rat.

Said , R. S., Ahmed, S. N. and El-Demerdash, E. 2012. Sodium Selenite Improves Folliculogenesis in RadiationInduced Ovarian Failure: A Mechanistic Approach PLOS ONE | www.plosone.org 1 December | Volume 7 | Issue 12 .

Salazar- Arredondo, E., Solis-Heredia, M. D. J., Rojas-G., E., HernandezO. I. and Quintanilla-V.B. 2008. Sperm chromatin alteration and DNA damage by methylparathion, chlorpyrifos and diazinon and their oxon metabolites in human spermatozoa. Reproductive Toxicology 25: 455–60.

Salem, S.M. and Kobeasy, M.I. 2006. Some Histological Responses New Zeland Rabbits To Drinking Water Polluted By Profenofos. Egyptian J. Anim. Prod., 43(2):127-138.

Sallam MA, Ahmad M, Ahmad I, Gul ST, Idrees M, Bashir MI and Zubair M. 2015. Toxic effects of Cypermethrin on the reproductive functions of female rabbits and their amelioration with Vitamin E and selenium. Pak Vet J, xx(x): xxx.

Schettler T, Solomon G, Kaplan J, Valenti M 2003. Generations at RiskHow Environmental Toxicants May Affect Reproductive Health in California Brisbane,CA: George Lithograph .

Sangha, G.K., Kamalpreet, K. and Khera, K.S. 2013.Cypermethrin induced pathological and biochemical changes in reproductive organs of female rats .Journal of Environmental Biology; 34: 99-105.

Selvaraju, S., Nandi, S., Gupta, P. S. P. and Ravindra, J .P. 2011.Effects of heavy metals and pesticides on buffalo (Bubalus bubalis) spermatozoa functions in vitro. Reproduction in Domestic Animals 46: 807–13.

Sendecor, G. W. and W. G. Cochran 1989. Iowa s Statistical methods.7th.ed tate Univ.press. Ames, Iowa.

Shaker, N.; Hassan, G.A.; El-Nouty. F.D.; Abo-El-Ezz, Z. and Abd-Allah, G.A. 1988. In vivo chronic effect of dimethoat and deltamethrin on rabbits. J. Environ. Sci. Health, B23 (4): 387-399.

Shalby, S. E. M. 2006. Comparative Haematological And Hepatorenal Toxicity Of Igr, Lufenuron And Profenofos Insecticide On Albino Rats J. Egypt. Soc. Toxicol.;34: 85-98 .

Sharma, Y., Bashir, S., Irshad, M.,Gupta, SD., and Dogra, TD. 2005. Effects of acute dimethoate administration on antioxidant status of liver and brain of experimental rats. Toxicology 206:49-57.

Shittu, M., Ambali, S. F., Ayo, J. O., Fatihu, M. Y, Sulaiman, M. M. and Yaqub L. S. 2013.Evaluation of chronic Chlorpyrifos-induced reproductive toxicity in male Wistar rat: protective effects of vitamin C. J Exp Integr Med.; 3(1): 23-30.

Song, S.B., Xu, Y., and Zhou, B.S., 2006. Effects of hexachlorobenzene on antioxidant status of liver and brain of common carp (Cyprinus carpio), Chemosphere.; 65:699– 706.

Suvarna, K. S., Layton, C. and Bancroft, J. D. 2013. Bancroft's Theory and Practice of Histological Techniques, by Suvarna, 7th Edition Churchill Livingstone.

Thomas, G. T., Rebecca, S. and Andre, B. A. 2008.The Natural History of Symptomatic Androgen Deficiency in Men: Onset, Progression, and Spontaneous Remission. J Am Geriatr Soc., 56(5):831-839.

Ullah, M.S., Ahmad, M., Ahmad, N., Khan, M.Z. and Ahmad, I. 2006. Toxic effects of cypermethrin in female rabbits. Pak Vet J, 26: 193-196.

Undeger, U., Institoris, L., Siroki, O., Nehez, M. and Desi, J. 2000. Simultaneous geno and immunotxicological investigations for earlly detection of organophosphate toxicity in rats. Ecotox. Environ. Saf., 45(1): 43-48.

Uzumcu, M. and Zachow, R. 2007. Developmental exposure to environmental endocrine disruptors: consequences within the ovary and on female reproductive function. Reprod. Toxicol., 23: 337-52.

Uzun, F. G., Kalender, S., Durak, D., Demir, F. and Kalender, Y. 2009. Malathion-induced hepatotoxicity in rats: The effects of vitamins C and E. Food and Chemical Toxicology, 47 (8), 1903-1908.

Vaskivuo, T.E., Stenb¨ack, F., Karhumaa, P., Risteli, J., Dunkel, L., Tapanainen, J.S. 2000. Apoptosis and apoptosis-related proteins in human endometrium. Mol Cell Endocrinol; 165 (1/2), 75–83.

Verma, R.S. and Srivastava, N.,(2003): Effect of chlorpyrifos on thiobarbituric acid reactive substances, scavenging enzymes and glutathione in rat tissues, Indian J. Biochem. Biophys.; 40: 423-428.

Wiltbank MC, Weigel KA, Caraviello, DZ,2007. Recent studies on nutritional factors affecting reproductive efficiency in U.S. dairy herds, Western Dairy Management Conference.

Young, DS. 2000.Effects of drugs on clinical laboratory tests . 5th edition . AACC Press.

Yugal, R. B., Sulochana S, N S. and Tara N. G .2013.Effects of nutrition on reproduction- A review Advances in Applied Science Research, 4(1):421-429

Zeng, H., Cao, J.J. and Combs, G.F. 2013. Selenium in bone health: roles in antioxidant protection and cell proliferation. Nutrients, 5: 97-110.

Zhang, XZ (1992): Crop Physiology Research Methods. China Agricultural Press Beijing, 131:207.

Zidan, H. Z., Mashhour, A. k., Zidan, A. A., Fawzy, A. A. and Okasha, Y. A.1998. "Toxicological effect of long term administration of minimal does of certain insecticides on white albino rats." Annals Agric. Sci., (3): 1085-11101.

Zidan, N.H. A. 2009.Evalution of the reproductive toxicity of Chlorpyrifos, Diazinon and Profenofos Pesticides in male rats. Int. J. Pharmacol., 5(1): 51-57.