Histological and DNA fragmentation impact of aluminum oxide nanoparticles in male albino rats and the role of fenugreek plant extract

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ABSTRACT

Background: Aluminum is an element that affects the functions of living organisms. It enters the body in several ways and has negative effects. Various nanoparticles are widely used in the field of industry and medical materials, especially metal oxides. Fenugreek (***T. foenum graecum***) is an herbal medicine that can reduce genotoxicity, the effects of inflammatory cells, and reduce oxidative stress because it contains beneficial chemical compounds, including sugars, saponins, flavonoids, fiber, choline, and trigonelline. However, their impact on public health is still not completely clear. **Objective:** Identify the extent of the effect of aluminum oxide nanoparticles in causing DNA- fragmentation in the male reproductive system and specific damage to testicular tissue and determine the effective preventive role of the action of the plant extract of fenugreek seeds in treating the condition and improving the results in all groups treated with aluminum oxide nanoparticles for two periods after the end of the period of dosing them with the nanoparticle, and then giving them aqueous extract of fenugreek seeds only for 14 days**. Methodology:** The study was conducted on 70 male albino rats divided into 14 groups including the control group, who took oral doses of the nanomaterial solution Al_2O_3 NPs in two different concentrations (70 mg/kg and 140 mg /kg) for two periods (21 days and 35 days) while the aqueous extract of fenugreek seeds was given in concentrations (2 gm/kg 4 gm /kg) for 14 days and the end of each experiment was followed by weighing the animals, blood sample of each animal was collected by heart puncture then directly centrifuged and the serum was kept at -80 ˚C for biochemical analysis and some histological standards, the animals were dissected then testes were excised and fixed in neutral buffered 10% formalin for histological preparation and evaluated the levels of DNA fragmentation by examining the Acridin Orange that take part in the Reproductive pathologies. **Results:** showed that Al_2O_3-NPs caused Testes sections to show seminiferous tubules with certain degeneration and necrosis of spermatogonia cells besides necrotic debris inside the lumen, no sperms appearing inside the lumen, as well as a significant increase ($P \leq 0.05$) in DNA fragmentation in sperms, fenugreek seed aqueous extract, on the other hand, improved testicular tissue and dramatically decreased DNA fragmentation. **Conclusions:** The current study concluded that Al_2O_3 caused through oral administration in high concentrations and for long terms causes tissue testicular damage and an increase the DNA fragmentation by generating oxidative stress and aqueous extract of fenugreek seeds succeeded in alleviating the harmful effects of Al_2O_3 and by curbing DNA-fragmentation.

Keywords: Aluminum , Antioxidants, Tests, fenugreek, DNA fragmentation .

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INTRODUCTION

Nanoparticles (NPs) are generally defined as particulate matter with sizes ranging from 1 to 100 nm. This broad class of materials can be classified into metallic and non-metallic nanoparticles. (1) The impact of NPs on health and the environment is still not completely understood. Aluminum is one of the most abundant elements on earth and is widely used in many consumer product applications due to its unique characteristics (2). Aluminum can enter the body through different ways, i.e., inhalation, consumption of food, and dermal contact with air, and water when the intake of Al exceeds the range of biological tolerance; it can cause adverse effects, including damage to the liver, kidney, reproductive system immune system, and gastrointestinal distress. Based on this and because the detection and quantification of Al in biological media is difficult, one should assume at least partial particle concentration in the body after oral uptake of Al. This raises questions about the potential hazard of particulate Al species. (3) Al accumulation in tissues and organs results in their dysfunction and toxicity (4). Studies conducted to determine the mechanisms of degeneration caused by aluminum oxide nanoparticles indicate that aluminum toxicity is caused by its production of reactive oxygen species and inducing cell membrane instability. (5) Although the knowledge of Al toxicity has markedly improved in recent years, information concerning the mechanism of male reproductive toxicity of this element is still minimal. Testes are small oval organs suspended within the scrotum, each weighing 10 to 12 grams in an adult human male; they produce sperm and secrete male sex hormones (Testosterone) (6). Reported that it can be concluded that Al causes reproductive dysfunction by creating oxidative damage. Al cytotoxicity may be mediated by free radicals derived from this element and its capability to induce apoptosis through a wide variety of mechanisms including the production of ROS, LPO, cell membrane damage, diminished activity of alkaline phosphatase, and cAMP reduction in various tissues(7). Thus, long-term exposure to high doses of aluminum has been shown to increase pro-oxidant activity and cause deterioration in the tissue of the testicle, the organ responsible for sperm production, thus affecting the process of sperm production and its quality. (8) Oxidative stress is a reaction to cell injury. Due to their larger surface area, nanoparticles have been found to produce more free radicals and reactive oxygen species. (9), Radical oxidative species (ROS) have an important effect on sperm quality and quantity. Oxidative stress (OS) occurs when the production of potentially destructive reactive oxygen species (ROS) exceeds the body's natural antioxidant defenses, resulting in cellular damage, Increased ROS generation and reduced antioxidant capacity are negatively correlated with sperm concentration and motility in males Sperm DNA fragmentation (SDF) refers to single- or double-stranded breaks in the spermatozoa genome. Due to the mature male gamete's inability to repair DNA damage (10) these breaks tend to persist and can hurt male reproductive potential and outcomes. Three primary mechanisms can cause SDF: (1) abortive apoptosis, in which spermatozoa destined for apoptosis fails to complete the process and are released with fragmented DNA due to the action of endonucleases; (2) defective chromatin maturation, in which DNA nicks are not repaired during the normal process of sperm chromatin compaction, resulting in persistent breaks as well as less compact DNA that is more susceptible to damage by exogen as spermatozoa move along the male reproductive tract (11).Damage to the sperm DNA can occur during passage through the testes .sperm Extrinsic factors (such as heat exposure, smoking, environmental pollutants, and chemotherapeutics) as well as intrinsic factors (such as defective germ cell maturation, abortive apoptosis, and oxidative stress [OS] can cause DNA fragmentation (SDF) (12) OS is a major contributor to male infertility, according to compelling evidence (13) ROS are required for physiological processes such as apoptosis and capacitation, but excessive production has several negative consequences, including SDF. Mismatch of bases, loss of base (abasic site), base modifications, DNA adducts and crosslinks, pyrimidine dimers, and single strand breaks (SSB) and double-strand breaks (DSB) (14). High levels of nano aluminum oxide that accumulate in testicular tissues after long-term exposure cause oxidative stress, which is a condition in which an imbalance in the production of free radicals occurs, and this permeation leads to increased production of reactive species of oxygen in tissues, damage to genetic material, and changes in protein and DNA fragmentation status, and thus a decrease in sperm quality. Aluminum levels in human testes, Leydig cells, spermatozoa, seminal plasma, and blood, have been linked to sperm quality and viability (15,16,17). Aluminum has also been linked to the formation of reactive nitrogen (RNS). Al-induced nitric oxide byproducts oxide (NO) could be a steroidogenesis inhibitory regulator. The functions of sperm NO is a type of free radical produced during the breakdown of food which has obvious harm to the male

reproductive system (18).Fenugreek is considered one of the most important types of natural medicinal plants and is rich in dietary fiber and many active phytochemicals (19) It is considered a herbal medicine with antioxidant properties. In a study (20) treatment with fenugreek showed lower levels of MDA And high SOD Of antioxidant enzymes, which in turn leads to testicular replacement and decreased apoptosis by improving testicular tissue. This study aims to clarify the role of aluminum oxide nanoparticles and the mechanisms of toxic exposure in the reproductive system, especially the testicles, by measuring antioxidant enzymes and histological features, and to demonstrate how the plant extract of fenugreek seeds works in treating damage and improving results.

METHODOLOGY

1- Animals

This study was carried out in the Biotechnology Center / Al-Nahrain University in the Department of Environmental Biotechnology / Baghdad, The seventy adult male albino rats weighing 180-200 g animals were obtained from the animal house at the University of Baghdad collected from, the animals were kept in clean cages with adequate ventilation a 12 hours light/dark cycle, and an average temperature of 22°C rates was provided with unfettered access to both tap water and normal rodent food throughout the experiment rats were given two weeks to get used to the experimental conditions before testing.

2- Materials

Nanopowder, white, solid Al_2O_3_NPs were obtained from (US. Research nonmaterial company) the properties of this product are: White powder in Appearance, Purity is 99.9% and the Particle size is (50 nm) in diameter, Energy Dispersive X-ray Spectroscopy (EDX) was used to verify the powder purity. the particle size was confirmed using X-ray diffraction (XRD) and the particle form was determined using Transmission Electron Microscopy (TEM). The stock aqueous extract of T. foenum graecum was prepared in two concentrations (2 gm/kg and 4 gm /kg) and dissolved in each concentration in (1000 ml D.W) and the animals were dosed orally (0.5 ml) once day.

3- Experiment design

Aluminum oxide nanoparticles (Al_2O_3-NPs) were suspended in 1 ml distilled water at a concentration of (70 mg/kg and 140 mg /kg) of body weight (21), Before being administered orally to rats, the suspensions were ultrasonically treated to avoid aggregation and to get the best size distribution for dispersed particles. Rats were randomly divided into 7 groups, each including five animals administered (0.5 ml)of each concentration of Al_2O_3 NPs (70 mg/kg and 140 mg /kg) once daily via a gastric tube for two periods 21 days and 35 days, Each period of exposure was divided into seven groups group 1: was the control group, group 2: received 70 mg/kg of Al_2O_3 NPs, group 3: 140 mg / kg of Al_2O_3 NPs, group 4: received 70 mg/kg Al_2O_3 NPs + 2 g of T. foenum graecum for 14 days, group 5: received 70 mg of Al_2O_3 NPs + 4 g of T. foenum graecum extract for 14 day, group 6 : received 140 mg/kg of Al_2O_3 NPs + 2 g of T. foenum graecum extract for 14 day , group 7: received 140 mg /kg of Al_2O_3 NPs + 4 g of T. foenum graecum extract for 14 day .

4- Histology Study

Fixed testes in neutral buffered 10% formalin were prepared according to the method used in a previous study (22). Samples were dehydrated through ethanol at a series of ascending concentrations and embedded in paraffin wax after clarifying in xylene. Sections (5 μm slices) were stained with hematoxylin and eosin (H&E). Slides were then examined and photographed by using the light microscope (22) to study the histological structure.

5- DNA fragmentation by Acridin orang assay

Two slides per subject were created after the sperm samples had been separated and liquefied. They were then left in the Carnoy solution for at least three hours. Rinsing, air-drying, and staining for five minutes with acridine orange solution (MERCK, USA) (10 ml) are all required after. 40 ml of 0.1 M citric acid and 2.5 ml of 0.3 M sodium hydroxide are combined with 1% AO in distilled water. Two of the stained-glass slides were then cleaned in distilled water and covered with coverslips while still damp. Following staining, the slides were examined using a 100-objective lens and immersion oil. Photographs were then shot using a fluorescent microscope (Leica DM 1000 Fluorescence Filter 13, Germany) and agitation of 450–490 nm. A total of 1000 sperm cells were counted for each subject (200 per swab). Sperm cell heads with normal (double-stranded) DNA fluoresced green, while those with denatured or single-stranded DNA chains fluoresced red or orange, respectively (together with the yellow-orange ones). (23).

6- Statistical analysis

The statistical analysis was conducted using SPSS (version 24). The information was displayed as mean ± standard error (SE). Variance analysis (two-way) ANOVA) was employed, with $P < 0.05$ considered significant. The difference between groups was represented as the least significant difference.

RESULTS

3-1 Histopathological effects of Al_2O_3 NPs. on reproductive organs

Histopathological changes were shown in the groups treated with aluminum oxide nanoparticles at two different concentrations (70 mg/kg and 140 mg/kg) at two periods (21 days and 35 days) to decrease the diameter of the seminiferous tubules in the microscopic field 40x Significantly ($P \leq 0.05$), The number of Leading cells deficiency significantly ($P \leq 0.05$) after the different doses of aluminum oxide, as well as with the length of the exposure period. There was also a decrease in the number of Sertoli cells after the single and double doses of Al_2O_3 NPs and in both periods compared with the control group, all of the above parameters were measured manually using (motic) an electron microscope with high resolution and contrast, the results better significantly ($P \leq 0.05$) when aqueous extract of fenugreek seeds was given according to two concentrations (2gm /kg and 4 gm/kg) for 14 days as the table shows (3-1) Figure (3-1,3-2,3-3,3-4).

Table (3-1): Histological Effects of Al_2O_3 on reproductive organs

Treatment	Time	Seminefrous Tubule diameter	Leading cell number	Sertoli cell numbers
Control	21&35	a 266.20 ± 18.80	a 30.90 ± 3.67	a 23.60 ± 1.70
70 mg Al_2O_3 NPs.	21	a 212.80 ± 5.91	a 20.20 ± 0.86	a 19.40 ± 1.03
70 mg Al_2O_3 NPs. + 2gm *T. foenum graecum*.	21	a 269.00 ± 5.72	a 29.20 ± 1.24	a 21.60 ± 1.17
70 mg Al_2O_3 NPs. + 4gm *T. foenum graecum*	21	b● 280.80 ± 6.39	b● 34.60 ± 1.81	b 24.80 ± 1.66
140 mg Al_2O_3 NPs	21	b 207.60 ± 5.11	a 20.80 ± 1.16	a 18.60± 0.93
140 mg Al_2O_3 NPs. + 2gm *T. foenum graecum*.	21	b 296.60 ± 5.30	a 26.80 ± 1.40	b● 24.60 ± 1.08
140 mg Al_2O NPs.$_3$+ 4gm *T. foenum graecum*.	21	b 301.40 ± 4.01	b● 36.00 ± 2.00	a 31.20 ± 1.36
70 mg Al_2O_3 NPs.	35	b◄ 212.40 ± 3.91	b◄ 21.60 ± 1.33	a 21.20 ± 0.97
70 mg Al_2O_3 NPs. + 2gm *T. foenum graecum*.	35	a● 272.00 ± 3.32	a● 27.00 ± 1.22	a 22.80 ± 0.58
70 mg Al_2O_3 NPs. + 4gm *T. foenum graecum*	35	b◄ 299.80± 6.99	a 32.80 ± 1.28	a 24.00 ± 0.71
140 mg Al_2O_3 NPs	35	b● 220.40 ± 2.73	b 21.60 ± 1.17	a◄ 20.40 ± 0.81
140 mg Al_2O_3 NPs. + 2gm *T. foenum graecum*.	35	b 302.60 ± 3.44	a 26.80 ± 1.02	a 22.00 ± 0.89
140 mg Al_2O_3 NPs. + 4gm *T. foenum graecum*.	35	b 318.00 ± 5.31	a● 36.40 ± 1.29	b● 29.20 ±0.66

-Data refers to mean ± S.E

-* It indicates that there is a significant difference in Different concentration of nanoparticles for the same period

-◄ indicates that there is a significant difference at the same concentration of nanomaterial for different periods.

- ●It indicates that there is a difference between the concentrations of nanoparticles and the concentrations of extracts

-Similar letters indicate that there is no significant difference between the concentrations of nanoparticles $P \leq 0.05$.

- Different letters indicate that there is a significant difference between the concentrations of the nanoparticles $P \leq 0.05$.

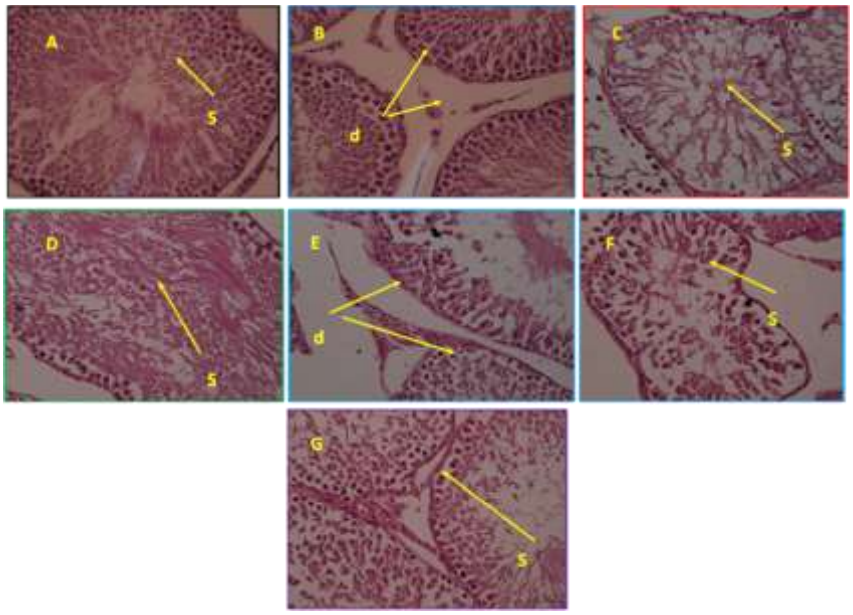

Figure (3-1): showed Testis histological structure in (A)control , (B) 70 mg/kg Al_2 O_3 NPS , (C) 70 mg/kg Al_2 O_3 NPS & 2 gm/kg T. foenum graecum, (D) 70 mg/kg Al_2 O_3 NPS & 4 gm/kg T. foenum graecum, (E) 140 mg/kg Al2 O3 NPS , (F) 140 mg/kg Al_2 O_3 NPS & 2 gm/kg T. foenum graecum, (G) 140 mg/kg Al_2 O_3 NPS & 4 mg T. foenum graecum for 21 days. normal histological structure of active mature functioning seminiferous tubules (S), marked degeneration of most seminiferous tubules with the absence of spermatogenic series in the tubular lumen (d). The arrow indicates mild disintegration of the seminiferous tubules with loss of spermatids and spermatozoa, marked degeneration (arrow) of most spermatocytes, spermatids, and congestion in the testis blood vessels (H&E) 40x.

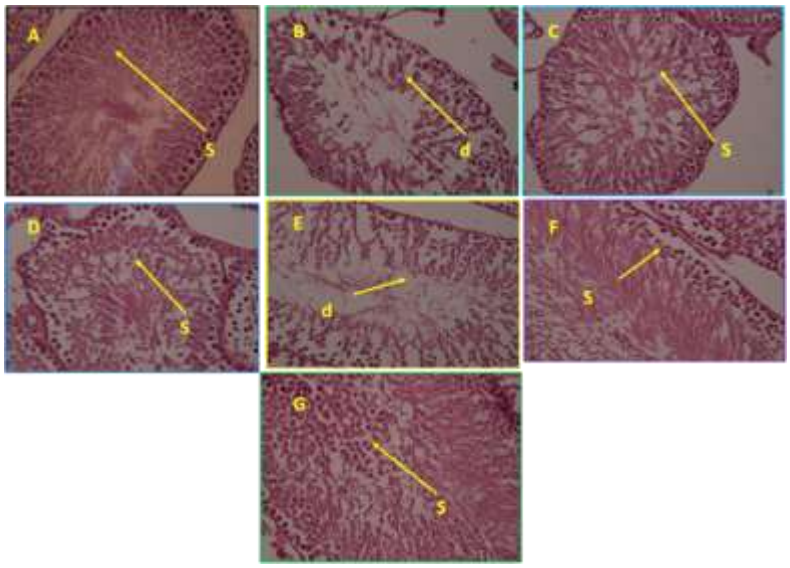

Figure (3-2): showed Testis histological structure in (A) control , (B) 70 mg/kg Al_2O_3 NPS , (C) 70 mg/kg Al_2O_3 NPS & 2 gm/kg T. foenum graecum, (D) 70 mg/kg Al_2O_3 NPS & 4 gm /kg T. foenum graecum, (E) 140 mg/kg Al_2O_3 NPS , (F) 140 mg/kg Al_2O_3 NPS & 2 gm/kg T. foenum graecum, (G) 140 mg/kg Al_2O_3 NPS & 4 gm T. foenum graecum for 35 days. normal histological structure of active mature functioning seminiferous tubules (S), marked degeneration of most seminiferous tubules with loose spermatogenic series in tubular lumen(d). The arrow indicates mild disintegration of the seminiferous tubules with loss of spermatids and spermatozoa, marked degeneration (arrow) of most spermatocytes, spermatids, and congestion in the testis blood vessels (H&E) 40 x.

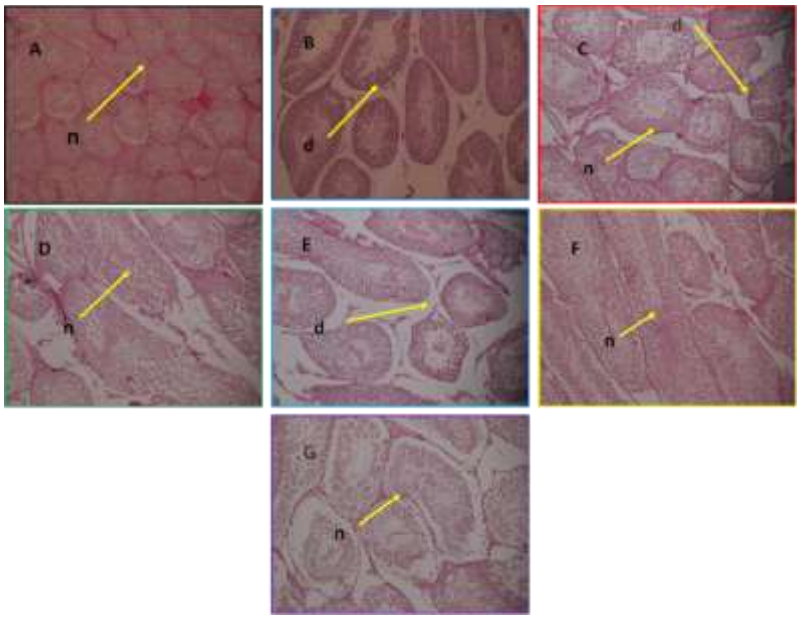

Figure (3-3): showed Testis histological structure in (A)control , (B) 70 mg/kg Al2 O3 NPS , (C) 70 mg/kg Al_2O_3 NPS & 2 gm/kg T. foenum graecum, (D) 70 mg/kg Al_2O_3 NPS & 4 gm/kg T. foenum graecum, (E) 140 mg/kg Al_2O_3 NPS, (F) 140 mg/kg Al_2O_3 NPS & 2 gm/kg T. foenum graecum, (G) 140 mg/kg Al_2O_3 NPS & 4 gm/kg T. foenum graecum for 21 days. normal histological structure of active mature functioning seminiferous tubules (n), marked degeneration of most seminiferous tubules with the absence of spermatogenic series in the tubular lumen, and the number of seminiferous tubules decreased (d) . The arrow indicates mild disintegration of the seminiferous tubules with loss of spermatids and spermatozoa, marked degeneration (arrow) of most spermatocytes, spermatids, and congestion in the testis blood vessels (H&E) 10 X.

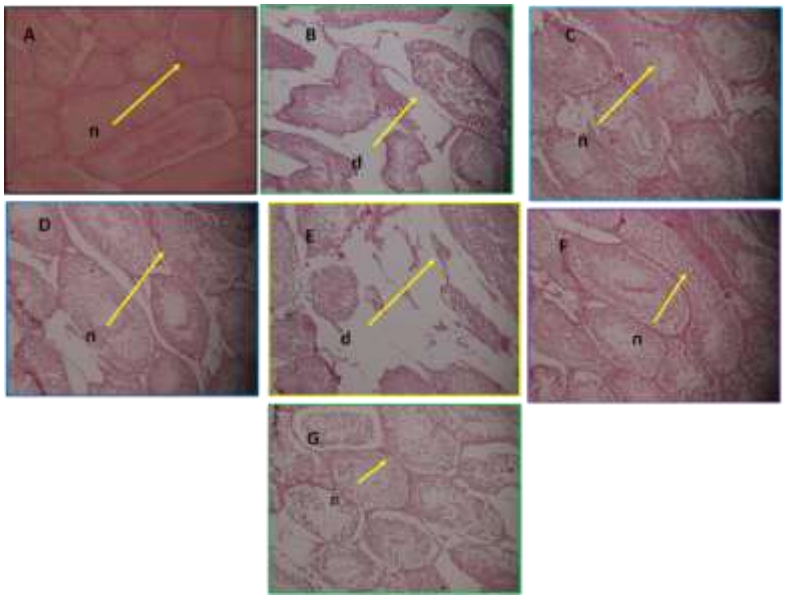

Figure (3-4): showed Testis histological structure in (A)control , (B) 70 mg/kg Al2 O3 NPS , (C) 70 mg/kg Al_2O_3 NPS & 2 gm/kg T. foenum graecum, (D) 70 mg/kg Al_2O_3 NPS & 4 gm/kg T. foenum graecum, (E) 140 mg/kg Al_2O_3 NPS, (F) 140 mg/kg Al_2O_3 NPS & 2 gm/kg T. foenum graecum, (G) 140 mg/kg Al_2O_3 NPS & 4 gm/kg T. foenum graecum for 35 days. normal histological structure of active mature functioning seminiferous tubules (n), marked degeneration of most seminiferous tubules with loose of spermatogenic series in tubular lumen and the number of seminiferous tubules decreased (d) . The arrow indicates mild disintegration of the seminiferous tubules with loss of spermatids and spermatozoa, marked degeneration (arrow) of most spermatocytes, spermatids, and congestion in the testis blood vessels (H&E) 10 x.

3-2 Effect of Aluminum oxide Al_2O_3 on DNA fragmentation in sperms:

The results of the statistical analysis recorded a significant increase ($P \leq 0.05$) in the number of degraded sperm during the 35 days when the concentration increased to(140 mg/kg) compared to the number of sperm degraded at a concentration(70 mg/kg) while there was a significant increase($P \leq 0.05$) at the concentration of (70 mg/kg) when the period was increased to 35 days compared to the period of 21 days after exposure to aluminum oxide nanoparticles and compared to the control group. When using the aqueous extract of fenugreek seeds (*T. foenum graecum*) in two concentrations(2,4 gm/kg) for 14 days the significant decrease ($P \geq 0.05$) at a concentration (70 mg/kg +2 gm/kg) and (70 mg/kg +4 gm/kg) at (21 days), With one exception, the increase significant ($P \leq 0.05$) at a concentration(140 mg/kg +2 gm/kg)and (140 mg/kg + 4 gm/kg) in the 35 days compared to the control group, (Table 3-2), Figure (3-5, 3-6).

Table (3-2): The effect of Al_2O_3 NPs. on DNA sperm fragmentation

Treatment	Time	Total degraded Sperm NO. M ± SE	Sperm NO. With frag. M ± SE
Control	21&35 days	a 221.7 ± 13.58	a 4.03 ± 0.040
70 mg Al_2O_3 NPs.	21	b 256.20 ± 14.62	b 22.54 ± 1.40
70 mg Al_2O_3 NPs. + 2gm *T. foenum graecum*.	21	a● 206.20 ± 7.074	b● 17.02 ± 1.08
70 mg Al_2O_3 NPs. + 4gm *T. foenum graecum*	21	a● 196.40 ± 6.19	b● 14.45 ± 0.58
140 mg Al_2O_3 NPs	21	b 254.40 ± 15.96	b 25.15 ± 1.31
140 mg Al_2O_3 NPs. + 2gm *T. foenum graecum*.	21	b 257.40 ± 15.11	b● 16.79 ± 0.76
140 mg Al_2O NPs.$_3$+ 4gm *T. foenum graecum*.	21	a 244.80 ± 9.74	b● 16.13 ± 0.89
70 mg Al_2O_3 NPs.	35	a◄ 193.60 ± 4.02	b◄ 28.87 ± 1.86
70 mg Al_2O_3 NPs. + 2gm *T. foenum graecum*.	35	a 216.00 ± 10.19	b● 17.09 ± 1.13
70 mg Al_2O_3 NPs. + 4gm *T. foenum graecum*	35	a 208.20 ± 5.04	b● 14.11 ± 0.86
140 mg Al_2O_3 NPs	35	a* 230.80 ± 10.83	b◄ 28.28 ± 1.29
140 mg Al_2O_3 NPs. + 2gm *T. foenum graecum*.	35	b● 293.80 ± 10.85	b● 13.98 ± 0.24
140 mg Al_2O NPs. + 4gm *T. foenum graecum*.	35	b● 297.40 ± 10.60	b● 13.18 ± 0.39

-Data refers to mean ± S.E

-* It indicates that there is a significant difference in Different concentrations of nanoparticles for the same period

-◄ indicates that there is a significant difference ($P \leq 0.05$) at the same concentration of Al_2O_3 NPs. for different periods.

- ●It indicates that there is a difference ($P \leq 0.05$) between the concentrations of Al_2O_3 NPs. and the concentrations of extracts

-Similar letters indicate that there is no significant difference between the concentrations of Al_2O_3 NPs. $P \geq 0.05$.

- Different letters indicate that there is a significant difference between the concentrations of the Al_2O_3 NPs. $P \leq 0.05$

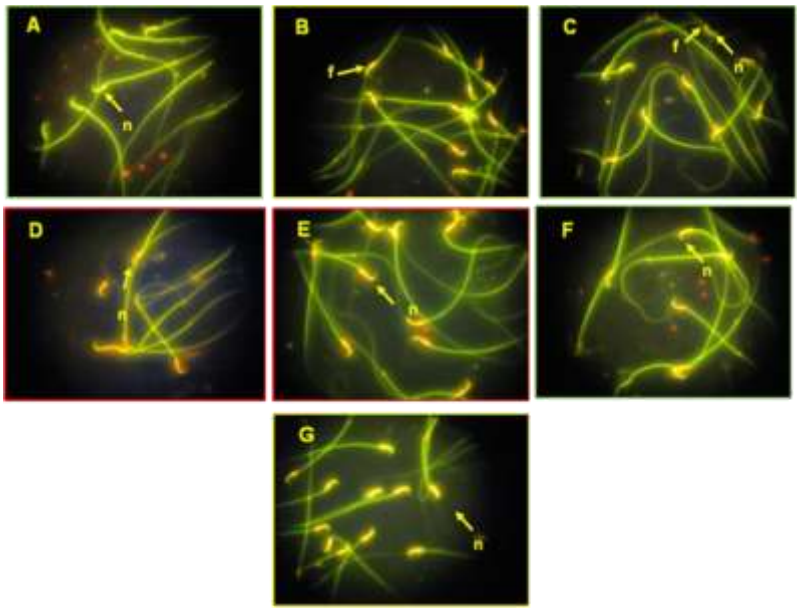

Figure (3-5): showed Sperm nuclear DNA fragmentation in (A) control , (B) 70 mg/kg Al_2O_3 NPs, (C) 70 mg/kg Al2 O3 NPS & 2 gm T. foenum graecum, (D) 70 mg/kg Al_2O_3 NPs & 4 gm T. foenum graecum, (E) 140 mg/kg Al_2O_3 NPs, (F) 140 mg/kg Al_2O_3 NPs & 2 gm T. foenum graecum, (G) 140 mg/kg Al_2O_3 NPs & 4 mg T. foenum graecumfor 21 days. Sperm heads with green fluorescence indicate intact DNA (n) while sperm heads with yellow and dark orange fluorescence indicate fragmented DNA (f). Sperm smears stained with freshly prepared acridine orange and viewed using a fluorescent microscope (oil immersion) and a 460 nm filter.

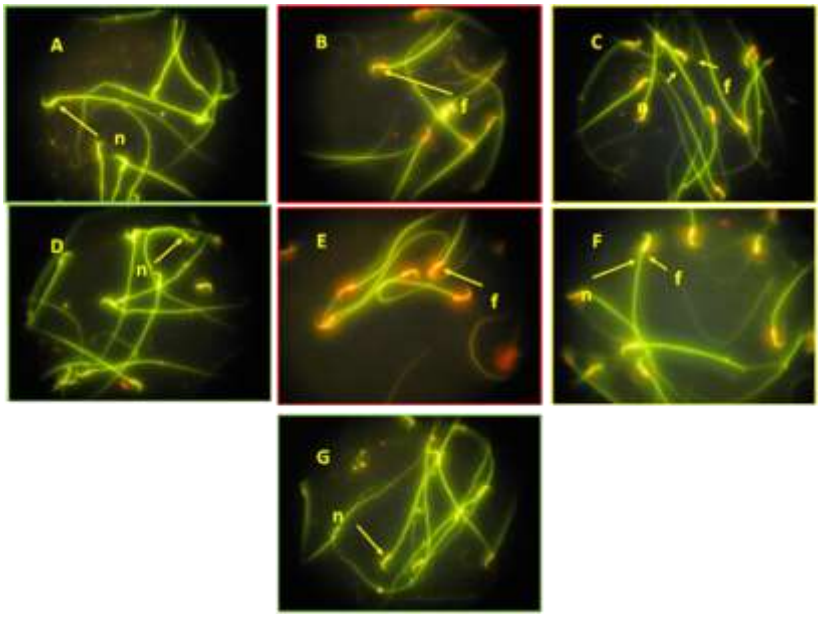

Figure (3-6): showed Sperm nuclear DNA fragmentation in (A) control , (B) 70 mg/kg Al_2O_3 NPs, (C) 70 mg/kg Al2O3 NPS & 2 gm T. foenum graecum, (D) 70 mg/kg Al_2O_3 NPs & 4 gm T. foenum graecum, (E) 140 mg/kg Al_2O_3 NPs, (F) 140 mg/kg Al_2O_3 NPs & 2 gm T. foenum graecum, (G) 140 mg/kg Al_2O_3 NPs & 4 mg T. foenum graecum for 35 days. Sperm heads with green fluorescence indicate intact DNA (n) while sperm heads with yellow and dark orange fluorescence indicate fragmented DNA (f). Sperm smears stained with freshly prepared acridine orange and viewed using a fluorescent microscope (oil immersion) and a 460 nm filter.

DISSCUSION

The histological evaluation of animal tissues has an important role in determining the harmful effects on reproductive health. It gives us information about the severity of the toxicity, the affected cellular site, and how to treat it. (25) The testicles are the organ responsible for the production of the hormone Testosterone, which is the main hormone of masculinity and the production of sperm (26,27) , The epididymis is one of the most important male sex ducts and its most obvious function is to transport sperm from the testicles to the vas deferens within 10-15 days (28), increasing the concentration of sperm in the semen, in addition to protecting the sperm during transit from damage caused by the external environment(29) ,the epididymis affects the characteristics of sperm, including metabolism and the ability to bind to the oocytes of the zona pellucida, and thus its direct effect on the ability of fertilization (30) , In our current study, the results of the histological evaluation of the control group of rats that were given distilled water only showed normal histological characteristics of the testis, as well as the arrangement of the seminiferous tubules lined with normal active sperms, , while the results of the group of rats that were given a low dose of Al_2O_3 (70 mg/kg) for 21 days the result was that the seminiferous tubules lost their normal distribution and appeared in different shapes than the control group, and when increasing the exposure period for 35 days at the same concentration (70 mg/kg) the shapes became more different and lost the normal distribution as well as the absence of a series generating sperms in the tubular cavity and the disintegration of the tubes with the loss of sperms and congestion of blood vessels in the testis, on the other hand, the rats that were treated with a high concentration of Al_2O_3 NPs (140 mg/kg) for 21 day The results showed disintegration of the seminiferous tubules, significant degeneration of most of the spermatozoa in the tubal cavity, and clear congestion of blood vessels in the testis, and when increasing the exposure period for 35 days with the high dose of Al_2O_3 NPs (140 mg/kg) the result was that the seminiferous tubules became deformed and lost their arrangement, the cytoplasm was empty, and the normal distribution of the epithelial lining was irregular. This indicates a clear deterioration in the testes, which in turn affects the production of healthy and active sperm, and this is consistent with the study (31) where his results showed that there are gaps, crusting, and degeneration in the seminal epithelial cells, and thus cell death and loss is noted by the presence of empty spaces in the epithelial lining, and that the loss of epithelial cells led to tubular shrinkage in the seminiferous tubules, and our study proved that exposure to aluminum oxide nanoparticles for a long time has a cumulative effect, and misuse can have a harmful and toxic effect on the testicular tissues, which leads to a state of infertility, as agreed with(32). In his study, which he conducted on four groups of rats, he dosed them with different concentrations of aluminum. The results of the histological dissection of the testicles showed that by increasing the dose, a loss of the normal distribution of the epithelial lining and a deformation of the seminiferous tubules occurred. The study also agreed with what it proved (33) whereas, the histological study on the testes of male white rats dosed with aluminum for 90 days showed variable focal areas and a deteriorating shape in the germ cells with a few number of broken sperms in the lumen and damage to the thick and irregular basement membrane of the seminiferous tubules, which led to the occurrence of histological changes in the seminiferous tubules of the testis, in the current study, it was discovered that exposure to aluminum oxide nanoparticles for two different periods caused tubular atrophy, cellular deterioration, and a decrease in the formation of mature spermatogonia in the testes of treated animals. In addition, Leydig cells are responsible for the production of testosterone, and the results show atrophy in the testicular tissue that was observed in animals treated with nanoparticles may explain the decrease in the level of testosterone, which was referred to previously, as confirmed (34). The results of our study showed that the testis of rats treated with two different concentrations of Al_2O_3 NPs (70 mg /kg and 140 mg/kg) within 21 days when she was given a aqueous extract of fenugreek seeds in concentration (2 gm/kg) for 14 day, there was a slight change in the texture of the testis and the arrangement of the seminiferous tubules, while the histological structure was clearly normal with treatment of deterioration in testicular tissue at the highest concentration of the plant extract of fenugreek seeds (4 gm/kg) ,and at the length of the exposure period to Aluminum 35 days and at both concentrations (70 mg /kg and 140 mg/kg) of Al_2O_3 NPs plant extract at both concentrations(2 gm/kg and 4 gm/kg) The results improved significantly when the histological structure of the testicle appeared normal, aqueous extract of fenugreek seeds in two different concentrations (2 gm/kg and 4 gm/kg) he worked to increase the thickness of the germinal layer and at the same time to reduce the diameters of the lumen of the seminiferous tubes and redistribute them naturally, as well as the presence of a large amount of germ cells responsible for the formation of active sperm, and this is consistent with the tissue areas of the

testicles, which he explained (35) Testosterone is essential for stimulating the stages of spermatogenesis and prolonging epididymal sperm life (36) , fenugreek seeds increase the production of testosterone due to the active substances they contain (37) ,Therefore, the changes in the testicular tissues in the experimental groups may be attributed at least partially to the rise in the hormone testosterone, and that the seeds of fenugreek contain plant estrogens, and that the low levels of dietary plant estrogens have an important biological effect in improving the tissues of the testis. therefore, the changes that occurred well due to the presence of estrogen in the fenugreek plant in addition to it contains antioxidants, so the increase in sperm in the seminiferous tubules may be due to the antioxidant activity of fenugreek seeds (38).

Sperm with DNA damage is a reliable sign of male infertility. Based on semen analysis, sperm DNA fragmentation hurts male reproduction. (39,40) Sperm DNA damage can occur due to errors in the packaging and separation of chromatin (39) Oxidative stress, abnormal programmed cell death, and hormonal deficiencies (41,42) In a study about DNA damage, it was determined that sperm with abnormal shapes are associated with cases of DNA damage (43) While other studies were not statistically significant between the abnormal shape and DNA damage to the sperm. (44,45) In our current study, the results indicated an increase in the state of DNA fragmentation in sperm with exposure to Aluminum oxide with different concentrations and an increase in the exposure period to the dose. The method of evaluating DNA fragmentation using the acridine orange dye is a method that is done by fluorescent microscopy of the dye that saturated cells with acridine orange. Single chain pigmented red or glowing orange (23), the results of our study are consistent with the study aimed at the toxic effect of nanoparticles, including aluminum oxide, on sperm cells and genotoxicity at three concentrations (0.1,10,100) kg/liter, it was noted that Al_2o_3 NPs. it is most dangerous at high concentrations, which leads to the generation of various reactive oxygen species (ROS) such as superoxide radical (O-2) and the hydroxyl radical (OH) lipid peroxidation leads to the emergence of various forms of chromosomal aberrations and decrease in the mitotic index (46). Another study showed that the process of forming sperm and maintaining the integrity of the DNA of the sperm is a process that takes place in a highly organized and strict manner by various factors and is strongly affected by environmental pollutants and overlaps in the stages of formation, which leads to cases of distortions and imbalances in the production of healthy sperm, including exposure to Aluminum oxide nanoparticles, as the results Our study is consistent with what has been proven (47), the high toxicity of Aluminum oxide led to a significant decrease in semen concentration, reduced motility, and changed the morphology, in addition to a significant increase in DNA damage, confirming the oxidative stress caused by the accumulation of nanoparticles of Aluminum (Al_2O_3). Some studies show the relationship between the formations of reactive oxygen species (ROS) and the fragmentation of sperm DNA, where the reactive oxygen species are a natural by-product of aerobic cellular metabolism and act as signaling molecules between one cell and another. (48) and that small amount of particles of (ROS) cause severe damage as it affects the cellular function of the sperm and damages the DNA in it (49), and excessive levels of ROS damage DNA by affecting its activity endonucleases cross path apoptotic cascade (50), may cause accumulation ROS to produce high levels of peroxides and free radical molecules that break down sperm DNA (51). The accumulation of aluminum oxide nanoparticles in cells led to interaction with cell components and binding to proteins and genetic materials DNA with the production of various other chemical compounds, which leads to cellular oxidative damage, disruption of mitochondrial integrity, and activation of programmed death (52). It is also believed that the main path leading to sperm DNA breakage is the process of programmed death, which is likely to result from poor chromatin maturation in the testicles and through oxidative stress during transit into the male reproductive system, and that oxidative stress is the primary cause of programmed cell death (53,54).The results of our study are consistent with a study conducted on 45 male albino rats, who were dosed daily orally for different periods (30, 45, and 60) days at a concentration of (34 mg/kg). It was observed that there was an increase in oxidative stress and deterioration in the properties of semen, including an increase in the number of damaged sperm with an increase in DNA fragmentation (55). In our current study, the results changed when the plant extract of fenugreek seeds was given to laboratory animals, as the numbers of damaged sperm and the state of DNA fragmentation decreased. The results of our study agree with a study conducted on male rats after they were exposed to aluminum toxicity and were given a plant extract of fenugreek seeds in different doses (25,50, 100 gm/kg) daily results showed that fenugreek reduced the rate of sperm damage and the level of gene expression (56).Fenugreek is considered one of the plants that contain substances that stimulate the action of antioxidant enzymes and have a positive effect on the integrity of the DNA in sperm through improving the methylation process of phospholipids,

proteins, DNA, RNA, synthesis, and reparation of nuclear matter. This may have happened because of stopping the programmed death process. (57) or a decrease in the effect of reactive oxygen species, and antioxidants have a role in reducing levels of ROS (58), also, The aqueous extract of fenugreek seeds has a preventive role in treating DNA fragmentation cases because it contains useful and effective chemicals such as steroidal sapogenins, dietary fiber, and antioxidants, as its biological components have an important role in regulating the stimulation of programmed cell death.(59,60). The plant extract of fenugreek seeds may have an antagonistic effect in some cases, as results showed an increase in the number of destroyed sperm at a high concentration of the plant extract, and this was proven by a study conducted by (24) when given aqueous extract of fenugreek seeds orally at a concentration of (600 mg/kg) for 28, 56 days Fenugreek caused degenerative changes in germ cells and increased the number of destroyed sperm in male mice. Al_2O_3 NPs. It can accumulate in the reproductive organs after passing through the blood and epithelial barriers protecting the reproductive tissues. As a result of its buildup, the testis, Sertoli cells, Leyding cells, and germ cells are destroyed. This results in a reproductive abnormality and a state of DNA fragmentation that impacts the quantity and quality of sperm.

CONCLUSION

Toxicity of Al_2O_3 NPs. in causing tissue changes and DNA fragmentation and the reasons behind this, which were explained in the current study in the male reproductive system of albino rats, which proved that the damage resulting from aluminum oxide nanoparticles when the dose was doubled and the length of the exposure period was more than required reducing the level of the safe dose for living organisms to a lower level at Their use in various fields of life. However, more investigations are needed to distinguish between the mechanisms followed and directly related to the cumulative effect of nanoparticles or the direct effect through oxidative stress upon exposure to nano-aluminum.

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التأثير النسيجي وتفتيت الحمض النووي لجزيئات الالمنيوم اوكسيد النانوية في ذكور الجرذان البيضاء ودور مستخلص نبات الحلبة

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قسم الاحياء المجهرية ، كلية العلوم ، جامعة الكرخ للعلوم

الخلاصة

خلفية البحث: الالمنيوم هو عنصر يؤثر على وظائف الكائنات الحية ويدخل الجسم بعدة طرق وله اثار سلبية وتستخدم الجسيمات النانوية المختلفة على نطاق واسع في مجال الصناعة والمواد الطبية وخاصة اكاسيد المعادن.الحلبة (T. foenum graecum) هي دواء عشبي يمكن ان يقلل من السمية الجينية وتاثيرات الخلايا الالتهابية ويقلل الاجهاد التاكسدي لانه يحتوي على مركبات كيميائية مفيدة بما في ذلك السكريات والصابونين والالياف والكولين والتريجونيلين ومع ذلك فان تاثيرها على الصحة العامة لايزال غير واضح تماما.**الهدف من البحث:** التعرف على مدى تاثير جزيئات اكسيد الالمنيوم النانوية في احداث تجزئة الحمض النووي في الجهاز التناسلي الذكري والضرر النوعي لانسجة الخصية وتحديد الدور الوقائي الفعال لعمل المستخلص النباتي لبذور الحلبة في علاج الحالة وتحسينها واظهرت النتائج في جميع المجموعات المعاملة باوكسيد الالمنيوم النانوية Al_2O_3 NPs لفترتين بعد انتهاء فترة جرعاتها بالجسيمات النانوية وثم اعطائها المستخلص المائي لبذور الحلبة فقط لمدة 14 يوما .**المواد وطرق العمل** : اجريت الدراسة على 70 جرذا البينو ذكرمقسمة الى 14 مجموعة بمافي ذلك المجموعة الضابطة التي تناولت جرعات فموية من محلول المواد النانوية بتركيزين مختلفين (70 ملجم/كجم و 140 ملجم/كجم) لفترتين (21 يوم و 35 يوم) بينما تم إعطاء المستخلص المائي لبذور الحلبة بتركيزات (2 جم/كجم 4 جم/كجم) لمدة 14 يوم وأعقب نهاية كل تجربة وزن الحيوانات وتم أخذ عينة دم من كل حيوان. تم جمعها عن طريق ثقب القلب ثم طردها مباشرة وحفظ المصل عند -80 درجة مئوية للتحليل الكيميائي الحيوي وبعض المعايير النسيجية، تم تشريح الحيوانات ثم تم استئصال الخصيتين وتثبيتهما في فورمالين محايد 10٪ للتحضير النسيجي وتقييم مستويات تجزئة الحمض النووي, من خلال فحص الاكردين البرتقالي الذي يشارك في امراض الجهاز التناسلي الذكري. **النتائج** : اظهرت النتائج ان Al_2O_3-NPs تسبب في ظهور أقسام الخصية في الأنابيب المنوية مع انحطاط ونخر معين للخلايا المنوية بالاضافة الى الى وجود بقايا نخرية داخل البطانة وعدم ظهور حيوانات منوية داخل البطانة بالاضافة الى زيادة معنوية ($P\geq 0.05$) في تجزئة الحمض النووي من ناحية اخرى ادى المستخلص المائي لبذور الحلبة الى تحسين انسجة الخصية وتقليل تجزئة الحمض النووي بشكل كبير. **الاستنتاجات** :خلصت الدراسة الحالية ان تجريع بمادة Al_2O_3 عن طريق الفم بتراكيز عالية وعلى مدى طويل يسبب تلف انسجة الخصية وزيادة تجزئة الحمض النووي عن طريق توليد الاجهاد التاكسدي ونجح المستخلص المائي لبذور الحلبة في تخفيف الاثار الضارة ل.Al_2O_3 .

الكلمات المفتاحية: الالمنيوم ، مضادات الاكسدة ، الخصية ،الحلبة ، تجزء الحمض النووي.