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RESEARCH ARTICLE

The Repro-prophylactic Impact of Synthetic Tocopherol Polyethylene Glycol, Succinate-Coated, Garlic-Selenium Nanoparticles against Lead Acetate Toxicity in Male Rabbits

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ABSTRACT

Because of industrial and human activities, lead is a hazardous heavy metal that is widely present in the environment and poses a threat to public health and ecosystems. Garlic extract (GE) demonstrates protective impacts against many disorders. Its effects on heavy metal toxicity require more research. A nanoparticle formulation could increase its effectiveness even more. This investigation examined the repro-protection impact of tocopherol polyethylene glycol succinate-coated garlic selenium (TPGS-GSNP) against lead acetate (LA) toxicity. For this purpose, 64 mature male rabbits were equally divided into eight groups (8 each). Male rabbits were treated three times a week for 12 weeks. The males received drinking water (G1), 30 mg of LA/ kg. BW (G2), 800 mg of GE/kg. BW (G3), GE and LA (G4), 1 mg of TPGS-Selenium/kg. BW (G5), TPGS-S and LA (G6), 1 mg of TPGS-GSNP/kg. BW (G7), and TPGS-GSNP and LA (G8). After treatment, serum levels of follicle stimulating hormone, luteinizing hormone, testosterone, and gonadotropin releasing hormone were assessed. Testicular histopathological findings and the expression levels of testicular FSH and LH receptors, ABP, 3β-HSD, 17β-HSD, and Inh-α, and pituitary LHβ and FSHβ genes were evaluated. Obvious testicular histopathological changes, decreased genital organ weights, reduced serum hormonal levels, and declined testicular and pituitary gene expression levels, were exhibited in LA group. The TPGS-GSNP group, however, displayed improvement in these findings. Finally, synthesized TPGS-GSNP showed a greater repro-protective effect against LA toxic-induced male rabbits. Further research is required to explore the mechanism of TPGS-GSNP action.

Keywords: Lead acetate, Reproduction, Selenium, Tocopherol, Testes, Toxicity

Introduction

Nanotechnology is a relatively new scientific subject that focuses on the synthesis and manufacture of different forms of nanoparticles. Due to their small size, nanoparticles might differ from the bulk material. They are minuscule, ranging in size from one to one hundred nanometers. Different metallic nanomaterials are now produced using Cu, Zn, Ti, Mg, Au, and Ag. NP is being used in many industries, including medical treatments.[1] Chemical or organic processes can be used to create nanoparticles.[2]

Lead compounds are found in many different parts of the environment since it is used in paints, fertilizers, pesticides, gasoline that contain lead, and metal objects. Lead exposure can occur in the workplace or in the environment. Furthermore, lead may build up and harm some organs, including the immune system, brain, kidney, liver, and heart.[3] After exposure, lead is known to cause biochemical, behavioral, and

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physiological abnormalities in both humans and animals. Lead is thought to induce male reproductive disorders in rats, along with weight loss in the testicles, destruction of the testis, a decrease in the quality of the sperm, and consequently male infertility.[4,5] Lead also clearly induces oxidative stress in testis tissue through increased reactive oxygen species (ROS) production and decreased activity of antioxidant enzymes.[6]

Selenium is one of the essential trace elements that benefits human and animal health. Selenium can promote its biological activities by scavenging peroxides.[7] Spermatozoa motility and activity, spermatogenesis, and testicular development are all specifically impacted by selenium. It primarily accomplishes this by regulating the synthesis of seleniproteins, which are necessary for sperm production and the development of post-testicular sperm.[8] According to research by Hamza and Diab,[9] low selenium levels are linked to sperm fragility and a reduction in sperm characteristics.

The annual plant garlic (*Allium sativum* L.) has been used for millennia as a spice and medicinal herb to prevent disease. Recent studies have demonstrated that garlic possesses several biologically significant qualities, such as antibacterial, anti-inflammatory, cardiovascular protective, antidiabetic, antioxidant, and anticancer effects.[10] Garlic's therapeutic properties may be linked to allicin's potent and pungent odor.[11] In addition to allicin, garlic has more than 100 beneficial components, including p-coumaric acid, cysteine, zinc, calcium, thiamine, niacin, beta-carotene, beta-sitosterol, folate, ferolic acid, oleanolic acid, geraniol, vitamin C, magnesium, manganese, selenium, and quercetin.[12]

Vitamin E is a powerful antioxidant found in plants. Vitamin E is the collective term for tocopherols and tocotrienols, which are further divided into alpha-, beta-, gamma-, and delta-isomers based on where the side chains are located on the chromanol ring.[13] As well as the antioxidant properties, vitamin E also appears to have a variety of functions, such as controlling the activity of monocytes, stopping platelet aggregation, inhibiting the proliferation of smooth muscle cells, and altering gene expression.[14]

The current study set out to determine the toxicological effects of lead acetate on male rabbit reproductive systems and how, after 6 and 12 weeks of use, selenium nanoparticles coated with garlic extract could lessen these negative effects.

Materials and methods

Animal ethics and care

All of the animals used in this study have been handled by national and international standards for animal welfare. The mature male rabbits, weighing between 1500 and 1800 g, were brought into the animal house environment before the beginning of the study; a room with a 12:12 light/dark ratio, a temperature of 22 ± 2 ˚C, and proper ventilation. The rabbits were given ad libitum access to water and regular diets.

Preparation of lead acetate solution

One liter of distilled water was used to dissolve thirty grams of LA powder (Thomas Baker, India) (30 mg/mL). The male rabbits were orally supplemented with 30 mg of LA/mL/kg of body weight (BW) three times a week for 12 weeks.[15]

Preparation of ethanolic extract of garlic

Using Soxhlet, 50 grams of garlic and absolute ethanol were used to prepare the ethanolic extract of garlic, at 65˚C. After extraction, the extract was then transformed into a ceramic container that was placed in an electric oven set at 60˚C. The extract was kept cold and in a dark container until use.[16,17]

Preparation of TPGS-S

To prepare the solution, 10 mg of D-α-Tocopherol polyethylene glycol 1000 succinate (CAS NO: 10102-18-8 and CAS NO: 9002-96-4, Sigma Aldrich, Darmstadt, Germany) was first liquefied at ˚C50 and then dissolved in 500 ml of distilled water. To create a 40 mM solution, 346 mg of sodium selenite (CAS NO: 10102-18-8, Sigma Aldrich, Darmstadt, Germany) was dissolved in 50 mL of distilled water. Then 1 and 5 mM solutions were created by dilution. To prepare TPGS-S, 5 mL of sodium selenite solution and 5 mL of TPGS solution are mixed under a magnetic stirrer at 600 rpm.[18]

Preparation of the reducing agent (ascorbic acid)

Ascorbic acid (1,32 g) was dissolved in 25 mL of distilled water to make a fresh 0.3 M solution. Ascorbic acid solutions should always be prepared fresh since exposure to air or light causes them to oxidize to dehydroascorbic acid.[19]

Preparation of TPGS-GSNP

Using thin-film rehydration method,[20] TPGS-GSNP was synthesized. NPs were synthesized at TPGS-S: GE ratio of 100:1 to 1:1 by mixing the stock solution of 10% (w/v) of GE with each TPGS-S solution concentration (0.01–1.00% w/v) as required, and evaporated at 50˚C. A stock solution of 10% (w/v)

GE was first prepared by dissolving 10 mg of GE in 100 ml ethanol. To make a final volume of 25 mL, 10 mL of deionized distilled water was added to 10 mL of prepared TPGS-S solution, and then 5 mL of ascorbic acid was added drop by drop while mixing at half speed. Throughout the 24-hour reaction, the solution gradually changed from colorless to a deep orange-red, ensuring consistently sized nano-sized NPs in a high yield. All operations were conducted at room temperature. [18,21]

Characterization of TPGS-GSNP properties

The synthesized TPGS-GSNP properties were characterized using the following techniques, [22,23] scanning electron microscopy (SEM-Tescan Vega III, Czech Republic); ultraviolet–visible spectroscopy (Metertech SP-8001 Taiwan), X-ray diffraction as described by (Shemadzu-6000 Japan); and infrared spectroscopy using the Fourier transform (Shimadzu 8400s, Japan).

Experimental design

A total of 64 mature male rabbits were randomly allocated to 8 equal groups (8 each). The males were gavaged three times a week for 12 weeks with distilled water, as a negative control group (G1), 30 mg of lead acetate (LA)/kg of body weight, [15] as a positive control (G2), 800 mg of GE/kg BW [21] (G3), a combination of GE and LA (G4), 1 mg of TPGS-S/kg BW (G5), a combination of TPGS-S and LA (G6), 1 mg of TPGS-GSNP/kg BW (G7), and a combination of TPGS-GSNP and LA (G8). Following treatment, male rabbits of each group were weighed. Blood samples were withdrawn using a cardiac puncture technique, and serum levels of LH, FSH, T, and GnRH were assessed. After anaesthetization and euthanization, the genital organs were dislocated. The relative weights (g/100 BW) of the testes, epididymis, prostate, and seminal vesicles were calculated. To analyze the gene expression levels of pituitary FSHβ and LHβ as well as testicular Inh-α and LHR, tissue samples from the pituitary and testicles were taken. Additionally, testicular sample tissues were collected and preserved in a neutral formalin buffer solution for histological examination and semi-quantitative analysis.

Assessment of serum hormonal levels

Rabbit LH, FSH, T, and GnRH ELISA kits (Sunlong company, China) were used for the assessment of serum LH, FSH, T, and GnRH concentrations. The assessment stages were according to the instructions provided by the manufacturer.

Gene expression analysis of pituitary FSHβ and LHβ, and testicular Inh-α and LHR

Using the AccuZol®total RNA Extraction solution (Bioneer, Korea), total RNA was extracted from pituitary and testicular tissues. cDNA was synthesized using the M-MLV reverse transcriptase kit (Bioneer, Korea) to produce cDNA from the extracted RNA. The procedure was performed according to the instructions provided by the manufacturer.

Quantitative real-time PCR

Using qRT-PCR, the analysis of pituitary FSHβ and LHβ, and testicular Inh-α, and LHR genes was carried out using procedures outlined by and normalized by the housekeeping gene GAPDH. [24] To analyze the qRT-PCR data, the fold changes were determined by evaluating the relative expression of GAPDH and target gene. [25]

Histological slide preparation of testis

After dissection, testes were fixed in a formalin buffer solution (10%), and microscopic slides were prepared at 5 μM of thickness and hematoxylin-eosin-stained according to Bilinska et al. [26]

Semi-quantitative analysis

Using such histological features in the histological sections, such as necrosis, cellular debris, and sperm density, the testis was evaluated semi-quantitatively. From each subgroup, three slides from eight rabbit's testis, with similar lesions were compared. The examination was done by three independent pathologists. Every characteristic was quantified using a random score: 0 represented absenteeism, 1 represented present in 0–25%, 2 represented present in 26–50%, 3 represented present in 51–75%, and 4 represented present in 76–100% of the lesion. A morphological study of the organs under investigation has been carried out in carefully selected lesions. [27]

A detailed description of the cellular component and structural features of the tissue was completed for each lesion that was being examined. To compare the experimental groups, a semi-quantitative analysis was then performed on the parameters. The morphological descriptions were subjected to the following standards: 1) the existence and degree of necrosis; 2) the existence and arrangement of cellular debris; and 3) the existence and arrangement of sperm. Three distinct thin slices were obtained, one from the midpoint of each organ, for the morphological examination. Finally, the distribution and existence

Table 1. Genital organ weights (g/ 100 g BW) in male rabbits treated with LA, GE, TPGS-S, and TPGS-GSNP.

Organ weight (g/100 g BW)	Groups							
	G1	G2	G3	G4	G5	G6	G7	G8
Testes	0.28 ± 0.024 B	0.11 ± 0.025 E	0.28 ± 0.027 B	0.19 ± 0.026 D	0.28 ± 0.021 B	0.18 ± 0.021 D	0.37 ± 0.027 A	0.23 ± 0.028 C
Epididymis	0.036 ± 0.003 B	0.019 ± 0.002 D	0.034 ± 0.003 B	0.025 ± 0.003 C	0.033 ± 0.003 B	0.026 ± 0.003 C	0.046 ± 0.003 A	0.034 ± 0.003 B
Prostate	0.063 ± 0.002 B	0.026 ± 0.003 D	0.068 ± 0.004 A	0.046 ± 0.003 C	0.061 ± 0.004 B	0.049 ± 0.003 C	0.068 ± 0.004 A	0.061 ± 0.004 B
Seminal vesicle	0.053 ± 0.003 B	0.022 ± 0.004 D	0.056 ± 0.004 AB	0.038 ± 0.004 C	0.052 ± 0.004 B	0.036 ± 0.003 C	0.059 ± 0.003 A	0.055 ± 0.003 AB

Male rabbits treated for 12 weeks with distilled water (G1), lead acetate; LA (G2), garlic extract; GE (G3), GE and LA (G4), tocopherol polyethylene glycol succinate-selenium; TPGS-S (G5), TPGS-S and LA (G6), TPGS coated garlic selenium nanoparticles; TPGS-GSNP (G7), and TPGS-GSNP and LA (G8).

The values were mean ± SE and represented the result of 8 observations. One-way analysis of variance (ANOVA-1) and the Newman-Keuls test were employed to analyze the values. Significant difference ($p < 0.05$) is shown by the different letters.

of sperm were used as a representation of the level of sperm density.

The findings were categorized into each category, as absent (score 0; absence of any of the parameters under consideration), scarcely present (score 1; found in 0–25% of lesions), low present (score 2; found in 26–50% of lesions), moderately present (score 3; parameter found in 51–75% of lesions), intensely present (score 4; parameter found in 76–100% of lesions). Then, the means and standard errors were compared statistically.

Statistical analysis

The results were presented as mean ± standard error (M ± SE). The data were compared by one-way analysis of variance (ANOVA 1), followed by the Newman- Keuls test to find out the significant differences between groups ($p < 0.05$). The statistical analysis was performed using GraphPad Prism V5 (USA).[28]

Results and discussion

Relative organs weights

The effects of GE, TPGS-S, and TPGS-GSNP alone or in combination with LA on genital organ weight (testes, epididymis, seminal vesicle, and prostate) in male rabbits are summarized in Table 1. The weights of all organs decreased significantly ($p < 0.05$) in rabbits receiving LA alone (G2) and significantly increased ($p < 0.05$) in rabbits receiving TPGS-GSNP alone (G7 group). Male rabbits in the G8 group showed no significant differences ($p > 0.05$) compared with the control (G1 group), but they had greater organ weights ($p < 0.05$) than those in the other LA-treated groups (G2, G4, and G6).

Serum concentrations of GnRH, FSH, LH, and testosterone

After 12 weeks of treatment, the serum concentrations of GnRH, FSH, LH, and testosterone in male rabbits treated with LA (G2) were considerably lower ($p < 0.05$) than in the other experimental groups. TPGS-GSNP-treated male rabbits (G7) recorded the highest levels ($p < 0.05$) among experimental groups Fig. 1. When comparing the male rabbits receiving LA (G2) and those receiving GE (G4), TPGS-S (G6), and TPGS-GSNP (G8) with each other, the levels of G8 male rabbits were higher ($p < 0.05$) among them. Hormonal levels were significantly higher ($p < 0.05$) in non-toxic male rabbits receiving GE (G3) and TPGS-S (G5) in comparison with control Fig. 1.

Molecular analysis

Pituitary gene expression levels

Fig. 2 shows that male rabbits treated with LA (G2 group) had the lowest expression levels ($p < 0.05$) of the pituitary FSHβ and LHβ genes, while non-toxic male rabbits treated with TPGS-GSNP (G7 group) had the greatest levels. The levels of male rabbits receiving a combination of TPGS-GSNP with LA (G8 group) were significantly higher ($p < 0.05$) than those receiving LA alone (G2) or those receiving a combination of GE with LA (G4) or TPGS-S with LA (G6).

Testicular gene expression levels

Fig. 3 demonstrates that non-toxic male rabbits treated with TPGS-GSNP (G7 group) had the highest levels ($p < 0.05$) of testicular FSHR, LHR, ABP, 3β-HSD, 17β-HSD, and inh-α gene expression, while male rabbits treated with LA (G2 group) had the lowest levels ($p < 0.05$). The male rabbits treated with TPGS-GSNP along with LA (G8 group) exhibited the

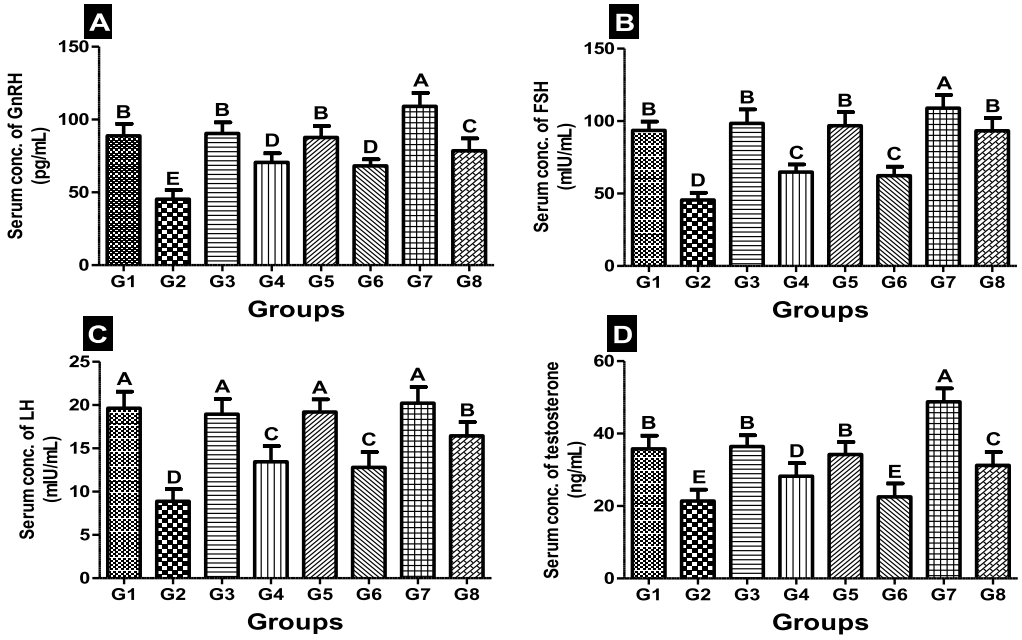

Fig. 1. Serum concentration of GnRH (A), FSH (B), LH (C), and testosterone (D) in male rabbits treated with LA, GE, TPGS-S, and TPGS-GSNP.

Male rabbits treated for 12 weeks with distilled water (G1), lead acetate; LA (G2), garlic extract; GE (G3), GE and LA (G4), tocopherol polyethylene glycol succinate-selenium; TPGS-S (G5), TPGS-S and LA (G6), TPGS coated garlic selenium nanoparticles; TPGS-GSNP (G7), and TPGS-GSNP and LA (G8).

The values were mean ± SE and represented the result of 8 observations. One-way analysis of variance (ANOVA-1) and the Newman-Keuls test were employed to analyze the values. Significant difference ($p < 0.05$) is shown by the different letters.

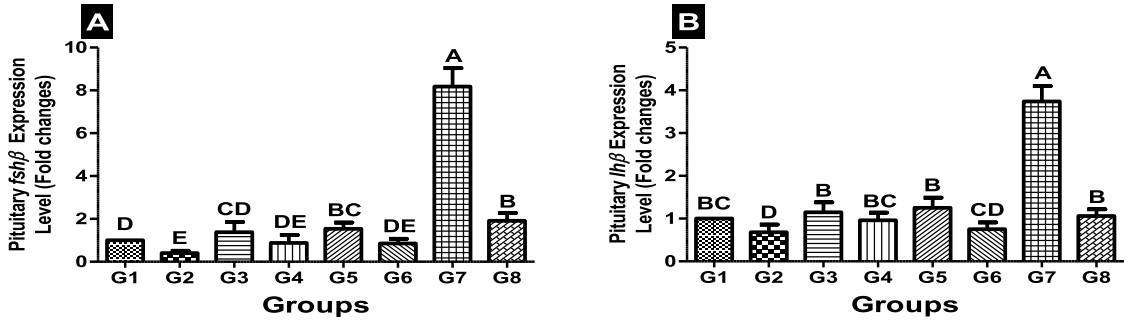

Fig. 2. Pituitary expression level (fold changes) of FSHβ (A) and LHβ (B) genes in male rabbits treated with LA, GE, TPGS-S, and TPGS-GSNP.

Male rabbits treated for 12 weeks with distilled water (G1), lead acetate; LA (G2), garlic extract; GE (G3), GE and LA (G4), tocopherol polyethylene glycol succinate-selenium; TPGS-S (G5), TPGS-S and LA (G6), TPGS coated garlic selenium nanoparticles; TPGS-GSNP (G7), and TPGS-GSNP and LA (G8).

The values were mean ± SE and represented the result of 8 observations. One-way analysis of variance (ANOVA-1) and the Newman-Keuls test were employed to analyze the values. Significant difference ($p < 0.05$) is shown by the different letters.

highest expression levels ($p < 0.05$) in comparison to the other LA-toxic male rabbits (G4 and G6 groups), treated with GE and TPGS-S, respectively.

Semi-quantitative analysis

When comparing the experimental groups, Table 2 showed significant differences in the scores for each histopathological feature, such as necrosis, the presence of cellular debris, and sperm density inside the seminiferous tubules, when testicular sections were subjected to a semi-quantitative analysis. In the LA-induced toxic groups (T2, T4, T6, and T8), the characteristics of the testes were significantly different ($p < 0.05$) than those of the corresponding non-toxic groups (T1, T3, T5, and T7). When the LA-toxic groups were compared to one another,

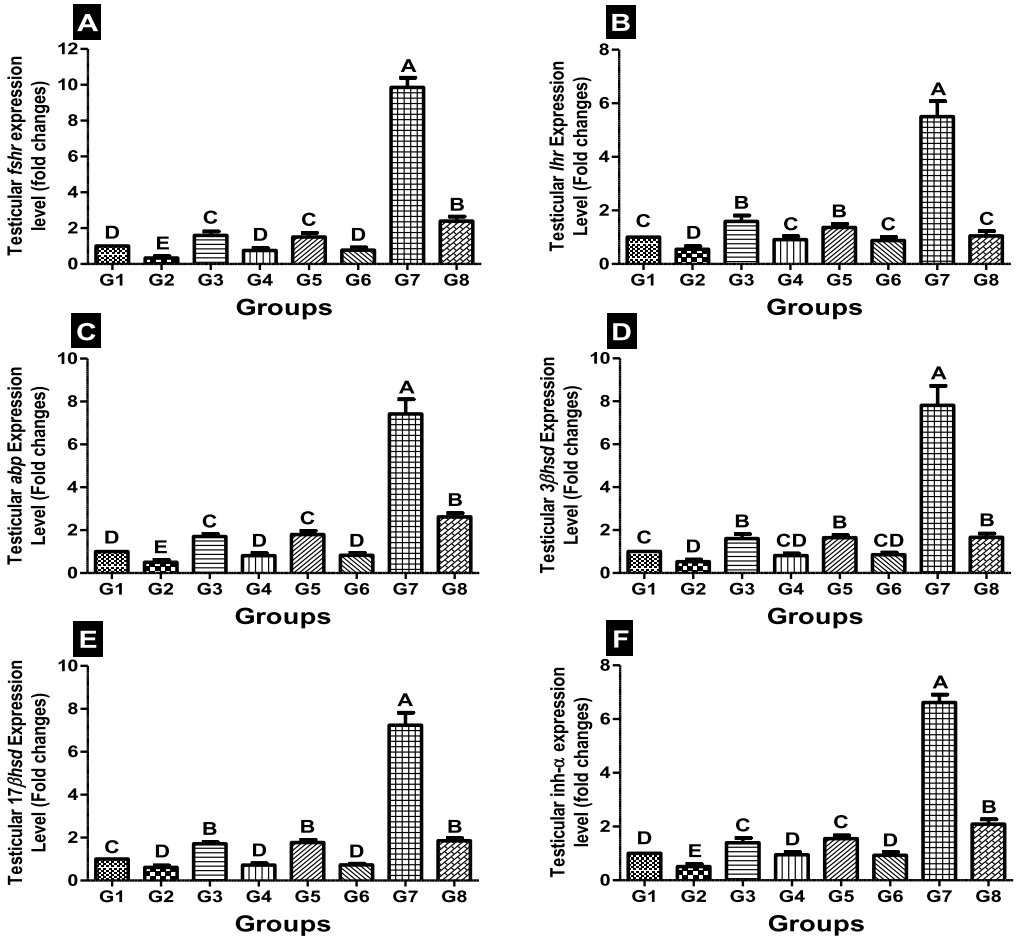

Fig. 3. Testicular expression level (fold changes) of FSHR (A), LHR (B), ABP (C), 3β-HSD (D), 17β-HSD (E), and Inh-α (F) genes in male rabbits treated with LA, GE, TPGS-S, and TPGS-GSNP.

Male rabbits treated for 12 weeks with distilled water (G1), lead acetate; LA (G2), garlic extract; GE (G3), GE and LA (G4), tocopherol polyethylene glycol succinate-selenium; TPGS-S (G5), TPGS-S and LA (G6), TPGS coated garlic selenium nanoparticles; TPGS-GSNP (G7), and TPGS-GSNP and LA (G8).

The values were mean ± SE and represented the result of 8 observations. One-way analysis of variance (ANOVA-1) and the Newman-Keuls test were employed to analyze the values. Significant difference ($p < 0.05$) is shown by the different letters.

Table 2. Semi-quantitative analysis (score) of testis histopathological lesions.

	Groups							
Lesion	G1	G2	G3	G4	G5	G6	G7	G8
Necrosis	0.46 ± 0.58 D	3.56 ± 0.53 A	0.49 ± 0.58 D	2.93 ± 1.16 B	0.51 ± 0.58 D	3.07 ± 1.25 B	0.47 ± 0.53 D	1.65 ± 1.09 C
Cellular debris	0.33 ± 0.48 D	3.61 ± 0.57 A	0.38 ± 0.49 D	2.61 ± 0.76 B	0.35 ± 0.48 D	2.65 ± 0.83 B	0.31 ± 0.46 D	1.54 ± 0.80 C
Sperm density	3.08 ± 0.77 B	0.56 ± 0.58 E	3.15 ± 0.69 B	1.38 ± 0.66 D	3.11 ± 1.23 B	1.24 ± 0.68 D	3.71 ± 0.46 A	2.65 ± 1.08 C

Male rabbits treated for 12 weeks with distilled water (G1), lead acetate; LA (G2), garlic extract; GE (G3), GE and LA (G4), tocopherol polyethylene glycol succinate-selenium; TPGS-S (G5), TPGS-S and LA (G6), TPGS coated garlic selenium nanoparticles; TPGS-GSNP (G7), and TPGS-GSNP and LA (G8).

The values were mean ± SE and represented scores of 72 observations (3 slides × 3 pathologists × 8 animals). One Way Analysis of Variance and the Newman-Keuls test were employed to analyze the values. Significant difference ($p < 0.05$) is shown by the different letters.

the LA-treated group (T8) had the highest score ($p < 0.05$) of necrosis and cellular debris and lower score ($p < 0.05$) of sperm density, while those treated with TPGS-GSNP, alone (G7 group) or in combination with LA (G8 group) had the lowest scores of necrosis and cellular debris, and highest score of sperm density, in comparison with the corresponding non-toxic groups (G1, G3, and G5 groups)

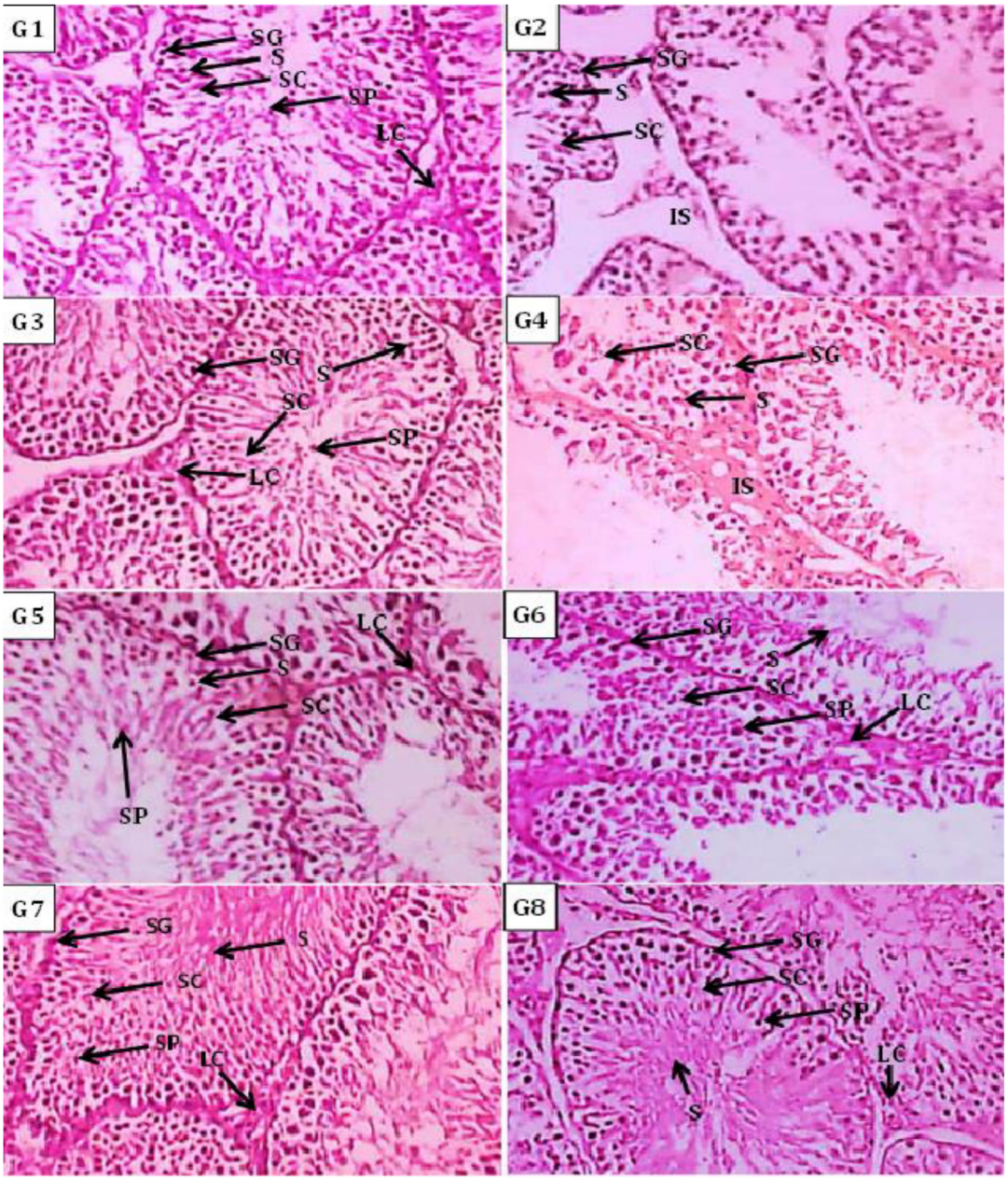

Fig. 4. Micrographs (H&E, 400x) from the testes of male rabbits after 12 weeks of treatment with distilled water (G1), lead acetate (G2), garlic extract (G3), combination of GE and LA (G4), tocopherol polyethylene glycol-succinate-selenium (G5), combination of TPGS-S and LA (G6), TPGS-GSNP (G7), and combination of TPGS-GSNP and LA (G8). G1, G3, and G5 groups showed normal seminiferous tubules (ST) with germinal epithelium (GEp) and the ST contained sperms in the lumen, whereas G7 group showed a high number of sperms inside the ST and improved GEp. G2, G4, and G6 showed an increase of interstitial space(IS), severe destruction of ST, loss of some germinal epithelium, different degrees of necrosis, and no sperm in the lumen of the ST, whereas G8 group showed improved spermatogenic cycle and sperm-filled ST. SG: spermatogonium, S: spermatocyte, SC: Sertoli cell, SP: sperm, LC: Leydig cell.

and LA-Toxic groups (G2, G4, and G6 groups), respectively.

Histopathological changes

Testes histological findings of G1, G3, G5, and G7 male rabbit testes Fig. 4 dominated the entirely typical structure of the germinal epithelium in the seminiferous tubules and interstitial tissues. It should be noted that G7 males were the most active in terms of active spermatogenesis and consisted of a regular arrangement of all forms of germ cells and Sertoli cells. The histological sections of testes from the LA treated male rabbits (G2, G4, and G6) revealed a

decline of germinal epithelial cell number and layers, as well as spermatogenesis arrest, as most of the seminiferous tubules showed germ cell disorganization or exfoliation and increased vacuoles inside the germ epithelium Fig. 4. An increased interstitial space while vascular obstruction was also observed in most LA treated testicular tissue sections. In male rabbits receiving TPGS-GSNP along with LA (G8 group), the testis structural architecture was significantly improved compared to other LA treated male rabbits (G2, G4, and G6 group), as a substantial reduction in the degree of germ epithelium degeneration of seminiferous tubules was observed in G8 group compared with G2, G4, and G6 group. Moreover, in addition to the observed active spermatogenesis and regular arrangement of all forms of germ cells and Sertoli cells, all seminiferous tubules were filled with a significant number of sperms.

Discussion

This study investigates the adverse impacts of lead acetate exposure in animal models as well as any potential benefits that TPGS-GSNP may have for male fertility.

The genital organ weights of the LA receiving group in the current investigation decreased. This outcome is comparable to that reported by Albarakati et al.[29] and Ibrahim et al.[30] Reactive oxygen species (ROS) and down-regulation of antioxidants caused by lead acetate may be the cause of this, resulting in oxidative stress and inflammatory reactions. Testicular dysfunction and infertility are caused by lead, a toxin that is harmful to the male reproductive system.[31] Therefore, testicular dysfunction due to lead toxicity may be related to lead's ability to induce membrane lipid peroxidation, which promotes oxidative stress and cell death.

In contrast to the lead acetate and control groups, our findings indicated that the TPGS-GSNP with LA that used a combination of garlic extract and nano-selenium had higher genital organ weights. According to Abdel-Wareth et al.,[32] selenium supplementation improves body weight gain in growing rabbits and minimizes the negative effects of free radicals due to its antioxidant properties, intestinal mucosa protection, and improved absorption capacity. Additionally, by lowering lead levels in tissues and neutralizing reactive oxygen species, the lysine and allicin content of GE has antioxidant qualities, as its actions should be either through increasing free radical scavenging activity or inducing endogenous antioxidant production. These findings align with the findings of Thompson et al.[33]

In the current investigation, male rabbits chronically exposed to lead acetate showed reduced serum levels of GnRH, FSH, LH, and testosterone. The impact of lead poisoning on the body's tissues, particularly the neurological system and the hypothalamus in particular, can be responsible for this decline, by disruption of neurological discharge.[34] The drop in serum GnRH levels could be attributed to this effect. Reduced blood levels of FSH and LH are caused by a decrease in GnRH secretion from the hypothalamus, as GnRH stimulates gonadotrophs, one of the secretory cells in the glandular pituitary, to release these hormones.[24] According to Sokol et al.,[34] lead enhances the expression of GnRH mRNA in the hypothalamus, but it also inhibits GnRH release from nerve terminals in the mediastinal eminence, which in turn reduces GnRH secretion. The rise may result from adaptation to the hypothalamic toxicity of lead and from the fact that molecular alterations in GnRH production do not cause increases in the levels of GnRH or LH in circulation.

According to the current findings, lead toxicity had a central effect on the hypothalamus and pituitary gland, as shown by a decline in GnRH, FSH, and LH levels, and a peripheral effect on the reproductive organs, as shown by a decrease in testosterone secretion. The observed decrease could potentially be attributed to two possible causes: either a direct effect on the testicles, including Leydig cells, as indicated by a decrease in the expression levels of testicular FSHR, LHR, ABP, 3β-HSD, 17β-HSD, and inh-α genes, or a decrease in pituitary FSHβ and LHβ gene expression levels. These findings coincide with those of Al-Wahab et al.[35] and Owumi et al.,[36] who revealed that mice given lead acetate had lower testosterone levels. Lead exposure disrupts the hypothalamic-pituitary-gonadal axis, causing an imbalance in hormones that lowers GnRH, FSH, and LH levels and impairs spermatogenesis, which lowers sperm count.

The present study looked at how lead acetate affected the mRNA expression of FSHβ in the pituitary, and the results indicated that FSHβ and FSH typically interacted in the pituitaries of rabbits given lead acetate. On the other hand, the amount of pituitary LHβ transcripts was lowered in male rabbits administered lead acetate, which decreased the pituitary's capacity to synthesis LH. The expression of the LHR gene was also reduced in the testicles, indicating that a limited amount of LH pulsed via the LHR to the Leydig cells in the testis, inhibiting the generation of testosterone. These results align with those of Al-Wahab et al.[35]

The male rabbits in our study who received TPGS-GSNP alone or in conjunction with LA showed significant improvements in GnRH, FSH, LH, and

testosterone levels in addition to expression levels of pituitary FSHβ and LHβ, and testicular FSHR, LHR, ABP, 3β-HSD, 17β-HSD, and inh-α genes. This proves the ameliorating role of TPGS-GSNP on the neurological level, which improves GnRH production and release and consequently risees FSH and LH secretions. According to Soleimanzadeh et al.,[36] male mice treated with lead acetate in addition to *Allium sativum* extract had significant blood levels of FSH, LH, and testosterone restored to control levels. These findings are consistent with our findings. Furthermore, the elevated expression level of testicular inh-α subunit in groups treated with TPGS-GSNP may indicate elevated inhibin synthesis as a result of enhanced Sertoli cell activity. Conversely, the decline in the expression level of the inh-α subunit in male rabbits treated with lead acetate was consistent with the findings published by Monsees et al.[37] The physiologic actions of LH and FSH, respectively, in gonadal tissue are mediated via testicular membrane receptors.[38] The majority of LHR expression occurs in the testicular Leydig cells, where it stimulates the manufacture of androgens in response to ligand contact.[39]

The significant testicular lesions caused by lead acetate that were observed were consistent with findings reported by M.I. Abdrabou et al.,[40] who noticed that rats exposed to lead had testes that showed clear signs of testicular degeneration, defective spermatogenesis, and vacuolation of the epithelial lining in addition to interstitial oedema. This suggests that lead can have cytotoxic effects on both Sertoli cells and spermatogenic cells. Asadpour et al.,[41] who found that Se-NPs administration decreases the degeneration in germinal epithelium of seminiferous tubules in male mice. Therefore, it can be suggested that the current alterations were reversed by the application of TPGS-G-SE via its antioxidant and free radicals scavenging properties which can protect the spermatogenic cells from oxidative stress and lipid peroxidation.[42]

Conclusion

This study highlights the repro-prophylactic potential of TPGS-GSNP against LA toxicity, and the efficacy of GE, TPGS-S, and TPGS conjugated with TPGS-GSNP in reducing LA toxicity in adult male rabbits. Our study's primary conclusion showed that the activity of the synthesized nanoparticles was higher than that of GE and TPGS-S. These benefits imply that TPGS-GSNP may be a promising therapeutic approach for LA-induced toxicity. Though more study is needed to fully understand the underlying mechanisms of TPGS-GSNP's activity, as well as to determine the drug's effectiveness in human and other animal models, these findings suggest the potential medical uses of TPGS-GSNP.

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Authors' declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images that are not ours have been included with the necessary permission for republication, which is attached to the manuscript.
- No human studies are present in the manuscript.
- The author has signed an animal welfare statement.
- Ethical Clearance: The project was approved by the local ethical committee at the University of Al-Qadisiyah.

Authors' contribution statement

The authors have contributed to writing, designing, compiling and editing the manuscript. Conceptualization, J. Al. and M. A.; Methodology, M. Y. and J. Al.; Validation, M. Y., M. A., and J. Al.; Analysis, J. Al.; Investigation, M. Y.; Data curation, J. Al.; Writing-original draft preparation, M. Y.; Writing-review and editing, J. Al. and M. A.; Supervision, J. Al. and M. A.

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تأثير الجزيئات النانوية المصنعة من التوكوفيرول كلايكول – ساكسينيت المغلف للثوم والسلينيوم الوقائي لتكاثر ذكور الأرانب المستحث فيها التسمم بالرصاص.

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الخلاصة

بسبب النشاطات الصناعية والبشرية ، يعد الرصاص من المعادن الثقيلة الخطرة الموجودة على نطاق واسع في البيئة، ويشكل تهديدا للصحة العامة والنظم البيئية. يظهر مستخلص الثوم أهمية وقائية ضد العديد من الاضطرابات الوظيفية، وعليه تتطلب دراسة تأثيره ضد سمية المعادن الثقيلة المزيد من البحوث. بإمكان الجسيمات النانوية المصنعة الحاوية على مركبات المستخلص أن تكون أكثر فعالية. تضمنت الدراسة الحالية التحري عن دور الجسيمات النانوية المصنعة من التوكوفيرول كلايكول –ساكسينيت المغلف للثوم والسلينيوم (TPGS-GSNP) لوقاية الجهاز التكاثري من التسمم بالرصاص. لتحقيق أهداف الدراسة، تم توزيع 64 أرنبا ذكرا ناضجا بالتساوي على ثمان مجموعات. عوملت الذكور ثلاث مرات اسبوعيا ولمدة 12 اسبوع. عوملت المجموعات بالماء المقطر (G1)، 30 ملغم من خلات الرصاص/كغم من وزن الجسم (G2)، 800 ملغم من خلاصة الثوم/ كغم من وزن الجسم (G3)، خلات الرصاص وخلاصة الثوم معا (G4)، 22.13 ملغم من مركب TPGS مع السلينيوم (G5)، خلات الرصاص و مركب TPGS مع السلينيوم (G6)، 22.13 ملغم من الجسيمات النانوية TPGS-GSNP/ كغم من وزن الجسم (G7)، خلات الرصاص و الجسيمات النانوية TPGS-GSNP (G8). بعد انتهاء المعاملة، تم قياس مستوى الهرمون المحفز للجريب والهرمون المصفر و التسيوستيرون و الهرمون المحرر لمحرضات القند. تم تقييم المعطيات النسجية المرضية للخصى و مستوى تعبير جينات FSHβ و LHβ في النخامية وجينات مستقبلات FSH و LH و جينات ABP و 3β-HSD و 17β-HSD و Inh-α في الخصى. أظهرت المجموعة المعاملة بخلات الرصاص تغيرات نسجية مرضية واضحة مع انخفاض ملحوظ في مستوى الهرمونات ومستوى تعبير الجينات للنخامية والخصى. أما المعاملة بالجسيمات النانوية TPGS-GSNP مع أو بدون خلات الرصاص، فقد أظهرت تحسنا ملحوظا. يستنتج أن للجسيمات النانوية TPGS-GSNP المصنعة تأثيرا كبيرا ضد آثار التسمم بالرصاص على الجهاز التكاثري المشتحث في ذكور الأرانب الناضجة. الموضوع يتطلب المزيد من البحوث لاستكشاف آلية عمل الجسيمات النانوية TPGS-GSNP.

الكلمات المفتاحية: خلات الرصاص، الخصى، الجسيمات النانوية، التكاثر، السلينيوم، توكوفيرول، التسمم.