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The effects of fenugreek seeds on the albino rat male reproductive system, MDA and SOD levels, and CD16 responses to Al_2O_3 NPs administration

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ABSTRACT. Aluminum is widely distributed in the environment and enters the body via air, water, food, medications, and manmade objects. Some studies suggest that aluminum toxicity increases the rate of lipid peroxidation and consequently the generation of free radicals. However, the impact of nanoparticles on human health is still not fully understood. An indicator of lipid peroxidation in serum and tissues is an increased MDA. At the same time, there was a decrease in the level SOD and detection CD16 using the immunohistochemistry technique, objectives evaluating the impact of Al_2O_3 NPs on oxidative stress markers (MDA and SOD) and immune responses (CD16) in the testes, as well as the protective effects of fenugreek seed extract it was observed that these changes in levels recovered when given the plant extract of fenugreek seeds,65 albino rats were divided into 13 groups, including the control group Two concentrations of fenugreek extract (2 and 4 gm kg^{-1}) and two concentrations of Al_2O_3 NPs solution (70 and 140 mg kg^{-1}) were given orally to the rats for 21 and 35 days, respectively. The amino-histochemistry method was used to detect CD16, and its presence was checked along with the concentrations of oxidative stress markers like SOD and MDA. Immunohistochemical findings revealed a significant increase in CD16 in testicular cells; fenugreek extract doses significantly decreased MDA and slightly raised SOD. Al_2O_3 NPs. were shown to significantly raise MDA and decrease SOD at ($p \leqslant 0.05$), according to the study's findings. and Immunohistochemical results detected a significant increase in CD16 in testicular cells, and doses of fenugreek extract reduced the MDA was significant, and a slightly significant increase in SOD. The study concluded Al_2O_3 NPs led to an imbalance in the testicle by generating oxidative stress and the fenugreek extract succeeded in alleviating the harmful effects of Al_2O_3 and by curbing MOD and strengthening the antioxidant defense system SOD, at the same time significantly influenced the histopathological and toxicological change responses through expression CD16.

Keywords: aluminum oxide; MDA; SOD; CD16; Fenugreek.

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Introduction

Nanotechnology is advanced scientific research with several utilizes in electronics, medical, and other industries. Small particles are known as nanoparticles. with a size of 1-100 nanometers. (Laurent et al., 2008) Aluminum it is a chemical element that is produced chemically in an unmodified form or as a starting point for combining with different oxides (Silva-Holguín, Ruíz-Baltazar, Medellín-Castillo, Labrada-Delgado, & Reyes-López, 2022), Although the exact mechanism by which nanoparticles exert their pro-inflammatory effects is unknown, it has been revealed that they work to produce reactive oxygen species and induce oxidative stress in cells (McCrank, 2009), the properties of nanoparticles, including their surface area, make them highly reactive and thus harm by an increase in oxidative stress in human and animal tissues (Ibraheem & Ibrahim, 2016), the damage that occurs in cells is caused by several mechanisms, including oxidation of fats, alteration of proteins, disturbance of DNA that disrupts signaling mechanisms and modification of gene expression, in addition to inflammation and the process of metabolizing foreign substances. Therefore, oxidative stress is a reaction to cell damage (Sioutas, Delfino, & Singh, 2005), the concentration and motility of sperm in males are inversely linked to a decrease in the amount of antioxidants needed to protect cells and an increase in the production of reactive oxygen species. One of the most important types of antioxidants is superoxide dismutase (SOD), which dismutates anion spontaneously to produce O_2 and H_2O_2. SOD prevents superoxide radical-induced hyperactivation and capacitation before ejaculating (Lamirande & Gagnon, 1995). Antioxidants such as SOD have been demonstrated to enhance sperm motility and membrane integrity while preserving sperm as opposed to the negative effects of ROS during sperm liquid storage bull sperm (El-Sisy, El-Nattat, & El-Sheshtawy, 2008; Michael et al., 2009; Cocchia et al., 2011), Immune checkpoints have a crucial part in preserving immunological homeostasis in a healthy body. However, they also show their negativity. Normal cells are considered immune checkpoints when combined with their corresponding ligands (Benson et al., 2010) Natural killer cells are a heterogeneous group of cells, several subtypes of which exist in both humans and animals, and five subgroups of which can be defined based on the relative expression of markers CD16 (Poli et al., 2009). NKT has immunoregulatory properties because it facilitates the cellular tumor and disease-associated immunological responses and inhibits cellular immunity associated with autoimmune diseases (Godfrey et al., 2010) previous research revealed that the testicles have a role in causing autoimmune diseases specific to the organs (Schuppe et al., 2008) Infection and inflammation of the reproductive system are among the most important factors that cause male infertility. Infertility occurs due to immune system impairment in the testicle through the activation of T cells via infections or antigens that compromise the testicular tolerance system for self-antigens. (Perez-Sepulveda, Torres, Khoury, & Illanes, 2014) Long after the immune system, testicular germ cells begin to develop. matures and the central tolerance system is established and possesses a new form of surface routine that can be distinguished either by the foreign antigens by the innate immune system or by the acquired immune system, which triggers a serious autoimmune reaction (Bolourian & Mojtahedi, 2017). Although aluminum is regarded as a valuable and necessary natural element used in significant fields, there are potential risks of nano-aluminum to reproductive health, particularly for those who are exposed to it professionally. Additionally, studies on the general toxicity of aluminum to reproduction are inadequate and of low quality. Here, we go over the possible poisoning mechanism as well as the functional and histological effects of aluminum on male reproductive capacity. The purpose of the study was to ascertain the effects of two distinct concentrations of aluminum oxide nanoparticles on antioxidants and their immune response in the testicular tissue of the male reproductive system in rats, as well as how fenugreek plant extract lessened the harm caused by Al_2O_3 NPs and enhanced outcomes.

Materials and methods

Animals

This study was conducted in the College of Sciences/ Al-mustansiriyah University in the Department of Biology department/ 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,they were left in these condition for two weeks to acclimate to the experimental conditions.

Materials

Al_2O_3 nanopowder, white, and solid were produced in) the 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 and 4 gm kg^{-1}) and dissolved in each concentration in (1000 mL D.W) and the animals were dosed orally (0.5 mL) once a day.

Experiment design

Al_2O_3-NPs, or aluminum oxide nanoparticles, were dissolved in 1 milliliter of distilled water at concentrations of 70 and 140 mg kg^{-1} of body weight Based on a study (Hamdi, 2020), the dose was doubled as an initial trial. The suspensions underwent ultrasonography before oral administration to rats to minimize particle accumulation and achieve the ideal size distribution for dispersed particles. There were 65 random rats, split up into 13 groups, and five animals in each group received 0.5 ml of each concentration of Al_2O_3-NPs (70 and 140 mg kg^{-1}). once daily via a gastric tube for two periods 21,35 days, one control group for both periods, and each period of exposure was divided into six groups, group 2: received 70 mg kg^{-1} of Al_2O_3 NPs, group 3: 140 mg kg^{-1} of Al_2O_3 NPs, The rats were dosed with the aqueous extract of fenugreek seeds for 14 days after completing the nanomaterial dose at the same doses for the fourth, fifth, sixth, and seventh groups for both period with two concentrations(2 and 4 gm kg^{-1}). Blood samples were collected from the right ventricle directly by heart stabbing of the rats for examination SOD, MDA, and Immunohistochemical analysis of CD16 and placed into a gel tube and then allowed to clot. The serum was separated by centrifugation of the blood sample at (3000) rpm for (10-15) and then kept frozen at (-20) C° until the time for use.

Measurement of oxidative stress (SOD)

In this study, plasma SOD, levels were determined using the Elisa Technique from (Sun Long Biotech) in the United Kingdom. For biochemical analyses, data are shown as mean and standard deviation (SD). The SPSS version was used to conduct statistical comparisons using the Student's t-test. Sandwich-ELISA is the technique used in this ELISA kit. This kit's Microelisa stripplate has been pre-coated with a sod-specific antibody. The appropriate Microelisa stripplate wells are filled with standards or samples, which are then combined with the designated antibody. A SOD-specific antibody that has been HRP-conjugated is then added to each Microelisa stripplate well before incubation. Free parts are removed through washing. To each well, the TMB substrate solution is added. Only the wells containing sod and HRP-conjugated SOD antibodies will initially appear blue before changing to yellow after the stop solution is added. At a wavelength of 450 nm, the optical density (OD) is measured spectrophotometrically—the relationship between the OD value and SOD concentration. By comparing the OD of the samples to the standard curve, one can determine the amount of SOD present in the samples.

Measurement of oxidative stress (MOD)

In this study, plasma MOD, levels were determined using the Elisa Technique from (Sun Long Biotech) in the United Kingdom. For biochemical analyses, data are shown as mean and standard deviation (SD). The SPSS version was used to conduct statistical comparisons using the Student's t-test. The Sandwich-ELISA method is used in this ELISA kit. This kit's Microelisa strip plate has been pre-coated with an antibody specific to MDA. Standards or samples are added to the appropriate Microelisa strip plate0 wells and combined with the specific antibody. Then, in each Microelisa stripplate well, a Horseradish Peroxidase (HRP)-conjugated antibody specific for MDA is added and incubated. The unneeded components are washed away. Each well receives the TMB substrate solution. Only the wells containing MDA and HRP conjugated MDA antibodies will appear blue and then turn yellow after the stop solution is added. At 450 nm, the optical density (OD) is measured spectrophotometrically. The OD is proportional about MDA concentration By comparing the OD of the samples to the standard curve, you can calculate the concentration of MDA in the samples.

CD16 is detected by immunohistochemistry in testes tissue

Immunohistochemistry Detection by kit from Biovision/USA. Samples embedded in paraffin were put onto charged slides in slices with a thickness of 5 μm. The slides were dewaxed, dried, and then dipped in an antigen retrieval solution and blocked with a bovine serum albumin solution (BSA 5%). Sections were incubated at 4°C for an entire night with CD16 primary antibodies at 1:100 dilutions. After that, sections were incubated for two hours at room temperature with secondary antibodies. A Hematoxylin stain was applied to sections after adding a substrate chromogen solution. The percentage of positive cells out of 100 total cells in 10 slide fields was used to calculate the expression of proteins of interest (Taylor et al., 2020).

Statistical analysis

The data were statistically analyzed using Minitab (Software package for statistical analysis - version 18). For numerical data, mean and standard Error (mean SE) were utilized to convey descriptive statistics. Two-way analysis of variance (ANOVA) and the least significant difference post-hoc test was employed to determine the significance of differences between groups. Statistics are significant at ($p \leqslant 0.05$; Griffith, 2007).

Results and discussion

Effect of Aluminum oxide nanoparticles on induced oxidative stress in the testicular tissue

1- Malondialdehyde enzyme (MDA)

The effect of aluminum oxide nanoparticles on the level of MDA in testicular tissues revealed a significant increase ($p \leqslant 0.05$) between all treated groups compared to the control group for different time points (Table 1). The exposure to different concentrations of each plant extract of fenugreek seeds T. foenum graecum and Al_2O_3 nanoparticle NPs. showed less increase in the level of MDA compared to the animal treated with different concentrations of Al_2O_3 NPs. for 21 and 35 days of exposure (Table 1).

2- Superoxide dismutase (SOD) enzyme

The statistical analysis of the current work showed a statistically significant decrease ($p \leqslant 0.05$) in the level of SOD in animals treated with 70 and 140 mg kg^{-1} of Al_2O_3 NPs. for 21 and 35 days of exposure compared to the control group with time effect (Table 1). The exposure to 2 and 4 gm of T. foenum graecum extract together with 70 and 140 mg kg^{-1} of Al_2O_3 NPs. for 21 and 35 days showed a statistically significant difference ($p \leqslant 0.05$) in the level of SOD compared with different concentration of Al_2O_3 NPs alone (Table 1).

Table 1. The Effect of Al_2O_3 on the SOD and MDA.

Treatment	Time	SOD umL	MDA ngmL
Control	21&35	a 7.15 ± 0.18	a 40.75 ± 0.92
70 mg Al_2O_3 NPs.	21	b 4.20 ± 0.19	b 92.69 ± 3.17
70 mg Al_2O_3 NPs.+ 2gm *T. foenum graecum*.	21	b● 5.20 ± 0 .20	b● 78.66 ± 3.42
70 mg Al_2O_3 NPs. + 4gm *T. foenum graecum*	21	b● 5.71 ± 0.31	b● 60.25 ± 2.60
140 mg Al_2O_3 NPs	21	b 3.36 ± 0.34	b 98.31 ± 3.41
140 mg Al_2O_3 NPs. + 2gm *T. foenum graecum*.	21	b● 4.39 ± 0.29	b● 79.72 ± 3.73
140 mg Al_2O NPs.$_3$+ 4gm *T. foenum graecum*.	21	b● 5.95 ± 0.41	b● 72.69 ± 2.78
70 mg Al_2O_3 NPs.	35	b◄ 4.06 ± 0.24	b 96.22 ± 3.70
70 mg Al_2O_3 NPs.+ 2gm *T. foenum graecum*.	35	b● 5.64 ± 0.28	b● 76.34 ± 3.64
70 mg Al_2O_3 NPs. + 4gm *T. foenum graecum*	35	a● 6.08 ± 0.37	b● 63.31 ± 2.99
140 mg Al_2O_3 NPs	35	b◄ 3.15 ± 0.34	B 102.63 ± 3.84
140 mg Al_2O_3 NPs. + 2gm *T. foenum graecum*.	35	b● 4.88 ± 0.26	b● 78.44 ± 4.29
140 mg Al_2O NPs. + 4gm *T. foenum graecum*.	35	a● 6.15 ± 0.43	b● 67.41 ± 3.05

-Data refers to mean ± S.E, (N-= 5 animals)◄ Indicates that there is a significant difference ($p \leqslant 0.05$) at the same concentration of Al_2O_3 NPs. for different periods.,- ● indicates a difference ($p \leqslant 0.05$) between the concentrations of Al_2O_3 NPs. and the concentrations of extracts,-Similar letters indicate no significant difference $p \geqslant 0.05$ between the concentrations of Al_2O_3 NPs.,- different letters indicate a significant difference $p \leqslant 0.05$ between the concentrations of the Al_2O_3 nanoparticle NPs.

It is known that testicular stress is the main feature of infertility in males (Bisht, Faiq, Tolahunase, & Dada, 2017). One of the most important damages caused by nano-aluminum particles is clear oxidative stress in the testicles, where SOD activity is reduced and MDA activity is increased. In a study (De et al., 2020) it was proven that nano-aluminum oxide has an antioxidant effect in the testicles of mice. Which received 70 mg kg^{-1} of aluminum oxide nanoparticles for 75 days, and it was proven that aluminum is involved in the generation of ROS, which leads to a reduction in antioxidant enzyme activities. which is consistent with our current study, nanoparticles can interact with organelles, such as energy homes, producing reactive oxygen as a result (Zhao, Wang, Wu, You, & Lv, 2013). suggesting that the oxidative damage may be caused by the antioxidant enzymes' incapacity to eliminate the oxidants generated in the vicinity of the testis. This makes the nanoparticles a useful biomarker for predicting tissue damage (Abduljabbar & Ismail, 2019). The properties of the nanoparticles, such as their surface area, make them highly reactive, which increases the risk of oxidative stress in human and animal tissues (Ibraheem & Ibrahim,2016). Antioxidants are unpaired electron compounds that can scavenge free radicals by either receiving or giving electrons. The antioxidant either directly or indirectly combats the free radical to provide protection (Mohsen, Abdula, Jassim, Rodhan, & Ayrim, 2021). The results came the study are consistent with the results of other studies that showed exposure to metal oxide nanoparticles occurs due to the occurrence of oxidative stress due to the occurrence of fat oxidation in tissues (Attarilar et al., 2020), these outcomes concur with his findings as well (Prabhakar et al., 2012) when giving Wistar rats aluminum oxide in doses (500, 2000, 1000) mg kg^{-1} orally, nanomaterials oxidative stress in a dose-dependent way, and the effect of the material on the level of antioxidant enzymes increased with increasing duration, as it decreased SOD level increased, and explain that SOD it is considered the first line of defense against oxygen toxicity, which inhibits the process by which free radicals are created, and when their level decreases, oxidative events occur. The results of the current study showed that increasing the dose of aluminum oxide inhibited the activity SOD allowing the flow of many free radicals resulting from the production H_2O_2 in cells Which is responsible for changing the effectiveness of antioxidant enzymes (Li, Hartono, Ong, Bay, & Yung, 2010). Many studies have shown MDA increases in tissue are brought on by oxidative stress and damage following exposure to metal oxide nanoparticles, and any rise in MDA levels encourages testicular autophagy and apoptosis (Al-Musawi, Al-Shmgani, & Al-Bairuty, 2022), Our results showed that aluminum oxide nanoparticles caused a rise in the level of lipid peroxidation in sperm and a decrease in the total antioxidant capacity. These outcomes align with a study (El-Gendy, 2011). After giving aluminum orally to five groups of mice at varying doses (5, 53.5, 100) mg kg^{-1} for thirty days, it was established that enhanced lipid peroxidation in the testicles causes an increase in MDA generation. $Al3O_3$ NPs are hazardous due to oxidative cell damage brought on by free radicals. It was also suggested that the toxic effects of aluminum oxide nanoparticles mainly lead to peroxidation activity, thus increasing oxidative stress, free radical attack, and oxidation of proteins and cellular lipids (Exley, 2013). The fenugreek plant is an herbal mixture that has broad therapeutic activity. Pharmacological studies have shown that fenugreek seed extract has the advantage of fighting oxidative stress because it contains active phytochemical compounds that work to suppress the formation of reactive oxygen species, activate antioxidant enzymes, and protect the cell from damage (Sindhu, Ratheesh, Shyni, Nambisan, & Helen, 2012; Chatterjee, Goswami, Bhatnagar, Kumar, & Kumar, 2013), in a study conducted on 40 adult rats for 8 weeks, they were given the plant extract of fenugreek seeds at a concentration (200, 400) mg kg^{-1} Fenugreek significantly reduced oxidative stress due to its antioxidant activity and the removal of free radicals because it contains phenolic compounds that are known to be secondary metabolic products of plants (Al Mashkor, 2014), in a research study, it was found that saponin, which is one of the components of fenugreek, reduces lipid peroxidation by enhancing the antioxidant defense system in mice (Jagadeesan, Nandakumar, Rengarajan, & Balasubramanian, 2012). In a study (Devasena & Menon, 2007). When 2 g kg^{-1} of fenugreek Plant extract was administered and added to the diet of a group of male Wistar rats, it was found that it elevated the concentrations of antioxidant enzymes and reduced lipid peroxidation. These results were consistent with the results of our study.

Immunohistochemical Results of Detection CD16

The results show reaction test CD16 in the tissue sections, there was a weak positive reaction in the testicular phagocytic cells of the control group. In contrast, the groups treated with aluminum oxide nanoparticles showed at 21 days at both concentrations (70, 140) mg kg^{-1} an effectively positive CD16 expression in testicular tissue, especially at a double concentration (140 mg kg^{-1}) Nevertheless, when the trial period is increased to 35 days the expression of the reaction in macrophages in testicle sections was very strong positive at both concentrations (70, 140) mg kg^{-1} compared with the control group.

When administered to animals, a plant extract of fenugreek seeds is given in two concentrations (2 and 4 gm kg^{-1}) the level of expression decreased, the results improved, and the differences were significant ($p \leq 0.05$) with the concentration of the extract (4 gm kg^{-1}) The difference was significant at ($p \leq 0.05$). Table 2, Figure 1 and 2.

Table 2. shows the immunoreactivity of CD16 proteins in male rats testes induced by treatment with Al_2O_3 NPs.

Treatment	Time	CD16
control	21&35	a 40.6 ± 1.47
70 mg Al_2O_3 NPs.	21	b 77.20 ± 1.77
70 mg Al_2O_3 NPs. + 2gm *T. foenum graecum*.	21	b● 65.20 ± 1.46
70 mg Al_2O_3 NPs. + 4gm *T. foenum graecum*	21	b● 49.80 ± 1.66
140 mg Al_2O_3 NPs	21	B 82.80 ± 2.44
140 mg Al_2O_3 NPs. + 2gm *T. foenum graecum*.	21	b● 68.80 ± 1.85
140 mg Al_2O NPs.$_3$+ 4gm *T. foenum graecum*.	21	a● 41.20 ± 9.29
70 mg Al_2O_3 NPs.	35	b 84.80 ± 1.98
70 mg Al_2O_3 NPs.+ 2gm *T. foenum graecum*.	35	b● 69.60 ± 2.34
70 mg Al_2O_3 NPs. + 4gm *T. foenum graecum*	35	a● 46.40 ± 2.84
140 mg Al_2O_3 NPs	35	b 87.20 ± 2.65
140 mg Al_2O_3 NPs. + 2gm *T. foenum graecum*.	35	b● 71.00 ± 1.64
140 mg Al_2O NPs. + 4gm *T. foenum graecum*.	35	b● 56.00 ± 2.65

-Data refers to mean ± S.E- ●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. $p \leq 0.05$.

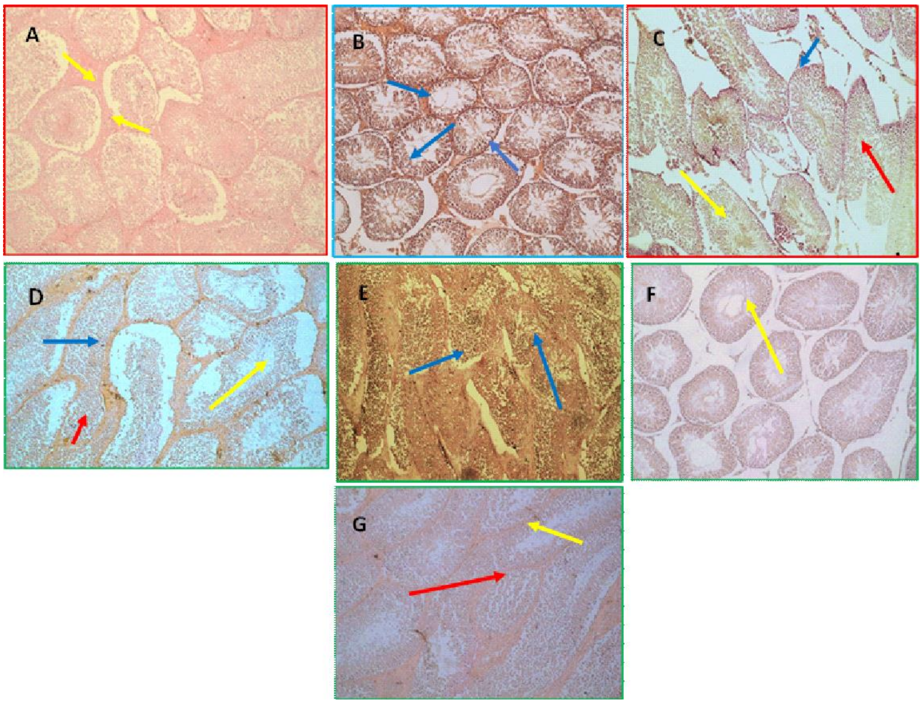

Figure 1. Immunohistochemical staining of activated CD-16 in rat testis (A control, (B) treated with Al_2O_3 NPs 70mg, (C) treated with given Al_2O_3 NPs 70 + 2 mg kg^{-1} T. foenum graecum. (D) treated with Al_2O_3 NPs 70 + 4 mg kg^{-1} T. foenum graecum. (E) treated with Al_2O_3 NPs 140 mg kg^{-1}. (F) treated with Al_2O_3 NPs 140 mg + 2 mg kg^{-1} T. foenum graecum. (G) treated with Al_2O_3 NPs 140 mg kg^{-1} + 4 mg T. foenum graecum. for 21 days ( ). The blue arrow indicates a strong positive chemokine immune reaction in the cytoplasm of macrophages located in the interstitial tissue of the testicle with the intensity of activated CD16 (deep brown). ( ) the red arrow indicates the moderate response to chemokine immune reaction, ( ) The yellow arrow indicates a weak negative chemokine immune reaction in the nuclei of sperm cells and the cytoplasm of macrophages located in the interstitial tissue of the testicle intensity of activated CD16 (antibody stain CD16 , 40X).

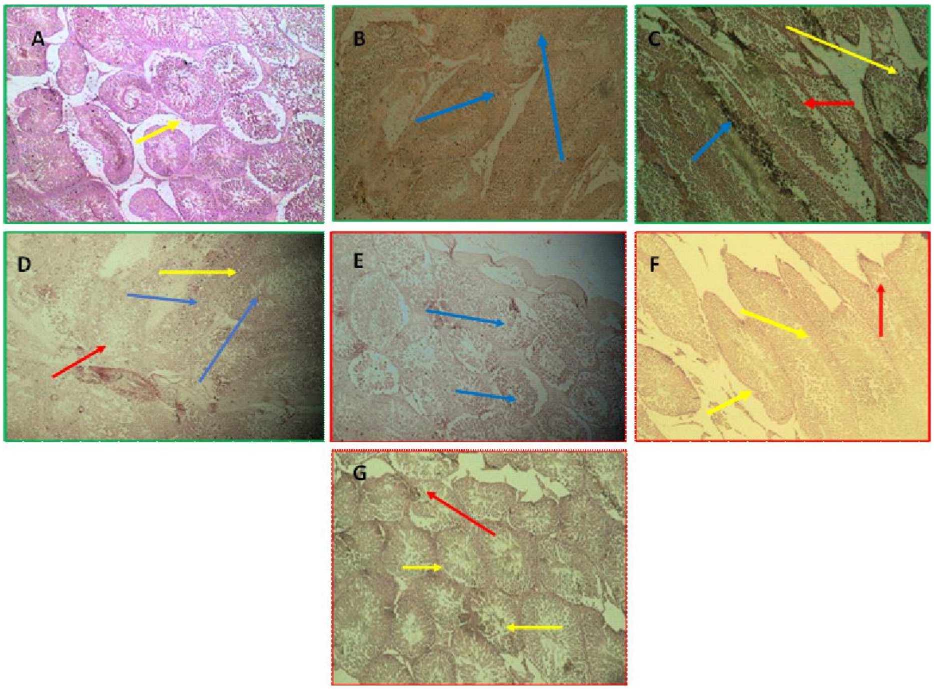

Figure 2. Immunohistochemical staining of activated CD-16 in rat testis (A control, (B) treated with Al_2O_3 NPs 70mg, (C) treated with given Al_2O_3 NPs 70 + 2 mg kg^{-1} T. foenum graecum. (D) treated with Al_2O_3 NPs 70 + 4 mg kg^{-1} T. foenum graecum. (E) treated with Al_2O_3 NPs 140 mg kg^{-1}. (F) treated with Al_2O_3 NPs 140 mg + 2 mg kg^{-1} T. foenum graecum. (G) treated with Al_2O_3 NPs 140 mg kg^{-1} + 4 mg T. foenum graecum. for 35 days ( ) The blue arrow indicates a strong positive chemokine immune reaction in the cytoplasm of macrophages located in the interstitial tissue of the testicle with the intensity of activated CD16 (deep brown). ( ) the red arrow indicates the moderate response to chemokine immune reaction, ( ) The yellow arrow indicates a weak negative chemokine immune reaction in the nuclei of sperm cells and the cytoplasm of macrophages located in the interstitial tissue of the testicle intensity of activated CD16. (antibody stain CD16 , 40X).

The immune response serves multiple functions, including protecting and repairing reproductive tissues that directly affect fertilization, such as producing antibodies to sperm (Boots, Donnelly, & White, 2013). Many original studies have shown that the reproductive and immune systems are interconnected and related in function It is linked to a physiological degree (Lawniczak et al., 2007; Siva-Jothy, 2009 ; Schwenke, Lazzaro, & Wolfner, 2016; Oku, Price, & Wedell, 2019; Wigby, Suarez, Lazzaro, Pizzari, & Wolfner, 2019). Detection of phagocytic cells in the interstitial tissue of the testicle using an immunohistochemical technique using antibodies CD16 showed a significant increase in expression CD16 in aggregates treated with aluminum oxide nanoparticles, the testicle is an immune-privileged organ. Sperm are shielded from immune system attacks by the blood-testis barrier (Terayama et al., 2014), in our current study, the accumulation of aluminum oxide nanoparticles in testicular tissue leads to the stimulation of oxidative stress and is responsible for increasing immune expression CD16 the interaction between oxidative stress and inflammation is stimulated by nanomaterials and generates reactive oxygen species directly on immune cells (McGarry, Biniecka, Veale, & Fearon, 2018), the presence of nanomaterials in the interstitium has harmful effects on germ cells in addition to the large concentration of unsaturated fatty acids in germ cell membranes, increasing the cell's susceptibility to oxidative stress brought on by a high aluminum dosage (Herman et al., 2020), Natural killer cells are immune response cells that are vital to the body. (O'Brien & Finlay, 2019) it communicates with surrounding cells by secreting chemokines and cytokines, and is expressed through receptors encoded in germ cells with inhibitory or stimulatory functions (Vivier, 2006) Natural killer cells are divided into groups based on their expression (Fu et al., 2017) It binds to the sperm immune complex IgG (gamma RIIIA CD16). When they are active, immune expression increases, and this perturbation on the surface of natural killer cells triggers a variety of early events to transduce signals between cells and increase immune expression (Trinchieri & Valiante, 1993). Natural killer cells are innate lymphocytes that can mediate a desensitized immune response and express receptors FC (fcγRs) that share an area FC of IgG It is equipped with a myriad of receptors that interact using ligands on targets to kill the target cell and leave the healthy cell intact (Aguilar et al., 2023), Natural killer cells have receptors to which antibodies bind, which is an important additional mechanism by which natural killer cells can kill target cells (Eggert-Kruse et al., 2002). The results of our study are consistent with a study conducted by him (Park et al., 2010). The mice were dosed with silver nanoparticles at two concentrations (0.05 and 1.00 mg kg^{-1}). Orally for 28 days, and the effects were observed at high doses (1.00 mg kg^{-1}). When analyzed by histochemistry, cytokines were significantly increased in a dose-dependent manner, increased expression of immune cells, and a high inflammatory response in mice. In a study (Habas, Demir, Guo, Brinkworth, & Anderson, 2021), nanoparticles' accumulation weakens immunity and increases the inflammatory response in testicular cells. In a study by (Gamal, Kortam, El Ghareeb, & El Rahman, 2022) Albino rats were injected with different doses (5 and 10 mg kg^{-1}) Three times a week for 60 days, using manganite nanoparticles (Mn NPs), there was a change in the rate of proliferation of lymphocytes, which indicates the effect of an immunotoxin. These particles affected the natural immune status of male rats. They facilitated the generation of reactive oxygen species, which subsequently led to oxidative damage to the testicles, and this was confirmed by the results of our study. Fenugreek seed extract can enhance secretion IgG as proven (Hossain, Begum, & Kim, 2018). In his study when using it increased significantly, while in our study, it was observed that the concentration of fenugreek seed extract had a significant positive regulatory effect on immune expression to CD16 production of immunoglobulins IgG. The reason is that it contains 50% sugars, and these sugar compounds enhance immune activity in the bodies of animals (Huang et al., 2022). It can stimulate the production of immunoglobulins in the blood and the ability to regulate innate and adaptive immunity (Ramesh & Reeves, 2002; Sharma, Suresh, Debnath, & Jha, 2017). Cytokines have vital physiological roles that include controlling immunity, promoting epithelial development, and healing damage, depending on their source and function (Banerjee & Saxena, 2014) and the fenugreek worked to improve the immune response in our study, as it affects the stimulation of the regulation of cytokines. Fenugreek contains flavonoids and polysaccharides, one of the biologically active anti-inflammatory and antioxidant components (Ktari et al., 2017 ; Pournamdari et al., 2018), antibodies were detectable against mature sperm in his test, IgG where you are located associated with sperm surface antigens (Shrestha et al., 2021).

Conclusion

Our research sheds light on the detrimental effects that exposure to nano-aluminum oxide can have on the male reproductive system at varying doses and times. It also determines the degree to which an aqueous plant extract of fenugreek seeds can be used as a prophylactic measure. Our study's findings demonstrate that exposure to nano-aluminum oxide not only affected the expression of CD16 in the immune system in testicular tissue but also disrupted the antioxidant defense system. Our recommendation is toT that further research on exposure to aluminum nano oxide at varying doses and times is warranted and use fenugreek seeds as a herbal remedy for infertility concerns.

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