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Selenium nanoparticles provoke the expression of proliferating cell nuclear antigen (PCNA) gene in testicular tissues of rats treated with deltamethrin insecticide

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Abstract:
Background*: Deltamethrin insecticide showed severe toxicity to testicular tissues regarding damages in their architecture, deterioration in fertility hormones secretion and severe anomalies in their structure. However, selenium nanoparticles exhibit protective role that can recover such damages, anomalies and strengthen the reproductive potency as well. The aim of this study was to investigate the effect of selenium nanoparticles on the expression of the proliferating cell nuclear antigen (PCNA) gene in testes of rats treated with 13.5 mg/ kg bwt and 27 mg/Kg bwt deltamethrin corresponding to its 1/10 and 1/5 LD_{50} using corn oil as a solvent for 28 days.*
Materials and Methods*: In this prospective five groups each of ten adult albino male rats were considered. One group was the control with rat fed on corn oil while in the second group rats were treated with 0.5mg/Kg bwt nanoselenium, rats in third and fourth groups were treated with 13.5 mg/ kg bwt and 27 mg/Kg bwt deltamethrin respectively. While, in the last group rats were treated with 0.5mg/Kgbwt nanoselenium and 27mg/Kg bwt deltamethrin.*
Results*: Results implied significant increase in the relative testes weight index among rats treated with nanoselenium. Levels of testosterone, luteinizing and follicle stimulating hormones were estimated showing a significant increase among groups treated with nano selenium compared to those that were untreated (P< 0.05). Histological studies were also carried out on treated testes along with estimating the enzymes of antioxidant status that also implied significant increase on the levels of catalase, superoxide dismutase enzymes and non significant increase in the glutathione reductase levels among nanoselenium treated groups.*
Conclusion: *General results implied a positive and protective effect of selenium nanoparticles towards the adverse effects of deltamethrin on the male reproductive potency regarding the expression of the proliferating cell nuclear antigen (PCNA) gene in treated testes.*
Key Word*: Deltamethrin, Proliferating cell nuclear antigen (PCNA), Selenium nanoparticles, Testicular immunocytochemistry, Testicular histology.*

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I. Introduction

Deltamethrin is a synthetic pyrethroid insecticide used in many fields including agriculture, household pest control and disease vector control. Although it was thought to be the least toxic pesticide, many reports revealed its toxic effects in mammalian and non-mammalian animal species 1. Many studies had pointed out that it is severely toxic, showing its particular harmfulness to the male reproductive health 2. Selenium (Se), is an essential trace element that can play a crucial role in the maintenance of diverse physiological functions, body metabolism and cellular homeostasis. Definitely, selenium nanoparticles (SeNPs), which are of growing importance not only because of their nutrients digestibility, medicinal therapy, targeted drug delivery system and production of vaccines, but also due to their high bioavailability and digestive efficiency 3. Recently, SeNPs have been introduced as alternatives for seleno compounds and many researchers have focused on the

excellent characteristics of SeNPs such as the lower toxicity and higher anti-oxidant effects 4 as well as their protective effects on the male reproductive system 5 compared to inorganic and organic seleno compounds. In fact, it has been determined that SeNPs not only possess protective potency but also, have positive effects on male reproduction 5. Interestingly, proliferating cell nuclear antigen (PCNA), also known as cyclin, is a 36 KDa nuclear protein and has been recognized as an auxiliary well conserved protein in all eukaryotic species 6. It acts as a processivity factor of DNA polymerase delta, which is required for DNA synthesis during replication 7. PCNA is believed to play a crucial role in regulating both DNA synthesis and cell proliferation 8. However, along with DNA replication, PCNA functions are associated with other vital cellular processes such as chromatin remodeling, DNA repair, sister-chromatid cohesion and cell cycle control 9. It was shown that expression levels of PCNA are associated with proliferation or neoplastic transformation 10. PCNA expression peaks at G1/S of interphase, and then drops during mitosis to a level that is too low to be detected using the immunohistochemical method. Thus, PCNA serves as an endogenous histological marker for the G1/S phases of the cell cycle 11. Genes encoding PCNA and/or its products have been identified in a wide variety of diverse organisms including animals 12. Analyses of all known PCNAs suggest that this protein is also conserved in structure and function 13. An important finding regarding the conservation of PCNA, described the ability of PCNA to interact and form a stable complex with human p21/WAF1 12, a p53-dependent protein involved in cell cycle regulation and stress response. Worthy to mention, is that the human anti-PCNA auto-antibody reacts with the nuclei of proliferating cells of all experimental animals examined so far 14.

Accordingly, the aim of this present study was to investigate the effect of selenium nanoparticles on the expression of the proliferating cell nuclear antigen (PCNA) gene in testicular tissues of rats treated with 13.5 mg/ kg bwt and 27 mg/Kg bwt corresponding to 1/10 and 1/5 LD50 of deltamethrin using corn oil as a solvent for 28 days. Relative testes weight index along with levels of testosterone, luteinizing and follicle stimulating hormones were estimated. Histological studies were carried out on treated testis along with estimating the enzymes of antioxidant status regarding superoxide dismutase, catalase and glutathione reductase.

II. Materials And Methods

This prospective comparative study was carried out on mature male albino rats (180+/- 10 g). A total 50 adult subject (males only) of aged 8-12 weeks old were for in this study. The rats were bred at the animal house of Research Institute of Medical Entomology (RIME), Egypt. All animal studies were performed according to the standards of animal care set out in Egyptian rules and the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals (Approval number: IME3060).

Study Design: Prospective open label observational study

Study Location*:* The study was carried out in Rodents Research Dept. (RRD), Research Institute of Medical Entomology, (RIME), Giza, Egypt.

Study Duration: May 2021 to July 2021.

Sample size: 50 male adult rats.

Sample size calculation: The sample size was estimated on the basis of a single proportion design. The target population from which we randomly selected our sample was considered 1000. We assumed that the confidence interval of 10% and confidence level of 95%. The sample size actually obtained for this study was 10 rats for each group. We planned to include 50 rats (Group I- Control, Group II, Group III, Group IV and Group V – deltamethrin and/or nanoselenium treated rats of 10 rats for each group) with 4% drop out rate.

Materials:

Chemicals:

Technical grade deltamethrin (98%) EGYPTCHEM INTERNATIONAL FOR AGROCHEMICALS, Egypt.
Sodium selenite, DIFCO LABORATORIES, DETROIT MICHIGAN, USA.
Ascorbic acid, KOCH – LIGHT LABORATORY LTD, UK.

Subjects & selection method: The study population was drawn from animal house male rats. Five groups of rats were involved in this study, 10 rats in each group (n= 10) where:

1- The first group was the negative control set. Where, neither the selenium nanoparticles nor deltamethrin were administered (corn oil only).
2- Second group (13.5 mg/Kg b.w. deltamethrin) and third group (27 mg/Kg b.w. deltamethrin) were orally administered with deltamethrin at doses (13.5 and 27mg/kg b.w. corresponding to 1/10 and 1/5 LD50 respectively for 28 days (The doses were determined on the basis of LD_{50} of deltamethrin in corn oil [15, 16].
3- The fourth group (0.5 mg/Kg b.w. nanoselenium) was exposed to selenium nanoparticles at dose of 0.5 mg/ kg b.w for 28 days.
4- Fifth group (27 mg/Kg b.w. deltamethrin+ 0.5mg/Kg b.w.nanoselenium) was exposed to deltamethrin (27mg/ kg) and SeNPs (0.5 mg/ kg) b.w for 28 days.

Procedure methodology

Preparation and Characterization of Nanoparticles

Appropriate amounts of selenium nanoparticles were synthesized following a modified method of **Li *et al,* 2010.** Thereafter, the prepared nanoparticles were characterized by their distinctive absorption band displayed by UV-visible spectrophotometer [17]**.**

Preparation of subject groups

Selected groups were prepared according to subjects and selection method then, rats were killed by mild ether anesthesia and the testes were removed and preserved in 10% neutral formalin solution to be used for further cellular, biochemical and histological studies. Meanwhile, rats' sera were isolated out of blood collected from the abdominal aorta, using a syringe puncture. The obtained testes and sera were used for further cellular, biochemical and histological studies. The weight of all the animals is measured prior to the different types of treatment and sacrifice.

Changes in the testes index of rats

The relative weight of testes was calculated according to the weight of the testes as follows: testis weight body weight / 100 [18]**.**

Estimation of serum fertility hormones

Serum testosterone, luteinizing hormone, LH and follicle stimulating hormone, FSH, of the control and treated rats were quantitatively measured by an enzyme linked immunosorbent assay (ELISA), following the instruction manual of PRE CHECK Bio INC. USA kit and using ELISA reader (STAT FAX, 2100- Awareness Technology INC.) Japan.

Preparation of testes homogenates

The testes were homogenized in ten volumes of ice-cold medium of Phosphate buffer solution, PBS (pH 7.4). Testes homogenates were centrifuged for 15 min at 4 C^o. The pellets were discarded while the supernatants were used for the investigations of antioxidant enzyme activities [19]**.**

Antioxidant status

The activities of three antioxidant enzymes were determined as indicators for the assessment of oxidative stress in the testes. Superoxide dismutase (SOD) was determined. The activity of testicular catalase (CAT), which is essential for removing the hydrogen peroxide produced by SOD, was also determined. And the glutathione reductase (GR) was also assayed indirectly based on the oxidation of NADPH to NADP+. [20, 21, 22]

Histological Studies

Isolated testes specimens were fixed in 10% neutral formalin, paraffinized and were processed for histological examination. Paraffin sections were prepared at 5 µm thickness from each testis. Tissue sections were stained with hematoxylin and eosin (H&E) and then are microscopically examined using Olympus imaging software for microscopy, Germany to detect the effect of deltamethrin and/ or nanoselenium on treated testicular tissues [23]**.**

Immunocytochemistry

The peroxidase/anti-peroxidase (PAP) method was used to assess the immune-cytochemical reactions in the testes. Formaldehyde-fixed, paraffin-embedded testicular tissue sections were used. To reduce the non-specific peroxidase reactions and background, tissue sections were incubated with methanol containing 3% H_2O_2 and normal goat serum for 30 min. Thereafter, the tissue sections were incubated with a specific first antibody that can recognize the proliferative cell nuclear antigen (PCNA) and successively with HRP-conjugated goat anti-mouse IgG as a secondary antibody. Successively, tissue sections were incubated with the PAP complex (dilution, 1:200) for 90 min. Diaminobenzidine was used as chromogen. Eventually, the samples were counterstained with haematoxylin and were analyzed using Olympus imaging software for microscopy, Germany [24] where PCNA gene expression was evaluated among test and control groups.

Statistical analysis

Statistical analyses were performed using the Statistical Package for Social Science (SPSS) version 25 (SPSS Inc., Chicago, IL, USA.). Data were presented as means ± SD. Differences in the various parameters in more than two groups were evaluated by a one-way analysis of variance (ANOVA). Differences between groups were considered significant at $p < 0.05$ [23]**.**

III. Results

Changes in the testes index of rats

Although results of 13.5 and 27 mg/kg b.wt. corresponding to 1/10 and 1/5 LD_{50} of deltamethrin treated rats showed significant reduction in the weight of their testes compared to control rats (Table and Figure 1), the weight of the testes of rats treated with 27 mg/kg bwt deltamethrin + 0.5 mg/kg selenium nanoparticles relative to the overall weight of control rats implied significant slight increase in the testes weight (Table and Figure 1).Selenium nanoparticles exhibited positive protecting effect on testes of deltamethrin treated rats.

Interestingly, four weeks of SeNPs treatment showed a highly significant ($p < 0.001$) effect in increasing the relative testis weight compared to the control rats.

Table 1: Average total body weight, testes weight and relative weight of testes among different groups treated with deltamethrin and/or nanoselenium.The difference in the values of all parameters in respect of five groups was statistically significant ($p<0.05$).

Groups	Average total body weight (g) ± SE	Average testes weight (g) ± SE	Average of relative testes weight index ± SE
Control (corn oil only)	221±3.48329**	1.67±.02667**	0.62±.00458**
0.5 nanoselenium mg/Kg	225±3.72678**	1.73±.01966**	0.65±.01202**
13.5 deltamethrin mg/kg	188±2.62890**	1.25±.03721**	0.6±.00526**
27 deltamethrin mg/kg	171±2.75278**	1.19±.09233**	0.52±.00869**
27 deltamethrin mg/kg + 0.5 nanoselenium mg/Kg	215±4.28434**	1.68±.03471**	0.63±.01660**

Values are represented as mean± SE, ** highly significant= P< 0.001, * significant = P<0.005, NS non significant= P>0.005. n=10.

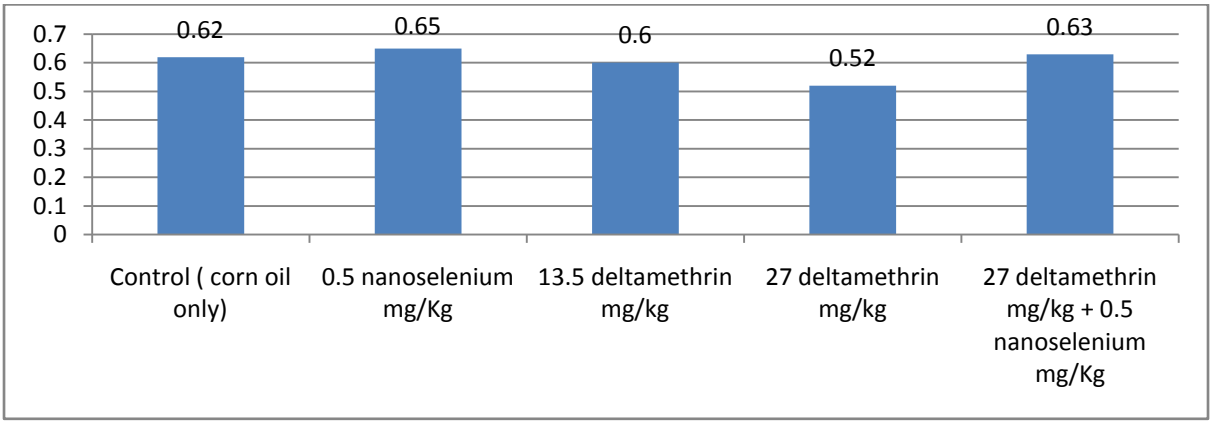

Figure 1: Average of relative weight of testes among different groups treated with deltamethrin and/or nanoselenium.

Estimation of serum fertility hormones

Levels of serum testosterone showed a highly significant ($p < 0.001$) reduction in 13.5 & 27mg/ kg bwt deltamethrin treated rats compared to the control rats. SeNPs significantly increased the serum testosterone levels in rats treated with 0.5 mg/kg bwt selenium nanoparticles, suggesting that SeNPs have a role in the synthesis of testosterone (Table2 and Figure2). Furthermore, four weeks of SeNPs administration could significantly recover the serum testosterone to the normal levels expected in rats treated with 27mg/kg bwt deltamethrin + 0.5 mg/kg bwt selenium nanoparticles (Figure 2).Luteinizing and follicle stimulating hormones levels showed also significant decrease in groups treated with deltamethrin compared to those treated with selenium nanoparticles and those of control group.

Table 2: Average levels of testosterone, luteinizing and follicle stimulating hormones in blood sera among different groups treated with deltamethrin and/or nanoselenium.

Groups	Testosterone mean levels in blood sera (ng/ml) ±SE	LH mean levels in blood sera (mIU/ml) ±SE	FSH mean levels in blood sera (mIU/ml) ±SE
Control (corn oil only)	6.3±0.16600**	1.64±0.02565**	0.9±0.01571**
0.5 nanoselenium mg/Kg	8.3±0.16600**	1.7±0.01106**	0.95±0.01938**
13.5 deltamethrin mg/kg	1.53±0 .01438**	1.61±0.01106**	0.85±0.00775**
27 deltamethrin mg/kg	0.4±0.00760**	1.58±0.00919**	0.78±0.00596**
27 deltamethrin mg/kg + 0.5 nanoselenium mg/Kg	4.2±0.06831**	1.68±0.01430**	0.89±0.00596**

Values are represented as mean± SE, ** highly significant= P< 0.001, * significant = P<0.005, NS non significant= P>0.005. n=10

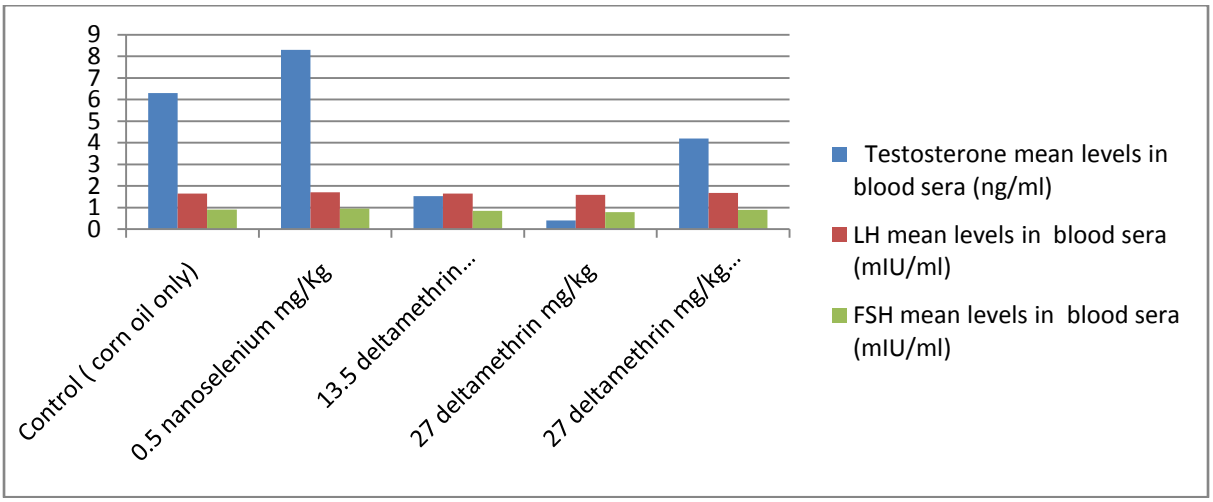

Figure 2: Average levels of testosterone, luteinizing and follicle stimulating hormones in blood sera among different control and treated groups treated with deltamethrin and/or nanoselenium.

Estimation of antioxidant status

To study the influence of deltamethrin on the oxidative damage in the treated testes, the action of the antioxidant defense system was assessed by estimating the activity of superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR) enzymes. The toxic effect of deltamethrin resulted in a significant reduction in the activities of superoxide dismutase (SOD), catalase (CAT) enzymes however; deltamethrin induced a non significant reduction in the activity of glutathione reductase (GR) enzyme (Table and Figure3). Accordingly, treatment of 27mg/kg deltamethrin with SeNPs increased the activity of SOD compared to the 13.5 and 27 mg/kg bwt deltamethrin treated rats (Table 3 and Figure 3). Deltamethrin treated rats significantly decreased catalase ($p < 0.001$) and non significantly decreased glutathione reductase levels in testicular tissue (Table3 and Figure3). This reduction in catalase and glutathione reductase contents was recovered by treatment with SeNPs compared to those that were untreated with SeNPs (Table3 and Figure4). 27mg/kg bwt deltamethrin rats treated with SeNPs exhibited increased activities of SOD, CAT and GR compared to the untreated with selenium nanoparticles (Table3 and Figure3). Thus, SeNPs seemed to help recover the impaired activity of antioxidant enzymes in deltamethrin treated rats.

Table 3: Average levels of Superoxide dismutase, catalase and glutathione reductase in testicular tissues among different control and treated groups treated with deltamethrin and /or nanoselenium.

Groups	**Super oxide dismutase mean levels in testicular tissues (U/ml tissue)** ±SE	**Catalase mean levels in testicular tissues (U/g)** ±SE	**Glutathione reductase mean levels in testicular tissues (mg/g tissue)** ±SE
Control (corn oil only)	314.13±1.9699**	0.183±0.003464**	1.17 ± 0.01350^{NS}
0.5 nanoselenium mg/Kg	366±1.5846**	0.266±0.002431**	1.25 ± 0.07958^{NS}
13.5 deltamethrin mg/kg	309.3±2.0913**	0.159±0.002006**	1.15 ± 0.04512^{NS}
27 deltamethrin mg/kg	290.6±9.1690**	0.125±0.002119**	1.1 ± 0.05899^{NS}
27 deltamethrin mg/kg + 0.5 nanoselenium mg/Kg	342.2±5.2513**	0.243±0.003127**	1.2 ± 0.08394^{NS}

Values are represented as mean± SE, ** is highly significant= P< 0.001, * is significant = P<0.005, NS is non significant= P>0.005. n=10

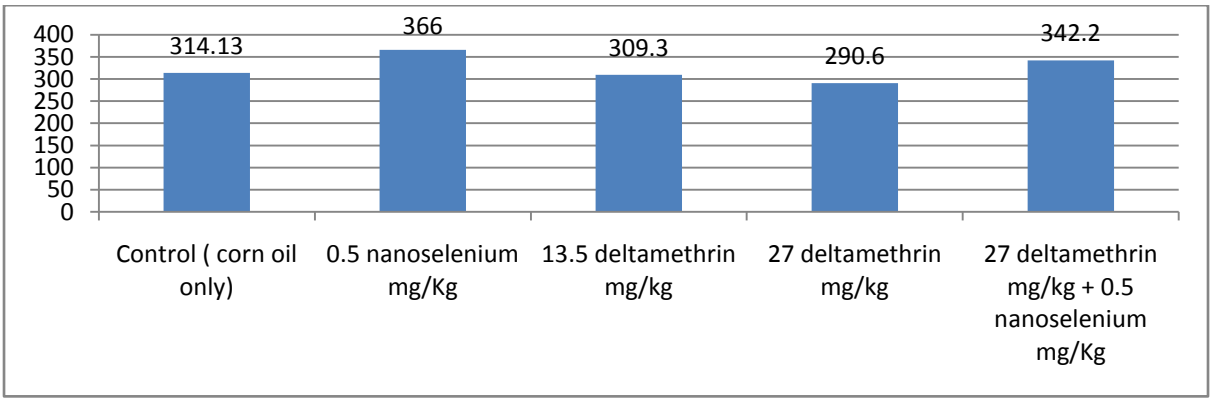

Figure 3: Average levels of Superoxide dismutase in testicular tissues among different control and treated groups treated with deltamethrin and/or nanoselenium.

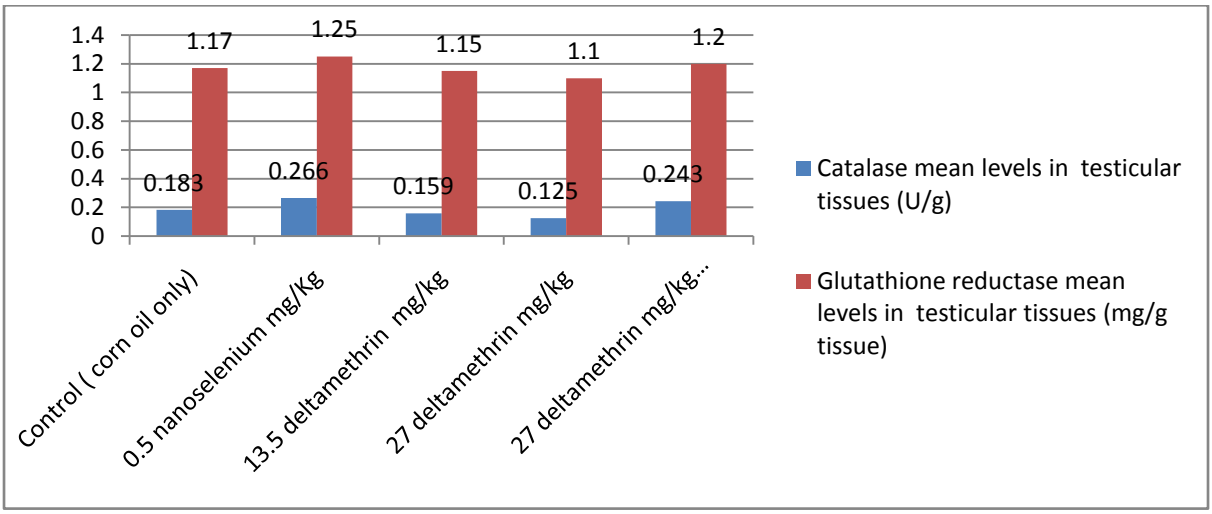

Figure 4: Average levels of catalase and glutathione reductase in testicular tissues among different control and treated groups treated with deltamethrin and/or nanoselenium.

Histological Studies:

H&E micrographs show seminiferous tubules (S.T), sperm (SP), exfoliation of spermatocytes (E.S.), vacuolization (V), separation of spermatogenic cells from germinal epithelium (G), tubular deformation (TD), rupture of germinal layer (black star), aspermatogenesis (black arrow), edema between the seminiferous tubule, absence of spermatocytes (red star), degeneration of interstitial cells (yellow star), vacuolated degeneration (V.D), and interstitial cells degeneration (I.C.D).These findings vary among different groups of rats treated with deltamethrin and/or nanoselenium where, nanoselenium treated groups showed the most preserved typical

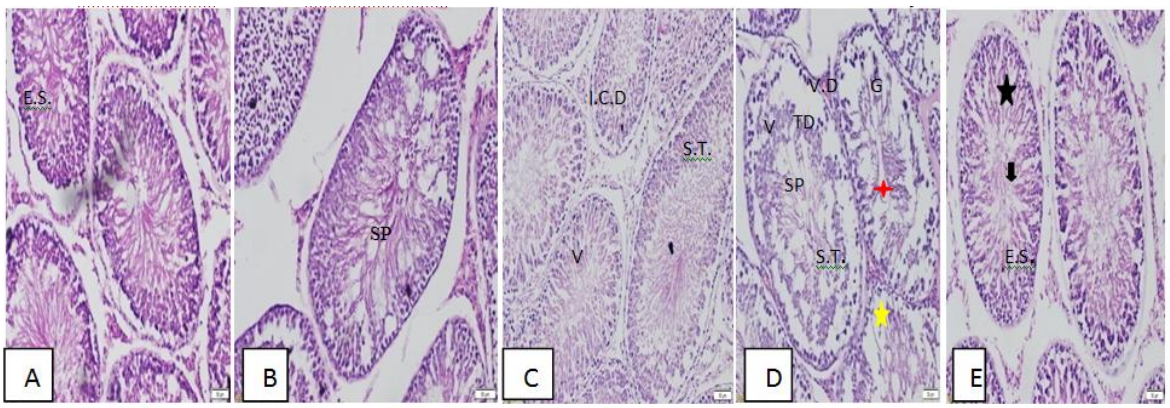

Figure 5: H &E stained photomicrograph showing the impact of SeNPs on the histology of the testes treated with deltamethrin.

(A) control rats; showed typical testicular architecture; (B) SeNPs-treated rats; showed typical spermatogenic cells in the seminiferous tubules; (C) 13.5 mg/kg bwt deltamethrin treated rats; showed mild testicular damage; (D) 27 mg/kg bwt deltamethrin treated rats; showed severe testicular damage; In particular, the layers of the epithelium were severely impaired varying from totally to partially disorganized and also with obviously impaired organization of the stages of spermatogenesis adding to this, treated testes exhibited atrophy in the seminiferous tubules with arrested spermatogenesis and necrotic cells evident in the luminal compartment associated with a lack of spermatogonia in the basal portion, in particular in the heavily damaged tubules . (E) 27 mg/kg bwt deltamethrin + 0.5 mg/kg bwt treated rats; showed recovered testicular architecture. SeNPs ameliorated the defects of the spermatogenic cells in the seminiferous tubules caused due to the adverse effect of deltamethrin exposure. Testes regained normal seminiferous tubules lined by several layers of spermatogenic cell series, similar to those of the control. Scale bar: 50ul.

Immunocytochemistry

The expression of the Proliferating Cell Nuclear Antigen Gene (PCNA) in testicular tissues was greatly varied among different groups of rats treated with deltamethrin and/or nanoselenium. The highest expression of PCNA was observed in tissues treated with nanoselenium while, the lowest expression was observed in tissues treated with deltamethrin.

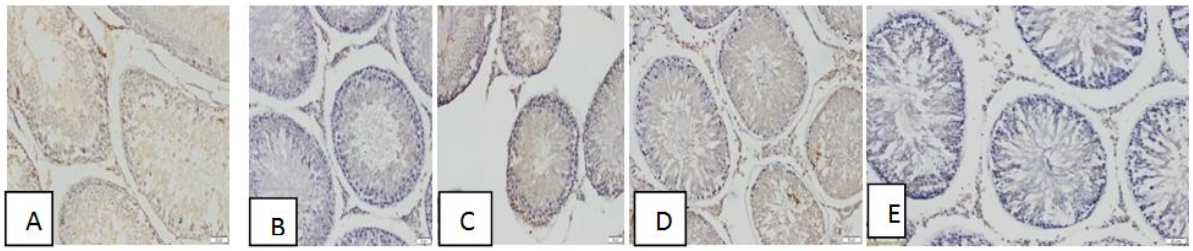

Figure 6: illustrates the cells that were immunereactive positive for the Proliferating Cell Nuclear Antigen Gene (PCNA) for all groups where, Figure 6A showed that both the seminiferous tubules and interstitial tissues were normally structured and had normal expression of PCNA in the control group and Figure 6B showed a significant spermatocytes and spermatogonia that have the highest content of PCNA in SeNPs treated groups. While, in groups that were treated with 13.5 and 27 mg/kg bwt deltamethrin (Figures 6 C&D) the number of PCNA-positive germinal cells was markedly reduced compared to those for groups A&B, and that PCNA staining was likely restricted to spermatogonia and primary spermatocytes with no PCNA content either in the secondary spermatocytes or spermatids. Interestingly, SeNPs treated 27 mg/kg bwt deltamethrin rats exhibited plenty of PCNA immunostained cells in the tissues of the testes, indicating high expression of PCNA (Figure 6 E compared to figure 6 C&D). While on the other hand, SeNPs treated 27 mg/kg bwt deltamethrin rats showed fewer positive nuclei for PCNA in the spermatogenic epithelium cells (Figure 6E, compared to figure 6B).

IV. Discussion

In spite of its stated hazardous effects, the administration of the toxic pyrethroid pesticide, deltamethrin in agriculture and public health sectors become an applicable and easy going way for controlling pests in the mean time. One of the most adverse effects of deltamethrin on males that experienced its oral or dermal exposure is the reduction in their reproductive potency and the production of significant reproductive toxicity in treated male rats [25]**.** On the other hand, recent studies confirmed the positive effect of selenium nanoparticles on the biology of human bodies generally and in protecting and increasing the reproductive potency particularly [5]**.**

Useful information on male reproductive capacity of laboratory animals can be obtained by measuring weights and the volume of testis [5]**.** Hence, the present study showed that the total body weight and absolute testes weight indices were the first toxicity markers that deltamethrin induced in male rats. Results of this study revealed significant decrease in the weight index of deltamethrin treated rats compared to control rats. However, selenium nanoparticles improved and increased the weight of deltamethrin treated testes. **Abdel- Maksoud *et al.*, 2018**[25] also showed results indicating that deltamethrin caused a significant reduction in testis weights, sperm count, motility and viability and fructose in semen.

Maintenance of weights of reproductive organs and accessory glands depends on testosterone level [26]**.** Consequently, the effect of selenium nanoparticles on the fertility hormones of rats treated with deltamethrin were regarded, results of the present study were in accordance with **Hozyen *et al.*, (2020)**[27] who revealed a significant reduction in the levels of testosterone hormone among rats treated with deltamethrin and an improved reproductive potency in groups treated with both deltamethrin and selenium nanoparticles. Worthy to mention is that testosterone in male bodies is a sex hormone that plays important roles in regulating sex drive

(libido), bone mass, fat distribution, muscle mass and strength, and the production of red blood cells and sperm [28]. Consequently, administration of deltamethrin, in the present study caused significant reduction in body weights, testes weights and serum testosterone level.

LH stimulates testosterone production from the interstitial cells of the testes (Leydig cells). FSH stimulates testicular growth and enhances the production of an androgen-binding protein by the Sertoli cells, which are a component of the testicular tubule necessary for sustaining the maturing sperm cell. This androgen-binding protein causes high local concentrations of testosterone near the sperm, an essential factor in the development of normal spermatogenesis. Sertoli cells, under the influence of androgens, also secrete inhibin, a polypeptide, which may help to locally regulate spermatogenesis. Hence, maturation of spermatozoa requires FSH and LH [29]. **Abdel- Maksoud *et al.*, 2018**[25] indicated that deltamethrin induced reduction in serum FSH and LH levels associated with increase in the sperm abnormalities. **Issam *et al.*, 2009**[30] showed also a significant decrease of FSH, LH and testosterone levels at the highest deltamethrin dose. These findings confirmed the present results that revealed a significant decrease in LH and FSH levels among the groups treated with 13.5 mg/kg bwt and 27 mg/kg bwt deltamethrin, while the reproductive picture of hormones was improved upon selenium nanoparticles intervention. These findings were in accordance with **Hozyen *et al.*, (2020)**[27] who showed that treatment with selenium nanoparticles improved deltamethrin-induced negative effects on sperm characteristics and sexual activity in general, as well as **Dekhil *et al.*, 2016**[18] who confirmed the protective effect of selenium nanoparticles exerted on the male reproductive organs.

Oxidative stress is considered as one of the most important mechanisms involved in cell damage [31]. Reactive oxygen Species, ROS play an essential role in the pathogenesis of many reproductive processes, they attack the sperm membrane lipids and the DNA in the sperm nucleus [32]. Recent studies have implicated ROS in male infertility in mammals [33]. Superoxide is one of the main reactive oxygen species in the cell. [34], it is produced as a by-product of oxygen metabolism and, if not regulated, causes many types of cell damage [35], hence it was used in the present study as a cellular marker to assess the antioxidant status upon treating rats with deltamethrin and selenium nanoparticles. However, Superoxide dismutases (SODs) constitute a very important antioxidant defense against oxidative stress in cells. They act as good therapeutic agents against reactive oxygen species-mediated diseases [36]. These enzymes catalyze the dismutation (or partitioning) of the superoxide (O^{-}_{2}) radical into ordinary molecular oxygen (O_2) and hydrogen peroxide (H_2O_2) and decrease O_2^- level which damages the cells at excessive concentration [37]. This may explain the leading cause of cellular damages that deltamethrin induced in the present study upon decreasing the activity of SOD in testicular tissues in rat groups treated with 1/10 and 1/5 of LD_{50} of deltamethrin. It was known that selenium nanoparticles enhances SOD activity and strengthen its therapeutic role [5]. Consequently, selenium nanoparticles which exhibit effective antioxidant potency could significantly increase SOD activity in testicular tissues treated with deltamethrin as it was previously described by **Hozyen *et al.*, (2020)** [27]and **Dekhil *et al.*, (2016)**[18].

Hydrogen peroxide is also damaging and is degraded by other enzymes such as catalase. This enzyme catalyzes the decomposition of hydrogen peroxide to water and oxygen [38]. It is a very important enzyme in protecting the cell from oxidative damage by reactive oxygen species (ROS) [39]. In the present study, deltamethrin treatment significantly decreases the activity of catalase which consequently increases the hydrogen peroxide in cells of testicular tissues causing sever damages in the cells. Decreased catalase activities in response to pesticide exposure were also observed during assessment of antioxidant status in pesticide-exposed farmers in several studies [40]. This could be attributed to the inhibition of thiol groups of catalase by the generated oxygen radicals. Increased ROS production at the cellular level might also inhibit CAT activity and result in the accumulation of excess hydrogen peroxide that may further inhibit SOD [5].

Meanwhile, glutathione reductase (GR), is responsible for maintaining the supply of reduced glutathione; one of the most abundant reducing thiols in the majority of cells. In its reduced form, glutathione plays key roles in the cellular control of reactive oxygen species [41]. In spite of the non significant decrease of GR upon groups treated with deltamethrin and/or nanoselenium, this could not deny the state of damage that deltamethrin induced in testicular tissues and the protective effect that selenium nanoparticles exerted upon treatment in the present study as it was previously described by **Dekhil *et al.*, 2016** [18].

Histological studies in the present work implied that testes of rats treated with deltamethrin exhibited various anomalies during their development. Multiple signs of vacuolization, decrease in number of spermatogenic cells and exfoliation of spermatocytes were also observed. The spermatogenic cells were reduced and the majority of spermatocytes and spermatids became degenerated compared to the control group. However, groups treated with SeNPs implied prominent increase in the number of spermatogenic cells associated with improvement in the general architecture of treated testes and observed reduced signs of vacuolization. These findings were confirmed by **Issam *et al.*, 2009** [30]**and Rashid *et al.*, 2012** [2]who mentioned severe damages within deltamethrin treated testes accompanied by some necrotic and apoptotic figures in particular cell fragments into the seminiferous tubules. Additionally, histological examinations revealed significant alterations in the testes of deltamethrin dosed groups as described by **Desai *et al.*, 2016** [28]. However, testes that were treated

with SeNPs in the present study showed typical testicular architecture or recovered one upon suffering deltamethrin exposure. As described by **Dekhil *et al.*, 2016** [18] who confirmed the protective potency and recovery effect that SeNPs can exert on damaged testes of treated rats.

Proliferating cell nuclear antigen, PCNA is believed to play a crucial role in regulating both DNA synthesis and cell proliferation [8]**.** However, along with DNA replication, PCNA functions are associated with other vital cellular processes such as chromatin remodeling, DNA repair, sister-chromatid cohesion and cell cycle control [9]**.** Since spermatogenesis is a complicated cell cycle of fast proliferating cells ending with the formation of sperm, PCNA was selected in this study as a tool to evaluate spermatogenesis. PCNA positive cells were strongly expressed in testicular tissues of control rats of the present study. In contrast, the density of PCNA positive testicular germ cells was significantly reduced in deltamethrin treated rats, which is an indication of disruption in proliferation and spermatogenesis. However, the density of PCNA positive testicular germ cells were highly observed in tissues of rats treated with selenium nanoparticles. Previous studies have demonstrated that the increase in PCNA expression in testicular tissue is an indication of high proliferative activity and stimulation of spermatogenesis, and this expression is down regulated in drug treated group [18]**.** This may explain the negative effect of lowered PCNA expression in deltamethrin treated rats in this study. However, SeNPs exerted protective effect on the deltamethrin treated testicular tissues in this study via increasing the secretion of fertility hormones and inducing the activities of the antioxidant enzymes thus decreasing ROS levels in cells and also via protecting the testicular tissues from being damaged as early mentioned. This can be attributed to the fact that Se compounds are implicated in modulating a variety of cellular activities leading to cell proliferation and survival [42]**.** These protective mechanisms that SeNPs exerted on testicular tissues may lead eventually to their recovery hence, the upregulation of PCNA. Thus, the upregulation of PCNA led to the promotion of cell cycle progression as described by **Dekhil *et al.*, 2016** [18]**.** Where, nano selenium could improve sperm count, motility and vitality in mice treated with nano selenium particles [5]. Therefore, the antioxidant effects of nano selenium may be a major reason for its positive impact on spermatic parameters and upregulation of PCNA.

V. Conclusion

In conclusion, oral deltamethrin administration for 28 days induces an arrest of spermatogenesis, a significant disharmony in sex hormones and reduction in enzymes of oxidative stress levels in rats that were treated with 13.5 mg/ kg bwt and 27 mg/Kg bwt deltamethrin corresponding to its 1/10 and 1/5 LD_{50} using corn oil as a solvent. Also, down regulation of the proliferative cell nuclear antigen (PCNA) gene, was detected which may be one of the molecular mechanisms involved in deltamethrin-induced gonads toxicity. However, selenium nanoparticles exhibit potent and effective mechanisms for protection and recovery, which lead eventually to up regulation of PCNA in deltamethrin treated testicular tissues.

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