j.Egypt.soc.med.Assoc 78, no 4. 717 – 731 (2018)

TOXIC EFFECTS OF PHOXIM ON FERTILITY AND GERM CELLS AND THE PREVENTIVE ROLE OF ZERON IN RATS

By

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ABSTRACT

The present study was designed to evaluate the ameliorative role of Zeron-20 as an antioxidant on Phoxim (organophosphorus insecticide) - induced genotoxicity and infertility in male rats. 20 rats were used for determination of acute oral LD50 of Phoxim. 40 animals were divided into four equal groups, each of 10 rats. Group (1) served as control. Group (2) given Zeron-20 at a dose of 25µl/kg BW, three times /week for 65 days. Group (3) orally administered Phoxim at a dose of 50mg/kg BW (equivalent to 1/40 LD50) three times/week for 65 days. Group (4) treated with Zeron-20 (25µl/kg BW, three times /week) in combination with Phoxim (50mg/kg BW, three times /week for 65 days). At the end of the experiment, sex organs (Testis, seminal vesicles and prostate gland) were taken for relative weight, spermatozoal examination and histopathological examination of the testes. In addition, chromosomal analysis in male spermatocytic cells was accomplished. The results of this study revealed that there was no difference in the relative weight of the testis, seminal vesicles and prostate gland following administration of Phoxim compared to the control group or any other treated group. As well, treatment with Zeron-20 didn't show any enhancement in these parameters compared to control. Administration of Phoxim reduced sperm cell count, life percent and increased percentage of abnormal sperm morphology compared to the control group. However, co-treatment with Zeron-20 significantly improved these parameters (sperm count and morphology). The frequency of chromosomal aberration in spermatocytic cells (both structural and numerical aberrations) was increased in Phoxim treated group; however, the treatment with Zeron-20 decreased these aberrations significantly and restored it near to control. In conclusion, Phoxim exposure for male rats caused reproductive and

genotoxicity, however, Zeron-20 co-treatment ameliorate these oxidative toxic effects. Therefore, it seems reasonable to try to support the treatment of male infertility with supplementation having the ability to neutralize ROS i.e. antioxidants.

Keywords:

Phoxim, Zeron -20, mutagenicity, germ cells, fertility, male, rats.

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INTRODUCTION

Male fertility disorder that is attributed to environmental factors such as exposure to certain chemicals, heavy metals, insecticides, and heat, or electromagnetic radiation **(Lahdetie, 1995).** The consequence of most of these factors is oxidative stress, which resulted from generation of reactive oxygen species (ROS). Oxidative stress affects spermatozoa activity, structural DNA damage, and cell apoptosis. The consequence is reduced sperm count, decreased motility and abnormal morphology as well infertility **(Henkel *et al.,* 2003).**

Organophosphorus compounds are widely used in agriculture as insecticides and acaricides. They are also frequently employed in medicine and industry. Residual amounts of organophosphate (OP) pesticides have been detected in the soil, water, rains, vegetables, fruits, and other food products **(Poet *et al.,* 2004**). Moreover, due to the wide availability of organophosphorus compounds, toxicity are common **(Garcia *et al.,* 2003)**. Phoxim is an organophosphorus insecticide used for topical treatment of cattle, sheep, goats, and pigs **(WHO, 2000).** Like other organophosphates, Phoxim is known to inhibit acetylcholinesterase (AChE) activity, an effect that is thought to underlie the neurotoxicity elicited by these compounds **(Haihua *et al.*2011).**

Previous studies reported that phoxim treatment induced significant decrease in the weights of male sex organs (testes, seminal vesicles and prostate glands), sperm cell count and motility. Whereas, the percent of dead and sperm abnormalities was increased. Histopathological examination revealed presence of clear degenerative changes in male sex organs. Testicular lesions characterized by moderate to severe degenerative changes of spermatogonia cells and by partial arrest of spermatogenesis **(Atef *et al.,* 1995, El-Yamanee *et al*., 2001).**

Zeron-20 is a synthetic and natural antioxidant, containing epigallocatchin (EGCG, as a main active principle, which present in green tea); proanthocyanidin (PAC) and L-carnitine (LC).

In vitro and *in vivo* studies demonstrate that antioxidants possess a beneficial effect on fertility and, therefore, their use is recommended as supportive therapy for the treatment of infertility **(Walczak-Jedrzejowska *et al.*, 2013).** The present study was designed to evaluate the ameliorative role of Zeron-20 as an antioxidant on Phoxim(organophosphorus insecticide) - induced genotoxicity and infertility in male rats.

MATERIALS AND METHODS

1-Chemicals:

1-Phoxim insecticide (Sebacil EC 50%) was acquired from Bayer Animal Health GmbH, D-51373 Leverkusen, Germany.

2- Zeron-20 was obtained from Microbiotech Corb Laboratories, USA. Each liter contains: Epigallocatechin-3 gallat (20g), Proanthocyanidin (20g), L-Carnitine (60g), Propylene glycol (50g) and Water added till 1000 ml.

2-Experimental animals:

Sixty adult male rats (130 - 140gm) were used in this study were obtained from the animal house of the National Research Center, Dokki, Giza, Egypt. Food and water were provided Ad-libitum.

3-Experimental design:

Twenty rats were used for determination of acute oral LD50 of Phoxim, which was performed mathematically according to the method described by **Finney (1964).** Forty rats were used for determination of the effect of Phoxim on male fertility and germ cells and the protective role of Zeron-20. They divided into four equal groups, each of 10 rats. Group (1) control group kept without treatment. Group (2) treated with 25µl/kg BW Zeron-20, three times /week for 65 days. Group (3) orally administered Phoxim at a dose of 50 mg/kg BW (equivalent to 1/40 LD_{50}) three times/week for 65 days. Group (4) treated with Zeron-20 (25µl/kg BW, three times /week) in addition to Phoxim (50mg/kg BW, three times /week).The period of the experiment was extended for 65 days to cover all the spermatogenic cycle. At the end of the experiment rats of each group were sacrificed for revealing the reproductive toxicity studies of male (5 rats from each group) as well as the cytogenetic analysis in spermatocytic cells (5 rats from each group).

4- Methods and sampling:

***Weight of sex organs*:**

Testes, seminal vesicles and prostate gland were taken out and weighed; the relative weight was calculated according to the 100 gm of body weight.

***Spermatozoa examination*:**

The sperm cells were obtained by maceration of the epididymis and vas deferentia and examined for sperm cell count **(Reddy and Bordekar, 1999).** Smears were prepared for assessment of live and dead sperm percent and spermatozoal abnormalities (sperm morphology) as proceded by **Bearden and Fluquary (1980).**

***Cytogenetic analysis*:**

At the end of the experiment, spermatocytes (obtained from the testis) were analyzed using standard protocols. The slides were stained with Giemsa stain and analyzed for structural and numerical chromosomal aberrations in germ cells (50metaphases/rat) as described by **Russo (2000).**

Histopathological examination:

Autopsy samples from testes of scarified rats were fixed in 10% neutral buffered formalin solution for at least 24 hours, routinely processed by the standard paraffin embedding technique and stained by hematoxylin and eosin **(Suvarna *et al.*, 2013).**

5-Statistical analysis:

Data were expressed as means ± standard errors and were subjected to one–way analysis of variance (ANOVA) using SAS program **(SAS, 2001)**.

RESULTS

The acute oral LD_{50} of Phoxim was calculated as 2007.13mg/kg BW in male rats (Table 1,2)., revealed that there was no difference in testis weight, seminal vesicles and prostate gland weight ($p < 0.05$) following administration of Phoxim compared to the control group or any other treated group. As well, co-treatment with Zeron didn't show any enhancement in these parameters compared to control. As shown in (Table 3), administration of Phoxim reduced significantly sperm cell count and life percent associated with significant increase in percentage of head and tail sperm abnormalities compared to the control group. However, treatment with Zeron-20 significantly improved these parameters and restore it near to control

specially for head and tail sperm abnormalities (2.80±0.58 and 4.80± 0.49 vs. 3.80 ±0.58 and 6.20±0.73, for head and tail for control and Zerone-20+ Phoxim, respectively. Cytogenetic analysis (Table 4) of germ cells showed that Phoxim treatment significantly induced autosomal univalents and breaks as structural aberrations (4.00±0.32and 3.00±0.32 % Vs 1.20±0.20^{b} and 1.00±0.32 for autosomal univalents and breaks in Phoxim and control groups, respectively), as well in total structural aberrations (7.00±0.54 vs 2.20±0.37 for Phoxim and control group, respectively) and numerical abnormalities (hypoploidy and total numerical variation) compared to control group (4.00±0.32vs.1.00±0.32) for both types of numerical abnormalities and control group, respectively).However, treatment with Zeron in combination with Phoxim reduced these aberrations especially for chromosomal breaks (restored to normal control), as well improved numerical variation. Histopathological examination of testes revealed that Phoxim treated group revealed sever damage and even desquamation of the spermatic series Fig. (2, 3). Phoxim and Zeron-20 treated group, there were significant improvement in form of repair of spermatogenic series in localized areas of the seminiferous tubules Fig. (4).

DISCUSSION

The previous studies have shown that pesticides can cause various histopathological and cytopathological changes in the reproductive system of male mammals.These changes include; Decreased spermatogenesis and sperm counts **(Mahgoub and El-Medany, 2001; Uzunhisarcikli *et al.,* 2007),** Significantly increased numbers of dead or abnormal sperm **(Contreras and Bustos-Obregón, 1999; Burruel *et al.,* 2000; Uzunhisarcikli *et al*., 2007)**. These changes are concentration-dependent and intensify the longer the animals are exposed **(Uzuna *et al.,* 2009).** The current study was planned to determine the effect of Phoxim on sperm cells, sex organs weight, and its genotoxic effects and the protective role of Zern-20 in male rats. The acute oral LD_{50} of Phoxim was calculated as 2007.13 mg / kg BW in male rats (Table 1), similar results were recorded by **WHO (2000)** which mentioned that, the LD_{50} of Phoxim in rats was 2000 mg/kg BW Regarding the effect of Phoxim and/or Zeron-20 on relative weights of testis, seminal vesicles and prostate gland of the treated rat groups, this study revealed no significant differences in these parameters compared to the control group. These results were in accordance with that of **Zhan *et al.,* (2000)** who found no changes of

these parameters in male treated rats. In contrast, **El-Yamanee *et al.* (2001)** found that Phoxim administration reduced significantly testis, seminal vesicle and prostate gland weights in the treated rats. Sperm cell examination in Phoxim exposed rats at a dose of 50mg/kg BW (equivalent to 1/40 LD50) three times/week for 65 days revealed significant decrease in sperm count, life percent and increase in the percentage of morphologically abnormal spermatozoa in the examined rats (Table 3) . The obtained results agreed with those of **Atef *et al.*, (1995)** who found an increased percentage of the dead and morphologically abnormal spermatozoa in the semen of Phoxim treated rats. Also **Xu *et al.* (2004)** reported significant decreases of the daily sperm production in rats treated with Phoxim (8.2 mg/kg, 5 days a week for 60 days) in comparison with the controls**.** In addition **Uzuna *et al*. (2009)** mentioned that male rats given malathion, an OP pesticide, (27 mg/kg; 1/50 of the LD50 for an oral dose) had significant lower sperm counts and sperm motility, and had significant higher abnormal sperm numbers, than that of the untreated control rats. However**, Farag *et al.*, (2000) and Khan *et al.*, (2001)** reported that OP pesticides decreases the number of spermatogenic cells in the testes and inhibits spermatogenesis. These findings were greatly supported by the histopathological examination of the testes of Phoxim administered group that revealed marked detachment and loss of spermatogenic series, scanty number of spermatids. The pathological changes of the testes agreed with those of **Uzuna *et al.* (2009) and Abo El-Soud *et al.* (2015)** in rat exposed to Malathion and pro fenofos, respectively. It is likely that these effects of Phoxim and other OPs relatives are related to their ability to cross the blood - testis barrier **(Uzunhisarcikli *et al.*, 2007)**, after which they induce oxidative stress and lipid peroxidation that damages the biological membranes in the testes. This in turn may cause the degeneration of the spermatogenic and Leydig cells, which disrupts spermatogenesis and reduces sperm counts. The sperms themselves may also be damaged by the oxidative effects of OPs, which affect the activities of mitochondrial enzymes and the structure of the microtubules in the sperm and this in turn reduces their motility. In addition, the reactive oxygen species may contribute to infertility caused by defective sperm function reported by **Latchoumycandane *et al.* (2002).** Supporting the notion that OPs like Phoxim exert their deleterious effects by oxidative stress within the testes, this study revealed that co-treatment of Phoxim exposed rats with Zeron-20 ameliorated the effects of Phoxim on sperm counts and morphology (Table 3) and the integrity of the testes. The positive effects of Zeron-

20 on sperm count could be linked to the anti-oxidative properties of its components. These results coincide with **Hassan *et al.* (2013)** who reported that administration of PAC increased serum testosterone and epididymyal fructose and sperm count. This beneficial effect was attributed to the reduction in lipid peroxidation potential and subsequently improved the testicular function **(Hassan 2005)**. Additionally, the positive effects of PAC on sperm count could be linked to its anti-oxidative properties **(Eid 2008).** Additionally, the protective effect of PAC on testis occurred probably through the enhanced degradation of the damaging oxygen free radicals leading to prevention of testicular tissue damage induced by it **(Hassan *et al*. 2013)** as well as to enhancement of the antioxidant defense system. Moreover, the improvement in sperm count and morphology (life % and approximately normal sperm counts) could be also attributed to the L-Carnitine content of Zeron. L-Carnitine also has great effects on spermatogenesis, sperm maturation and sperm motility. It is a small water-soluble particle important in fat metabolism **(Dehghani *et al.,* 2013).** It plays an important role in long- chain fatty acids oxidation in mitochondria then, producing energy. In addition, adjustment of acyl- CoA/CoA ratio, store energy as acetyL-Carnitine and revising the toxic effects of the poorly metabolized acyl groups by releasing them as carnitine esters **(Ahmed *et al.,* 2011).** L-Carnitine, with its extensive physiological roles is an essential nutrient for the body health. It is concentrated in the epididymis and sperm **(Pruneda *et al.,* 2007)**. Despite of the blood-testis barrier, carnitine is highly concentrated in testis **(Kobayashi *et al*. 2005)**. "It plays an important role not only in initiating sperm motility, promoting sperm maturation and enhancing sperm fertilizing property, but also plays a role in regulating Sertoli cell functions and protecting sperms against oxidative damage, reducing apoptosis of the spermatogenic cells and inhibiting sperms aggregation" **(Abdelrazik and Agrawal, 2009).** L-Carnitine is known to have antioxidant, anti- inflammatory and anti- apoptotic effects on various pathophysiological conditions **(Miguel - Carrasco *et al.,* 2008).** On the other hand, reactive oxygen and nitrogen species (ROS and RNS) are implicated in the diagnosis of male infertility **(Abd-Elrazek and Ahmed- Farid, 2017).** Therefore, L-Carnitine as an antioxidant protects sperm membranes against toxic reactive species by removing the toxic acyl- CoA and substitute fatty acid in cell membrane **(Vicari and Calogero, 2001),** thereby it extends the life span of sperms leading to increased male fertility **(Neuman *et al.,* 2002; El-Sherbini *et al.,* 2016)**. **Abd-Elrazek and Ahmed - Farid (2017)** reported significant improvement in

sperm morphology, motility, velocity and count in the group of rats treated with L-Carnitine. **Dehghani *et al.,* (2013)** also found that, the sperm cell count significantly increased in animals treated with L-Carnitine in comparison with the busulfan-treated group. **Lenzi *et al.* (2004)** believed that L-Carnitine affected the sperm quality by its positive effect on the epididymal environment, that leads to reduced phagocytosis of gametes and, therefore, increased sperm count. Other studies have suggested that L-Carnitine improved sperm viability (motility) and chromatin quality via its antioxidant properties and the enhanced glucose uptake by sperm **(Moretti *et al*, 2002 and Aliabadi *et al*, 2012).** The ameliorative role of Zeron against the adverse effects induced by Phoxim in the male reproductive system was proved by the histopathological examination of the testes of rats administered co-treatment of Zeron-20 and Phoxim, which revealed evidence of improvement in the spermatogenic series, with focal areas of completed serpmatogenic series Fig. (4).

Another way in which OPs affect male reproductive function is to damage DNA **(Sarabia *et al.,* 2009)**. Increases in abnormal sperm counts and the disruption of spermatogenesis are important indicators of genetic damage in pesticide-exposed mammals **(Burruel *et al.,* 2000).** Since sperm morphology is controlled by various autosomal and Y-specific genes **(Uzuna *et al.,* 2009).** Phoxim like other OPs was found to promote destructive oxidation of lipids, proteins and DNA within the testes like the observed spematocytic structural aberration (breaks and autosomal univalents) in our study, however, Zeron-20 co-treatment with Phoxim exposed rats ameliorated these toxic effects of Phoxim on the testes spermatocytic cells and restored it near to control level (Table4). That positive effect of Zeron could be linked to its antioxidative properties and coincide with those reported by **Walczak–Jedrzejowska *et al.,* (2013) and Abd-Elrazek and Ahmed-Farid (2017)**. In addition,L-carnitine content of Zeron enhances the activity of DNArepairing enzymes and other related repair mechanisms possibly through its antioxidant action **(Thangasamy *et al.,* 2009).** In conclusion, Phoxim exposure of male rats caused reproductive and geno-toxicity, however, Zeron-20 co-treatment ameliorate these oxidative toxic effects. Therefore, it seems reasonable to try to support the treatment of male infertility with supplementation having the ability to neutralize the oxidative stress of reactive oxygen species (ROS) produced by the treatment with organophosphate insecticides, i.e. antioxidants like Zeron-20.

Table (1): Determination of acute oral LD_{50} of Phoxim in male rats.

Group	Dose (mg/kg)	Number of rats in each group	Number of dead animals	Mortality %
1	500	5	0	0
2	1000	5	1	20
3	2000	5	3	60
4	4000	5	5	100

Calculation of LD_{50} was as follows: M= x1+1/2d-dr1/N

Log LD_{50} =Log 4000+1/2 Log 2-9/20 = 3.60206+0.150515-0.45 = 3.302575

Therefore, the LD_{50} = 2007.13 mg Phoxim /kg BW (2.007g phoxim/kg BW).

Table (2): Effect of Phoxim and/or Zeron -20 on relative weight of sex organs (g/100g BW) of male rats.

Groups	Testis	Seminal Vesicles	Prostate gland
Control	2.86±0.05	2.10±0.08	0.204±0.007
Zerone-20	2.91±0.14	2.26±0.06	0.209±0.012
Phoxim	2.81±0.05	2.13±0.09	0.206±0.004
Zerone-20+ Phoxim	2.79±0.09	1.97±0.04	0.198±0.009

Values represents means ± standard errors (SE). Number of animals/group = 5

Table (3): Effect of Phoxim and/or Zeron-20 on sperm count and morphology.

Groups	Countx10^6/ml	Life %	Abnormalities %		
			Head %	Tail %	Total %
Control	13.56±0.69^a	89.00±0.50^a	2.80±0.58^b	4.80± 0.49^b	7.60±0.40^c
Zerone-20	15.00±1.80^a	91.80±1.88^a	2.00±0.55^b	1.80±0.58^c	3.80±0.58^d
Phoxim	2.68±0.66^c	63.20±2.76^b	5.40±0.81^a	7.00±0.55^a	12.40±0.75^a
Zerone-20+ Phoxim	6.08±0.52^b	81.20±2.33^c	3.80 ±0.58^{ab}	6.20±0.73^{ab}	10.00±0.31^b

Values represents means ± standard errors (SE). Number of animals/group = 5

Values in the same Colum with different superscript letters are differing significantly (p<0. 05).

Table (4): Frequency of chromosomal aberration in male spermatocyte cells treated with Phoxim insecticide and/or Zeron (antioxidant).

Groups	Structural aberrations			Numerical variation		
	Autosomal univalent	Break	Total aberrations	Hypoploidy	polyploidy	Total numerical variation
Control	1.20±0.20^{b}	1.00±0.32 ^{b}	2.20±0.37^{b}	1.00± 0.32^{d}	0.00 ± 0.00	1.00±0.32^{d}
Zerone-20	1.20±0.20^{b}	1.20±0.20 ^{b}	2.40±0.24^{b}	2.00±0.32^{c}	0.00 ± 0.00	2.00±0.32^{c}
Phoxim	4.00±0.32^{a}	3.00±0.32^{a}	7.00±0.54 ^{a}	4.00±0.32^{a}	0.00 ± 0.00	4.00±0.32^{a}
Zerone-20+ phoxim	2.00±0.32^{b}	1.20±0.20 ^{b}	3.20±0.49^{b}	3.00±0.32^{b}	0.00 ± 0.00	3.00±0.32^{b}

Values represents means ± standard errors (SE). No. of examined cells 50/rat (250/group).

Values in the same Colum with different superscript letters are differing significantly (p<0. 05).

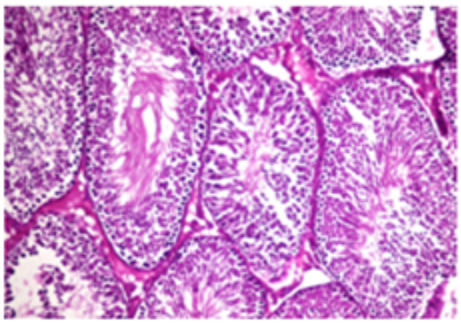

Fig.(1)

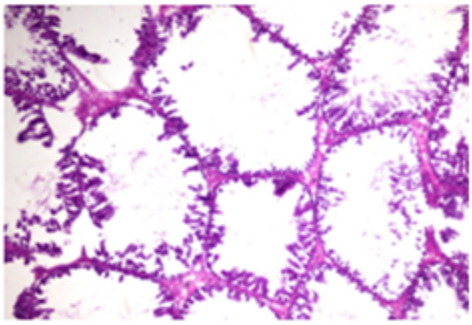

Fig. (2)

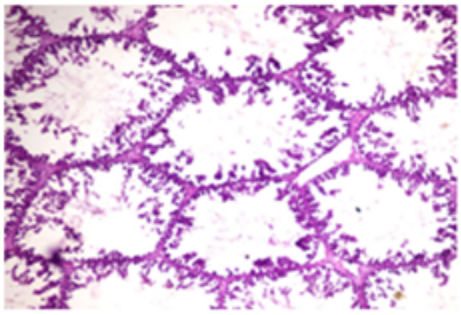

Fig.(3)

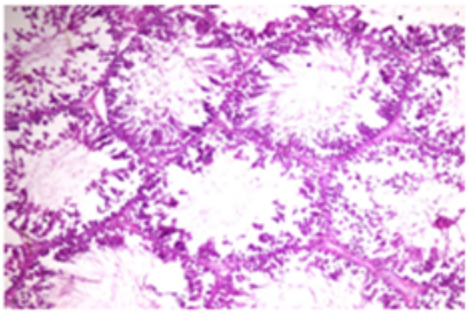

Fig.(4)

Fig.(1): Seminiferoustubules of control group revealing normalspermatogenic series. **(H&E X 200).**

Fig. (2): Seminiferous tubules of Phoxim group revealing marked damage of sermatogenic series. **(H&E X 200).**

Fig. (3): Seminiferous tubules of Phoxim group revealing marked detachment and loss of spermatogenic series. **(H&E X 200).**

Fig. (4): Seminiferous tubules of Phoxim and Zeron group revealing preservation of spermatogenic series with product of spermatid in some tubular lumens. **(H&EX 200).**

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التاثير السمي للفوكسيم علي الخصوبة و الخلايا التناسلية والدور الواقي للزيرون في ا لجرذان

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1-قسمالكيمياء والنقص الغذائى والسموم –معهد بحوث الصحة الحيوانية 2-قسم بيولوجيا الخلية المركز القومى للبحوث

الملخص العربى

صُممت الدراسة الحالية لتقييم الدور الواقي للزيرون -20 كمضاد للأكسدة عند المعاملة بالفوكسيم (مبيد فوسفوري عضوي) الذي يتسبب في- السمية الجينية والعقم عند ذكور ا لجرذان. إستخدم 20 فأر لتحديد الجرعة النصف مميتة لمبيد الفوكسيم. تم تقسيم 40 حيوان إلى أربع مجموعات متساوية ، كل منها 10 جرذان. المجموعة (1) بمثابة مجموعة ضابطة. المجموعة (2) تعطى زيرون-20 بجرعة مقدارها 25 ميكرولتر / كيلوجرام من وزن الجسم ، ثلاث مرات في الأسبوع لمدة 65 يوماً. مجموعة (3) تجرع بالفوكسيم عن طريق الفم بجرعة مقدارها 50 مجم / كجم وزن حي (متساوية إلى 40/1 من الجرعة النصف مميتة) ثلاث مرات في الأسبوع لمدة 65 يوماً. المجموعة (4) عولجت بزيرون (25 ميكرولتر / كيلوجرام من وزن الجسم ، ثلاث مرات في الأسبوع) بالإضافة إلى الفوكسيم (50 مجم / كجم وزن الجسم ، ثلاث مرات في الأسبوع لمدة 65 يوماً) . في نهاية التجربة ، تم أخذ الأعضاء التناسلية (الخصية ، الحويصلات المنوية وغدة البروستاتا) للوزن النسبي وفحص الحيوانات المنوية كما تم الفحص الباثولوجي للخصية ، بالإضافة إلى ذلك ، تم إجراء تحليل الكروموسومات في خلايا الخصية للذكور. وكشفت نتائج هذه الدراسة عن عدم وجود اختلاف معنوي في وزن الخصية والحويصلة المنوية وغدة البروستاتا بعد إعطاء الفوكسيم مقارنة بالمجموعة الضابطة أو أي مجموعة أخرى معالجة. بالإضافة إلى ذلك ، لم تظهرالمجموعة المعاملة بالزيرون أي اختلاف مقارنةً بالمجموعة الضابطة . تعاطي الفوكسيم خفض معنويا نتائج فحص الحيوانات المنوية من حيث العدد و الشكل الطبيعي مقارنة مع مجموعة الضابطة و قد حسنت المعاملة المشتركة مع الزيرون بشكل كبير هذه النتائج (عدد الحيوانات المنوية و الشكل المورفولوجى). تم زيادة الأختلالات الكروموسومية (النوعية والعددية على حد سواء) في المجموعة المعرضة الفوكسيم ، ولكن العلاج بالزيرون قلل من هذه الانحرافات بشكل كبير واستعادها بالقرب من المجموعة الضابطة. في الختام ، اثر تعرض ذكور الجرذان للفوكسيم علي الخصوبة والسمية الوراثية ، ومع ذلك ، فإن العلاج بالزيرون قلل من هذه التأثيرات السمية التأكسدية و الناتجة عن الشوارد الحرة (ROS). لذلك توصى الدراسة باضافة مضادات الأكسدة كالزيرون عند المعاملة بالمبيدات الفوسفورية العضوية لتفادى حدوث العقم و الأختلالات الوراثية عند الذكور.