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Effect of graphene nanoparticles on the histological structure of seminal vesicles of male albino mice

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Abstract

Graphene nanoparticles (GNPs) have drawn a lot of attention recently for their potential use in cutting-edge technology because of their outstanding physical characteristics. Graphene materials' nanotoxicity, on the other hand, has quickly grown to be a significant issue, particularly in the area of occupational health. Information on the toxicity of GNPs on the male reproductive system accessory gland is lacking. Consequently, this work aims to ascertain how Graphene nanoparticles affected the structure of seminal vesicles in male albino mice. Mice were given 0.1 ml oral gavages containing 10 mg/kg, 20 mg/kg, and 30 mg/kg of GNPs for 14 days. After the end of treatment, seminal vesicles were collected for histological processing. When compared to the controller group, the findings revealed a drop in the mean body weight and seminal vesicle index weight, as well as a reduction in the folded and lined epithelium of seminal vesicles height. As the concentration increased, damage to the seminal vesicles was observed, including a reduction in interstitial tissue, a reduction in fold height, blood vessel congestion, cell shrinkage, and cell destruction. In conclusion, the finding of this study determined that oral GNPs gavages at different concentrations have a detrimental impact on the activity of the male reproductive system.

Keywords: Graphene nanoparticles, seminal vesicles, Nanoparticles, histopathology.

تأثير الجسيمات النانوية للكرافين في التركيب النسجي للحويصلات المنوية في ذكور الفئران البيض

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الخلاصة

جذبت جسيمات الكرافين النانوية (GNPs) الكثير من الاهتمام مؤخرًا لاستخدامها المحتمل في التكنولوجيا المتطورة بسبب خصائصها الفيزيائية المتميزة. من ناحية أخرى، تطورت السمية لمواد الكرافين النانوية بسرعة لتصبح مشكلة كبيرة، خاصة في مجال الصحة المهنية. المعلومات عن سمية جسيمات الكرافين النانوية على الغدة الملحقة في الجهاز التناسلي الذكري تكون قليلة. وبالتالي، فإن الهدف من هذا العمل هو التأكد من كيفية تأثير جسيمات الكرافين النانوية على البنية النسجية للحويصلات المنوية في ذكور

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الفئران البيض. أعطيت الفئران 0.1 مل عن طريق الفم التراكيز 10 ملغم / كغم، و 20 ملغم / كغم، و 30 ملغم / كغم من النانوغرافين لمدة 14 يومًا. بعد انتهاء المعاملة، تم جمع عينات الحويصلات المنوية من اجل التحضيرات النسيجية. عند مقارنتها بالمجموعة المتحكمة، كشفت النتائج عن انخفاض في متوسط وزن الجسم ووزن مؤشر الحويصلة المنوية بالإضافة إلى انخفاض في ارتفاع ظهارة الطياة المبطنة للحويصلات المنوية. مع زيادة التركيز، لوحظ تضرر الحويصلات المنوية، بما في ذلك انخفاض في النسج الخلالية، وانخفاض في ارتفاع الطية، واحتقان الأوعية الدموية، وانكماش الخلايا وتحطم الخلايا. في الختام، توصلت نتائج هذه الدراسة إلى أن التراكيز المختلفة من النانوكرافين المعطى عن طريق الفم احدثت تأثيرات ضارة على نشاط الجهاز التناسلي الذكري.

Introduction

A mother carbon atom that separates from crystalline graphite is called graphene (GNPs). GNPs are composed of a two-dimensional structure, a nanosized, single- atom thick sheet with a hexagonally organized arrangement in the form of a honeycomb lattice [1, 2]. Along with its use in electronics, catalysis and energy storage, graphene has drawn a lot of attention to its possible application in biomedicine [3, 4]. Reduced graphene oxide (rGO) and graphene oxide (GO) are the most widely utilized forms of graphene because of their high water solubility and availability of functional groups. Recently, rGO and GO have been used in biological sensing [5, 6], bioimaging [7], drug delivery [8,9], and cancer photothermal therapy [9,10]. The toxicity of nano-graphene has drawn more interest due to the potential *in vivo* biomedical applications of this carbon-based nanomaterial (such as cancer phototherapy) [11,12]. Numerous investigations have been conducted to estimate the *in vivo* and *in vitro* cyto- and biocompatibility of graphene nanomaterials [13, 14]. These findings indicate that the intricate interaction of several physiochemical properties, such as size, shape, functional groups, synthesis methods, oxidative state, dispersion state, route and dose of administration, and exposure durations, determines the toxicity of nanographene [14, 15].

GNPs can be administrated through intratracheal instillation [16], orally [17], intravenously [18], intraperitoneally [19], or subcutaneously [20]. Nanographene can cause acute and chronic tissue damage by breaching several barriers, such as the blood-air, blood-brain, blood-testis, and blood placenta, as well as accumulating in organs such as the lung, spleen, liver, or reproductive organs. Intratracheally administered graphene was mostly retained in the lung, with 47% still present after 4 weeks, causing pulmonary edema and acute lung damage that was dose-dependent. However, these effects faded with time despite the persistence of graphene [21]. Furthermore, it has been found that intratracheally administered nanographene was redistributed to the spleen and liver across the air-blood barrier. Nanomaterials triggered the formation of intracellular reactive oxygen species (ROS) based on the cellular absorption, intracellular reaction, and releases of intracellular metal ions [22, 23]. Cells are adversely affected by ROS, which also leads to mitochondrial malfunction [24]. ROS harms biological macromolecules , such as proteins, lipids, and DNA. High ROS levels can also cause a variety of physiopathological consequences, including genotoxicity, apoptosis, inflammation, hypertrophy, necrosis, fibrosis, and even cancer [25]. Numerous studies have demonstrated that, depending on the dose and duration of exposure, exposure to graphene material may result in the production of reactive oxygen species (ROS), which may lead to histopathological damage in a variety of organs [14, 26]. Research has demonstrated that oral gavages of 60 mg/kg nanographene for 7 or 9 days to male albino mice led to liver injury, a drop in body weight, and an elevation in blood GOT enzyme levels [27]. Whereas, Al-kazomy *et al.* [28] reported that oral administration of 0.1 ml from different concentrations (10, 20, and 30 mg/kg) of nanographene for 14 days caused reductions in the weight of body and weight of some reproductive organs, declined concentration of sperm in

the epididymis tail and the proportion of live sperm, increased proportion of sperm abnormalities, and histopathological changes in the structure of testes and epididymis of the mice.

Most males of mammals have seminal vesicles, which are important accessory glands and are crucial to the male reproductive process [29]. Seminal vesicle secretion has been reported to be vital for enhancing the integrity of sperm chromatin and inhibiting immunological activity in the female reproductive tract [29]. Morgan *et al.* [30] revealed that oral administration of 100 mg/kg TiO2 NPs for 8 weeks decreased animal body weight and relative weights of sex organs (testes, epididymis, prostate, and seminal vesicle); decreased the percentage of sperm viable, motile, and concentrated sperm; increased the proportion of sperm aberrations; and induce histological changes in the testis (odema with sloughing of germinal epithelium, and apoptosis), epididymis (blood congestion, vacuolation and infiltration of inflammatory cell); seminal vesicle (widespread congestion); and prostate (inclusive spread congestion, hyperplasia and epithelial desquamation and edema) gland. According to a study by Taha [36], exposure to 100 mg/kg of Cu NPs for seven, 14, and 21 days resulted in foci of cell atrophy, degeneration of lining epithelium with pyknotic nuclei, and a diminution in the thickness of the surrounding layer of seminal vesicles. It also caused a decline in the weight of seminal vesicles and the levels of testosterone.

However, no information regarding the impact of graphene nanoparticle toxicity on the male seminal vesicle structure has been provided. Therefore, this study examines how GNPs affect these organs in male albino mice.

2. Materials and Methods

2.1 Chemical and Materials

Nano graphene powder was supplied from the American company Sky Spring Nanomaterials (Inc.) and had the following characteristics: purity of 99.5%, graphene sheets with a thickness of 6–8 nm, an average diameter of 15 nm, laminar shape, and black powder as well as the size of this material was 26 nm.

2.2 Design of Experiment

The current study employed twenty-four male albino mice (*Mus musculus*), with ages between eight and twelve weeks, and weighing between twenty-six and thirty-four grams. Animals were transferred to the animal house at the Microbiology Department of the College of Science in Alkarkh University of Science after being bought from the Ministry of Industry and Minerals, Industrial Research and Development Authority (Al-Razi center) in Baghdad, Iraq. All of the mice were fed with commercial pellets and given tap water. Animals were first kept in normal laboratory conditions of temperature, light, and air before being divided into four groups of six mice each. The first one was a control group that drank distilled water. The second, third, and fourth groups were orally administrated with 10, 20, and 30 mg/kg of nanographene for 14 days, respectively. The dosages of nanographene were chosen based on information from a previous mouse study (data not shown). The Alkarkh University of Science Ethics Committee of the College of Science, Iraq gave its approval to this study with approval No.3 on 2-10-2022.

2.3 Preparation of nano graphene.

GNPs solution was created using the same material powder that was used in a previous study [27]. Briefly, the GNPs were created in a stock solution. The different concentrations of GNPs (10, 20, and 30 mg/kg) were made by dissolving 150, 300, and 450 mg of nanographene in fifteen ml of distilled water, respectively. After the concentrations preparation, the solution mixture of nanographene was placed in the ultrasonic bath for 30 min. to mix and prevent the particles from clumping together. The particle morphology and

sizes were determined by Transmission electron microscopy (TEM). Energy Dispersive X-ray spectroscopy (EDX) was used to determine the purity of the powder. X-ray diffraction spectroscopy (XRD) was used to reveal the particles size.

2.4 Histology Preparation and Organ Index Recorded.

After the GNP treatment period of 14 days, the animals were weighed using an electronic balance before being sacrificed under anesthetic. The seminal vesicles were collected and weighed to calculate the organ index, as described in the equation below [32]:

Organ indexes (%) = weight of the organ(mg) /weight of the animal body(g)× 100.

The seminal vesicle was then fixed in a fixative solution (10% formalin) for histology preparation according to the method used in [33]. Samples of seminal vesicles were dehydrated in rising concentrations of ethyl alcohol, purified in xylene solution, and then embedded in paraffin wax. Finally, 5-7 μm thick sections were obtained using a rotary microtome, and then the sections were stained with hematoxylin Harris and Eosin (H&E). The light microscope was then used to examine and photograph the slides in order to study and calculate the histology structure and the biometric changes.

2.5 Biometric calculation

The biometric changes in the seminal vesicles section were calculated by using Image J version 1.53.

2.6 Statistical analysis

Version 24 of SPSS was performed for the statistical analysis. All data were written as Mean ± SE. One-way analysis of Variance (ANOVA) with the least significant difference (LSD) at a probability was achieved to analyze the differences between treatments groups. When the value of probability was less than 0.05, the value was deemed statistically significant Average mean ± standard error (SE) was used to represent the value.

3. Results and Discussion

3.1 Nano Graphene Characterization

The characterization of nano graphene was described in a previous study [27]. Briefly, the nanographene purity was detected using an Energy EDX device, which was found to contain carbon at 96.27% and oxygen at 3.73% (Figure 1A). An X-ray diffraction device was employed to confirm the particle size, which was 26 nm (Figure 1B). The laminar structure of nanographene was determined by using the TEM (Figure 1C). The outcomes matched corporate descriptions almost exactly as described by the company.

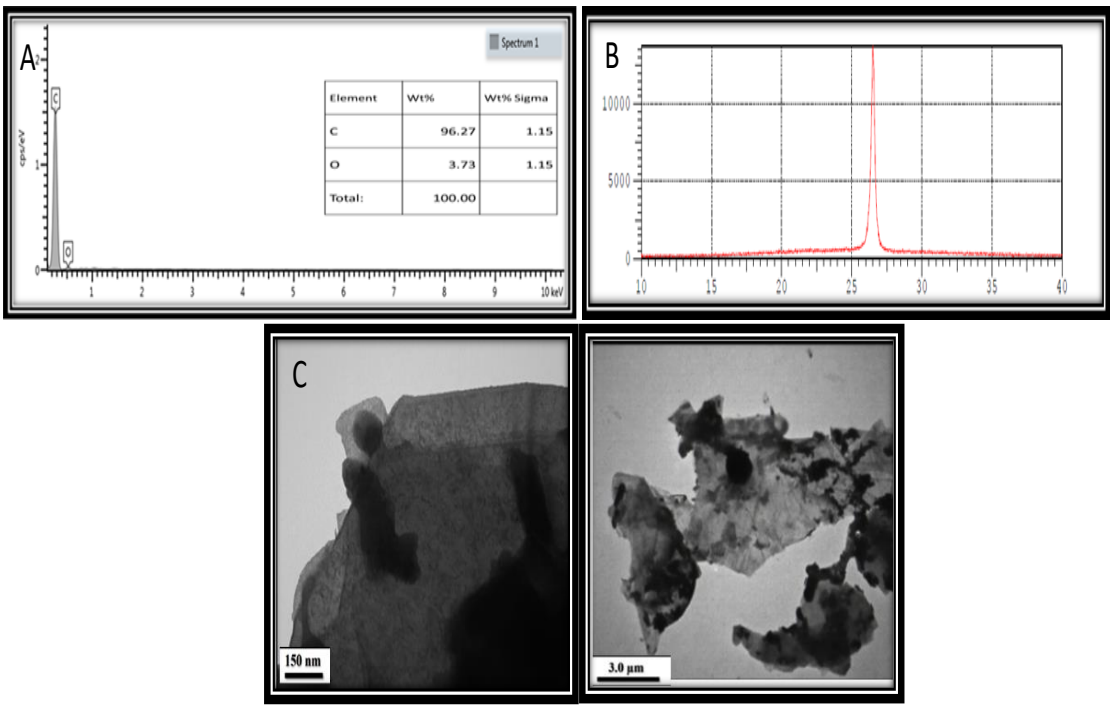

Figure 1: (A) Nanographene purity that was 96.27% of carbon and 3.73% of oxygen (impurities) by using an Energy Dispersive X-Ray Spectroscopy device. (B) The scale size of the nanographene was 26 nm by using XRD. (C) The laminar shape of nanographene was obtained using TEM [27].

3.2 Weights of Animal Body and Seminal Vesicles' Index.

The current results revealed a significant decrease ($P \leq 0.05$) in the average body weight and seminal vesicles weight index after 14 days of treated animals in all concentrations of nanographene (10, 20, and 30 mg/kg) compared to the control (Figures 2 and 3). Conversely, there were no statistical differences between the different concentrations of nanographene, as revealed in (Figures 2 and 3). The toxicity features, including loss of body weight and organs, made by nanographene have been reported by several studies [27,28]. Reduced body weight may be due to the accumulation of GNP in the stomach or intestine, which disrupts the process of absorption by having an adverse effect on the mucosal membrane of the gastrointestinal system and leads to an appetite reduction in animals. This finding is in line with the study of Tabish *et al.* [34], which reported that rats treated for 27 days with 5 and 15 mg/kg of GNPs resulted in a drop in body weight at both concentrations. At the same time, a study by [31] revealed that oral administration of 100, 200, 300, 400 and 600 mg ZnO NPs/kg bw/day (30 ±5 nm size of particle) for 10 weeks caused a decrease in the animal body weight that starting in the sixth week. The oral gavage of 0.1 ml of 0, 10, 20, and 30 GNPs mg/kg bw /day for 14 days in mice caused reduced body weight and certain reproductive organs (testes and epididymis) [28].

Reduced seminal vesicle weight in the current study may have contributed to lower testosterone levels, which is consistent with the findings of Sastry and Gupta [35], or it may have been caused by a histological lesion in these organs as a result of treatment with NPs [36].

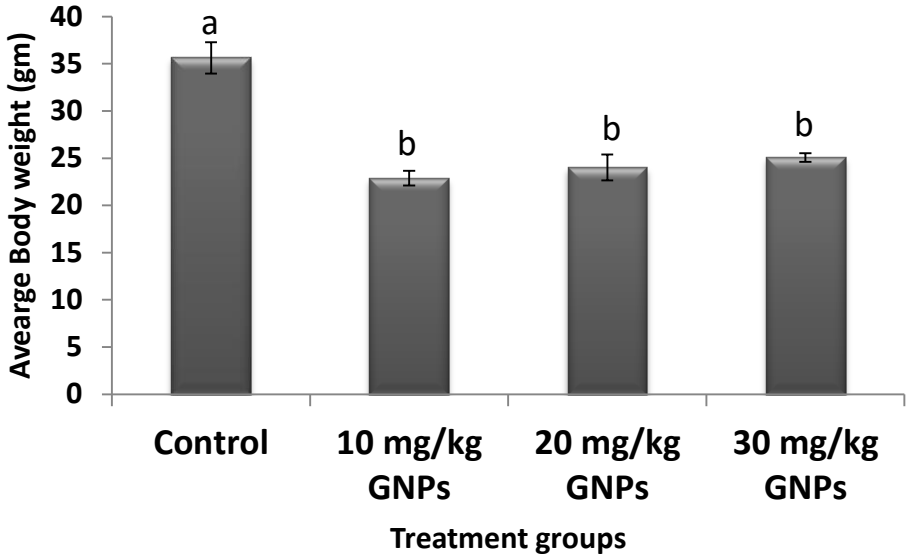

Figure 2: The body weight average of mice after fourteen days of oral administration of 10, 20, or 30 mg/kg of nanographene compared to the control group. Data represent the average mean ± SE, dissimilar small letters mean significant difference (P≤0.05) between treatments.

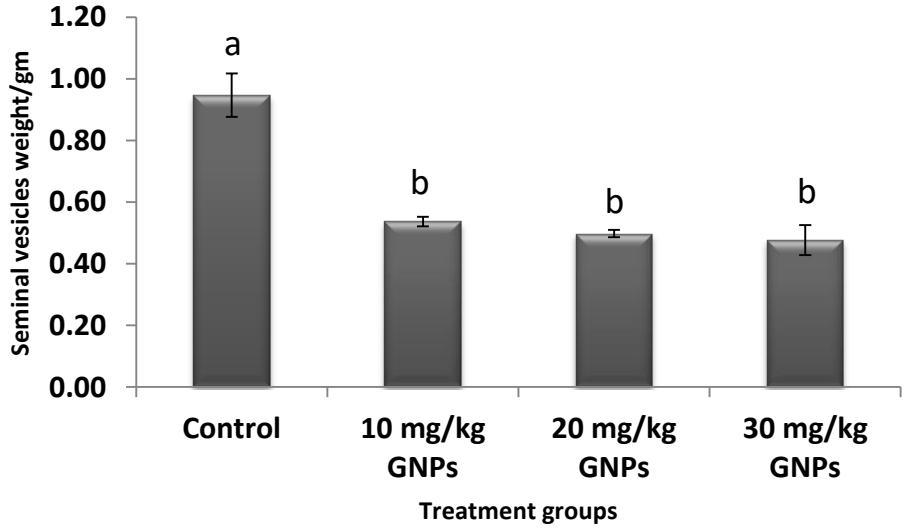

Figure 3: Seminal vesicles weights index of treated animals with different concentrations of nanographene for 14 days compared to the control group. Data represent the average mean ± SE, dissimilar small letters mean significant difference (P≤0.05) between treatments.

3.3 Histological changes in the seminal vesicle

Histological sections of the seminal vesicle from mice in control animals showed the normal structure of the seminal vesical folds that were lined with simple or pseudo- stratified columnar epithelium (Figure 4). The treated mice with nano graphene at different concentrations (10, 20 or 30 mg/kg) for fourteen days revealed changes in the lining epithelial layers, high folds, cell shrinkage and destruction, congestion of blood vessels, as well as decreased interstitial tissue between seminal vesicles (Figures 5, 6, and 7). Exposure to 100 mg/kg of Cu-NPs resulted in atrophy, epithelial cell degeneration with pyknotic nuclei, and a diminution in the thickness of the seminal vesicles surrounding the layer [34]. After 8 weeks of oral exposure, Morgan *et al.* [30] examined the histopathological changes caused by 100 mg/kg/day of TiO2NPs in the seminal vesicle gland such as congestion in this

vesicle. Al-Mashta and Al-Murshidi found that exposing rats to 40 and 80 µg/kg of Au NPs for 60 days induced epithelial shrinkage with necrosis and cytoplasm vacuolation as well as degeneration in the lining epithelial cell of seminal vesicles [37]. In contrast, another study found that the oral gavage of Cu NPs (10, 100, and 200 mg/kg) in mice for 70 days caused degenerative alteration in the seminal vesicle structure such as hyperplasia, atrophy and scant the secretary substance [41]. It has been revealed that NPs penetrate through the testicular and brain barriers with ease. In male animals, the seminal vesicle gland is one of the accessory glands that secrete around 60 % of the proteins, complex carbohydrates and fructose [38].

The seminal vesicle is extremely reliant on androgenic hormones, such as testosterone, to maintain its form and function, making it highly responsive to androgen levels in the blood. This agrees with Nishino *et al.* [39]. The researchers confirmed that testosterone plays a function in suppressing chemicals that alter the activity of the seminal vesicle, which is in agreement with Sastry and Gupta [35]. The decrease in testosterone hormone secretory activity of the leydig cells is the cause of the weight loss and structural degeneration of seminal vesicles. Al-kadomy found that fourteen days of exposure to 10, 20 or 30 mg/kg nanographene resulted in decreased levels of testosterone and other sex hormones [40].

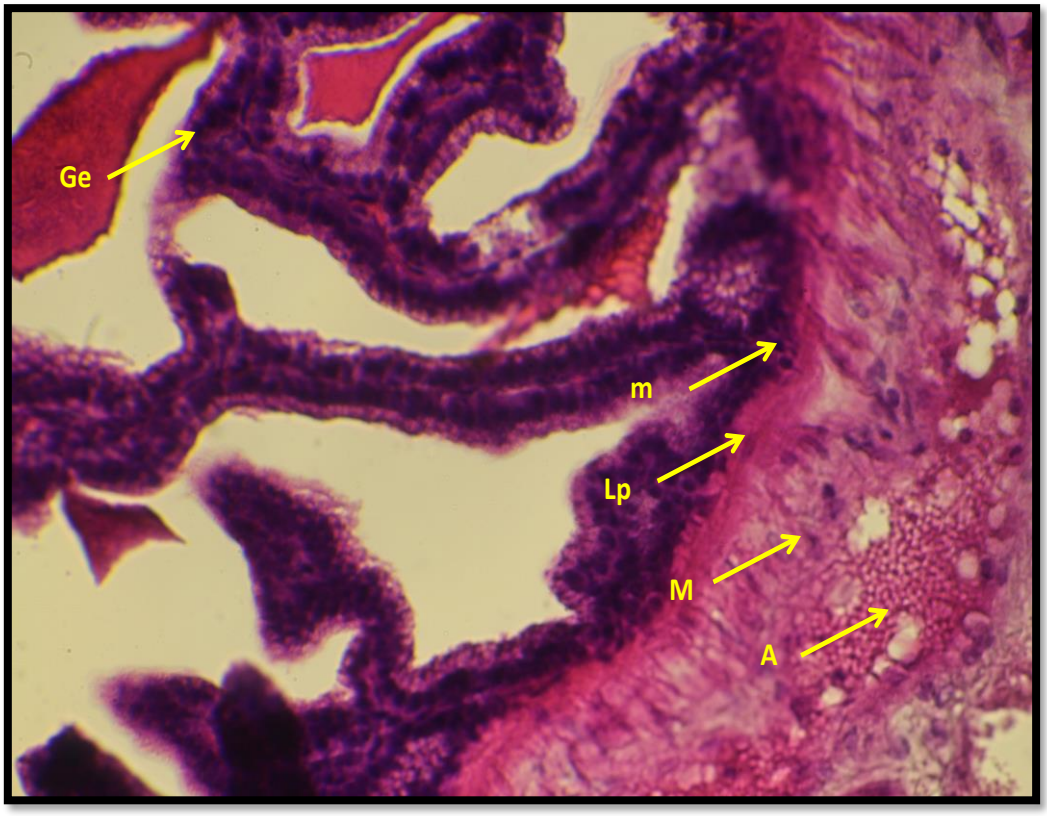

Figure 4: Cross sections of seminal vesicle in male albino mice of control animals showed normal structure of glandular epithelium (Ge); Mucosal layer (m); lamina propria (Lp); Muscular layer (M) and Adventitia (A). Hematoxylin and eosin (H&E) stain, X40.

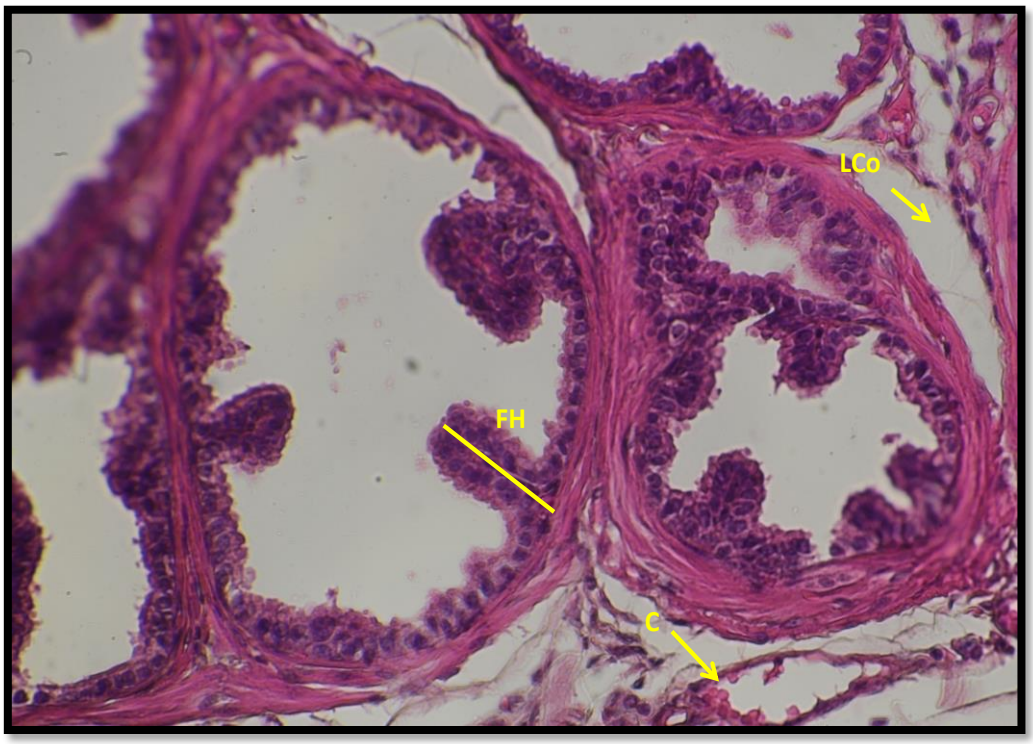

Figure 5: Cross sections of seminal vesicle in albino male mice administrated with 10 mg/kg of nanographene for 14 days displayed histological alteration, such as decreased interstitial tissue (connective tissue) (LCo), reduced fold height (FH), blood vessel congestion (C). Hematoxylin and eosin (H&E) stain, X40.

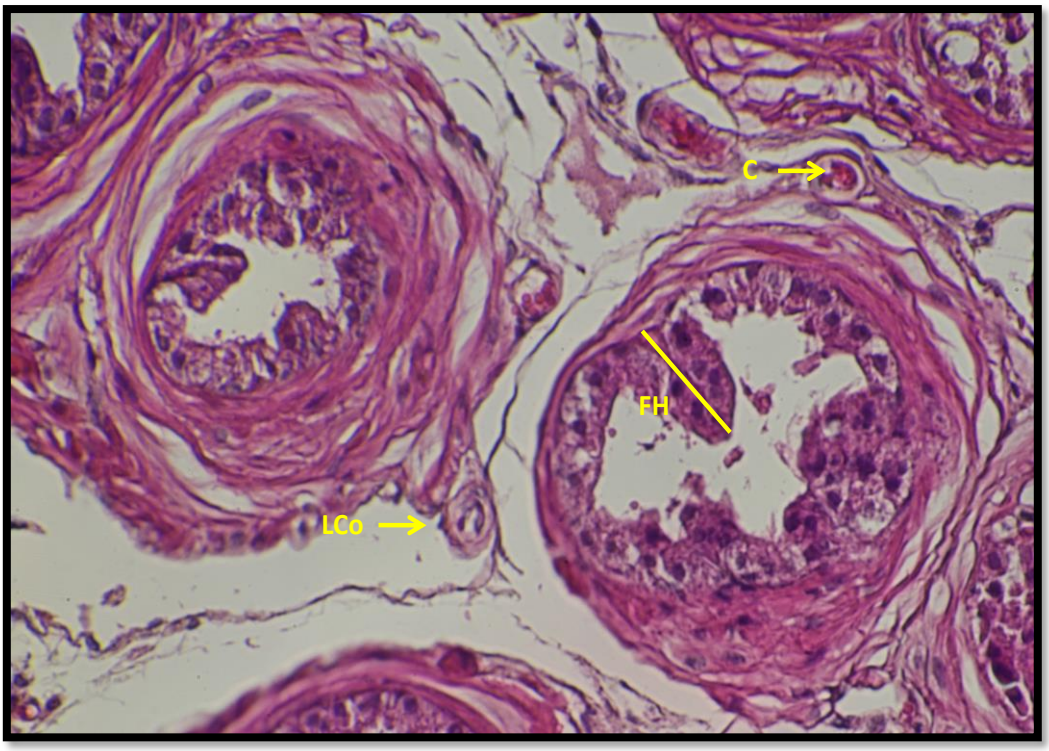

Figure 6: Cross sections of seminal vesicle in albino male mice administrated with 20 mg/kg of nanographene for 14 days displayed histological alteration, such as decreased interstitial tissue (connective tissue) (LCo), reduced fold height (FH), blood vessel congestion (C); Hematoxylin and eosin (H&E) stain, X40.

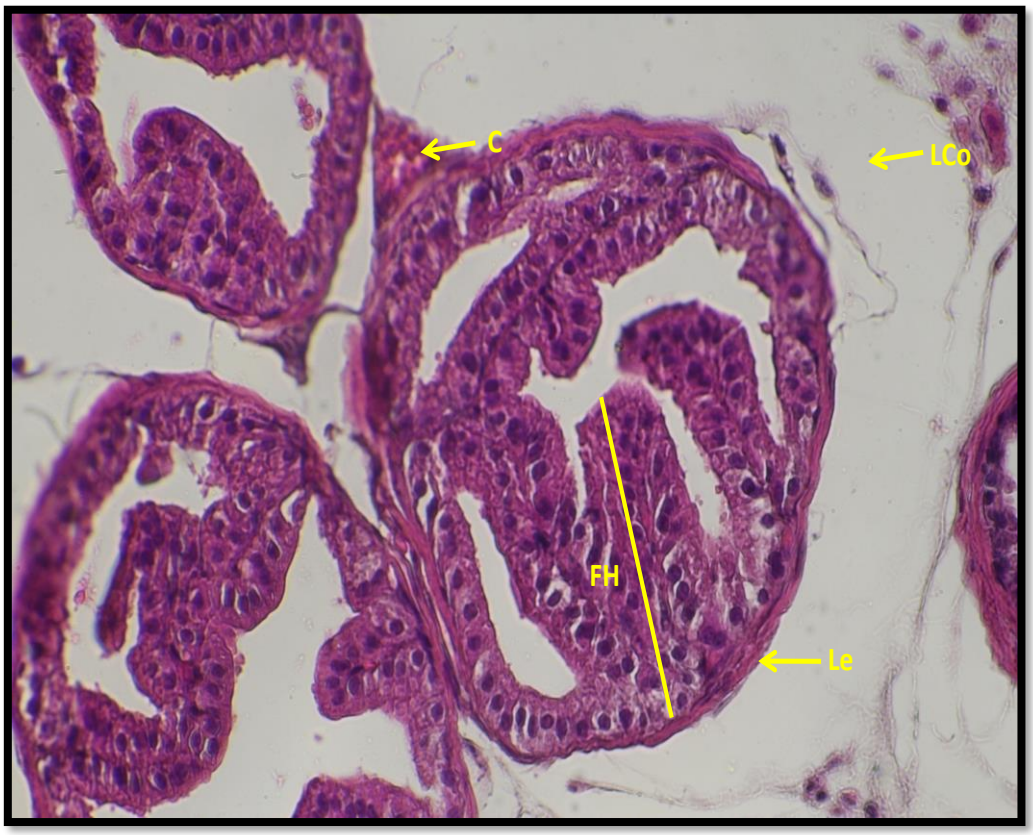

Figure 7: Cross sections of seminal vesicle in albino male mice administrated with 30 mg/kg of nanographene for 14 days displayed histological alteration, such as decreased interstitial tissue (connective tissue) (LCo), reduced fold height (FH), blood vessel congestion (C), a shorter cell in lining epithelium (Le) and damage to the seminal vesicle; Hematoxylin and eosin (H&E) stain, X40.

3.4 Biometric Changes

The height of folding and the height of lining epithelium of seminal vesicles treated groups with 10, 20, and 30 mg/kg of nanographene showed a significant decrease ($P \leq 0.05$) in comparison to the control, with a concentration effect (Table 1). In comparison, there were no differences between the control group and the treatment animals in terms of the diameter of the cell nuclei($P > 0.05$) (Table 1). The current finding agreed with a study by Taha, which found that exposure to 100 mg/kg of Cu-NPs resulted in a decrease in the lining epithelial cells height and the diameter of the epithelial cell nuclei, as well as a decrease in the thickness of the muscular fibrous layer that is surrounding the seminal vesicle gland [37].

Table 1: Biometric changes in the seminal vesicles of albino mice after fourteen days of exposure to nanographene at different concentrations (10, 20 and 30 mg/kg).

Treatments	N	duration of exposure (days)	Seminal vesicle (micrometer)		
			height of folded	height of lining epithelium	diameter of cell nuclei
Control	6	-	98.12 ± 17.07a	19.03±1.76a	3.67±0.21a
10 mg/kg GNPs	6	14	38.42 ± 3.40b	10.49±0.44b	3.30±0.17a
20 mg/kg GNPs	6	14	40.49 ± 6.12b	11.18±1.17b	3.63±0.28a
30 mg/kg GNPs	6	14	67.88 ± 6.81b*	13.29±1.12b	3.49±0.13a

Values represent means ± standard error, N represents the number of animals.

Similar vertical small letters reveal no significant change (P >0.05)
Vertically, different small letters reveal significant changes between treatments (P ≤0.05)
*Represents the presence of significant differences (P<0.05) between various concentrations

Conclusion

The current *in vivo* investigation using male albino mice has examined the toxicity of G NPs on seminal vesicles. GNPs were shown to affect seminal vesicle structure and function by reducing weight and altering histology. Therefore, GNPs have a detrimental impact on the activity and secretion of seminal vesicles.

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