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PROTECTIVE ROLE OF VITAMIN E AGAINST THE HARMFUL IMPACT OF CHLOROPYRFIOS ON SEMEN QUALITY AND TESTOSTERONE LEVELS OF RABBITS.

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ABSTRACT

Chlorpyrifos (CPF) is a broad spectrum organophosphate pesticide used for agricultural health purposes. Vitamin E has been reported to be important antioxidant. Therefore, this study aimed at elucidating the protective effects of V.E against reproductive toxicity of CPF in male rabbits. Therefore, the present experiment was undertaken to determine the effectiveness of vitamin E in alleviating the toxicity of chloroyfiros on body weight, reproductive performance and testesteron of male rabbits. Animals were assigned to 1 of 4 groups: control; 33.3 mg CPF mg/kg bw; 100 mg V.E/kg bw; CPF (33.3 mg/kg bw) plus V.E (100 mg/kg bw), respectively. Results showed that live body weight (LBW), testes weight (RTW), and serum testosterone were significantly reduced ($P<0.05$) by treatment with CPF treatment also decreased ($P<0.05$) ejaculate volume, sperm concentration, total sperm output, sperm motility index, and semen initial fructose concentration. The negative effects of CPF on semen characteristics were dose-dependent. Treatment with V.E increased ($P<0.05$) LBW, RTW andserum testosterone concentration, improved semen characteristics, and alleviated the negative effects of CPF treatment increased ($P<0.05$) the numbers of abnormal and dead sperms in a dose-dependent manner. Treatment with V.E alleviated the negative effects of CPF during treatment . Results demonstrated the beneficial influences of V.E in reducing the negative effects of CPF on production and reproduction of male rabbits.

Keywords: Chloropyrfios, Rabbits, Semen, Vitamin E.

INTRODUCTION

Pesticides are substances or mixture of substances intended for preventing, destroying, repelling or mitigating any pest[1]. A pesticide may be a chemical substance, biological agent (such as a virus or bacterium), antimicrobial, disinfectant or device used against any pest. Subclasses of pesticides include: herbicides, insecticides, fungicides, rodenticides, pediculicides, and biocides[2]. Insecticides are pesticides used particular against insects. One of the most widely used groups of insecticides in the world is organophosphate compounds. Chlorpyrifos is an organophosphate insecticide, acaricide and miticide used to control foliage and soil-borne insect pests on a variety of food and feed crops[3]. The most common trade name is Dursban. Chlorpyrifos is effective by direct contact, ingestion and inhalation[4]. The toxicological profile of chlorpyrifos demonstrates that, chlorpyrifos like other organophosphates, has anticholinesterase activity in all species tested[5]. Inhibition of brain acetylcholinesterase (AChE) at synapses by organophosphorus pesticides (OPP) results in accumulation of acetylcholine and over activation of acetylcholine receptor at neuromuscular junction and in the autonomic and central nervous system. This will manifested in convulsions and even tremors leading in severe cases to death [6]. Chlorpyrifos toxicity is predicted from LD50 (a dose that expected to cause death in 50% of animals). Acute oral LD50 of chlorpyrifos in rats was estimated to be 135-163, guinea pigs 504, rabbits 1000-2000 mg/kg body weight [4]. However, no previous study assessed the exact or narrow range of oral LD50 in male domestic rabbit. Chlorpyrifos is very low in toxicity via dermal exposure; the dermal LD50 in rats was estimated at greater than 2000 mg/kg. Inhalation LC50 (4-6 hours) of chlorpyrifos for rats >0.2 mg/I [4]. The reproductive toxicity of male rats with CPF was in the form of a marked reduction in testicular sperm counts, motility and significant growth of sperm malformation rate in

exposed males. Histopathological examination of testes showed mild to severe degenerative changes in seminiferous tubules. The levels of testosterone showed a decreasing tendency in association with CPF administration [7]. Some studies identified reactive oxygen species (ROS) as a cause of toxic effects exerted by OP pesticides. These ROS are responsible for inducing oxidative stress in the tissues and chronic, permanent damage[8]. This raised the interest of scientists to search for antioxidants, which might alleviate oxidative stress caused by pesticides. Several substances including OP pesticides-induced oxidative stress in experimental animals as an essential oil, wheat germ oil and grape seed oil, vitamin E (α-tocopherol) and zinc were used against CPF induced oxidative stress in rats[9]. The mammalian cells reduced the adverse effect of lipid peroxidation via the utilization of both enzymatic and non-enzymatic antioxidants, which scavenge for free radicals in the system. Oxidative stress results when the endogenous antioxidants have been over whelmed by the rate and extent of free radical generation. Therefore, during oxidative stress, an increase in the exogenous supply of antioxidants improves the capacity of the tissue to cope with high antioxidant as zinc and vitamin E demands [10]. Vitamin E may have various roles Many biological functions have been postulated, including a role as a fat-soluble antioxidant[11]. In this role, V.E acts as a radical scavenger, delivering a hydrogen (H) atom to free radicals. At 323 kJ/mol, the O-H bond in tocopherols is about 10% weaker than in most other phenols [12]. This weak bond allows the vitamin E to donate a hydrogen atom to the peroxyl radical and other free radicals, minimizing their damaging effect. The thus-generated tocopheryl radical is recycled to tocopherol by a redox reaction with a hydrogen donor, such as vitamin C [13]. As it is fat-soluble, V.E is incorporated into cell membranes, which are therefore protected from oxidative damage.

MATERIALS AND METHODS

In this study chlorpyrifos and Vitamin E were used. The CPF insecticide (purity 99.0%) was obtained from Dr Ehrenstorfer GmbH (Augsburg, Germany). Vit. E (Dietvit E, 53% α- atocopherol acetate) was purchased from Neolait SA, France (Manufactured by Codislait Sarl, 22120 Yffiniac).The doses of vitamin E was 100 mg/kg BW every day. These doses were used because previous studies showed that 100 mg vitamin E/kg BW were effective against the toxicity of pesticides (fenvalerate) and carbon tetrachloride [14,15]. Mature male New Zealand White rabbits (age of 6 months and initial weight of (2.247±0.04Kg) were used. Animals were individually housed in cages and weighed weekly throughout12- weeks experimental period. Feed and water were provided ad libtum. Rabbits fed pellets which consisted of 30 % berseem (Trifolium alexandrinum) hay, 25 % yellow corn, 26.2% wheat bran, 14 % soybean meal, 3 % molasses, 1 % $CaCl_2$, 0.4 % NaCl, 0.3 % mixture of minerals and vitamins, and 0.1 % methionine. The vitamin and mineral premix per kg contained the following IU/gm for vitamins or minerals: vit A-4000,000, vit D3-5000, 000, vit E-16,7 g, K-0.67 g, vit B1-0.67 g, vit B2-2 g, B6-0.67 g, B12-0.004 g, B5-16.7 g, Pantothinc acid-6.67 g, Biotein-0.07 g, Folic acid-1.67 g, Choline chloride-400 g, Zn-23.3 g, Mn-10 g, Fe-25 g, Cu-1.67 g, I-0.25 g, Se-0.033 g, and Mg-133.4 g (Rabbit premix produced by Holland Feed Inter. Co.). The chemical analysis of the pellets [16] showed that they contained 15.8 % crude protein, 11.3 % crude fiber, 3.7 % ether extract, 7.2 % ash, 92.9 % organic matter and 62.4 % nitrogen free extract % as DM basis. Twenty mature male rabbits were randomly divided into four equal groups (each five rabbits) as follows:- Group I: Rabbits were used as control daily for 12 weeks. Group II: Rabbits were treated with V.E. V.E was given daily by gavage at a dose of 100 mg/kg B.W, [17] for 12 successive weeks. Group III: Rabbits were treated daily with CPF by gavage at a dose of 33.3 mg/kg B.W/day [18]. Group IV: Rabbits were given with CPF daily at a dose of 33.3 mg/kg B.W./day by gavage like group III and given the V.E concurrently daily at a dose of 100 mg/kg B.W./day by gavage like group II for 12 successive weeks. The doses of the CPFand V.E were calculated according to the animal's body weight on the week before dosing. The tested doses of CPF and V.E were given daily for 12 weeks. Body weight of each animal was recorded weekly throughout the 12-week of the experimental period. The weight measurements were carried out in the morning before access to feed and water. At the end of treatment period, all animals of each group were slaughtered. Weights of liver, lung, heart, kidney, spleen and testis were also recorded. These organs were individually identified and kept frozen (–20°C) until assays performed. Blood samples were collected from the ear vein of all animals every other week throughout the 6-weeks experimental period. Blood samples were obtained in the morning before accesses to feed and water and placed immediately on ice. The blood samples were collected in tube containing heparin to obtain plasma. Semen collection was done weekly and continued

throughout the 12-weeks experimental period, so 60 ejaculates obtained per treatment. Ejaculates were collected using an artificial vagina and a teaser doe. The volume of each ejaculate was recorded (using a graduated collection tube) after removal of the gel mass. A weak eosin solution[19] was used for evaluation of sperm concentration by the improved Neubauer haemocytometer slide (GmbH + Co., Brandstwiete 4, 2000 Hamburg 11, and Germany). Total sperm output calculated by multiplying semen ejaculate volume and semen concentration. Determination of initial fructose concentration in seminal plasma was determined immediately after semen collection according to [20]. Assessments of dead and normal spermatozoa were performed using an eosin-nigrosine blue staining mixture[21]. The percentages of motile sperm were estimated by visual examination under low-power magnification (10x) using light microscope. Total number of motile sperm was calculated by multiplying the percentage of motile sperm and total sperm output. Reaction time was determined as the moment of subjecting a doe to the buck until the completion of erection; it was measured in seconds. Initial hydrogen ion concentration (pH) was determined immediately after collection using pH cooperative paper (Universalindikator pH 0-14 Merck, Merck KgaA, 64271 Darmstadt, Germany). Packed sperm volume (PSV) was recorded. Total functional sperm fraction (TFSF) was calculated as the product of total sperm output (TSO), sperm motility (%), and normal morphology (%) [22]. Statistical analysis: Where applicable, statistical analysis was carried out in Minitab software (version17) statistical significance was assessed using ANOVA analysis with Tukey multiple comparison test after detection normal distribution to the information and suitable $P < 0.05$ consider critical.

RESULTS

Body weight (BW) relative weight of testes and testosterone were significantly ($P < 0.05$) decreased in rabbits treated with CPF compared to control animals (Table 1). Results obtained showed that CPF significantly ($P < 0.05$) decreased libido (by increasing the reaction time), ejaculate volume, sperm concentration, total sperm output, sperm motility (%), total motile sperm per ejaculate (TMS), packed sperm volume (PSV), total functional sperm fraction (TFSF), normal and live sperm and semen initial fructose. While initial hydrogen ion concentration (pH) and dead and abnormal sperm were increased ($P < 0.05$). Live body weight (LBW) and relative weights of testes (RTW) were significantly ($P < 0.05$) decreased. Concentrations of thiobarbituric acid-reactive substances (TBARS) were significantly ($P < 0.05$) increased in plasma of rabbits treated with CPF compared with control (Table 2). Vitamin E alone significantly ($P<0.05$) increased on all parameters. The presence of vitamin E with CPF caused significant increase in the reduction of all parameters , and this means that vitamin E alleviated its toxicity.

Table 1: The overall means (±SE) of body weight, relative testes weight , blood plasma testosterone concentration and Thiobarbituric acid-reactive substances (TBARS) in plasma and testes during treatment of male rabbits with CPF, V.E .

Parameters	***Groups***			
	Control	***V.E***	***CPF***	***CPF+V.E***
BW (Kg)	2.247 ± 0.04^{ab}	2.699 ± 0.09^{a}	1.975 ± 0.06^{b}	2.240 ± 0.02^{ab}
RTW (g/100 g BW)	7.500 ± 0.354^{a}	7.00 ± 0.64^{a}	5.800 ± 0.464^{b}	6.300 ± 0.200^{ab}
Testosterone (ng/mL)	1.629 ± 0.039^{b}	2.751 ± 0.204^{a}	1.105 ± 0.112^{c}	1.636 ± 0.088^{b}
TBARS (nmol/ml)	2.655 ± 0.034^{b}	2.381 ± 0.049^{c}	3.113 ± 0.096^{a}	2.578 ± 0.040^{bc}
Testes TBARS (nmol/g tissue)	13.7 ± 1.78^{a}	9.6 ± 0.50^{a}	20.0 ± 5.88^{a}	14.322 ± 1.69^{a}

[abc] Within row, means with different superscript letters differ significantly ($p < 0.05$).

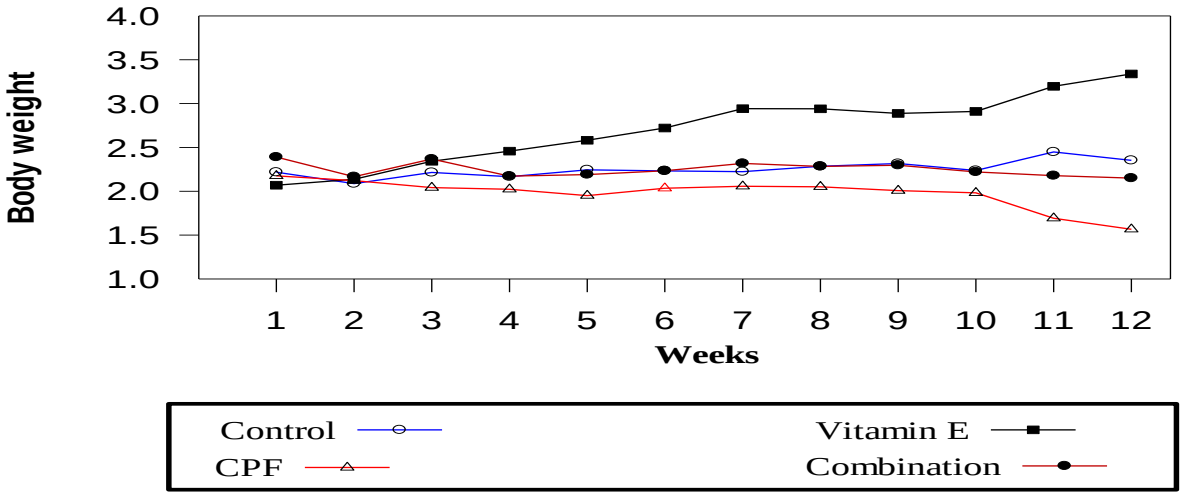

Figure1. Change in body weight during treatment of male rabbits with V.E, CPF, and/or their combination..

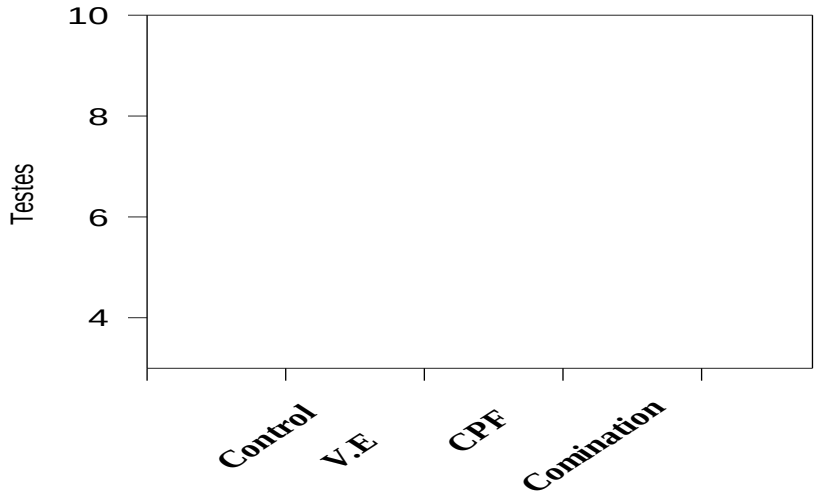

Figure2. Change in testes weight during treatment of male rabbits with V.E, CPF, and/or their combination.

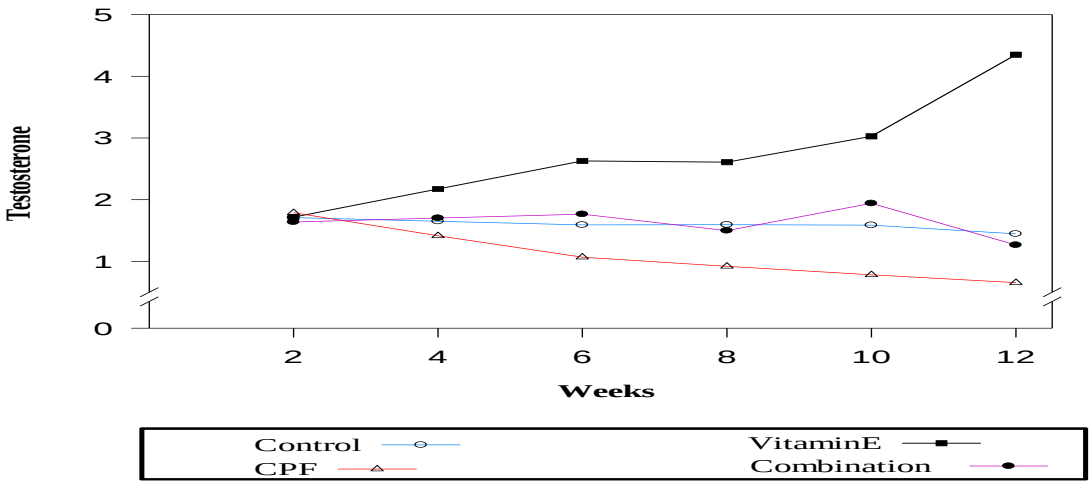

Figure 3. Change in Testesteron during treatment of male rabbits with V.E, CPF, and/or their combination.

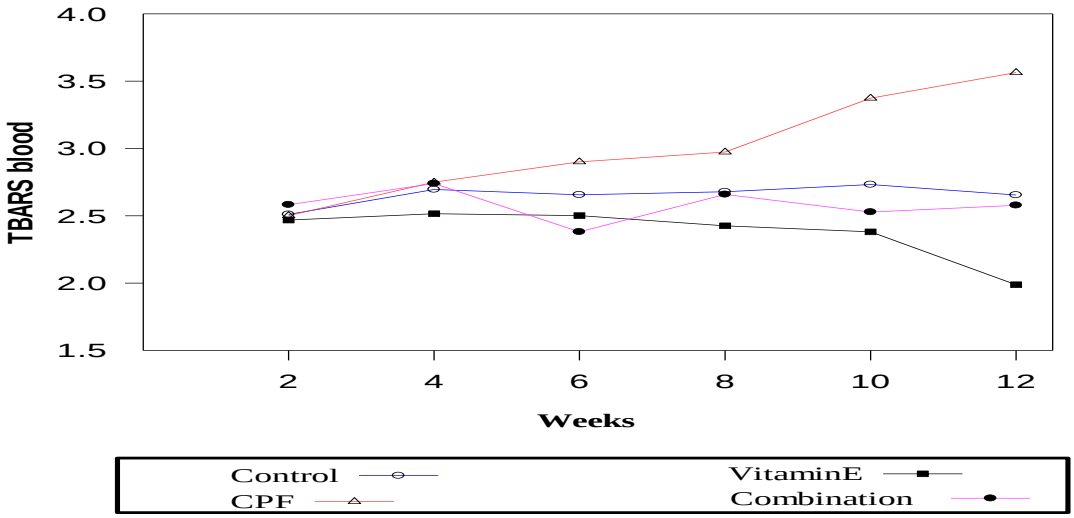

Figure Error! No text of specified style in document. **Change in the TBARS of plasma during treatment of male rabbits with V.E, CPF, and/or their combination.**

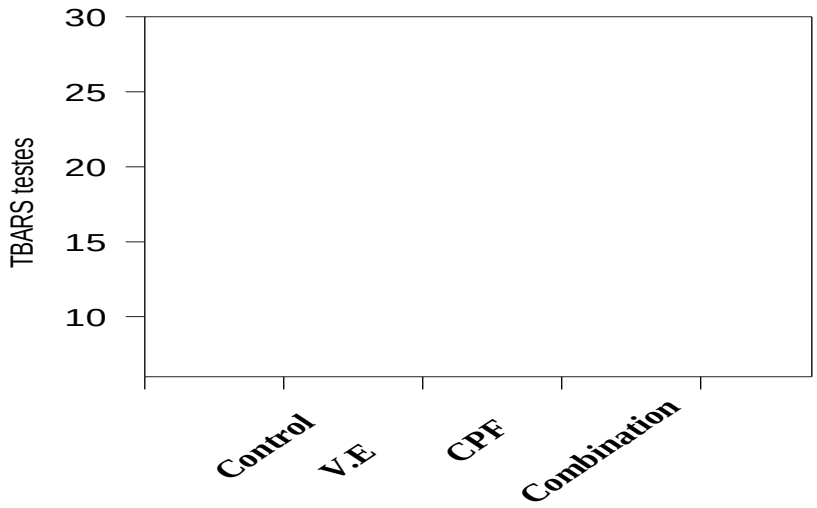

Figure5. Change in the activity of testes TBARS during treatment of male rabbits with V.E, CPF, and/or their combination.

Table 2: The overall means (±SE) of semen characteristics during treatment of male rabbits with V.E, CPF, and/or their combination

Parameters	Animal Groups			
	Control	**V.E**	**CPF**	**V.E + CPF**
Ejaculate volume (ml)	1.24± 0.017[a]	1.32± 0.018[a]	1.13± 0.018[a]	1.20± 0.017[a]
PH	8.33±0.022[ab]	8.25±0.044[b]	8.53±0.032 [a]	8.37±0.035 [ab]
Reaction time (s)	5.28±0.104[a]	4.21±0.144[a]	6.13 ±0.204 [a]	5.23±0.107 [a]
Packed sperm volume (%)	15.8±0.16[ab]	18.1±0.38 [a]	13.42±0.21 [b]	14.8±0.15 [b]

Sperm concentration (×10^6 ml^{-1})	264±4.5^b	320± 7.0^a	229± 4.6^b	258±4.3^b
Total sperm output (×10^6)	328±6.6 ^{b}	426± 12.5^a	263±8.1 ^{b}	309± 6.9^b
Sperm motility (%)	68.7±0.7 ^{ab}	73.8± 0.9^a	61.8±0.9 ^{b}	68.2±0.7 ^{ab}
Total motile sperm (×10^6)	225±5.4 ^{b}	318±11.8 ^{a}	164±6.2 ^{b}	213±6.0 ^{b}
Live sperm (%)	73.9± 0.8 ^{a}	82.2±1.1 ^{a}	63.0±1.1 ^{b}	71.8±0.7 ^{a}
Dead sperm (%)	26.6±0.82^b	18.3 ±1.11^b	37.5 ±1.10^a	28.7±0.69^b
Normal sperm (%)	82± 0.3^{ab}	85± 0.4^a	78±0.7 ^{b}	80±0.3 ^{ab}
Abnormal (%)	20±0.3 ^{ab}	16±0.4 ^{b}	24±0.7 ^{a}	21±0.3^{ab}
Total functional sperm fraction (×10^6)	182± 5.0^b	173± 11.2^a	130±5.5 ^{b}	169±4.4 ^{b}
Initial fructose (mg/dl)	257± 3.9^a	277± 3.7^a	201 ±5.9^b	222±3.5^b

^{abc}Within row, means with different superscript letters differ significantly ($p < 0.05$).

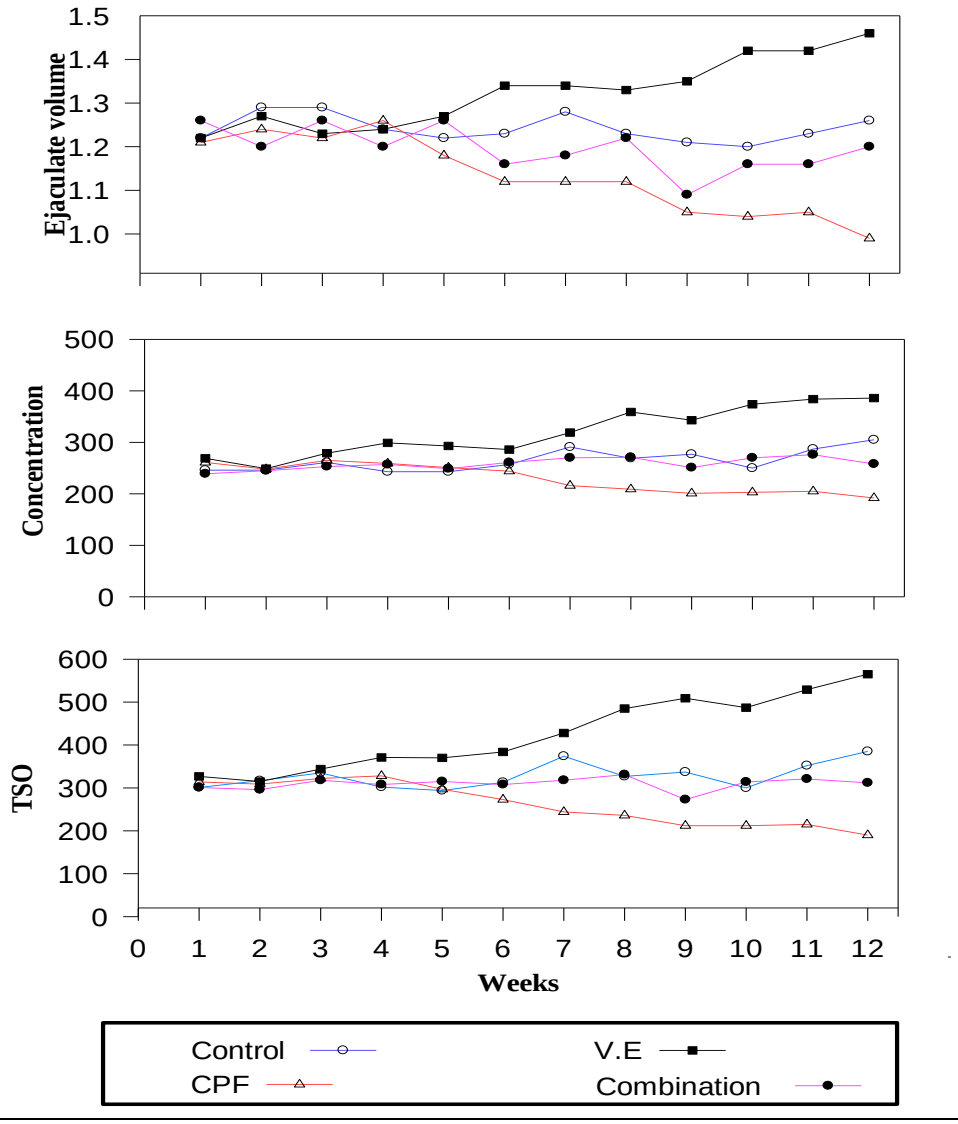

Figure 6. Change in ejaculate volume, sperm concentration, TSO during treatment of male rabbits with V.E, CPF, and/or their combination.

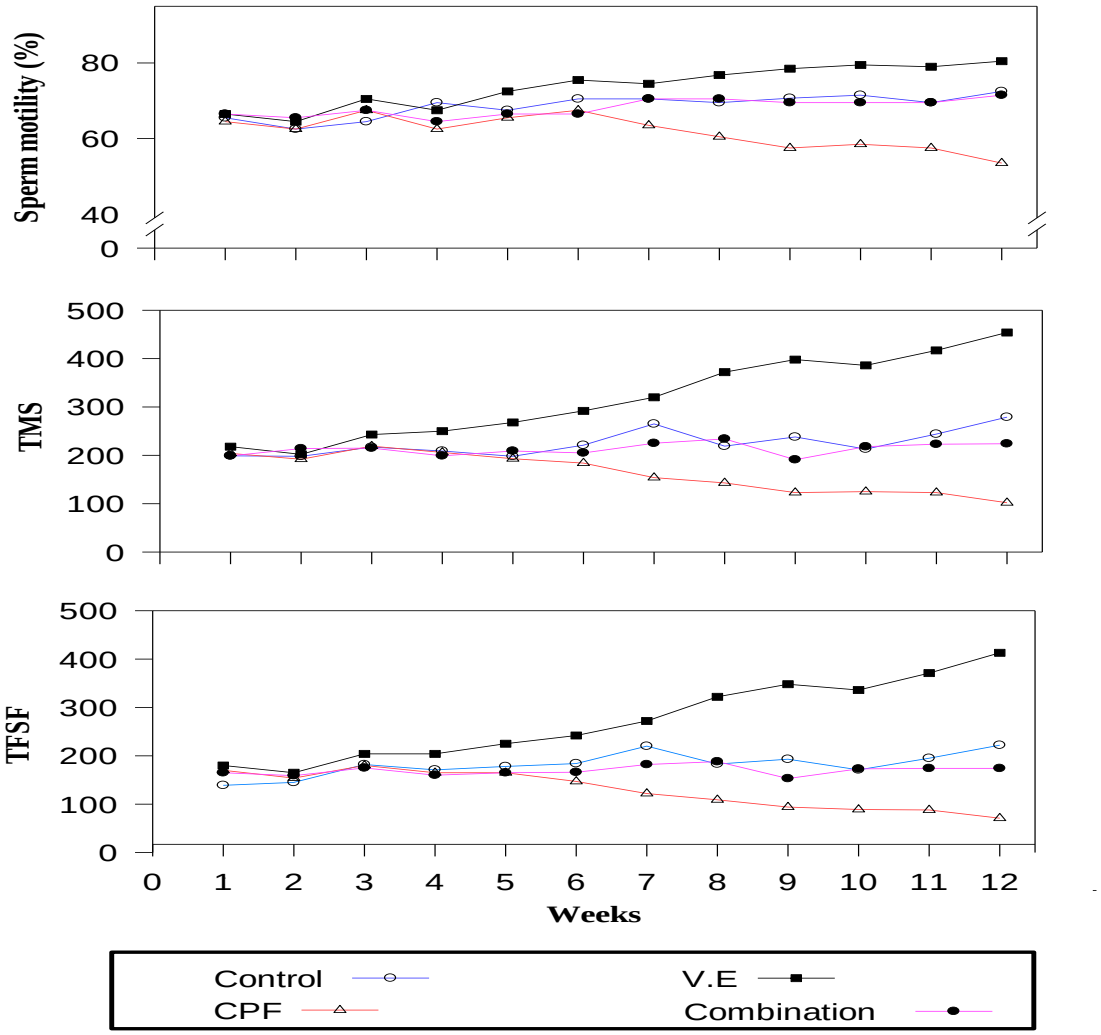

Figure 7. Change in sperm motility, total motile sperm, total functional sperm fraction during treatment of male rabbits with V.E, CPF, and/or their combination.

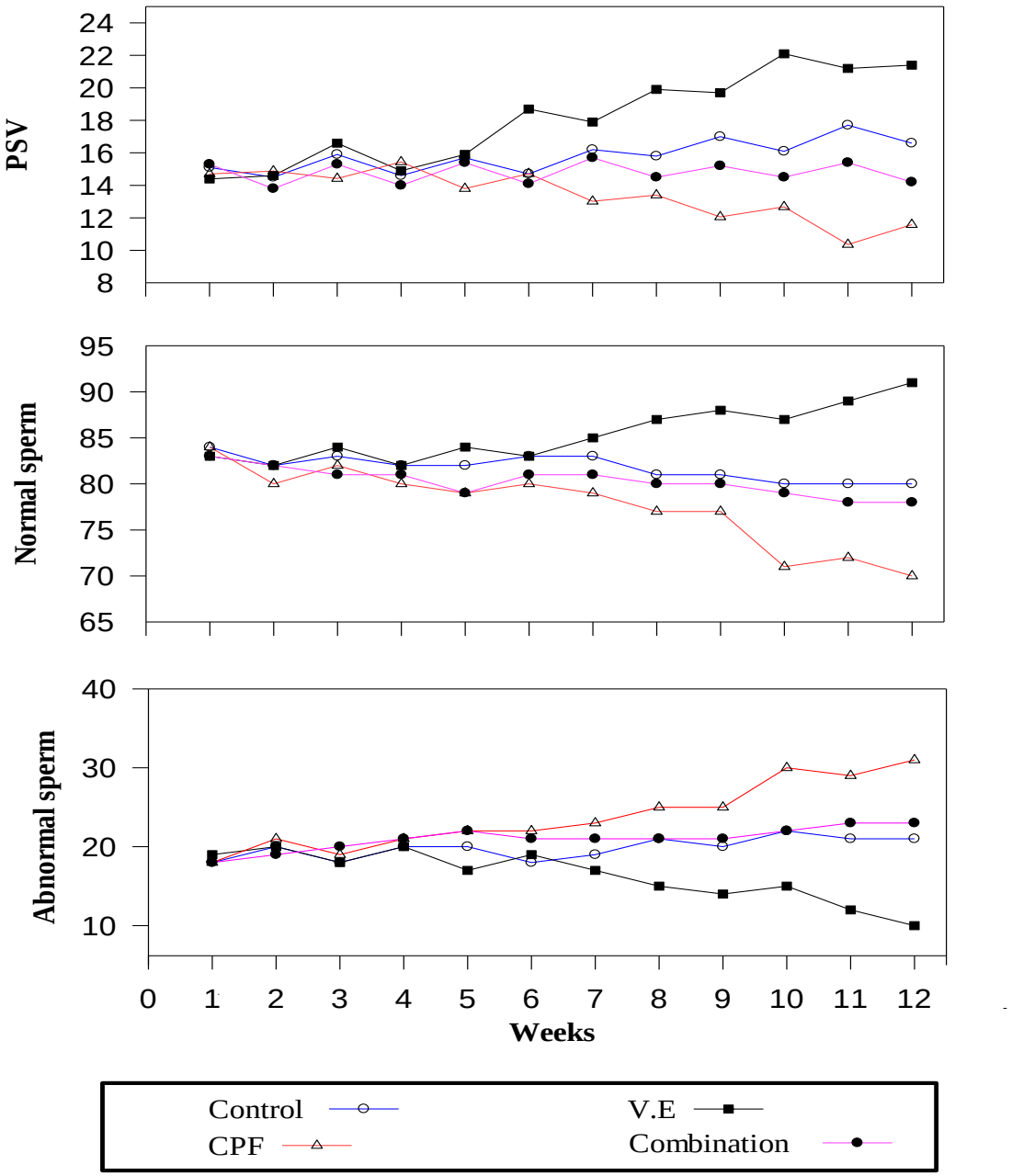

Figure 8. Change in Normal sperm ,Abnormal sperm , packed sperm volume during treatment of male rabbits with V.E, CPF, and/or their combination.

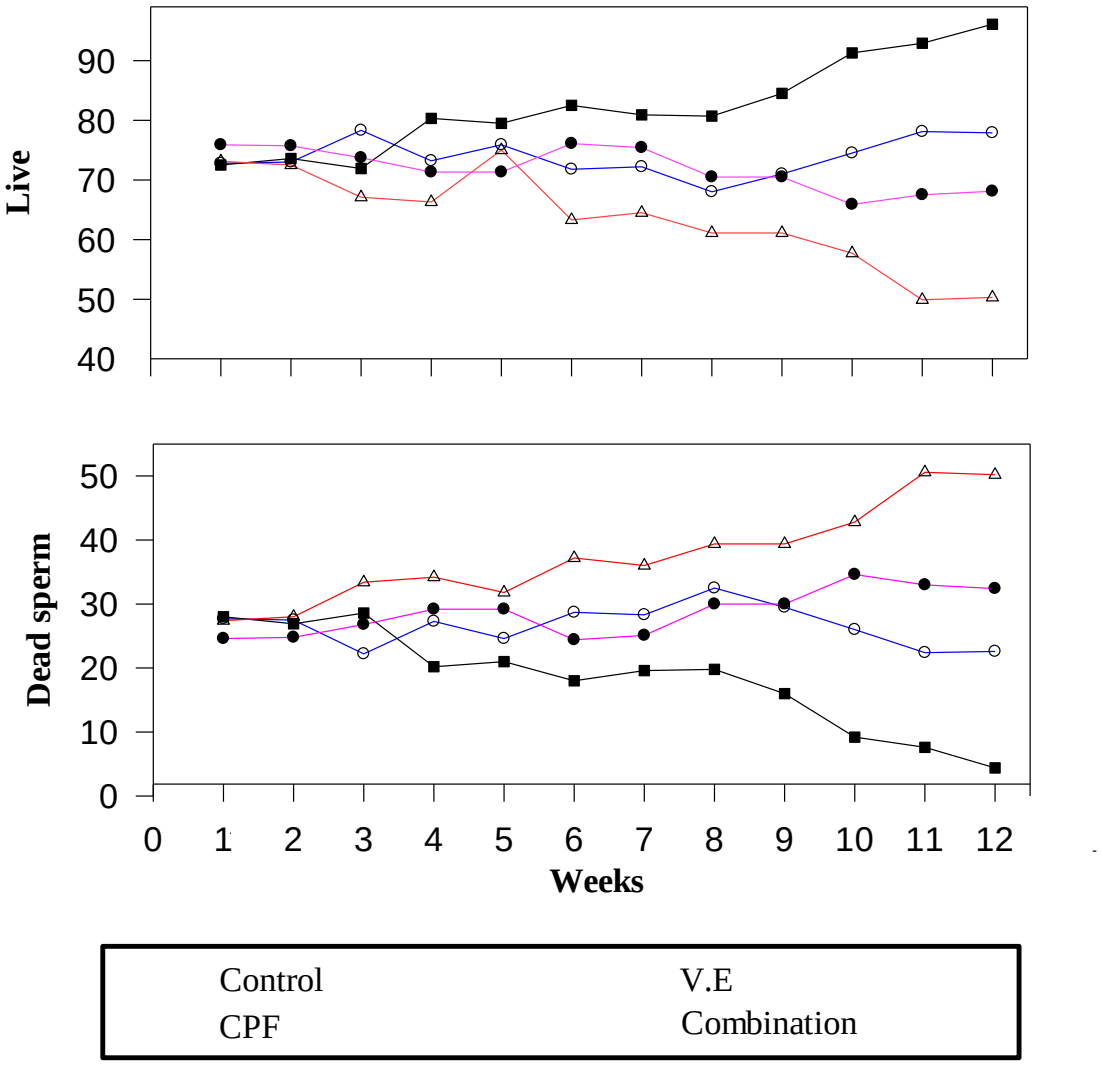

Figure 9. Change in dead sperm ,Live during treatment of male rabbits with V.E, CPF, and/or their combination.

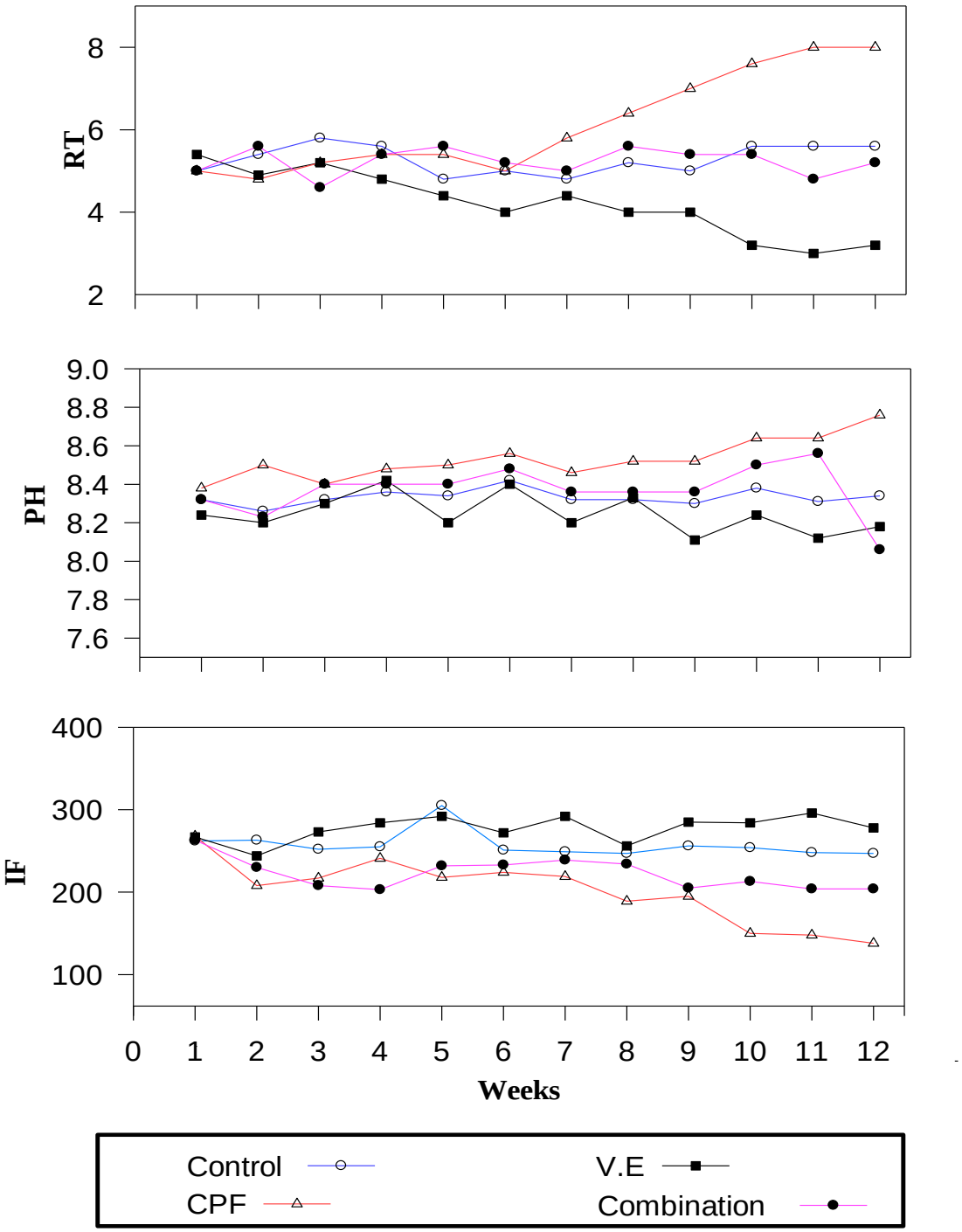

Figure 10. Change in initial fructose, initial hydrogen ion concentration, reaction time during treatment of male rabbits with V.E, CPF, and/or their combination.

DISCUSSION

The present results indicate that treatment with (CPF) caused significant reductions in body weight (BW) and relative organs weight (ROW) of testes (Table 1 and Figures 1 to 2). The reduction in BW and ROW of the (CPF) treated rabbits is in agreement with that obtained by [23,24]. The reduction in body weight in response to chlorpyrifos intake may be a result of the combined action of cholinergic and oxidative stress and/or due to increase degradation of lipids and proteins as a direct effect of organophosphours compound exposure [25-27]. The results are in agreement with [28] who reported that the net weight of testes were decreased significantly after oral intubation with CPF for 30 days. The increase body weight observed in the present study due to treatment with V. E is agreements with [29,30]. Vitamin E scavenges for reactive oxygen species (ROS), prevents cellular damage and improves growth [31]. This observation is in line with the report of [32] who observed that dietary vitamin E addition in rabbits'

diet resulted in higher body weight and feed conversion efficiency in rabbits. The present study showed that CPF decrease plasma testosterone, in rabbits (Tables 1and figures 3). These results are consistent with[28] who demonstrated that chlorpyrifos at dose levels 7.5, 12.5, and 17.5 mg kg/day for 30 days produced reduction in serum testosterone concentration. The present study showed that CPF increased free radicals (Tables 1 and Figures 4) and these results are in agreement with the finding of[33] who suggested that In humans, ROS can cause lipid peroxidation in sperm cell membranes, changes in the structure of membrane receptors, and in enzyme and transport proteins. It can also increase anomalies in chromatin structure and breaks in sperm DNA strands. Although the role of free radicals is well established in many chronic disorders, the implication of ROS in a condition like OP pesticide poisoning has not yet been investigated. However, ROS produced by OP may be involved in the toxicity of various pesticides[34]. The effects of OP on rabbis studied revealed that changes characteristic of oxidative stress, namely in the elevation of MDA levels in the plasma, brain and testes following CPF administration. Decrease in free radicals of rabbits treated with V.E (Tables 1 and Figures 4) is in accordance with other published studies[35]. Vitamin E protects critical cellular structures against damage caused by oxygen-free radicals and reactive products of lipid peroxidation. It has been reported that lipid peroxidation was prevented by vitamin E[36]. Vitamin E as antioxidant [37] was observed to prevent membrane damage mediated by free radicals [38]. In addition,[35] reported reduced oxidative stress in the testis following administration of vitamin E. Oxidative stress is one of the major factors that affect male fertility[39]. Oxidative stress is known to play a major role in the sperm malfunctions through induction of lipid peroxidation to biomembranes [40]. Vitamin E (a-tocopherol) inhibits peroxidation of membrane lipids by scavenging lipid peroxyl radicals, as a consequence of which it is converted into a tocopheroxyl radical. This radical is thought to be either recycled to a-tocopherol by interacting with soluble antioxidants, such as ascorbic acid, or irreversibly oxidized to a-tocopherylquinone. In fact, a-tocopherylquinone may act as a potent anticoagulant, and as an antioxidant through its reduction to hydroquinone[41]. Also,[42] reported that the protective role of vitamin E against the toxicity of oxidants may be due to the quenching of hydroxyl radicals. Our results (Table 1) indicate that treatment of vitamin E alone or in combination with CPF produces a significant reduction in CPF-induced increase in TBARS in different rabbits tissues. Thus, the present results indicate that adequate antioxidant status may attenuate CPF-induced oxidative stress and cellular damage. The present study showed that chlorpyrifos (CPF) caused deterioration in semen characteristics (ejaculate volume, sperm concentration, total sperm output, sperm motility, normal sperm, total motile sperm, total functional sperm fraction and semen packed sperm volume, dead sperm, semen initial fructose, semen initial hydrogen ion concentration (pH) and reaction time) of male rabbits (Tables 2 and Figures 3 to5- 10**)** . This means that CPF has reproductive toxicity in male rabbits. Also, previous studies demonstrated that semen of different animal species was adversely affected by CPF. The obtained results were in agreement with those reported by[43-45] in rat who reported that exposure to environmental toxicants including pesticides is a proven factor in impairment of male reproductive system and infertility. Also, One of these pesticides is chlorpyrifos (CPF), an organophosphorus pesticide, which is used extensively in urban and agricultural crop pest control[44]. The results of the present study were in agreement with [46] who found that adult male mice that were treated by gavage with CPF at doses 0, 5, 15, or 25 mg kg/day for 4 weeks, their sperm motility, and count were markedly decreased and the percentage of morphologically normal spermatozoa was also affected. Also, [47] mentioned that CPF when orally administered to male rats at the doses 3, 6, and 9 mg kg/day for 90 days produced adverse effects along with mild testicular and spermatotoxic effects in male rats. Similar results were obtained by[28] who reported that CPF produced marked reduction in epididymal and testicular sperm counts in exposed albino rats. This is because low testosterone production could result in suppression of spermatogenesis. Inhibition of spermatogenesis may also be as a result of low FSH and LH levels[45]. Furthermore, reduction in sperm motility could be due to decreased mitochondrial activity, altered fructose synthesis, or corrosion of microtubule structure of spermatozoan[48]. The inhibition of sperm motility in pesticide treated rats could be because of low ATP level[49]. The present results also indicated that V.E alone caused significant increases in ejaculate volume, sperm concentration, total sperm output, sperm motility, normal sperm, total motile sperm, total functional sperm fraction and semen packed sperm volume. While, significant decreases dead sperm, semen initial fructose, semen initial hydrogen ion concentration (pH) and reaction time. In addition, the presence of V.E with CPF counteracted its

reproductive toxicity and these results were in agreement with other published studies by [17] who reported that vitamin E observed to increase sperm viability and reduced lipid peroxidation when

subjected to oxidative stress induce. Treatment with vitamin E alone and/or in combination caused a significant (P < 0:05) increase in semen quality, and minimized the toxic effects of CPF (Table 2). [50] reported that the formation of TBARS was significantly (P < 0:05) decreased by treatment with vitamin E alone. Similarly, [51] reported that vitamin E supplementation reduced ROS generation and protected spermatozoa from loss of motility. [52] found that oral treatment with 200 mg vitamin E daily decreased ROS significantly and increased fertilization rate of fertile normospermic human male after one month of treatment.[53] reported that in vivo experiments, seven weeks of oral vitamin E (1000 IU/d/animal) administration in boar caused a significant fall in the level of semenal plasma TBARS from 2.2 to 1.2 nmol/ml. Therefore, the improving and protective effect of vitamin E and and/or their combination against the toxic effect of CPF on semen characteristics may be due to the reduction in lipid peroxidation.

CONCLUSION

It is clear from the obtained results that CPF induced pronounced hazardous effects in several physio-metabolic functions including body weight, semen characteristics and testosterone. This effect may decrease the productive and reproductive performance of animals. Also, the measured parameters could be used as bioindicators for the negative effect and reproductive toxicity of the exposure to CPF in adult rabbits. Using vitamin E in combination with CPF minimized and alleviated the hazardous effects of CPF on most of the tested parameters and this may be attributed to the vital role of vitamin E as antioxidant. In addition, treatment with vitamin E alone reduced the generation of free radicals and caused an improvement in the productive and reproductive performance of male rabbits.

REFERENCES

1. **United States Environmental Protection Agency, EPA (2007):** What is apesticide?Information on Profile http://www.epa.gov/fedrgstr/EPAPEST/October/Da17/p20442.htm.
2. **Gilden R.C., Huffling K. and Sattler B. (2010):** Pesticides and health risks. Journal of Obstetric Gynecologic and Neonatal Nursing 39 (1): 103-110.
3. **United States Environmental Protection Agency, EPA (2002):** Pesticides: Reregistration, Chlorpyrifos Facts Information onProfilehttp://www.epa.gov/oppsrrd1/REDs/factsheets/chlorpyrifos fs.htm.
4. **Tomlin C.D.S. (2006):** The Pesticide Manual, A World Compendium, 14th ed ., British Crop Protection Council: Alton, Hampshire, UK 186 187.
5. **Slotkin T.A., Olivier C.A. and Seidler F.J. (2005a):** Critical periods for the role of oxidative stress in the developmental neurotoxicity of chlorpyrifos and terbutaline, alone or in combination. Brain Research, Developmental Brain Research 157 (2): 172-180.
6. **Lotti M. (2001):** Clinical toxicology of anticholinesterase agents in humans. In Handbook of Pesticide Toxicology (Krieger, R. I., Ed), 2nd edition, Academic Press, San Diego 1043–1085.
7. **EL-SHARKAWY E.; YAHIA D. AND EL-NISR A. (2014):** Chlorpyrifos induced testicular damage in rats: Ameliorative effect of glutathione antioxidant. Environ Toxicol, 29; 1011-1019.
8. **OLORUNSHOLA K.V.; ACHIE L.N. AND AKPOMIEMIE M.L. (2011):** Ascorbic acid ameliorates the toxic effects of chlorpyrifos on testicular functions of albino rats. British Journal of Pharmacology and Toxicology, 2, 5; 262-269.
9. **Mansour S.A. and Mossa A.H. (2010):** Adverse effects of lactational exposureto chlorpyrifos in suckling rats. Human and Experimental Toxicology 29 (2):77-92.
10. **AMBALI S.; AKANBI D.; SHITTUA M.; GIW A.; OLALEKAN O. AND AYOA O. (2010):** Chlorpyrifos-Induced Clinical, Hematological and Biochemical Changes in Swiss Albino Mice- Mitigating effect by co-administration of vitamins C and E. Life Science Journal, 7, 3; 37-44.
11. **Traber, M. G., Leonard, S. W., Ebenuwa, I., Violet, P. C., Wang, Y., Niyyati, M., ... & Levine, M. (2019):** Vitamin E absorption and kinetics in healthy women, as modulated by food and by fat, studied using 2 deuterium-labeled α-tocopherols in a 3-phase crossover design. The American journal of clinical nutrition, 110(5), 1148-1167.
12. **Lide, David R., ed. (2006):** CRC Handbook of Chemistry and Physics (87th ed.). Boca Raton, FL: CRC Press. ISBN 0-8493-0487-3.
13. **Traber MG, Stevens JF September (2011):** "Vitamins C and E: beneficial effects from a mechanistic perspective". Free Radical Biology&Medicine. 51 (5):13.

doi:10.1016/j.freeradbiomed.2011.05.017. PMC 3156342. PMID 21664268.
14. **El-Demerdash, F.M., Yousef, M., Sheweita, S.A., (1999):** Ameliorating effect of some antioxidants on carbon tetrachloride-induced biochemical alterations in rats. Environmental and Nutritional Interactions 3, 245–255.
15. **El-Demerdash, F.M., Yousef, M.I., Kedwany, F.S., Baghdadi, H.H., (2004):** Role of a-tocopherol and b-carotene in ameliorating the fenvalerate-induced changes in oxidative stress, hemato-biochemical parameters and semen quality of male rats. Journal of Environmental Science and Health B 39, 443–459.
16. **AOAC, I. (1990).** AOAC: Official Methods of Analysis (Volume 1). K. Helriched.. Association of Official Analytical Chemists. Inc., Arlington.
17. **Bansal, A.K., Bilaspuri, G.S., (2009):** Antioxidant effect of vitamin E on motility, viability and lipid peroxidationof cattle spermatozoa under oxidative stress. Animal Science Paper Reports 27 (1), 5-14.
18. **Adas, T. O. (2013).** Toxic Effects of Chlorpyrifos on Liver and Kidney of Male Domestic Rabbit.
19. **Smith, J. T., & Mayer, D. T. (1955).** Evaluation of sperm concentration by the hemacytometer method: Comparison of four counting fluids. Fertility and Sterility, 6(3), 271-275.
20. **Mann, T. (1948).** Fructose content and fructolysis in semen. Practical application in the evaluation of semen quality. The Journal of Agricultural Science, 38(3), 323-331.
21. **Blom, E. (1950).** A one-minute live-dead sperm stain by means of eosin-nigrosin. Fertility and sterility, 1, 176-177.
22. **Correa, J. R., & Zavos, P. M. (1996).** Preparation and recovery of frozen-thawed bovine spermatozoa via various sperm selection techniques employed in assisted reproductive technologies. Theriogenology, 46(7), 1225-1232.
23. **Carr R.L., Chambers H.W., Guarisco J.A., Richardson J.R., Tang J. and Chambers J.E. (2001):** Effects of Repeated Oral Postnatal Exposure to Chlorpyrifos on Open-Field Behavior in Juvenile Rats. Oxford Journals Toxicological Sciences 59 (2): 260-267.
24. **Ahmad K.R. and Asmatullah. (2007):** Teratological Effects of Chlorpyrifos in Mice. Iranian Journal of Toxicology 1 (2): 1-3.
25. **Goel, A.; Dani, V.; Dhawan, D.K.(2005):** Protective effects of zinc on lipid peroxidation, antioxidant enzymes and hepatic histoarchitecture in chlorpyrifos-induced toxicity. Chem. Biol. Interact. 2005, 156, 131–140.
26. **Mossa A.H., Refaie A.A. and Ramadan A. (2011):** Effect of Exposure to Mixture of Four Organophosphate Insecticides at No Observed Adverse EffectLevel Dose on Rat Liver: The Protective Role of Vitamin C. Research JournalEnvironmental Toxicology 5: 323-335.
27. **Heikal T.M., Mossa T.H., Marei G.I.KH. and Rasoul M.A.A. (2012a):** Cyromazine and Chlorpyrifos Induced Renal Toxicity in Rats: The Ameliorating Effects of Green Tea Extract. Journal Environmental Analytical Toxicology 2 (5): 146-152.
28. **Joshi CS, Mathur R, Gulati N.(2007):** Testicular toxicity of chlorpyrifos (an organophosphate Pesticide) in albino rats. Toxicol Ind Health. 2007;23(7):439–444.
29. **Eiben, Cs. Végi, B., Virág, Gy., GódorSurmann, K., Kustos, K., Maró, A., Odermatt, M., Zsédely, E., Tóth, T., Schmidt, J. and Fébel, H. (2011):** Effect of level and source of vitamin E addition of a diet enriched with sunflower and linseed oils on growth and slaughter traits of rabbits. Livestock Science, 139: 196-205.
30. **Marai I.F.M, El-Darawany A.A, AbouFandoud E.I, Abdel-Hafez, M.A.M. (2009):** Reproductive & physiological traits of Egyptian Suffolk rams as affected by selenium dietary supplementation during the subtropical environment of Egypt. Livestock Research for Rural Development. 21 (10): 102-124.
31. **Wolf, G. (2005).** The discovery of the antioxidant function of vitamin E: the contribution of Henry A. Mattill. The Journal of nutrition, 135(3), 363-366.
32. **Corino, C., Lo Fiego, D.,Macchioni, P., Pastorelli, G., Di Giancamillo, A., Domenghini, C. and Rossi, R. (2007):** Influence of dietary conjugated linoleic acids and Vitamin E on meat quality and adipose tissue in rabbits. Meat science, 76: 19- 28.
33. **HUGHES C.M.; LEWIS S.E.; MCKELVEY-MARTIN V. AND THOMPSON W.A. (1996):** comparison of baseline and induced DNA damage in human spermatozoa from fertile and infertile men, using a modified comet assay. Mol. Hum. Reprod., 2: 613-619.

34. **Gultekin, F., M. Ozturk, and M. Akdogan. (2000):** The effect of organophosphate insecticide chlorpyrifos-ethyl on lipid peroxidation and antioxidant enzymes (in vitro). Archives of Toxicology 74: 533–38.
35. **Kutlubay, R., Oguz, E.O., Can, B., Guven, M.C., Sinik, Z., Tuncay, O.L.,(2007):** Vitamin E protection from testicular damage caused by intraperitoneal aluminium. International Journal of Toxicology 26, 297-306.
36. **Meydani, M., (1995):** Vitamin E. Lancet 345, 170–175.
37. **Yue, D.B., Yan, L.Y., Luo, H.L., Jin, X.X., Xu, X., (2010):** Effect of Vitamin E supplementation on semen quality and the testicular cell membranal and mitochondrial antioxidant abilities in Aohan fine-wool sheep. Animal Reproduction Science 118, 217-222.
38. **Gurel, A., Coskun, O., Armutcu, F., Kanter, M., Ozen, O.A., (2005):** Vitamin E against oxidative damage caused by formaldehyde in frontal cortex and hippocampus: biochemical and histological studies. Journal of Chemistry and Neuroanatamy 29, 173-178.
39. **Agarwal, A., Prabakaran S.A., (2005):** Mechanism, measurement and prevention of oxidative stress in male reproductive physiology. Indian Journal of Experimental Biology 43, 963-974.
40. **Arabi, M., Seidaie, S.R., (2008):** Assessment of motility and membrane peroxidation of bull permatozoa in the presence of different concentration of vitamin C. Veterinary Medical Journal 2, 39-46.
41. **Arita, M., Sato, Y., Arai, H., Inoue, K., (1998):** Binding of atocopherylquinone, an oxidized form of a-tocopherol, to glutathione S-transferase in the liver cytosol. FEBS Letters 436, 424–426.
42. **Boldyrev, A.A., Bulygina, E.R., Volynskaya, E.A., Kurella, E.G., Tyulina, O.V.,(1995):** Effect of hydrogen peroxide and hypochlorite on the activity of Na, K-ATPase from brain. Biochemistry 60, 1293–1298.
43. **Hussien H, Abdoub H, Yousef M et al (2011)** :Cypermethrin induced damage in genomic DNA and histopathological changes in brain and haematotoxicity in rats: the protective effect of sesame oil. Brain Res Bull 87(5):402–419.
44. **Eaton DL, Daroff RB, Autrup H, et al.(2008):** Review of the toxicology of chlorpyrifos with an emphasis on human exposure and neurodevelopment. Crit Rev Toxicol 2008; 38: 1-125.
45. **Sharma P, Huq AU, Singh R (2014):** Cypermethrin-induced reproductive toxicity in the rat is prevented by resveratrol. J Hum Reprod Sci 7(2):99–106.
46. **Farag AT, Radwan AH, Sorour F, El Okazy A, El-Agamy ES, El-Sebae AE (2010)**: Chlorpyrifos induced reproductive toxicity in male mice. Reprod Toxicol 29(1):80–85.
47. **Akhtar N., Srivastava M.K. and Raizada R.B. (2009):** Assessment of chlorpyrifos toxicity on certain organs in rat, Rates norvegicus. Journal ofEnvironmental Biology 30 (6): 1047-1053.
48. **Uzunhisarcikli M, Kalender Y, Dirican K, Kalender S, Ogutcu A,Buyukkomurcu F (2007):** Acute, subacute and subchronic administration of methyl parathion-induced testicular damage in male .
49. **Heikal TM, Mossa AT, Ibrahim AW, Abdel-Hamid HF (2014):** Oxidative damage and reproductive toxicity associated with cyromazine and chlorpyrifos in male rats: the protective effects of green tea extract. Res J Environ Toxicol 8:53–67.
50. **Yousef, M.I., Abdallah, G.A., & Kamel, K.I. (2003):** effect of ascorbic acid and a vitamin A supplementation on semen quality and biochemical parameters of male rabbits. Animal Reprod. Sci., 20,76(1-2), 99-111.
51. **Hsu, P.C., Liu, M.Y., Hsu, C.C., Chen, L.Y., Guo, Y.L., (1998):** Effects of vitamin E and/or C on reactive oxygen species-related lead toxicity in the rat sperm. Toxicology 128 (3), 169–179.
52. **Geva, E., Bartoov, B., Zabludovsky, N., Lessing, J.B., Lerner-Geva, L., Amit, A., (1996):** The effect of antioxidant treatment on human spermatozoa and fertilization rate in an in vitro fertilization program. Fertility and Sterility 66, 430–434.
53. **Brzezinska-Slebodzinska, E., Slebodzinski, A.B., Pietras, B., Wieczorek, G.,(1995):** Antioxidant effect of vitamin E and glutathione on lipid peroxidation in boar semen plasma. Biology and Trace Element Research 47, 69-74..