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Effects Of Polyvinyl Chloride Sub-Chronic Toxicity On Fertility In Adult Male Rats And The Possible Protective Role Of Omega-3

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ABSTRACT

Background: Infertility is a worldwide health problem. Polyvinyl chloride (PVC) is a toxic agent and can induce endocrinal and reproductive disruption. Omega-3 is a polyunsaturated fatty acid with anti-oxidative effects. This study aimed to investigate whether omega-3could protectthe testes against polyvinyl chloride (PVC) – induced disruption and preserve fertility in adult male rats and if so, what is the possible mechanism?**Design:**Thirty-five rats were used, they were divided into five groups; control, corn oil, omega -3, PVC and PVC & omega-3. Oral treatment was continued for 40 days. Serum testosterone (T), luteinizing hormone (LH), follicle stimulating hormone (FSH) and sperm parameters were evaluated. Total antioxidant capacity (TAOC), catalase (CAT) and malondialdehyde (MDA) were also assessed in testicular homogenate. Testes were subjected to histological examination by the light and electron microscopes.**Results:** The study revealed that PVC decreased significantly the fertility hormones levels, TAOC and CAT activity, sperm count, vitality, motility, increased MDA and the sperm abnormal forms. In addition, alternation of the normal histological structure of the testes was found. Omega-3 improved PVC-induced alterations in the previous parameters.**Conclusions:** PVC reduced fertility potential in male rats, probably through lipid peroxidation of the testes and reduction of the antioxidant capacity. While omega-3 could protect the testes from the PVC-inducedsuch toxicities due to its antioxidant properties.

Keywords Polyvinyl chloride; Omega-3; Oxidative stress; Testes; Fertility.

1. INTRODUCTION

Infertility is a public health priority, defined as the inability to conceive within one year of unprotected intercourse **(Centers for Disease Control and Prevention, 2014).** Globally, infertility affects 15% of couples of the reproductive age **(Gerrits et al., 2017).** About 50% of couple's infertility is due to male factors **(Campagne, 2013).** Male factors could be classified into genetic and non-genetic **(Sharma, 2017).** Regarding non genetic factors, at least 10% are exogenous and reversible such as environmental, lifestyle and psychological impacts, most cases of male infertility are caused by an intrinsic disorder in the testes **(Sharma, 2017).**

Numerous exogenous chemicals have been released into the environment due to marked expansion of chemical industries in the past 50 years. Most of these chemicals and xenobiotics are endocrinal disruptors and affect the male reproductive system which is highly sensitive **(Singh and Pakhiddey, 2015).**

Among these chemicals is the polyvinyl chloride (PVC), the world's third-most widely produced synthetic plastic polymer**(Allsopp and Vianello, 2012).** It is used as a raw material in multiple chemical industries as making plastic containers, food packaging, toys, coated wires, medical devices and in automotive industry **(Sadeghi et al., 2019).** PVC is a dangerous pollutant of biological environments, **(Chiellini et al., 2013).** PVC is not chemically bonded with polymer and can be separated easily **(Hartmann and Klaschka, 2017),** so it can be transmitted to the human through water, food, air and even medical devices **(Latini et al., 2010).** It is considered as one of the most abundant poisons in the human environment, and it leads to toxicity through production of oxidative stress enzymes and disruption of some antioxidant enzymes **(Sadeghi et al., 2017).** Previous studies had shown that PVC exerts hepatotoxic, teratogenic, mutagenic and carcinogenic effects and produces renal, pulmonary, immune and reproductive dysfunctions **(Chen et al., 2016; Dong et al., 2017; Lieshchova et al., 2019).**

The reactive oxygen species (ROS) can damage the sperm DNA in a variety of ways, such as changing the structure of DNA strands, the abnormal corrosion of DNA and re-matching the chromosomes **(Lobascio et al., 2015; Aitken, 2017).**

Omega-3 is an essential nutrient throughout the animal kingdom **(Innis, 2014).** It is one of important dietary fatty acids, which has significant effects on male fertility **(Reza Safarinejad and**

Abberviations: PVC, polyvinyl chloride; ROS, reactive oxygen species; ALA, α-linolenic acid; T, testosterone; LH, luteinizing hormone; FSH, follicle stimulating hormone; H&E,hematoxylin-eosin; TAOC, Total antioxidant capacity; FRAP, Ferric Antioxidant Power Test; CAT, catalase; MDA, malondialdehyde.

Safarinejad, 2012). Omega-3 contains eicosapentaenoic acid, docosahexaenoic acid and α-linolenic acid (ALA), which is found in vegetable sources such as linseed oil and soybean, and is found in fish such as salmon, halibut, tuna, sardines, and other seafood as krill and algae **(Gammone et al., 2019).** Omega-3 has antioxidant, anti-inflammatory and anti-apoptotic effects on various tissues stressed by ischemia reperfusion damage and it has a potential cytoprotective effect against various extrinsic toxic stimuli **(McDonald et al., 2013).** It has a lot of health benefits and antioxidant activity in human seminal fluid and it enhances sperm count, sperm morphology, and sperm motility. Large number of infertile men may be benefit from administration of omega 3 fatty acids **(Reza Safarinejad and Safarinejad, 2012**).

Because of the extensive exposure to PVC and its potential toxic effects on the male reproductive system, the current study aimed to investigate the toxic effect of PVC on the testes and fertility parameters in adult rats, and whether omega-3 supplementation could protect against the PVC toxic assault.

2. MATERIALS AND METHODS

a. Drugs and chemicals

PVC was purchased from Sigma-Aldrich Company, Egypt as a fine white, odorless powder, stable under normal condition, insoluble in water and soluble in oil.The powder was dissolved in corn oil as a vehicle **(Barakat et al., 2014).**

Omega-3 was purchased from Arab Company for Drug Industries and Medical Appliances, Abidin, Cairo, Egypt.

b. Experimental animals

A total of thirty-five adult male albino rats, weighing 200-250 g were used in this study. The animals were obtained from Helwan animal breeding farm, Cairo, Egypt. They were housed in stainless steel cages in a well-ventilated animal house at normal temperature (22°C ± 5°C) under 12 hours alternating light/ dark cycle. They were fed commercial rat pellets (El-Nasr Company, Abou-Zaabal, Cairo, Egypt) with water ad libitum. They were kept under standard conditions for one week for adaptation prior to the start of the experiment.

c. Ethics statement

The handling of animals was following the rules for the experimental research ethics approved by Research Ethics Committee at faculty of Medicine for Girls Al-Azhar University, Egypt (No. 201912290), and conformed to the Guide for the Care and Use of Laboratory Animals **(National Health Institute, 1996).**

d. Experimental design

The rats were divided randomly into five groups, 7 rats each, as follows:

- **Group 1 (G1):**normal control, received normal feeding and distilled water only.
- **Group 2 (G2):** received corn oil.
- **Group 3 (G3):**received omega-3 (400 mg/kg/day) according to **Uygur et al. (2014).**
- **Group 4 (G4):** PVC treated group, received PVC (200 mg/kg per day) according to **Sadeghi et al. (2017).**
- **Group 5 (G5):** PVC and omega-3 treated group, received the same dose as group (3) and group (4).

Administration route was oral, and the experimental period was 40 days according to **Sadeghi et al. (2017).**

e. Serum and tissue collection

At the end of the experiment, blood samples were taken from rats' retro-orbital sinuses using glass capillaries while they were under anesthesia by diethyl ether, the samples were collected in clean dry test tubes and left at room temperature for 20 minutes to clot. The blood samples were centrifuged at 4,000 rpm for 15 minutes to separate the sera. The sera were then stored at -20 °C for biochemical analysis of the fertility hormones; testosterone (T), luteinizing hormone (LH) and follicle stimulating hormone (FSH).

All anesthetized animals were sacrificed, and the testes were dissected. The left testes were subjected to histological examination by the light microscope using hematoxylin-eosin (H&E) stain and the electron microscope. The right testes were homogenized for subsequent assaying of oxidative stress markers. The epididymis of each rat was processed for sperm characteristics.

f. Testes homogenate preparation

Testes were homogenized according to **Akondi et al. (2011).** They were rinsed in ice-cold PBS (0.01mol/L, pH 7.0-7.2), sliced into pieces then minced and homogenized in 5-10 ml cold buffer per gram tissue using a crusher (HeidolphSilentCrusher M type – Germany) to obtain 10% homogenate (w/v). The homogenates were centrifuged at 4000 rpm for 15 min. The supernatant was used for estimating the oxidative stress markers.

g. Biochemical analysis

i. The fertility hormones

Serum T level was measured according to **Wheeler, (1995)** by enzyme-linked immuno-absorbent assay (ELISA) using a specific kit (Diaplus, USA; Diagnostic System Laboratories Inc., STAT FAX, Webster,

TX) as recommended by the manufacturer. Serum LH and FSH levels were measured according to **Butt, (1983)** and **Wide, (1976)** respectively by chemiluminescence immunoassay method using specific kits (Roche Diagnostics, Germany).

ii. Oxidative stress markers in testicular homogenate

Total antioxidant capacity (TAOC) was measured by using Ferric Antioxidant Power Test (FRAP) **(Benzie and Strain, 1996).** Catalase (CAT) activity was determined based on its ability to decompose H_2O_2 using the Aebi method **(Aebi, 1984).** Testicular malondialdehyde (MDA) level was evaluated by thiobarbituric method and calculated by using the MDA absorption coefficient according to**Esterbauer and Cheeseman, (1990).**

h. Sperm characteristics

All steps of sperm analysis were performed at 37°C according to **Wennemuth et al. (2000)** as followings:

1. The epididymis was processed for sperm count: The cauda epididymis was dissected out: The excised pieces were placed in Petri dish containing 4 ml of normal (0.9% sodium chloride). Gently minced with a fine scissor, incisions of about 1 mm were made. The dishes were incubated at 37 C for 10 min to make spermatozoa swim out.
2. The extract was diluted: A dilution ratio of 1:20 with 0.9% NaCl or buffered saline from each well-mixed sample by diluting 50 µl of epididymal spermatozoa suspended in physiological saline with 950 µl diluents in white blood cell pipette.
3. The counting chamber of improved Neubauer hemocytometer was filled with the diluted extract.
4. The number of spermatozoa in 5 small squares were counted. The total count was obtained by multiplying the number with one million.
5. Two samples were counted from each epididymis within one hour of sacrifice and the mean value was taken. The average of all the four counts, two from each side, from single rat was constituted as an observation.
6. Sperm motility, count, morphology and viability (%) for at least 200 spermatozoa from each animal were determined and counted through microscopic examination.
7. The microscopic field was scanned systematically, and each spermatozoon encountered was assessed and carried out at room temperature between 24 - 28, motility was classified as either motile or non-motile.

8. Motility indices were expressed as the percentage of progressive motility (rapid and slow), non-progressive and immotile spermatozoa.
9. The procedure was repeated, and average reading was taken.
10. The morphologically normal spermatozoa and the percentage of viable sperm cells were assessed by Eosin staining.

i. Histological study

- **Light microscopic study**

The testes specimens were fixed using 10% neutral buffered formaldehyde. After proper fixation, the specimens were dehydrated in ascending grades of ethyl alcohol (70%, 90%, 100%), cleared in xylol, impregnated and then embedded in paraffin wax. 5-µm thick sections were cut using rotatory microtome. Testes sections were stained with H&E and examined by light microscope **(Bancroft and Gamble, 2002).**

- **Electron microscopic study**

The testicular samples were fixed 4% glutaraldehyde for 2 hours. Then washed by 0.01 M sodium cacodylate buffer at PH7.3. The specimen then post-fixed with a 1% osmium tetroxide in the same cacodylate buffer; the specimens were then dehydrated, cleared and embedded in epoxy resin. Ultrathin sections (60a nm) were cut and examined using a transmission electron microscope (JEOL1010 EX II, Japan) at the Regional Mycology and Biotechnology center, Al-Azhar University, Cairo, Egypt.

Statistical analysis

Data were statistically presented as means ± standard deviation (SD). They were determined by using SPSS software version 20 for windows. Differences among groups were detected by analysis of variance using one-way (ANOVA) test followed by Tukey's post-hoc test. Values with $p < 0.05$ or lower was considered as statistically significant.

3. RESULTS

a. Biochemical measurements

i. Changes in fertility hormones' levels

Table 1 showed that PVC administration resulted in significant decrease ($P< 0.05$) in the fertility hormones levels including serum T, LH and FSH as compared to the control group. On the other hand, co-administration of omega-3 and PVC resulted in a significant increase ($P<0.05$) in serum T and LH and insignificant increase ($P> 0.05$) in FSH level as compared to the PVC- administered group.

ii. Changes in antioxidant markers in testicular homogenate

Table 2 showed that PVC caused significant decrease (P< 0.05) in TAOC andCAT activityand significant increase (P< 0.05) in MDA levels in the testicular homogenate when compared to the control group. While co-administration of omega-3 and PVC caused significant increase (P< 0.05) in TAOC andCAT activityand significant decrease (P< 0.05) in MDA level in the testis as compared to the PVC-administered group.

b. Changes in sperm parameters

Table 3 showed that administration of PVC to rats resulted in significant decrease (P< 0.05) in the sperm count, vitality and total motility and significant increase (P< 0.05) in the abnormal forms as compared to the control group. On the other hand, co-administration of omega-3 with PVC produced significant increase (P< 0.05) in the sperm count, vitality and total motility and significant decrease (P< 0.05) in the abnormal forms when compared to the PVC administered group.

c. Histological examination by the light microscope

i. Control group

The testicular parenchyma of rats in the control group showed active spermatogenesis and normal-size seminiferous tubules; each one was surrounded by an outer thin layer of connective tissue and lined with spermatogenic and Sertoli cells. The spermatogenic cells consisted of many layers, namely, the spermatogonia, primary and secondary spermatocytes and spermatids rested on the thin basal lamina. The interstitial cells were found mostly in clusters between the seminiferous tubules with characteristic large and ovoid nuclei **(Fig. 1 A&B).**

ii. Polyvinyl chloride group

The structure of testes in PVC administered group showed disorganization of the normal histological structure with overall different degrees of atrophy in the seminiferous tubules and congestion of blood vessels between tubules**(Fig.2-a)**. Some seminiferous tubules appeared with irregular outlines with eosinophilic edematous fluid in the interstitial tissues**(Fig2-b)**. Sertoli cells were seen resting on the thin, irregular basement membrane. Some spermatogenic cells showed vacuolated cytoplasm; pyknotic nuclei and spermatogenic cellular loss (**Fig.2-c)**. Reduction of Leydig cells numbers was also noticed (**Fig.2-d).**

iii. Polyvinyl chloride & omega-3 group

The testicular parenchyma of rats co-treated by PVC and omega-3 showed improvement of testicular damage, appeared as active spermatogenesis in normal-size seminiferous tubules (**Fig.3 a**). The spermatogenic cells consisted of many layers of spermatogenic cells, and spermatids rested on the thin

basal lamina **fig.3 b-c**. The interstitial spaces showed clusters of interstitial cells between the seminiferous tubules **fig.3 d**.

d. Histological examination by the electron microscope

i. Control group

The ultrastructural observation of the testes of the control rats revealed normal testicular architecture. Well-developed Sertoli cells, were resting on the basal lamina of the seminiferous tubules with oval shaped large nucleus, prominent nucleolus (**Fig.4a)**. The germinal epithelium showed different stages of maturation. Primary spermatocytes which appeared as large rounded cells, rounded nuclei with distinct chromatin networks and well-defined nuclear membranes (**Fig.4b)**. The rounded spermatids showed pyriform nucleus with fine granular chromatin and clear nuclear membranes. Acrosomal vesicle is apparent to form acrosomal cap **Fig.4c**. Clumps of normal interstitial cells (Leydig cells) showed nuclei with thin peripheral rim of chromatin (**Fig.4d).**

ii. Polyvinyl chloride group

Examination of PVC- treated testis showed abnormal structure of Sertoli cells. The nuclei appeared with irregular nuclear membranes, and the cytoplasm contained large clear vacuoles**(Fig.5a)**. The spermatogonia showed irregular nuclear membrane and clumped chromatin**(Fig.5b)**. Round spermatids showed indented nuclei with dense clumped chromatin and complete loss of acrosomal caps**(Fig.5c)**. Some abnormal forms of elongated spermatids were also noticed. The Leydig cells appeared with indented nuclei and clumped chromatin. The cytoplasm contained lipid droplets and vacuoles**(Fig.5d).**

iii. Polyvinyl chloride & omega-3 group

The ultrastructure of the co-treated testis revealed normal Sertoli cells which have triangular nuclei with prominent nucleoli and fine granular chromatin (**Fig.6a)**. The cytoplasm contained some dilated mitochondria. The spermatogonia appeared large rounded cells, with regular nuclear membranes, the cytoplasm contained ballooned mitochondria**(Fig.6b)**. Rounded spermatids appeared with spherical nuclei and complete acrosomal caps**(Fig.6c)**. Leydig cellsappeared with slight indentation in the nuclear membranes. The cytoplasm contained mitochondria, lysosomes and vacuoles**(Fig.6d)**.

4. DISCUSSION

Several factors are identified to underlie male infertility. Exposure to environmental chemicals as PVC could be one of these factors, as the testis is vulnerable to damage by such toxicant. PVC is an

endocrinal disruptor and can affect human body through plastic food containers, water bottles and medical instruments **(Latini et al., 2010; Sadeghi et al., 2017).**

In the present study, adult rats exposed to PVC exhibited significant decrease in in fertility hormones, including serum T, FSH and LH in comparison to control group. Sperm quality was also affected as sperm count, vitality and motility were significantly reduced while abnormal forms were increased significantly. Our results indicated that male reproductive system is adversely affected upon exposure to PVC. Similar findings were obtained by **Huang et al. (2014).** Also, **Sadeghi et al. (2020)** found that oral administration of PVC for 40 days to adult rats decreased T level, sperm count, motility, vitality and increased sperms with abnormal DNA.

Our findings were confirmed by histopathological examination of the testes in PVC exposed rats. Seminiferous tubules were decreased in diameter with various degrees of atrophy, also basement membrane showed irregularity and decreased thickness. Leydig cells decreased in number and exhibited nuclei indentation and clumped chromatin. Similar findings are observed by **Sadeghi et al. (2017).** The decrease of T level in PVC exposed rats, mostly reflected the destruction of Leydig cells in the testes. In addition, the observed atrophy of seminiferous tubules may be related to the reduction in T level as T is necessary for increasing tissue mass and growth **(Srinivas-Shankar and Wu, 2009).** Other histological disorder was abnormal Sertoli cells that may be due to the decreased FSH level, the hormone that is needed for support of Sertoli cells and their maintenance. Moreover, abnormal spermatogonia, rounded spermatids and elongated forms were noticed. The reduction of sperm quality in the present study could be explained by the decreases of T levels. The process of spermatogenesis is a T- dependent and it is essential for its initiation and maintenance; completion of meiosis, differentiation of the spermatids, maturation and conversion of round spermatids to elongated ones **(O'Donnell et al., 1994; Norazit et al., 2012).**

Despite of the decreased serum T level, LH and FSH levels also decreased, which means that the negative feedback of the pituitary-testicular hormonal axis was interrupted. Additionally, the decrease of LH and FSH secretion may be explained by their inhibition by T metabolites **(Steinberger and Chowdhury, 1977).** This finding puts a light on neuronal degeneration caused by PVC besides its toxic effect on the testicular tissue **(Dhanya et al., 2004; Sadeghi et al., 2019).**

In addition, the present study revealed significant decreases in TAOC and catalase while MDA increased significantly in comparison to control group. These findings explained that the toxic effect of the PVC on the rat testicular tissue was via production of reactive oxygen species (ROS) and lipid peroxidation through the increase of MDA and reduction of the radical scavengers. Such findings were

consistent with studies of **Mathurand D'Cruz, (2011) & Wang and Su, (2018)**. The decrease of TAOC and catalase after exposure to PVC were probably due to protein inactivation by ROS **(Al-Olayan et al., 2014).** One possible mechanism is that ROS production might be correlated with PVC-induced calcium entry, potentially through the calcium-mediated activation of the nicotinamide adenine dinucleotide phosphate complex **(Palleschi et al., 2009).** A study by **Erkekoglu et al. (2010)** had shown that in vitro exposure to PVC induces DNA damage of mouse Leydig cells through ROS generation.

The resultantOxidative stress can be highly damaging to testes which have a high level of metabolism and cell proliferation. So, key processes including apoptosis, spermatogenesis, and steroidogenesis are disrupted beside destruction of Leydig cells. Such findings agreed with **Mathurand D'Cruz, (2011) andSadeghi et al. (2017),** who found that exposure to PVC fragments testicular DNA, lead to gamete death, spermatogenesis disorder and causes apoptosis in rat testes.

Fortunately, in the present study, administration of omega-3 to PVC- exposed rats caused increase in serum T, FSH and LH levels in comparison to PVC- exposed group. Sperm quality indices in the form of sperm count, vitality and motility were significantly increased while abnormal forms were decreased significantly in comparison to PVC- exposed rats. Moreover, the histological picture improved and showed normal sized seminiferous tubules, increased spermatogonia layer thickness, increased clusters of Leydig cells in comparison to PVC exposed rats. Also, Sertoli cells showed normal structure and. Rounded spermatids appeared with spherical nuclei and complete acrosomal caps. These results are in line with **(Ahmed, 2017).**Our findings indicated that omega-3 could protect the testes from the PVC- induced testicular disruption and preserve the Leydig cells.

The use of omega-3 in protecting the reproductive system was confirmed by others **(Yan et al., 2013;Bostani et al., 2014;Esmaeili et al., 2014; Aleksandra et al., 2015). Mahmoud et al. 2019** showed increase in T, LH and decrease in prolactin by administration of omega-3 in bisphenol A exposed rats. Additionally, a study by **Khavarimehr et al. (2017)** revealed that omega-3 could protect against diabetes-induced rat testicular damages, sperm parameters and preimplantation embryo development in rat model.

The increase in FSH and LH by administration of omega-3 could be explained according to recent studies by **Ross et al. (2016) and Ahmed, (2017)** who reported that omega 3 increases nitric oxide which activates guanylate cyclase that causes the release of cyclic guanosine monophosphate and eventually increase gonadotropin releasing hormone, LH and FSH levels. LH and FSH enhance sperm motility and other sperm parameters **(Esmaeiliet al., 2014).** Another possible mechanism is the preservation of Leydig cells and prevention of the reduction of T. Omega-3 contains alpha linolenic acid, which can be

converted to arachidonic acid, a precursor to make prostaglandin E2, **(Uygur et al., 2014).** Arachidonic acid seems to play an important role in testicular steroid genesis. As researches indicated that, arachidonic acid increases cyclic adenylate cyclase and enhances the rate of cholesterol side-chain breakage, thus stimulating the T production **(Esmaeiliet al., 2014).**

Administration of omega-3 to PVC-exposed rats increased TAOC and catalase while it decreased MDA significantly. CAT is an important line of the enzymatic antioxidant system of defense in cells against the toxicity caused by ROS. It reacts with resultant H_2O_2, neutralizes its toxicity, and turns it into H_2O and O_2**(Khan et al., 2011).** The decrease in MDA level indicates that omega-3 could compete lipid peroxidation and ROS production in testicular tissue. Hence, Leydig cells preserved their function and T secretion. A study by **Uygur et al. (2014**) found that omega-3 decreased germ cell apoptosis and oxidative stress induced by doxorubicin.Our findings are agreed with **Elshaari et al. (2010),** they found that antioxidants usage in the culture medium improved the fertility ratio and implantation. Moreover, **Sadeghi et al. (2017)** confirmed the protective effect of antioxidants like vitamin E on rat testicular damage by PVC.

5. CONCLUSION

The current study reveals that the administration of PVC disrupts the testicular tissue, fertility hormones and adversely affects sperm characteristics. Moreover, it causes lipid peroxidation of testicular tissue and reduces antioxidant capacity. The disturbance of oxidant /antioxidant system upon exposure to PVC could be one possible mechanism of testicular disruption and the reduction of fertility in adult male rats. Fortunately, co-administration of omega-3 in PVC exposed rats could effectively protect the rat testes and preserves fertility hormones and sperm parameters. It seems that the antioxidative properties of omega-3 can prevent the oxidative assault and lipid peroxidation caused by PVC and compete testicular damage. Therefore, increasing the antioxidant capacity of testicular tissue is very important particularly to those exposed to environmental toxicants. Omega-3 can be a promising potential cytoprotective agent against various extrinsic toxicants like PVC. Further studies are needed to explore other encountered mechanisms.

6. DECLARATIONS OF INTEREST

The authors declare no conflicts of interest in the current study.

7. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to

submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

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Table 1. Mean changes of the fertility hormones levels (serum testosterone, luteinizing hormone and follicle stimulating hormone) in the different studied groups.

Parameters	T (ng/ml)	LH (mIU/ml)	FSH (mIU/ml)

Groups		Mean ± S.D.		
G1 (Control)		1.95±0.47	0.13±0.02	0.22±0.02
G2 (Oil group)		1.96±0.36 [a]	0.12±0.02 [a]	0.23±0.01[a]
G3 (Omega-3 group)		1.90±0.23 [a]	0.13±0.01 [a]	0.23±0.02 [a]
G4 (Polyvinyl chloride group)		0.90±0.19 [a*]	0.04±0.01[a*]	0.19±0.01[a*]
G5 (Polyvinyl chloride + omega-3 group)		1.63±0.21 [b*]	0.08±0.01 [b*]	0.20±0.01 [b]
ANOVA	**F**	14.503	60.213	8.221
	P-value	<0.05	<0.05	<0.05

T, testosterone hormone; LH, luteinizing hormone; FSH, follicle stimulating hormone. [a]: values compared to the control group; [b]: values compared to the polyvinyl chloride group. *: indicates significant values (P < 0.05).

Table 2. Mean changes of oxidative stress markers levels (total antioxidant capacity**,** catalase activityand malondialdehyde) in testicular homogenate in the different studied groups.

Parameters / Groups		TAOC (mmol/g tissue)	CAT (U/g tissue)	MDA (mmol/g tissue)
		Mean ± S.D.		
G1 (Control)		191.86±11.87	8.44±1.01	21.51±2.37
G2 (Oil group)		190.70±6.58 [a]	8.21±1.31[a]	21.19±1.87 [a]
G3 (Omega-3 group)		212.99±18.67 [a]	12.63±2.07[a]	21.23±3.91[a]
G4 (Polyvinyl chloride group)		165.67±8.24 [a*]	5.30±0.54 [a*]	28.96±4.85 [a*]
G5 (Polyvinyl chloride + omega-3 group)		185.20±4.35 [b*]	7.47±1.47 [b*]	23.41±2.00 [b*]
ANOVA	**F**	16.166	26.242	7.411
	P-value	<0.05	<0.05	<0.05

TAOC, total antioxidant capacity; CAT, catalase; MDA, malondialdehyde. [a]: values compared to the control group; [b]: values compared to the polyvinyl chloride group. *: indicates significant values (P < 0.05).

Table 3. Mean changes of sperm parameters (sperm count, abnormal forms, vitality and total motility) in the different studied groups.

Parameters / Groups		Sperm Count	Abnormal forms	Sperm Vitality	Total motility
		Mean ± S.D.			
G1 (Control)		65.57±4.83	9.86±1.07	40.43±2.99	65.57±4.43
G2 (Oil group)		62.71±3.50 [a]	9.71±1.11[a]	41.43±2.37 [a]	64.14±4.53 [a]
G3 (Omega-3 group)		65.29±4.72[a]	9.64±2.54[a]	47.29±3.55 [a]	65.28±4.62[a]
G4 (Polyvinyl chloride group)		40.43±5.74 [a*]	17.43±3.21[a*]	25.43±4.43 [a*]	41.00±5.13[a*]
G5 (Polyvinyl chloride + omega-3 group)		49.14±4.49 [b*]	12.43±3.10 [b*]	31.14±2.04 [b*]	50.43±5.41 [b*]
ANOVA	**F**	40.289	13.645	52.472	32.326
	P-value	<0.05	<0.05	<0.05	<0.05

[a]: values compared to the control group; [b]: values compared to the polyvinyl chloride group.

*: indicates significant values (P < 0.05).

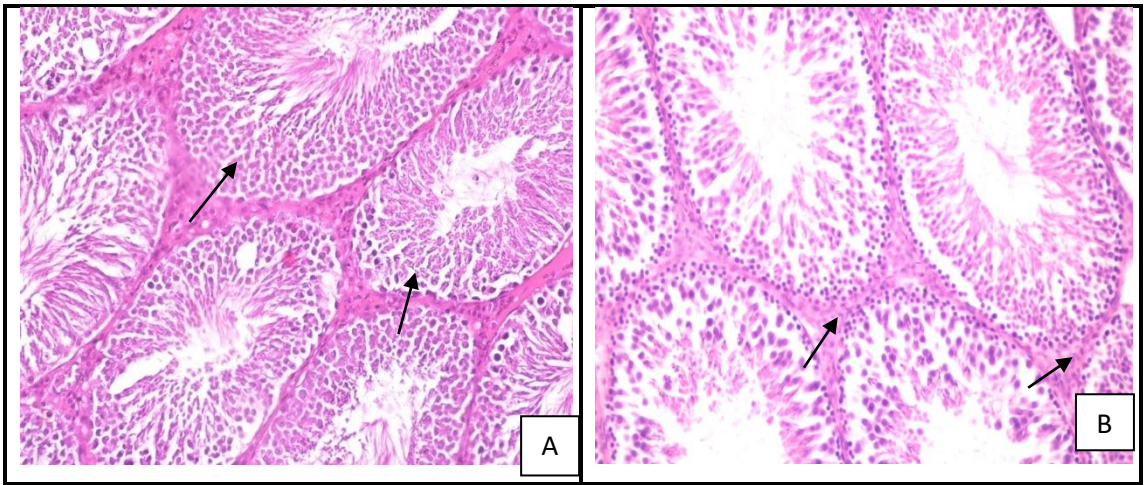


Fig. 1 Photomicrograph of rat testis showing **(A)** active spermatogenesis in normal-size

seminiferous tubules with thin basement membranes **arrow** (H&EX200) and **(B)** interstitial cells in-between tubules **arrow** (H&EX400).

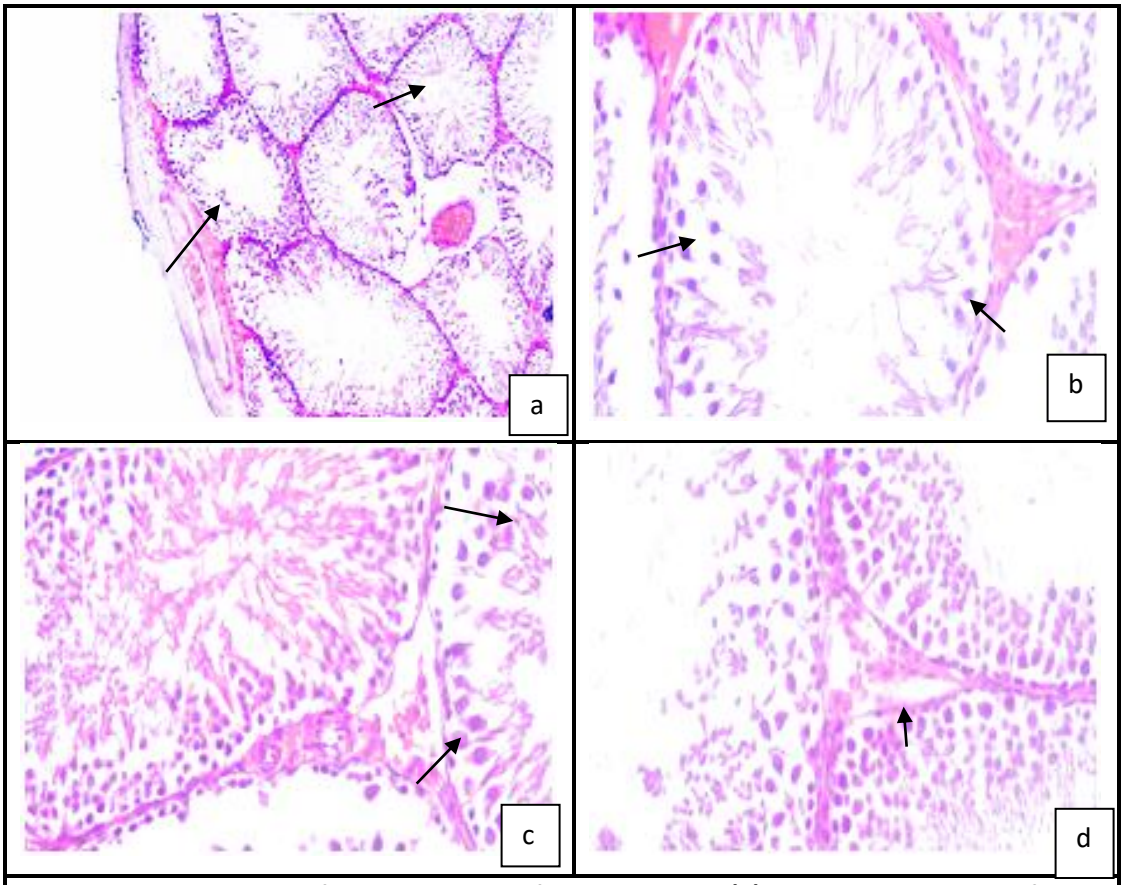


Fig.2 Photomicrograph of tissue sections of testis showing **(a)** atrophy in the seminiferous tubules and congestion of blood vessels between tubules **arrow** (H&EX100) **(b)** irregular outline of seminiferous tubules with eosinophilic edematous fluid in the interstitial tissues **arrow** (H&EX400) **(c)** vacuolated cytoplasm; pyknotic nuclei of spermatogenic cells **arrow** (H&EX200) **(d)** Reduction of Leydig and spermatogenic cells numbers **arrow** (H&EX400).

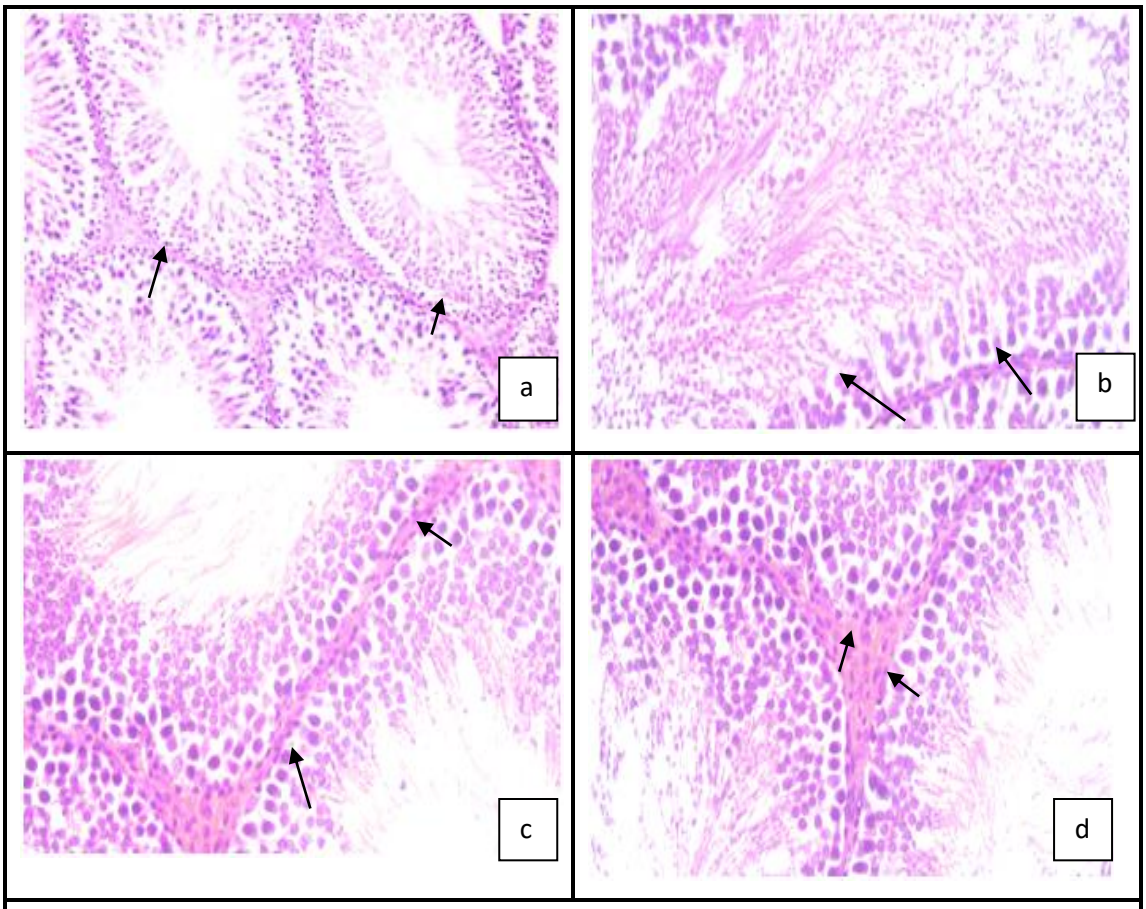
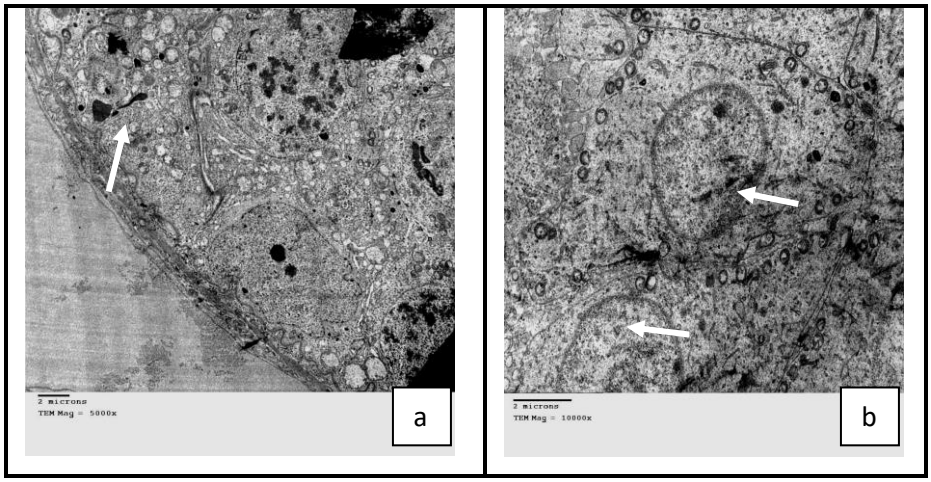


Fig.3 Photomicrograph of tissue sections of testis showing **(a)** active spermatogenesis in normal-size seminiferous tubules **arrow** (H&EX200) **(b)** regularly arranged spermatogenic cells within the seminiferous tubules **arrow** (H&EX400) **(c)** spermatogenic cells and spermatids rested on the thin basal lamina **arrow** (H&EX400) **(d)** clusters of interstitial cells between the seminiferous tubules **arrow** (H&EX400).



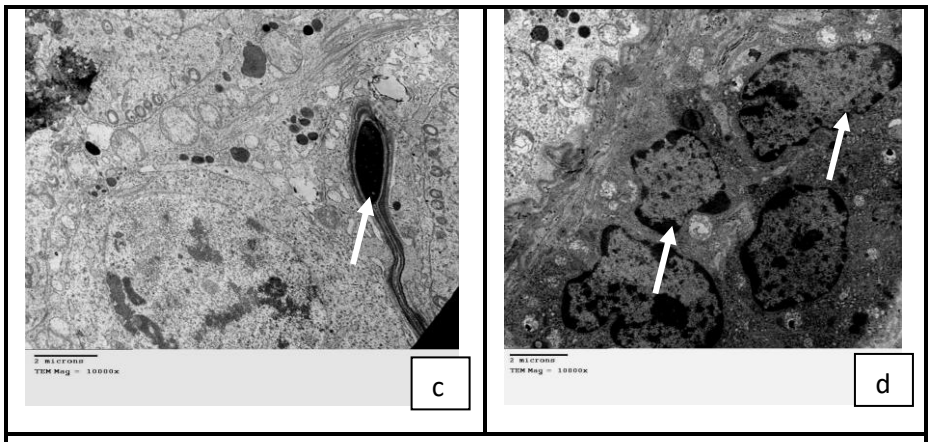
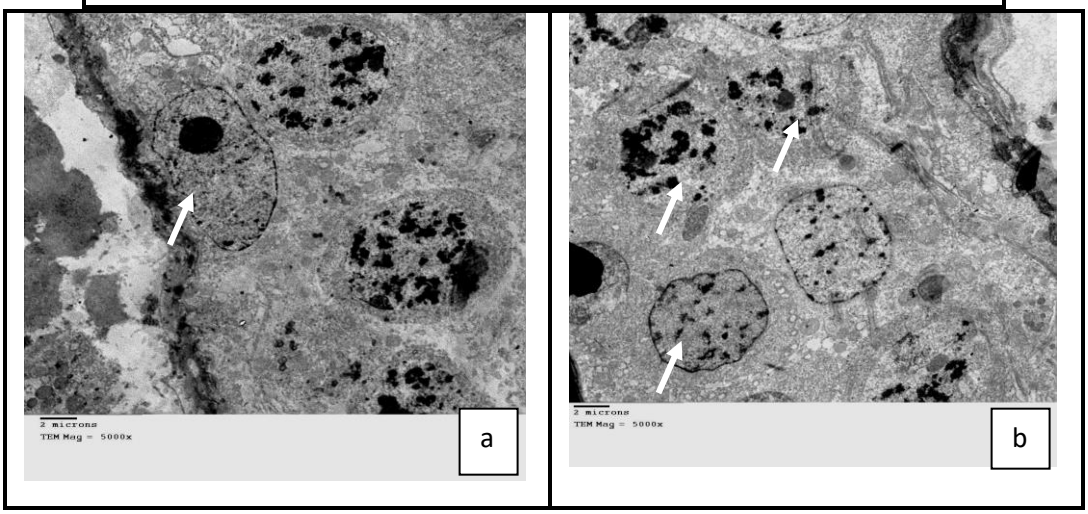


fig.4 Photomicrograph of ultrastructure of the control testis showing(a) Well-developed Sertoli cell rested on the basal lamina of the seminiferous tubules with oval shaped large nucleus **arrow(b)** Primary spermatocytes which appeared as large rounded cells **arrow (c)** Rounded spermatids with acrosomal cap, pyriform nucleus and fine granular chromatin **arrow(d)** Clumps of normal interstitial cells (Leydig cells) with thin peripheral rim of chromatin **arrow.**



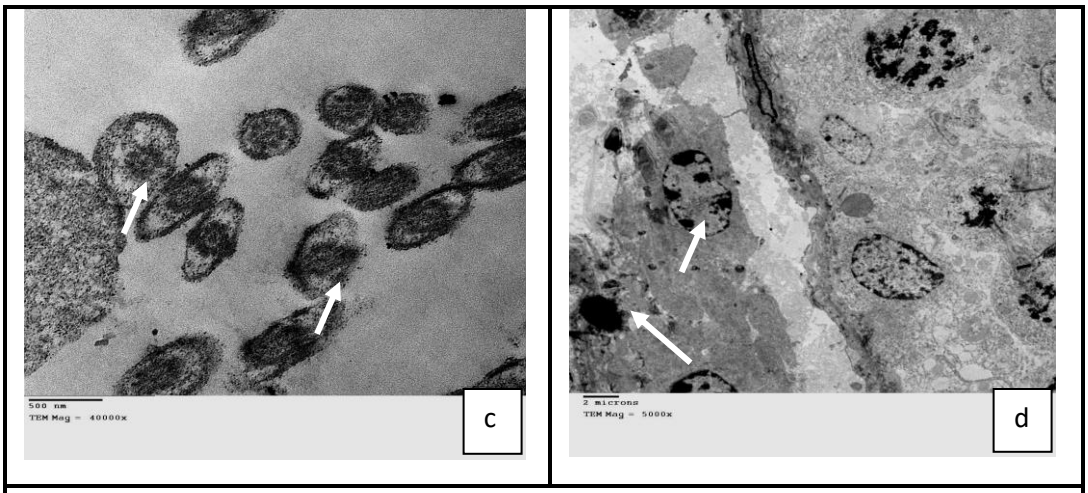
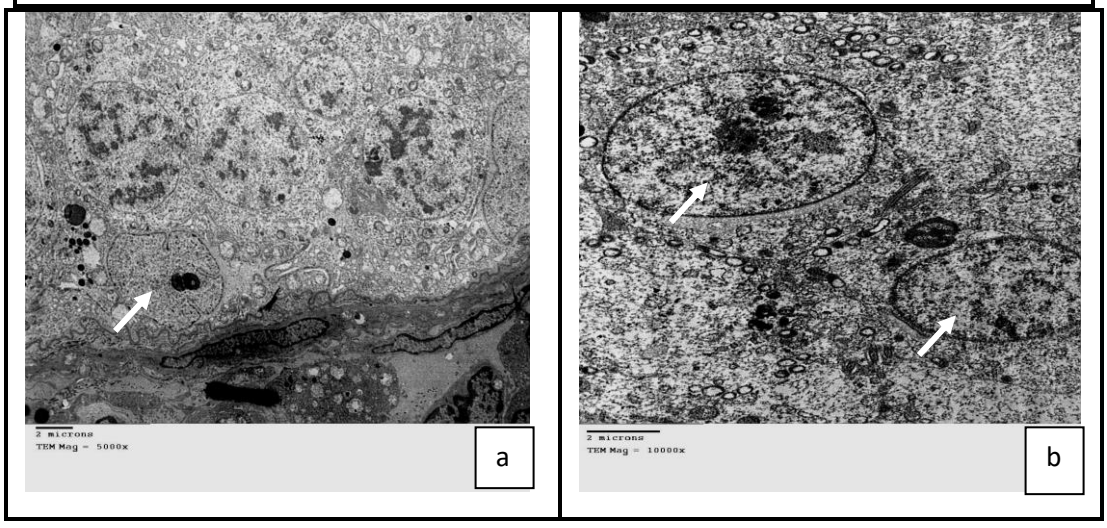


fig.5 Photomicrograph of ultrastructure of the affected testis showing(a) Sertoli cells with irregular nuclear membrane **arrow** and intra-cytoplasmic vacuoles **(b)** spermatogonia with irregular nuclear membrane and clumped chromatin **arrow(c)** spermatids with loss of acrosomal caps, indented nuclei and dense clumped chromatin **arrow(d)** interstitial cells with indented nuclei and clumped chromatin **arrow.**



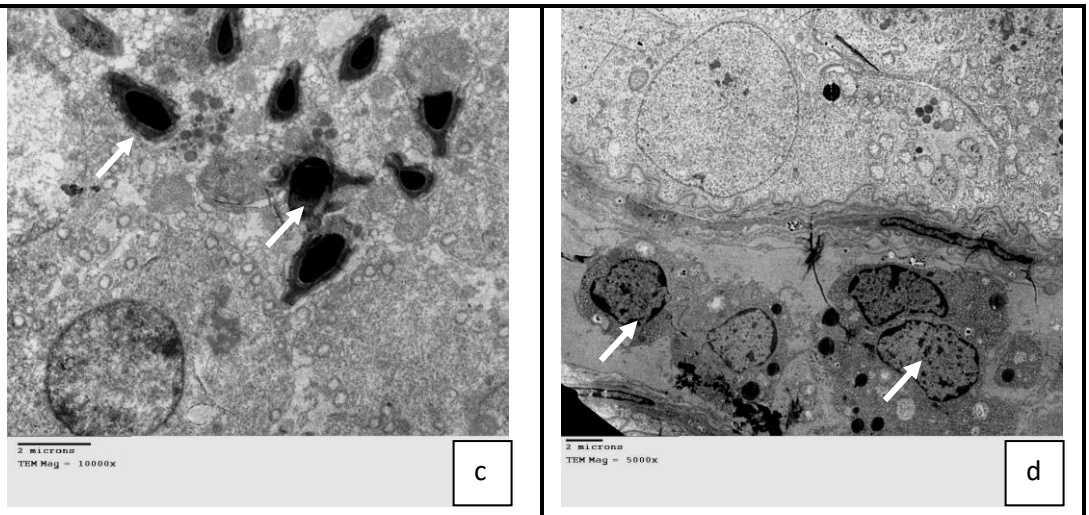


Fig.6 Photomicrograph of ultrastructure of the affected testis showing **(a)** Sertoli cells have triangular nuclei with prominent nucleoli and fine granular chromatin **arrow(b)** spermatogonia appeared large rounded cells, with regular nuclear membranes **arrow(c)** Rounded spermatids appeared with spherical nuclei and complete acrosomal caps **arrow(d)** Leydig cells appeared with slight indentation in the nuclear membrane **arrow.**