Effect of Lanthanum Strontium Manganese Oxide (LaSMnO₃) Nanoparticle on mouse Testosterone and Fertility

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1.0. INTRODUCTION

Considerable research interest in the area of drug delivery using particulate delivery systems as carriers for small and large molecules. Nanoparticles system have been used as a physical approach to alter and improve the pharmacokinetic and pharmacodynamics properties of various types of drug molecules especially most of the anti-cancer drugs, hence used in-vivo to protect the drug entity in the systemic circulation, restrict access of the drug to the chosen sites and to deliver the drug at a controlled and sustained rate to the site of action. Various polymers have been used in the formulation of nanoparticles for drug delivery research to increase therapeutic benefit, while minimizing side effects. Polymeric nanoparticles offer some specific advantages over liposomes, they help to increase the stability of drugs/proteins and possess useful controlled release properties. However, the safety of these carriers has been questioned. This study is therefore designed to evaluate the effect of this new nanoparticle on male fertility.

2.0. MATERIALS AND METHODOLOGY

Lanthanum strontium manganese oxide (LaSMnO₃) (synthesized in the laboratory), Nitro blue tetrazolium (NBT), Sodium chloride (NaCl), Sodium azide, Bradford reagent, Sodium pyrophosphate, Sodium acetate & Sodium hydroxide (NaOH) Na₂HPO₄ (Disodium hydrogen phosphate; dibasic), Potassium dihydrogen phosphate (KH₂PO₄), Glycine, Sodium citrate, NaH₂PO₄·2H₂O (Sodium dihydrogen Phosphate hydrated monobasic) were procured from Sigma, USA.

2.1. Experimental animals

Male and female mice were obtained from animal house of Ekiti-State University Ado Ekiti, Nigeria. The animals were maintained under standard conditions of humidity (50 ± 5 %), temperature (25 ± 2°C) and dark and light cycles (12 hrs each) with free access to food and water.

2.2. Dose grouping

The animals were divided into 4 groups (n=5). Group I served as control while groups II, III, and IV received 5, 10, and 20 µg/kg/day of LaSMnO₃ respectively. Five animals from each group were sacrificed at interval of 0, 7, 14 and 21 days, however, after twenty-one days of the treatment, animals in all groups were allowed to cohabited with untreated female mice for fertility study. Toxic effects of LaSMnO₃ on the testosterone and sperm parameters were analyzed. Effect on ROS and anti-oxidative biomarkers were also measured. Significant decrease (p<0.05) of epididymal spermatozoa motility and numbers was measured revealing the cytotoxicity effects of this nanomaterial. Light microscopic study revealed changes in the cauda epididymal sperm morphology. Failure of the fertility in LaSMnO₃-treated mice as evidenced by the significant reduction in the average number of implantation in females mated with the treated males. Depletion of testicular testosterone hormone level by high dose of LaSMnO₃ (20µg/kg/day) shows a reduced testicular androgen synthesis. This study therefore, shows the potential adverse effect of LaSMnO₃ on male fertility.

2.3. Tissue Sampling

At autopsy, Epididymis and testis were removed, blotted free of blood and adhering tissues, kept in tube and immediately stored in -20°C for biochemical studies.

2.4. Testosterone estimation

The testicular testosterone level from each group was measured. Briefly, testicular proteins were extracted with phosphate buffer (50 mM, pH 7.4) and centrifuged at 10,000 g for 20 min. The supernatant was used to estimate testosterone level using ELISA method and were expressed in ng/ml.
2.5. Measurement of Reactive Oxygen Species (ROS) Level

Briefly, 50 µl of epididymis homogenate and 1400 µl sodium acetate buffer were transferred to a cuvette. After then, 1000 ul of reagent mixture (N, N-diethyl para phenylenediamine 6 mg/ml with 4.37 µM of ferrous sulfate dissolved in 0.1 M sodium acetate buffer pH- 4.8) was added at 37 °C for 5 min. The absorbance was measured at 505 nm using spectrophotometer (Molecular Devices.) ROS levels from the tissue were calculated from a calibration of H$_2$O$_2$ and expressed as U/mg of protein (1 unit=1.0 mg H$_2$O$_2$/L).

2.6. Biomarkers enzymes analyses

10% epididymis homogenates (w/v) was prepared in chilled 100mM Phosphate buffer (pH 7.4) and the homogenate was used to measure the levels of GSH and CAT measured according to the standard protocols 4,5. Protein contents in the samples were estimated by the Bradford method. All the parameters were expressed as per mg protein.

2.7. Sperm parameters

Cauda epididymidis was removed from each mouse and cleaned off from the epididymal fat pad, and minced in a pre-warmed petri dish containing 500 µl phosphate buffer saline solutions (PBS, pH 7.4) at 37°C. Sperm motility was estimated and expressed as percentage incidence 6. For sperm count, an aliquot of this suspension was charged into the Neubauer’s counting chamber and the spermatozoa were counted under light microscope. Total sperm count was calculated as the average of the spermatozoa count (N) in each chamber X multiplication factor (106) X dilution factor 7. 8. The sperm morphology was also evaluated 9. Briefly, a smear of sperm was made on a clean slide and stained with hematoxylin and eosin and were examined under a light microscope with an oil immersion lens. The morphology of spermatozoa was scored according to Qureshi et al., 9.

2.8. Incidence of Implantation and dominant lethality

Male fertility was checked in mice of groups V, VI, VII, and VIII following treatment according to standard method scored 8. Each male was caged with one female per week for four weeks. The females were sacrificed on 13 days of mating to check implantation. The number of pregnant mice was recorded to determine percent of fertility 11,12. The incidence of pregnancy was established after counting the number of implants. The dead implants per female were determined to obtain the post-implantation loss Agrawal 13. Fertility index was calculated by the ratio of the number of pregnant females to number of females cohabited with males multiplied by 100.

2.9. Statistical analysis

Statistical comparisons between the groups were analyzed using Statistical Package (SPSS version 22). The results were expressed as mean ± SEM. Significant differences among means of the groups were determined using one-way analysis of variance (ANOVA) and where significant difference existed, it was followed by Duncan’s multiple range tests. Mean was considered significant when p ≤ 0.05.

3.0. RESULTS

3.1. Testicular testosterone level was found to be reduced in all LaSMnO$_3$-treated mice. Significant dose-dependent depletion of testicular testosterone was seen across all the groups (Table 1).

| Testosterone (ng/ml) | Dose Groups | Day 0 | Day 7 | Day 14 | Day 21 |
|----------------------|-------------|-------|-------|--------|--------|
| Group I              | 7.82 ± .29  | 7.97 ± .12 | 8.02 ± .02 | 8.07 ± .12 |
| Group II             | 7.99 ± .23  | 5.17 ± .12* | 4.78 ± .02** | 4.37 ± .12** |
| Group III            | 7.85 ± .11  | 4.18 ± .17*** | 3.52 ± .23*** | 2.75 ± .32*** |
| Group IV             | 7.54 ± .17  | 2.48 ± .25** | 1.89 ± .01*** | 0.83 ± .25*** |

Note: *, **, and *** indicate significant difference as compared to control at (p<0.001), (p<0.01) and (p<0.05) respectively.

3.2. Effect of LaSMnO$_3$ on Reactive Oxygen Species

Significant raised level of Reactive Oxygen Species (ROS) in groups II, III and VI compared to the control was measured across all the groups in dose-dependent pattern confirming generation of reactive oxygen species following LaSMnO$_3$ exposure.

3.3. Effect of LaSMnO$_3$ on GSH and CAT

Significant decrease (p <0.01) in the concentration of reduced glutathione and catalase was seen in the epididymal homogenate of LaSMnO$_3$-treated mice following 21 days exposure in dose-dependent manner across all the groups. Oxidative stress was induced by LaSMnO$_3$ treatment as confirmed by the depletion of these biomarkers (Figure 2 a & b).

Figure 1: Barchat showing effect of LaSMnO$_3$ on reactive oxygen species. Note: *, **, and *** indicate significant difference as compared to control at (p<0.001), (p<0.01) and (p<0.05) respectively.
Figure 2: Bar chart showing effect of LaSmO$_3$-treatment on (a) GSH, (b) CAT in epididymal tissue of mice. Note: *, **, and *** indicate significant difference as compared to control at (p<0.001), (p<0.01) and (p<0.05) respectively.

3.4. Effect on Sperm Parameters
LaSmO$_3$ treatment caused a significant decrease in the number of epididymal spermatozoa (Figure 3a) and motility (3b) (p<0.001) when compared to control and a marked increase in abnormal spermatozoa morphology (3c) was observed in epididymal spermatozoa of mice following 14 and 21 days treatment of LaSmO$_3$ nanoparticle.

Figure 3: Effect of LaSmO$_3$ treatment on sperm parameters. Figure 3(a) shows percentage rate of spermatozoa motility, (b) sperm count and (c) abnormal morphology of the spermatozoa.

3.5. Male fertility and dominant lethality
Low incidence of pregnancy and implantation in females mated with LaSmO$_3$ nanoparticle–treated males was observed after 21 days exposure confirming the infertility potential of this nanoparticle (Table 2).

Table 2: Effect of LaSmO$_3$ Nanoparticle on Incidences of pregnancy in female cohabited with treated male mice

| Dose Groups/ Day 21 | Pregnant female | Male Fertility Index (%) | Average No of Implants/ Female ± S.E.M |
|---------------------|-----------------|--------------------------|---------------------------------------|
|                     |                 |                          | Total | Live | Dead |
| Group I 18/20       | 90              | 16.35 ± 0.22             | 14.34 ± 0.16 | 2.01 ± 0.01 |
| Group II 12/20      | 60              | 13.11 ± 0.14**           | 11.29 ± 0.11** | 1.82 ± 0.03 |
| Group III 5/20      | 25              | 6.54 ± 0.07***           | 5.22 ± 0.06*** | 1.32 ± 0.01 |
| Group IV 1/20       | 02              | 2.00 ± 0.01***           | 0      | 2.00 ± 0.01 |

Note: *, **, and *** indicate significant difference as compared to control at (p<0.001), (p<0.01) and (p<0.05) respectively.

DISCUSSIONS
Nanomaterials are integral components of daily-used products including food, sunscreens, cosmetics and pharmaceutics and have been reported to be among factors that influence development of various medical conditions including reproductive disorders. Despite the benefits of nanoparticles, various applications of nanotechnology have exposed humans and animals to their potential toxicities. Exposure of nanoparticle into the body can be through inhalation, ingestion, skin uptake, injection, or implantation and nanoparticles uptake which could be intentional or non-intentional. Thus, the wide use of nanomaterials has raised concerns about the negative impact to human health, mainly on the reproductive systems of both men and women. Research studies on nanoparticles are associated with different disorders in animals, including, hepatotoxicity, neurotoxicity, renal toxicity, and irreversible
testis damage, with few reports on the effect of nanoparticles on male fertility. This study therefore shows significant depletion of the testicular testosterone hormone across all the groups treated with Lanthanum Strontium Manganese oxide (LaSm03) nanoparticle. This reduction was significant at 7 days dosing with the peak after 21 days treatment which implies that Lanthanum nanoparticle destroys the functions of testis of the treated mice which are mainly the productions of sperm and testosterone. This study also revealed that micrograms concentration of Lanthanum nanoparticles in mice decreases epididymal sperm motility and marked reduction of spermatozoa numbers in dose-dependent patterns was also measured. The abnormal spermatozoa morphology significantly increased across all the dose groups. Similar report on nanoparticles showed decreases motility of human sperm. The decrease in the numbers of spermatozoa indicates the cytotoxicity effects of this nanomaterial, Lanthanum Strontium manganese oxide (LaSm03) resulting to testicular tissue dysfunction. Report have shown that TiO2 nanoparticle leads to alteration in testicular morphology and reduction in daily sperm production. Similar study also showed maternal exposure to diesel exhaust particles enhances mutations in male germ lines during development. More so, decreased sperm production has been associated with alteration in the overall expression of genes involved in spermatogenesis.

CONCLUSION

This study established that the reproductive health of male mice used in this study is severely impaired following Lanthanum Strontium Manganese Oxide (LaSm03) treatment. The study clearly shows reduction of testosterone, epididymal sperm motility and increased abnormal spermatozoa morphology after the administration of this Lanthanum nanomaterial. This study also revealed the generation of oxidative stress in the epididymis after the treated as confirmed by the increase level of reactive oxygen species and simultaneous depletion of anti-oxidant biomarkers which could be the reason for the cytotoxicity of spermatozoa seen in the treated mice. This study further shows that LaSm03-treatment results in male infertility. Finally, this study has been able to give insight on the reproductive toxicity of Lanthanum Strontium Manganese Oxide (LaSm03) nanoparticles. Further study is therefore required to clearly understand the mechanism involved.

Competing Interests: The authors have declared that no competing interests exist.

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