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Comparative impact of selenium and Nano-selenium on cypermethrin induced toxicity in experimental rabbits

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ABSTRACT

The study's goal is to show comparative effects of selenium and Nano-selenium in reduction of the negative side effects of cypermethrin in male rabbits. Forty rabbits were divided into four groups: normal control (G1), groups (G2, G3, and G4) that were given cypermethrin (1/100 of LD50, equivalent to 24 mg/kg b. wt) three times per week via stomach tube.G3 and G4 received oral treatments with selenium and Nano-selenium at doses of 0.5 mg/kg b.wt. after two hours of cypermethrin for four weeks.
The administration of selenium and Nano-selenium (G3&G4) significantly reduced liver function enzymes and significantly increased growth, total protein (TP), albumin, and globulin levels compared to G2 levels, in addition to normalizing the lipid profile. Prominent downregulation of NO and pro-inflammatory cytokines (IL-6 and TNF-α) levels and upregulation of lysozyme were elicited in Nano-selenium (G4) treatment than selenium (G3). Selenium and Nano-selenium (G3 and G4) increase testosterone and estrogen levels and decrease LH and FSH compared to G2. Nano-selenium administration (G4) could restore hormone levels to normal (G1).As opposed to Nano- selenium, administration of selenium (G3) showed a lower level of cypermethrin residue in the liver, kidney, and muscle. According to those findings, Nano-selenium outperformed selenium in its capacity to reduce cypermethrin adverse effects.

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INTRODUCTION

Cypermethrin, a pyrethroid insecticide, is still often used in agriculture and animal husbandry. Despite having a wide margin of safety, many side effects have been noted in the published literature, including hepatorenal toxicity, DNA damage, and anaemia **(Khan et al. 2009; Sharaf et al. 2010; Ahmad et al. 2011; Mossa et al. 2018)**. Some pyrethroids or their metabolites may affect hormonal balance by having endocrine disruptive characteristics. Cypermethrin can also alter hormonal concentrations through a variety of target sites and processes including receptors, synthesis, or release of hormones (**Sun et al. 2007; Wojciechowska et al. 2016**).

Previous research has linked cypermethrin exposure to oxidative stress, inflammation, immunotoxicity, and apoptosis induction **(Afolabi et al. 2019; Ambwani et al. 2018)**. It has also been reported to induce immune suppression and inhibit neutrophil phagocytic activity in rats **(El Elaimy et al. 2013)**. Furthermore, chronic exposure to various doses of alpha cypermethrine has been shown to alter nonspecific humeral immunity and decrease lysozyme activity in various bird genotypes (**Yarkov and Sortirov 1995)**. Furthermore, cypermethrin was found to increase inflammatory damage by stimulating the pro-inflammatory cytokines interleukin 6 (IL-6) and tumor necrosis factor-alpha (TNF-α), as well as increasing NO levels **(Afolabi et al. 2019)**.

Selenium is essential for energy control, protein anabolism, oxidative stress, and other processes in the body to support healthy animal development and reproduction **(Liu et al. 2020)**. Recent researchers have found that selenium nanoparticles are safer and have higher catalytic efficiency, bioavailability, and absorbability than conventional selenium sources while being less dangerous. Because of their larger surface area and smaller particle size, nanoparticles outperform traditional particles in terms of intestine absorption and tissue deposition (**Boostani et al. 2015; Hosnedlova et al. 2018)**. Nano-selenium has the potential to be used as a chemopreventive agent with antifungal and antibacterial properties in addition to its use as a low toxicity antioxidant. Additionally, it stops metal intoxication. Furthermore, it was demonstrated that selenium has a nanoscale immunostimulatory impact **(Eid et al. 2019; Nabi et al. 2020)**.

The research aimed to investigate the side effects of cypermethrin and ameliorative effects of selenium and Nano-selenium on a multidisciplinary approach including growth, some reproductive hormone levels, liver function, lipid profile, innate immune mediators, and pro-inflammatory cytokines, in addition to tissue cypermethrin residue in rabbits.

MATERIALS AND METHODS

Materials:

Forty healthy New Zealand white male rabbits, weighted (1200 ± 200 g), were procured from a farm in Cairo. Cypermethrin (10%), Vetarin®, was gifted by Advanced Agrochemicals & Veterinary products Industrial (Chemvet), Jordan. Oral cypermethrin LD50 is 2400 mg/kg b. wt in rabbits. Selenium was obtained from Riedel- DE Haen AG Seelze Hannover while Nano-selenium (3 - 5 nm size) was obtained from Nanotechnology Unit, Animal Health Research Institute, Egypt. Cypermethrin standard was purchased from Sigma-Aldrich. HPLC grade methanol, acetonitrile, isoctan, and glacial acetic acid were obtained from Fisher Scientific. The Purified deionized water was prepared using Milli-Q system. Anhydrous magnesium sulfate (MgSO4) was purchased from Merck- Germany and anhydrous sodium acetate were obtained from BDH Chemicals-England, and anhydrous sodium sulfate (Na2SO4) was purchased from Elnasr Pharmaceutical Chemicals-Egypt. QuEChERS clean-up tubes had 50 mg of both primary and secondary amine exchange material, 150 mg MgSO4, and 50 mg C18 packing material (P.N. UCTCUMPSC18CT) were procured from Chromatographic Specialties-Canada.

Experimental Design:

After being observed for a week, rabbits were allocated equally into four groups (n = 10). The first group (G1) was given normal saline as a control, while the other groups (G2,

G3, and G4) were given 1/100 of LD50 cypermethrin (three times per week) by stomach tube for 28 days **(Anwar et al. 2020)**. G3 and G4 received oral treatments with selenium and Nano-selenium at doses of 0.5 mg/kg b.w.t. after two hours of cypermethrin, according to **Szulc-Musiol et al. (2004)** and **Gouda et al. (2021)**. Rabbits were given unlimited access to food and drink **(NRC 1977)**. Throughout the study, rabbits were weighed weekly, daily weight gain was recorded, and any clinical symptoms were noted. At the end of the four-week experiment, rabbits were sacrificed to obtain blood and organs. The experimental procedure was carried out at AHRI in conformity with the ARC and IACUC committee (ARC, IACUC, 22/32).

Laboratory methods:

According to **Anderson and Cockayne (1993)**, serum aminotransferase (AST& ALT), alkaline phosphatase (ALP) enzymes, total proteins (TP), albumin, globulin, total cholesterol, low and high-density lipoprotein cholesterol (LDLc & HDLc), and triglyceride were estimated. Additionally, according to **Evans (2009)**, Quantitative determination of serum hormones levels using ELISA kit, testosterone Catalog No. ABIA Dk 040 013; DBC, Canada; estrogen, Estradiol BIOS.10009, lutenizing hormone (LH) and follicle-stimulating hormone (FSH) Monobind Lake Forest CA 92630, USA, with sensitivity 0.2 ng/ml; 5.0 pg/ml, 0.1 ng/ml and 0.1ng/ml respectively. Lysozyme levels were determined using the **Peeters and Vantrapen (1977)** method on agarose gel plate lyses, and serum nitric oxide levels were determined using the **Yang et al. (2010)** method.

Determination of mRNA IL-6 and TNF-α in liver tissue by quantitative real time-PCR (qRT -PCR) were achieved in Biotechnology Unite, Animal Health Research Institute. RNA extraction and purification from liver samples were applied using QIA amp RNeasy Mini kit (Qiagen, Germany, GmbH). IL-6 and TNF-α oligonucleotide primers (Metabion, Germany) were clarified in table 1. The SYBR green qRT -PCR reaction was achieved in a Stratagene MX3005P real-time PCR machine and analysis of RT-PCR results were determined by using the amplification curves and threshold cycles (CT) values (Stratagene MX3005P software). To evaluate differences of fold changes in mRNA gene expression of the diverse samples, the CT of each sample was matched with cypermethrin group sample rendering to the "$2^{\Delta\Delta Ct}$" method as reported by **Yuan et al. (2006)**.

Table 1. qRT-PCR Primer sequences

Target gene	Primers sequences	Reference
GAPDH*	TGACGACATCAAGAAGGTGGTG	**Schnupf and Sansonetti (2012)**
	GAAGGTGGAGGAGTGGGTGTC	
IL-6	CTACCGCTTTCCCCACTTCAG	
	TCCTCAGCTCCTTGATGGTCTC	
TNF-α	GTCTTCCTCTCTCACGCACC	**Godornes et al. (2007)**
	TGGGCTAGAGGCTTGTCACT	

GAPDH* (Glyceraldehyde 3-phosphate dehydrogenase) served as an internal control for sample normalization

Cypermethrin residue in liver, kidney, and muscle was estimated by HPLC system (Agilent 1200 series, Software – Agilent Chemi-station) (Agilent Technologies, Germany), with a pump, degasser, autosampler, DAD detector, and Agilent C18, 100 A ,4.6 × 250 mm, 5 µm Chromatographic column as stationary phase was used. The chromatographic condition was set according to **Supraja et al. (2022)**. Standard Preparation was performed by a stock solution in isoctan that used for an intermediate solution (100 mg/L preparation. Working standards was prepared by using the inter-mediate solution in blank, liver, kidneys and muscle (normal control group) at a range of 10 to 10000 µg/L of cypermethrin. The standard curve was a good linearity with ($r^2 \geq$ 0.999).

Samples extraction was performed by using a QuEChERS method for the analysis of pyrethrins according to the procedure described by **Rawn et al. (2010)** and validated according to **USP (2021**) via the determination of method precision, recovery, linearity, the limit of detection (LOD), and quantification (LOQ). HPLC method was accurate with high recovery (95- 99%) with a low LOD and LOQ; as LOD was 0.17 mg/kg and LOQ was 0.52mg/kg. Specificity and selectivity were demonstrated **(**Fig.1) with a retention time of 7.0 minutes for cypermethrin. Liver, kidney, and muscle known to be free of cypermethrin was run as normal control within each set of samples analyzed. Moreover, each set contained a reagent blank consisting of distilled water and reagents; Preparation of quality control samples at 3 levels was conducted according to **USP (2021)** for cypermethrin. The working standards and Quality control sample were extracted as mentioned below.

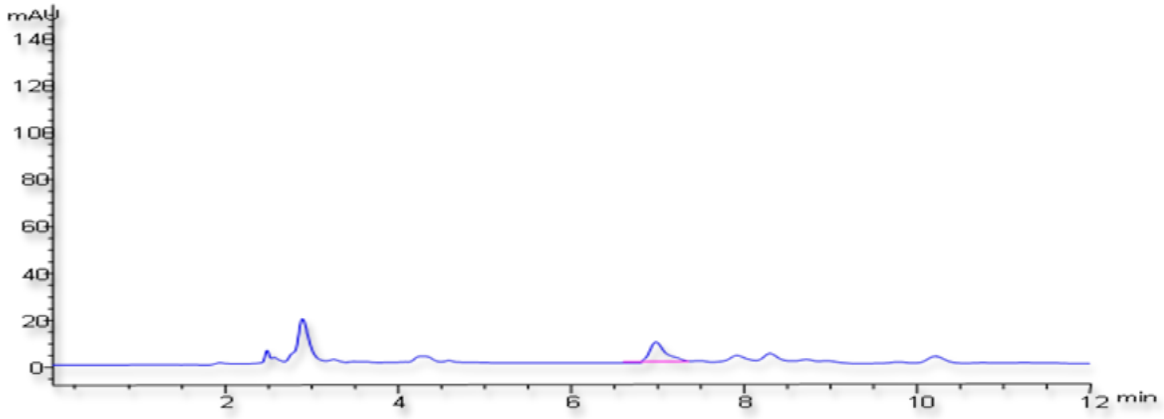

Fig (1): Chromatogram of cypermethrin showed retention time 7.0 minutes in tissue sample (2.54 mg/kg of hepatic tissue)

Statistical analysis:

Obtained data was exposed to analysis of variance (one-way ANOVA test) by IBM SPSS 23 Statistics for Mac OS, Armonk, NY, USA. The differences were considered significant when $p \leq 0.05$. All the data are stated as mean ± SD.

RESULTS:

It has been noted in Table 2 that oral administration of cypermethrin (G2) decreased final weight, weight gain, and average food intake, and subsequently feed efficiency ratio, compared to the normal control group (G1). When compared to the cypermethrin group (G2), the administration of selenium and Nano-selenium improved nutritional parameters such as

weight gain, average food intake, and feed efficiency ratio. Administration of Nano- selenium (G4) showed better effects in the above-mentioned parameters than selenium administration (G3).

Table 2. Nutritional indicators of experimental rabbit groups

	G1	G2	G3	G4
Initial weight(g)	1735±33.11^{a}	1733.22±39.22^{a}	159.01±40.17^{a}	1655.50±38.46^{a}
Final weight(g)	2545.22±78.11^{a}	1843.33±70.14^{cd}	1908.55±59.11^{c}	2090.44±80.33^{ab}
Weight gain (g)	810.22±20.77^{a}	110.11±8.22^{d}	312.55±15.20^{c}	434.94±19.18^{b}
Food intake(g)	280.33±20.42^{a}	178.82±17.71^{b}	175.22±18.11^{b}	187.29±16.15^{bc}
Feed efficiency ratio	0.1032±0.0331^{a}	0.0219±0.0017^{d}	0.0637±0.0021^{c}	0.0829±0.0220^{b}

The different alphabetical letters in each raw were significantly (P≤ 0.05)

Liver enzyme parameters (ALT, AST, and ALP) of cypermethrin-intoxicated rabbits (G2) were significantly elevated compared to a normal control group (G1). Administration of selenium and Nano-selenium (G3 & G4) significantly decreased these enzymes compared to the levels of G2 and showed a non-significant difference compared to the normal control (G1). Administration of cypermethrin significantly decreased total protein (TP), albumin plus globulin in G2 compared to G1. Supplementation of selenium and Nano-selenium with cypermethrin showed a significant increase in these parameters compared to G2 and achieved normal levels as in G1 as illustrated in table (3).

Table 3. Liver function parameters in experimental rabbit groups

	G1	G2	G3	G4
ALT (U/L)	58.68 ± 5.50^{bd}	84.31±11.47 ^{a}	59.70 ± 4.50^{bc}	60.99 ±6.76^{b}
AST (U/L)	41.81±4.985 ^{bd}	78.31± 7.41 ^{a}	43.49± 4.848^{b}	42.09 ±4.76^{bc}
ALP (IU)	210.74±25.49^{bd}	358.51±45.84^{a}	219.13±22.49^{bc}	222.81±29.66^{b}
T. Protein (g/dl)	6.33 ±1.32 ^{a}	4.27 ± 0.30^{c}	5.78 ±0.66 ^{ab}	6.09 ± 0.331^{ab}
Albumin (g/dl)	3.09 ±0.33^{a}	2.51± 0.12^{c}	2.98 ± 0.34 ^{ab}	2.91± 0.28a^{b}
Globulin (g/dl)	3.15± 0.53^{a}	1.755 ± 0.15^{c}	2.8±0.21 ^{ab}	3.17 ±0.40 ^{a}

The different alphabetical letters in each raw were significantly (P≤ 0.05)

Following a 28-day cypermethrin treatment, the G2 had substantially higher levels of serum NO, mRNA of IL-6, and TNF- α (P≤0.05) than the other groups. In both groups (G3 & G4), supplementation with selenium and Nano-selenium significantly decreased NO levels to reach close to the extent of a normal value, especially in the G4, in addition to a significantly greater decline in the mRNA of IL-6 and TNF-α levels than the cypermethrin control group (G2). Nano-selenium administration showed more down-regulation of IL-6 and TNF-α cytokines (G4) than selenium (G3). Serum lysozyme levels significantly declined in G2 compared to other groups. However, supplementation with selenium and Nano-selenium in both groups (G3 & G4) significantly increased lysozyme levels above G2 levels and brought them close to normal. Nano-selenium in G4 triggered more upregulation of lysozyme levels than selenium in G3, where its values were insignificantly different from those of the normal values, as shown in table (4) and figure (2).

Table 4. Serum nitric oxide and lysozyme levels in experimental rabbit groups

	G1	G2	G3	G4
NO (µmol/ml)	8.71 ± 0.17 ^{c}	33.59 ± 2.08^{a}	21.82 ± 0.44 ^{b}	14.68 ± 0.39^{c}
Lysozyme(µg/ml)	100.37 ± 4.77 ^{a}	40.67 ± 4.95 ^{c}	85.0 ± 7.01 ^{b}	110.5 ± 5.02 ^{a}

The different alphabetical letters in each raw were significantly (P≤ 0.05)

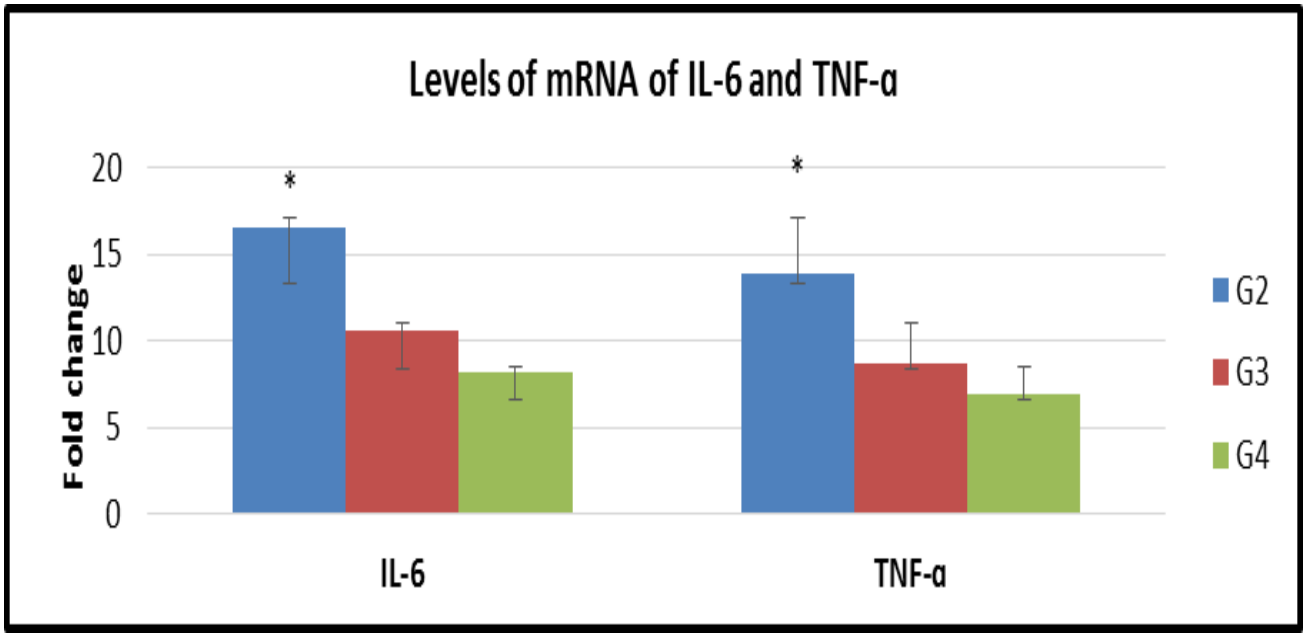

Fig. (2): Quantitative real-time PCR for mRNA expression of IL-6 and TNF-α genes in rabbit liver
$2^{-\Delta\Delta Ct}$ method is used to calculate the relative expression fold change.
Column with an asterisk indicates significantly different than the other column within the same cytokine.

Table (5) revealed that oral administration of cypermethrin in G2 induced a significant rise in serum total cholesterol, TG, and LDL-c and a significant decrease in HDL-c compared to G1. Moreover, lipid profile parameters were enhanced by a decline in total cholesterol, TG, and LDL-c and a significant increase in HDL-c by selenium and Nano-selenium in G3 and G4 compared to G2 but without reaching normal levels in G1.

Table 5. Lipid parameters in experimental rabbit groups

	G1	G2	G3	G4
T-Cholesterol (mg/dl)	74.23 ± 6.66^{c}	104.22 ±11.95a	82.10 ±8.52^{b}	83.40 ±7.61^{b}
TG (mg/dl)	90.20 ±9.56^{c}	133. 11±13.85^{a}	114.33 ±14.01^{b}	112.30±12.93^{b}
HDL-c (mg/dl)	27. 11± 2.79^{a}	20.7± 3.17^{d}	22.20 ± 2.82^{bc}	21.9 ± 3.56^{c}
LDL-c (mg/dl)	29.08±4.021^{d}	56.90± 5.77^{a}	37.04± 3.60^{bc}	39.04 ±3.29^{b}

The different alphabetical letters in each raw were significantly (P≤ 0.05)

The cypermethrin control rabbit group (G2) had lower testosterone and estrogen levels and higher LH and FSH levels than the normal control group (G1). On the other side, administration of selenium and Nano-selenium (G3&G4) could ameliorate these hormones, as represented by increased testosterone and estrogen levels and decreased levels of LH and FSH compared with G2. Nano-selenium administration (G4) could maintain the same levels as normal control (G1), as illustrated in table 6.

Table 6. Reproductive hormones in experimental rabbit groups

	G1	G2	G3	G4
Testosterone(ng/ml)	1.73±0.07^{c}	0.819 ±0.01^{d}	2.27±0.14^{b}	4.84±0.91^{a}
Estrogen (Pg/ml(	57.59±2.78^{a}	38.41±3.01^{d}	49.89±3.436^{bc}	54.62 ±3.30^{ab}
LH(ng/ml)	1.559±0.09^{cd}	3.18 ±0.04^{a}	2.178±0.04^{b}	1.77 ±0.04^{c}
FSH(ng/ml)	7.281±1.06^{cd}	15.62±3.12^{a}	10.65±2.08^{b}	8.22±1.06^{c}

The different alphabetical letters in each raw were significantly (P≤ 0.05)

According to the data in Table 7, the muscles of the experimental rabbit groups were the ideal location for cypermethrin residue accumulation. Group 2 had the highest cypermethrin residue levels in the liver, kidney, and muscle, while administration of selenium (G3) and Nano-Selenium (G4) both reduced cypermethrin residues. Administration of selenium (G3) showed lower levels of cypermethrin residue in the examined organs and muscles compared to the values of G4.

Table 7. Liver, kidney, and muscle cypermethrin residues in experimental rabbit groups

	G2	G3	G4
Liver (µg/g)	2.54 ±0.16[a]	1.30±0.13[c]	1.78±0.18[b]
Kidney (µg/g)	4.80±0.27[a]	1.35±0.10[c]	1.82±0.24[b]
Muscle (µg/g)	4.82±0.26[a]	1.58±0.16[c]	2.00±0.23[b]

The different alphabetical letters in each raw were significantly (P≤ 0.05)

DISCUSSION:

The body weight fall of the cypermethrin-treated group might be attributed to pesticide effects on the gastrointestinal tract manifested by appetite reduction and nutrient absorption from the stomach **(Sangha et al. 2013**; **Mahdi et al. 2016)**. No signs of cypermethrin toxicity (salivation, increased startle response, splayed gait, ataxia, and tremors or convulsions as signs of central nervous system toxicity) were observed during the whole period of our experiment, such symptoms were observed upon administration of lethal dose levels or prolonged repeated doses of more than 3 months **(EMEA 2001)**. Cypermethrin has cholinergic effects (lower feed intake and induced diarrhoea), oxidative stress, and an increase in lipid and protein breakdown. The reduced appetite and growth rate are resulting from alteration in the mucosal layer and epithelial cells of the digestive system **(Adjrah et al. 2013, El-Sheshtawy et al. 2019)**. Selenium administration improved nutritional parameters of cypermethrin-induced toxicity in rabbits because of the antioxidant effects that prohibited the free radicals and pathogens damaging influence, protected the intestinal mucosa, and enhanced the absorptive capacity by diminishing the movement of the intestine **(Marounek et al. 2009**). Because of their tiny particle size plus vast surface area, greater mucosal permeability, better intestinal absorption, and up-regulated activity of seleno-enzymes compared to selenite, nanoparticles were able to accomplish their performance benefits. **El-Kazaz et al. (2020)** concurred with **Mohamed et al. (2016) and Emara et al. (2019)** regarding the influence of Nano-selenium findings.

Because the liver is in charge of pesticide metabolism, Cypermethrin causes oxidative stress, resulting in hepatocyte inflammation and damage, disruption of the outflow of examined enzymes (ALT, AST, and ALP) from hepatocytes to the blood circulation, as well as a reduction in total protein, albumin, and globulin levels, all of which may indicate liver damage **(Ahmad et al. 2011; Kanbur et al. 2015; El-Sheshtawy et al. 2019)**. Pyrethroids block the formation of adenine triphosphate by inhibiting of cell's mitochondrial complex and oxygen consumption. The malfunction of the Na+/K+ pumps results in the transfer of water and sodium into cell cytosol and disturbs protein synthesis, which leads to hypoproteinemia. Also, pyrethroids may degrade proteins as an effect of oxidative stress **(Gassner et al. 1997; Khan et al. 2009)**. Pesticide toxicity has a negative effect on the process of globulin synthesis by disrupting the endoplasmic reticulum in plasma cells. It has been pro-

posed that the endoplasmic reticulum stores calcium via the Ca^{2+} pump before releasing it via cyclic adenosine diphosphate ribose or inositol 1, 4, 5-trisphosphate **(He et al. 2006)**. The primary contribution of selenium and Nano-selenium is the development of antioxidant defense systems against oxidative stress and DNA damage, as well as apoptosis. Because Nano-selenium can reduce cypermethrin-induced oxidative stress by reducing free radical production, it can preserve the integrity of hepatocytes or promote the regeneration of injured hepatocytes. Liver enzymes, total protein, albumin, and globulin levels all returned to normal after selenium and Nano-selenium were given together with cypermethrin. Nano-selenium was extra effective than sodium selenite in enhancing liver antioxidant action in rabbits **(Zhu et al. 2010; Zachara 2015; Qin et al. 2016**; **Rashid et al. 2023)**.

NO, a pro-inflammatory cytotoxic mediator, regulates a variety of immunological and physiological processes **(Brennan et al. 2002)**. The increased NO level in G2 was consistent with the results of **Wang et al. (2009**) who mentioned that administration of either moderate or high doses of cypermethrin induced an increase in the NO level of male mice testes. Excessive quantities of NO induced by cypermethrin can be detrimental as it has the potential to generate harmful reactive nitrogen species that cause cellular oxidative stress **(Brennan et al. 2002; Ambwani et al. 2018)**.

Pro-inflammatory cytokines (IL-6 and TNF - α) are effective in regulating immune response, inflammation, and endothelial function. The increased production of these cytokines in G 2 was agreed with **Afolabi et al. (2019**), who found that, the oral administration of a sub-lethal dose of cypermethrin increased the expression of the examined cytokines in rats, demonstrating cypermethrin ability to initiate an inflammatory response. This result may be attributed to the increased NO production that in turn triggers the redox-sensitive transcription factors, such as NF-κB which is responsible for the activation of TNF-α, IL-6, and iNOS genes **(Forrester et al. 2018)**, which may explain the induced higher mRNA of IL-6 and TNF-α as well as the serum NO in G2. The decreased lysozyme level in G2 may be attributed to the immuno-toxic effect of cypermethrin on the viability of immune cells including macrophages and neutrophils **(Huang et al. 2016)**.

However, the treatment with selenium and its nano form can ameliorate the adverse immunotoxic and inflammatory effects of cypermethrin as found in G3 and G4. The downregulation of NO, IL-6, and TNF-α in these groups was agreed upon by **Rupil et al. (2012) and Abdou and Sayed (2019)**. Selenium may have an anti-inflammatory impact via down-regulating the p38 mitogen-activated protein kinase and NF- Kb signaling pathways, which in turn suppress the expression of the examined cytokine genes and reduce the inflammatory response **(Morris et al. 2003; Kim et al. 2004)**.

Moreover, the upregulation of lysozyme by selenium compounds (G3 and G4) was consistent with **Sheiha et al. (2020),** who found that Nano-selenium supplementation significantly amplified serum lysozyme activity and increased the innate immunity of heat-stressed rabbits. This enhanced lysozyme level may arise because of the activating effect of selenium on the immune cells and its lysozyme production. Selenium is found in the structure of a number of selenoenzymes, has antioxidant and immunomodulatory characteristics, and can shield proteins, DNA, and chromosomes from oxidative effects of free radicals **(Li et al. 2011)**.

Interestingly, the greater absorption and bioavailability of Nano-selenium may account for the greater immunological and inflammatory regulatory effects **(Saffari et al. 2018)** as shown in Nano-selenium G4 compared to selenium (G3).

Results of the current investigation of hypercholesterolemia in cypermethrin-administered groups were consistent with those of **Yousef et al. (2003), Dahamna et al. (2009), and Boumezrag et al. (2021)**, indicating an inhibitory impact of cypermethrin on the he-

patic CP450 enzymes and alteration of hepatic cell membrane permeability, in addition to cypermethrin accumulation in the liver, which disrupts lipid metabolism and increases cholesterol level.

Pyrethroids have the potential to damage the mitochondrial membranes of Leydig cells, which would interfere with testosterone biosynthesis by decreasing cholesterol delivered to the mitochondria and slowing down the conversion rate of cholesterol to pregnenolone in cells. This would then result in a decrease in testosterone production. Pyrethroids are also capable of interfering with estrogen signaling, affecting male reproductive organs, and altering the quality of semen **(Kilian et al. 2007**; **Sharma et al. 2018)**. Furthermore, Cypermethrin had a toxic effect on the rabbit reproductive system, as evidenced by changes in endocrine hormones as a result of direct impact on testes androgen biosynthesis pathways, or gonadotropin levels changes as a result of Leydig cell damage or direct influence on 3ß-hydroxysteroid dehydrogenase (3ß-HSD) enzyme gene expression that converts pregnenolone to progesterone. Reduced activities of the steroidogenic enzymes 3- and 17-hydroxysteroid dehydrogenases alter the production of testosterone. The decline of testosterone is followed by rises in LH and FSH levels after cypermethrin administration **(Jin et al. 2011; Hu et al. 2013; Ikpeme et al. 2016)**.

As is well-known, the withdrawal period is the amount of time that must pass between the last administration of veterinary medicine and the slaughter or production of food from that animal to ensure that the food does not contain levels of the medication that exceed the maximum residue limit. In the case of the current study, the repeated oral administration of cypermethrin dramatically increased the levels of liver, kidney, and muscles because of its lipophilic nature and rapid absorption after oral administration **(EMEA 2001)**. Our findings contradict those of **Nekovi et al. (2013)**, who claimed that cypermethrin did not accumulate in organs and tissues after administrated to hens for 28 days at nominal doses of 150, 300, and 600 ppm. The discrepancies in species, doses, delivery intervals, and chemical makeup of the utilized cypermethrin may be to blame for the discrepancy between the results. It was demonstrated that using selenium and Nano-selenium (G3 & G4) had a favorable impact on cypermethrin residues, with selenium having a preference over Nano-selenium giving indication the usage of such medications may shorten the withdrawal period and accelerate the fall of residue concentration below the maximum residue limit (MRL) of cypermethrin. The most notable decrease in cypermethrin residue in the liver, kidney, and muscles in G3&G4 following selenium treatment is linked to efficient detoxification processes, such as rapid cypermethrin metabolism and elimination **(Imura and Naganuma 1991; EMEA 2001; Fang et al. 2022)**.

CONCLUSION:

The present study demonstrated that cypermethrin-induced toxic effects mainly alter reproductive hormones, biochemical indices, lipid patterns, innate immune mediators, and pro-inflammatory cytokines. Administration of Nano-selenium showed a better effect than selenium in ameliorating growth, biochemical, immunological, and inflammatory parameters. On the other side, selenium had the ability to lower cypermethrin residue in the liver, kidney, and muscle more than Nano-selenium. Thus, we advise administering Nano -selenium to breed rabbits exposed to cypermethrin toxicity, whereas selenium is much better to provide to fatten rabbits exposed to cypermethrin toxicity. More researches are necessary to ascertain the precise selenium mechanism action and to identify cypermethrin withdrawal period when selenium and Nano-selenium are supplied in such circumstances.

REFERENCES:

Abdou RH, Sayed N. 2019. Antioxidant and anti-inflammatory effects of Nano-selenium against cypermethrin-induced liver toxicity. Cell Bio., 8 (04): 53-65.

Adjrah Y, Karou SD, Agbonon A, Ameyapoh Y, de Souza C, Gbeassor M. 2013. Effect of cypermethrin-treated lettuce (Lactuca sativa)

on wistar rat liver. *J of App Pharm Sci.*, *3* (1), 128-132.

Afolabi OK, Aderibigbe FA, Folarin AA, Wusu AD .2019. Oxidative stress and inflammation following sub-lethal oral exposure of cypermethrin in rats: mitigating potential of epicathchin. Heliyon, 5(8): e02274.

Ahmad L, Khan A, Khan MZ. 2011. Cypermethrin Induced Biochemical and Hepatorenal Pathological Changes in Rabbits. 13 (60): 865-872.

Ambwani S, Ambwani TK, Singh R. 2018. Immunotoxic effects of cypermethrin in mitogen stimulated chicken lymphocytes due to oxidative stress and apoptosis. J Entomol Zool Stud., 6(2): 37-42.

Anderson SC, Cockayne S. 1993. Clinical Chemistry: Concepts and applications. Philadelphia, W.B. Saunders Company.pp:248-264.7.

Anwar M, Muhammad F, Akhtar B, Ur Rehman S, Saleemi MK. 2020. Nephroprotective effects of curcumin loaded chitosan nanoparticles in cypermethrin induced renal toxicity in rabbits. Environ Sci Pollut Res Int., 27(13):14771-14779.

Boostani A, Sadeghi A, Mousavi S, Chamani M, Kashan NE. 2015. Effects of organic, inorganic, and nano-Se on growth performance, antioxidant capacity, cellular and humoral immune responses in broiler chickens exposed to oxidative stress. Livest Sci., (178):330–6.

Boumezrag A, Hemida H, Boumezrag FA, Smail F, Cisse S. 2021. Pathological and biological effects of treatments with lambda -cyhalothrin in rabbits. Iraqi J Vet Sci., 35 (3): 443-450.

Brennan ML, Wu W, Fu X, Shen Z, Song W, Frost H, Hazen SL. 2002. A tale of two controversies: defining both the role of peroxidases in nitrotyrosine formation in vivo using eosinophil peroxidase and myeloperoxidase-deficient mice, and the nature of peroxidase-generated reactive nitrogen species. J Biol Chem., 277(20): 17415-17427.

Dahamna S, Harzallah D, Guemache A, Sekfali N. 2009. Biochemical investigation of cypermethrin toxicity in rabbits. Commun Agric. Appl Biol Sci., 74(1): 149-153.

Eid SY, El-Zaher HM, Emara SS, Farid OA, Michael MI. 2019. Nano selenium treatment effects on thyroid hormones, immunity and antioxidant status in rabbits. World Rabbit Sci., 27(2): 93-100.

El Elaimy IA, Bayomy MF. Salem TA . 2013. Immune-Toxicity of Synthetic Pyrethroids: Modulation of Cypermethrin and Permethrin Toxicity by Tamoxifen. Delta J Sci., 36(3): 123-138.

El-Kazaz SE, Abo-Samaha MI, Hafez MH, El-Shobokshy SA, Wirtu G. 2020. Dietary supplementation of nano-selenium improves reproductive performance, sexual behavior and deposition of selenium in the testis and ovary of Japanese quail. J Adv Vet Anim Res., 7(4):597–607.

El-Sheshtawy SM, El-Gobary GI, Omar NA, Shawky NA. 2019. Ameliorating the toxic effects of cypermethrin by sesame oil in male rabbits. Slovenian Vet Res., 56 (22): 51–9.

Emara SS, El-Zaher HM, Michae MI, Eid SY. 2019. Comparative effects of nano-selenium and sodium selenite supplementation on blood biochemical changes in relation to growth performance of growing New Zealand White rabbits. Arab J Nucl Sci Appl., 52(4):1–114.

EMEA. 2001, Cypermethrin - Summary Report. The European Agency for the Evaluation of Medical Products, Veterinary Medicines and Inspections, Committee for Veterinary Medical Products, MRL/801/01-Final

Evans GO. 2009. Animal clinical chemistry: A practical Guide for Toxicologists and Biomedical Researchers (2nded) CRC Taylor and Francis group, Boca Raton, London, New York .pp.17-66.

Fang M, Hu W, Liu B. 2022 .Protective and detoxifying effects conferred by selenium against mycotoxins and livestock viruses: A

review. Front Vet Sci. 2(9):956814.

Forrester S J, Kikuchi DS, Hernandes MS, Xu Q, Griendling KK. 2018. Reactive oxygen species in metabolic and inflammatory signaling. Circulation Res., 122(6): 877-902.

Gassner B, Wüthrich A, Scholtysik G, Solioz M. 1997. The pyrethroids permethrin and cyhalothrin are potent inhibitors of the mitochondrial complex I. J. Pharmacol. Exp. Therapeut., 281(1): 855–860.

Godornes C, Leader BT, Molini BJ, Centurion-Lara A. Lukehart SA. 2007. Quantitation of rabbit cytokine mRNA by Real-Time RT-PCR. Cytokine, 38(1): 1–7.

Gouda N, El Kelawy H, Abd El Rahim M, El-Gaafary N, Ibrahim H. 2021. Effect of treatment with nano-Se and vitamin E on semen quality and some blood parameters of buck rabbits. J. Product. & Dev., 26(4): 903-922.

He J, Chen J, Liu R, Song L, Chang HC, Wang X .2006. Fenvalerate induced alterations in calcium homeostasis in rat ovary. Biomed and Environ Sci., 19(1): 15.

Hosnedlova B, Kepinska M, Skalickova S, Fernandez C, Ruttkay-Nedecky B, Peng Q, Kizek, R. 2018. Nano-selenium and its nanomedicine applications: a critical review. Int J Nanomed., (13):2107.

Hu JX, Li YF, Li J, Pan C, He Z, Dong HY, Xu L C. 2013. Toxic effects of cypermethrin on the male reproductive system: with emphasis on the androgen receptor. J Appl Toxicol., 33(7): 576-585.

Huang F, Liu Q, Xie S, Xu J, Huang B, Wu Y, Xia D. 2016. Cypermethrin induces macrophages death through cell cycle arrest and oxidative stress-mediated JNK/ERK signaling regulated apoptosis. Intern J of Mol Sci., 17(6): 885.

Ikpeme EV, Okono LE, Udensi OU. 2016. Detrimental effect of chlorpyrifos and cypermethrin on reproductive physiology of male albino rats. Res J Environ Toxicol., 10 (1): 68-7.

Imura N, Naganuma A. 1991. Possible Mechanism of Detoxifying Effect of Selenium on the Toxicity of Mercury Compounds. In: Suzuki, T., Imura, N., Clarkson, T.W. (eds) Advances in Mercury Toxicology. Rochester Series on Environmental Toxicity. Springer, Boston, MA. https://doi.org/10.1007/978-1-4757-9071-9_17

Jin Y, Wang L, Ruan M, Liu J, Yang Y, Zhou C, Fu Z. 2011. Cypermethrin exposure during puberty induces oxidative stress and endocrine disruption in male mice. Chemosphere, 84(1): 124-130.

Kanbur M, Eraslan G, Ince S, Altintas L, Liman BC, Bayram L. 2015. The effects of propetamphos, cypermethrin and propetamphos-cypermethrin combination on some biochemical and histopathological parameters in mice. Kafkas Üniversitesi Veteriner Fakültesi Dergisi, 21(2): 187-194.

Khan A, Faridi HA, Ali M, Khan MZ, Siddique M., Hussain I, Ahmad M. 2009. Effects of cypermethrin on some clinico-hemato-biochemical and pathological parameters in male dwarf goats (Capra hircus). *Exp Toxicol Pathol.*, 61(2): 151-160.

Kilian E, Delport R, Bornman M S, Jager CD. 2007. Simultaneous exposure to low concentrations of dichlorodiphenyl trichloroethane, deltamethrin, nonylphenol and phytoestrogens has negative effects on the reproductive parameters in male Spraque-Dawley rats. Andrologia, 39(4): 128-135.

Kim SH, Johnson V, Shin YT, Sharma P. 2004. Selenium attenuates lipopolysaccharideinduced oxidative stress responses through modulation of p38 MAPK and NF-kappa B signaling pathways. Exp Biol Med. 229(2):203–213.

Li X, Xu H, Chen ZS, Chen G. 2011. Biosynthesis of nanoparticles by microorganisms and their applications. J. Nanomater., 2011 (8): 1-16.

Liu YJ, Zhang ZD, Dai SH., Wang Y, Tian XF, Zhao JH, Huo WJ. 2020. Effects of sodium selenite and coated sodium selenite addition on performance, ruminal fermentation, nutrient digestibility and hepatic gene ex-

pression related to lipid metabolism in dairy bulls. Livestock Sci., 237 (2020):104062.

Mahdi YS, Falih IB, Zaid NY. 2016. Toxicological effects of cypermethrin on sperm morphology in male rabbit. Int J Adv Res Biol Sci.,3(10),46-51.

Marounek M, Dokoupilová A, Volek Z, Hoza I. 2009.Quality of meat and selenium content in tissues of rabbits fed diets supplemented with sodium selenite, selenized yeast and selenized algae. World Rabbit Sci. ,(17):207 – 12.

Mohamed AH, Abd-ElGhany TF, Mahgoup AS. 2016. Influence of supplementation of some trace minerals in form of nanoparticles on productive and reproductive performance of rabbit. Egypt J Nut and Feeds. 19(2):325–35.

Morris KR, Lutz RD, Choi HS, Kamitani T, Chumura K. Chan ED. 2003. Role of the NF -kappaB signaling pathway and kappaB cis-regulatory elements on the IRF-1 and iNOS promoter regions in mycobacterial lipoarabinomannan induction of nitric oxide. Infect Immun., (71):1442-1452.

Mossa AT, Mohafrash SM, Chandrasekaran N. 2018. Safety of natural insecticides: Toxic effects on experimental animals. Biomed. Res. Int. 2018, 4308054.

Nabi F, Arain M A, Hassan F, Umar M, Rajput N, Alagawany M, Liu J. 2020. Nutraceutical role of selenium nanoparticles in poultry nutrition: a review. Worlds Poult Sci J., 76(3): 459-471.

Nešković N, Gašić S, Brkić D, Pavlovski Z, Cmiljanić R. 2013. Effects of dietary cypermethrin on chickens. Acta veterinaria, 63(2-3), 325-335.

NRC 1977.Nutrient Requirement of Rabbits. Committee on Animal Nutrition. National Academy of Sciences, Second Edition, National Research Council, Washington, DC., USA.

Peeters T L, Vantrapen GR. 1977. Factors influencing lysozyme determination by lysoplate method. Clin Chim Acta. 74(3):217 –55.

Qin S, Chen F, Zhao F, Jin T, Ma J. 2016. Effects of Nano-selenium on Blood Biochemistry, Liver Antioxidant Activity and GPx-1 mRNA Expression in Rabbits. In International Conf on Biomed and Biolog Eng., 166-171. Atlantis Press.

Rashid U, Qureshi IZ, Jan S, Khalid T, Khan DA. 2023. Protective effects of selenium against cypermethrin induced hepatorenal damage in Sprague Dawley rats. World J Biol Pharm and Health Sci., 22(01): 284–298.

Rawn DF, Judge J, Roscoe V. 2010. Application of the QuEChERS method for the analysis of pyrethrins and pyrethroids in fish tissues. Anal Bioanal Chem., 397(6):2525-31.

Rupil LL, de Bem AF, Roth GA. 2012. Diphenil diselenide-modulation of macrophage activation: downregulation of classical and alternative activation markers. Innate Immun., 18(4): 627-637.

Saffari S, Keyvanshokooh S, Zakeri M, Johari SA, Pasha-Zanoosi H, Mozanzadeh MT. 2018. Effects of dietary organic, inorganic, and nanoparticulate selenium sources on growth, hemato-immunological, and serum biochemical parameters of common carp (Cyprinus carpio). Fish Physiol and Biochem, (44): 1087-1097.

Sangha GK, Kaur K, Khera KS. 2013. Cypermethrin induced pathological and biochemical changes in reproductive organs of female rats. *J Enviro Biol*., *34*(1): 99-105.

Schnupf P, Sansonetti PJ. 2012. Quantitative RT-PCR profiling of the Rabbit Immune Response: Assessment of Acute Shigella flexneri Infection. PLoS One, 7(6):e36446.

Sharaf S, Khan A, Khan MZ, Aslam F, Saleemi MK, Mahmood F. 2010. Clinico-hematological and micronuclear changes induced by cypermethrin in broiler chicks: Their attenuation with vitamin E and selenium. *Exp. Toxicol. Pathol*. , 62(4): 333-341.

Sharma A, Yadav B, Rohatgi S, Yadav, B. 2018. Cypermethrin toxicity: A review. J Forensic Sci Crim Investig., 9(4): 555767.

Sheiha AM, Abdelnour AS, Abd El-Hack ME, Khafaga AF, Metwally KA, Ajarem J S, Maodaa SN, Allam AA, El-Saadony MT. 2020. Effects of dietary biological or chemical-synthesized nano-selenium supplementation on growing rabbits exposed to thermal stress. Animals, 10(3): 430.

Sun H, Xu XL, Xu LC, Song L, Hong X, Chen JF, Wang XR. 2007. Antiandrogenic activity of pyrethroid pesticides and their metabolite in reporter gene assay. Chemosphere, 66(3): 474-479.

Supraja B, Giridhar C, Naidu V .2022. Analytical method development and validation analysis for quantitative assessment of cypermethrin by HPLC procedure." World J Adv Res and Rev., 14 (1): 541-553.

Szulc-Musiol B, Gadomska-Nowak M, Danch A, Ryszka F. 2004. Pharmacokinetics of selenium following oral administration selenium preparation in rabbits. Boll Chim Farm., 143(2):62-64.

USP. 2021. Pharmacopeia, Validation of compendial procedures, United States Pharmacopeial Convention Rockville, 2021.

Wang XZ, Liu SS, Sun Y, Wu JY, Zhou YL, Zhang J H 2009. Beta-cypermethrin impairs reproductive function in male mice by inducing oxidative stress. Theriogenology, 72 (5):599-611.

Wojciechowska M, Stepnowski P, Gołębiowski M. 2016. The use of insecticides to control insect pests. Invertebr Surviv J, 13(1): 210–220.

Yang X, Sun Q, Raza A, Jian X, Zhong B, Shahzad M, Zhang F, Han Y, Lu S. 2010. Nitric oxide in both bronchoalveolar lavage fluid and serum is associated with pathogenesis and severity of antigen induced pulmonary inflammation in rats. J Asthma, 47(2): 135–144.

Yarkov DJ, Sortirov LK. 1995. Effects of the pyrethroid insecticide Fastac 10EC Alphcypermethrin on lysozyme activity in birds of different genotypes. Conference: Aspects on Environmental Toxicology. At: Thessaloniki, Greece

Yousef MI, El-Demerdash FM, Al-Salhen KS. 2003. Protective role of isoflavones against the toxic effect of cypermethrin on semen quality and testosterone levels of rabbits. J Environ Sci and Health, part B, 38(4): 463-478.

Yuan JS, Reed A, Chen F, Stewart CN .2006. Statistical analysis of real-time PCR data. BMC bioinformatics, (7): 1-12.

Zachara BA. 2015. Selenium and selenium-dependent antioxidants in chronic kidney disease. Advan in Clin Chem., (68): 131-151.

Zhu F, Zhu L, Sun J, Meng X, Zou L. 2010. Effects of dietary supplemental nano-se levels on liver selenium content and antioxidant abilities in hens. Chinese J Vet Sci., 30(11): 1537-1539.