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Docosahexaenoic Acid Prevents SiNPs Induced Alterations in Male Reproductive Hormones in Rats

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Abstract **Original Research Article**

Silica nanoparticles (SiNPs) are found to be toxic during excessive exposure for both human and animals (Fan *et al*., (2006). The main routes of exposure are air, drinking water and other environmental factors (Leung *et al*., 2012). It is reported that SiNPs decreased the sperm number and sperm motility rate, sperm malformation and apoptosis in the testicle spermatogenic cells in rats (Ying et al., 2014). Aim of the present study is to evaluate deterioration induced by SiNPs in male reproductive hormones. In the present study, 40 and 80 mg SiNPs were exposed to rats for 60 days. At the end of the exposure period serum were removed from the blood for the investigation of reproductive hormones such as Folicle stimulating Hormones (FSH), Leutinizing Hormones (LH), Testosterone (TT) and Prolactin (PROL) in both control and experimental rats. In the present study it was found that LH, FSH, Testosterone and Prolactin values significantly alters SiNPs exposed groups when compared with control groups. We also found that epididymal sperm count was decreased significantly in the experimental groups. On the basis of results it may be concluded that the interperitoneal injection of SiNPs alters the male reproductive hormones, which further deteriorate the spermatogenesis.

Keywords: Silica nanoparticles, Folicle stimulating Hormones, Leutinizing Hormones, Prolactin, Testosterone.

INTRODUCTION

Air pollution has been a serious social problem that poses the threat to human health and wildlife. SiNPs in the air are mainly from road dust, construction dust and different kinds of industrial wastes (Maurya *et al*., 2021). Natural silica nanoparticles (SiNPs) have been documented as the main inorganic ingredient of particulate matter. SiNPs are associated with different disorders in animals and humans namely pulmonary injury, hepatotoxicity, immuno-nanotoxicity neurotoxicity, renal toxicity, and irreversible testis damage (Maurya *et al*., 2021; Brohi *et al*., 2017). The wide exposure of SiNPs has raised concerns about the negative impact on human health, mainly on the reproductive systems (Olugbodi *et al*., 2020) of both men and women. Moreover, it is also affected by SiNPs due to small size of SiNPs, their ease of penetration and biocompatibility and their potential ability to breach the placental barrier. Recent studies have indicated an increased incidence of male reproductive defects, including low sperm production in adulthood, hypospadias, cryptorchidism, and testicular cancer. This increased incidence of male reproductive defects may be partly attributable to environmental contaminant exposure. Silica nanoparticles are able to pass the cell membrane and penetrate the blood barrier in the brain and the blood barrier in the testis (Suker *et al*., 2013), therefore its effect on all organs of the body (Yousefi *et al*., 2012; Balasubramanian *et al*., 2010). Various studies have been reported that the silica nanoparticles decreased the number of sperm and percentage of sperm motility and damage of sertoli cells and spermatogenic cells in male rat testis (Chauhan et al., 2013; Lin et al., 2007; Fan *et al*., 2006). In addition to causing dysfunctional and oxidative stress, this is led to reproductive toxicity (Komatsu *et al*., 2008) and alteration in the levels of follicle-stimulating hormone

(FSH), luteinizing hormone (LH), Testosterone (TT) and Prolactin (PROL).

Docosahexaenoic acid (DHA), an important ω-3 fatty acid, is abundantly present in the central nervous system and is important in every step of development of human beings. Docosahexaenoic acid (DHA) is essential for the growth. DHA is also required for maintenance of normal brain function in humans. The inclusion of plentiful DHA in the diet improves learning ability, whereas deficiencies of DHA are associated with deficits in learning.

In this study, because of the spermatogenesis time course, we investigated the effects of silica nanoparticles on the spermatogenesis process along with the alteration in reproductive hormones and potential mechanism DHA in reference of toxicity in rats, our study will provide a scientific basis for evaluation the risk of silica particles in the ecosystem to human health (Ying Xu *et al*., (2014). In view of the aforementioned information in this study was designed to investigate the SiNPs induced alterations in male reproductive hormones and modulation of DHA.

MATERIAL AND METHOD

A. MATERIALS

I. Animals

Thirty healthy adult male albino rats weight (120±10gm), were taken from Nims University Rajasthan, Jaipur India. All these animals were housed during the period of experiment in the animal house, under controlled temperature (21± 1^0C) and constant light-dark schedule (12 hours light and 12 hours dark cycle), food and water were available. After experimental period the blood serum was taken for the hormonal study.

II. Silica nanoparticles (SiNPs)

The SiO_2 nanoparticles that were used in this study are white nanopowder, with particle size of 10-30 nm (TEM). It was purchased from Sigma-Aldrich Chemical Co. (Germany).

B. METHODS

Groups of Animals and Experimental protocol

Thirty male rats were randomly divided into five identical groups, each group involved (06) animals as follow:

Group 1- Control rats treated with normal saline.
Group 2- Rats treated with 40mg/kg bw of SiNPs.
Group 3- Rats treated with 80mg/kg bw of SiNPs.
Group 4- Rats treated with 40mg/kg bw of SiNPs along with 100 mg/kg bw of DHA
Group 5- Rats treated with 80mg/kg bw of SiNPs along with 100 mg/kg bw of DHA

The Rats were administered injections of the vehicle control and silica nanoparticles intraperitonially. Doses of amorphous silica nanoparticles were 40 mg/kg bw and 80 mg/kg bw, which had diameters of 10-30 nm, were dissolved in physiological saline. Because silica nanoparticales were used as a carrier system in various drug deliveries as well as getting in blood from environmental exposure, the intraperitonial administration route was selected. The dosage of SiNPs was based on the results of previous acute toxicity studies (Yu *et al*., 2013).

Ethics statement

The experiments were strictly conducted in accordance with the institutional guidelines for animal welfare. The protocols were reviewed and approved by the Institutional animal ethical committee of Nims University Rajasthan, Jaipur.

Blood, testes and epididymides were collected from each animal for analysis. Plasma samples were obtained from blood by centrifugation at 4500 rpm for 15 min at 4^0C. The testicles were collected and weighed for the calculation of organ index. For the fertility assessment, sperm from the epididymides of all groups were collected.

Hematological parameters

The hematological tests were carried out using commercially available by HAVet CLINDIAG (B-1177-78, G.D. Colony Mayur Vihar Phase-III, Delhi-110096, India) system blood auto-analyzer. The hematological parameters namely hemoglobin (Hb; g/dl), white blood corpuscles (WBC; X 10^3 cells/ mm^3 of blood), Packed cell volume (PCV; %), red blood corpuscles (RBC; X 10^6 cells/ mm^3 of blood), Partial thromboplastin time (PTT; Sec) and Erythrocyte sedimentation Rate (ESR; mm/h) were carried out in blood samples of experimented and control rats.

Determination of gonadal hormones in plasma

The testosterone, luteinizing hormone (LH), follicle stimulating hormone (FSH) and prolactin levels in the plasma were determined using RIA kits provided by Diagnostic Products Corporation, USA and a Multiskan Ascent Microplate Reader (Thermo Multiskan MK3, USA) at 450 nm.

RESULT

Haematological investigations

The haematological parameters namely, Hb (g /dl), RBCs (x10^6mm3), WBCs (x10^3mm3), PCV (%), PTT (Sec.) and ESR (mm/h) were investigated in the control and experimental groups and data are presented in Table 1.

The Hemoglobin (Hb) count was found considerably decreased in SiNPs intoxicated rats i.e. group-2 by 17% ($p<0.01$) and group-3 by 31% ($P<0.001$). The DHA administered groups i.e. group-4 and group-5 exhibited remarkable recovery by 13%

(P<0.05) and 28% (P<0.001) respectively. The comparison between control and experimental rats, the RBCs count were found markedly decreased in group-2 and group-3 by 24% (p<0.01) and 32% (P<0.001) respectively. After the co-administration of DHA along with SiNPs, there was an increment was found in the concentration of RBCs in the group-4 and group-5 by 20% (P<0.01). The white blood cells (WBCs) counts were found considerably upraised in group-2 and group-3 by 22% (p<0.01) and 40% (P<0.001). A reduction was found in the concentration of WBCs after the treatment of DHA in group-4 and group-5 by 14% (P<0.05) and 18% respectively. The Packed Cell Volume (PCV), also termed as haematocrit were found considerably increased in group-2 and group-3 by 14% (p<0.05) and 21% (P<0.01). A reduction was found in the PCV concentration after the administration of DHA in group-4 and group-5 by 14 % (P<0.05) and 18% respectively. The partial thromboplastin time (PTT) were observed significantly increased in SiNPs treated groups i.e. in group-2 and group-3 by 14% (p<0.05) and 21% (P<0.01). After the administration of DHA along with the treatment of SiNPs, the PTT exhibited remarkable (P<0.05) recovery in group-4 and group-5 by 14% and 18% respectively. The erythrocyte sedimentation rate (ESR) were considerably increased in group-2 and group-3 by 22% (p<0.01) and 51% (P<0.001) respectively. A reduction was found in the erythrocyte sedimentation rate after the administration of DHA in group-4 and group-5 by by 14% (P<0.05) and 19% (P<0.01) respectively.

Follicle Stimulating Hormone (FSH)

The results of our study showed that FSH levels decreased significantly in group-2 and group-3 by 40% (p<0.01) and 60% (P<0.001) respectively as compared with control. While SiNPs treated groups along with supplementation of DHA exhibited remarkable increment in the level of FSH by 33% (P<0.01) in group-4 and 70% (P<0.001) in group-5 (Fig 1).

Luteinizing Hormone (LH)

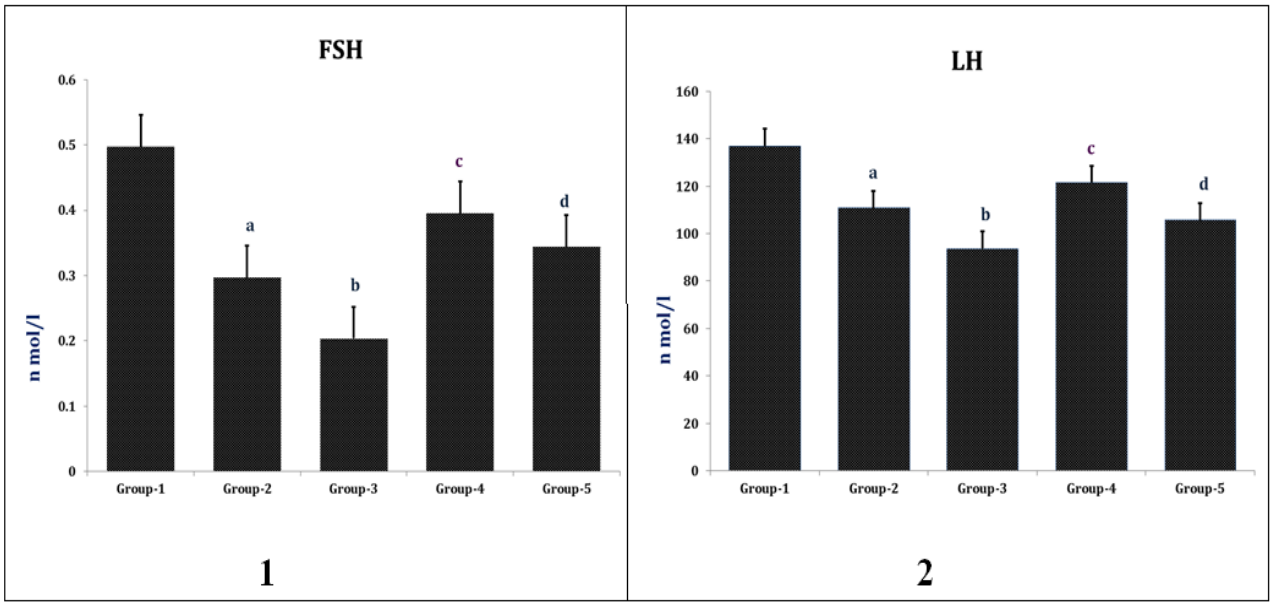
The results of study revealed significant decreased in the level of LH in silica treated groups i.e. group-2 and group-3 by 19% (p<0.05) and 31% (P<0.01) respectively as compared with control, whereas the silica treated groups recovers in the level of LH along with co-administration of DHA and its increased in group-4 and group-5 by 10% (P<0.05) and 13% (P<0.05) respectively (Fig 2).

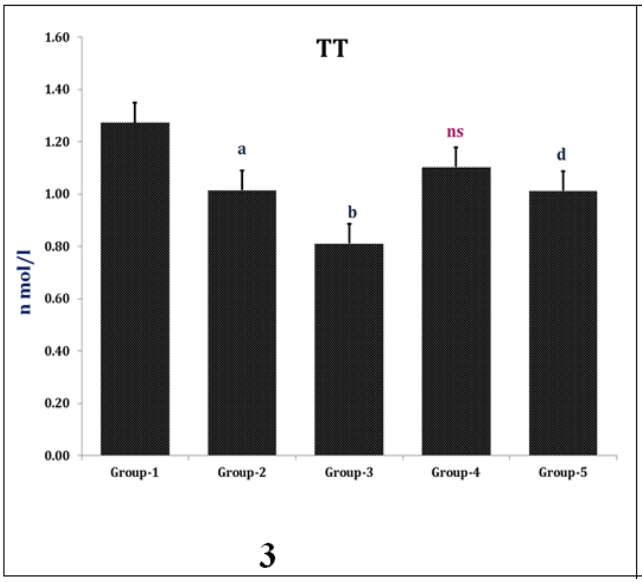
Testosterone Hormone

The results of our study showed significant changes in the level of testosterone in the both control and experimented groups A remarkable reduction was observed in silica administered groups i.e. group-2 and group-3 by 20% (p<0.05and 36% (P<0.001) respectively. Moreover, along with DHA supplementation the groups exhibited remarkable increment in the level of TT by 25% (P<0.01) in group-5 (Fig 3).

Prolactin Hormone

The results of our study showed that prolactin levels increased significantly in SiNPs treated groups viz. group-2 and group-3 by 14% (p<0.05) and 25% (P<0.01) respectively. Whereas the DHA treated group exhibited reduction in the level of prolactin by 14% (P<0.05) in group-4 and 16% (P<0.05) in group-5 (Fig 4).





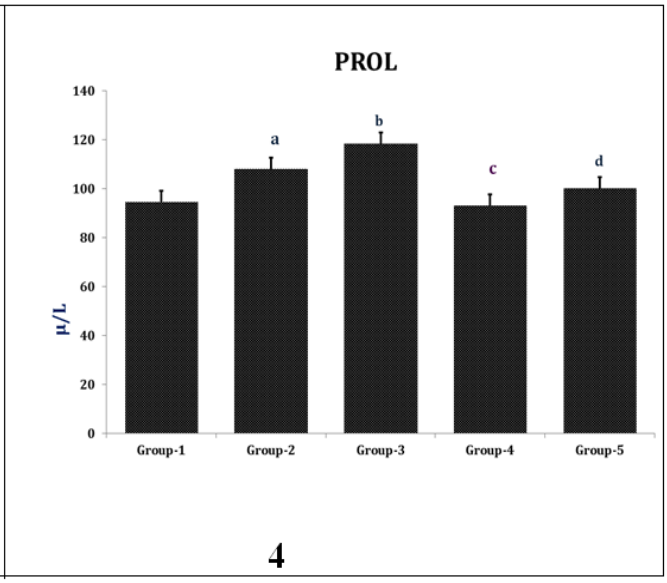


Fig 1, 2, 3 & 4: The level of FSH, LH, TT (n mole/l) and Prolactin (µ/L) was expressed as mean ± SEM of control and experimental groups. Whole data were analysed only through one way ANOVA along with the methods of Newman–Keuls. The remarkable comparisons were denoted as (a), (b), (c) and (d) for Group-1 & Group-2, Group-1 & Group-3, Group-2 & Group-4 and Group-3 & Group-5 respectively. The minimum remarkable value was considered as p<0.05

Table 1: Haematological investigations in control and Experimental groups

Parametrs	Group-1	Group-2	Group-3	Group-4	Group-5
Hb (g /dl)	12.55±0.58	10.35±0.38 [a]	8.57±0.23 [b]	11.65±0.35 [c]	10.01±0.12 [d]
RBCs ($x10^6$mm3)	4.38±0.1	3.31±0.2 [a]	2.94±0.3 [b]	3.98±0.1 [c]	3.53±0.2 [NS]
WBCs ($x10^3$mm3)	6.10±0.1	7.46±0.3 [a]	8.57±0.2 [b]	6.38±0.1 [c]	7.00±0.1 [d]
PCV (%)	62.92±1.6	71.59±1.2 [a]	76.08±1.0 [b]	63.03±0.92 [c]	64.92±1.5 [d]
PTT (Sec.)	13.57±1.1	15.44±1.4 [a]	17.83±1.6 [b]	14.09±1.0 [c]	15.73±0.95 [d]
ESR (mm/h)	4.23±0.04	5.20±0.08 [a]	6.40±0.10 [b]	4.45±0.04 [c]	5.18±0.08 [d]

The remarkable comparisons were denoted as (a), (b), (c) and (d) for Group-1 & Group-2, Group-1 & Group-3, Group-2 & Group-4 and Group-3 & Group-5 respectively. The minimum remarkable value was considered as p<0.05.

DISCUSSION

Silica nanoparticles (SiNPs), which is recognized as main inorganic components of air pollution (Duan *et al*., 2016). In humans exposure of SiNPs via the atmosphere is the most common way for different exposure (Liang *et al*., 2014). Occupational exposure of silica may increase the rate of silica induced reproductive toxicity (Pacey, 2010; Zhang *et al*., 2018). The present study focuses on the preventative measure to silica induced reproductive toxicity and protective potential of docosahexaenoic acid (DHA). The objective of this analysis was to estimate the potential toxicity and the general mechanism concerned in SiNPs and the protective potential of DHA against the particular toxicity. In this study the reproductive toxicity examined in rat testes along with the effect of DHA against these doses. In the present study, it was found that exposure of Silica nanoparticles at different dosage levels expressed

various biochemical changes. We observed that reproductive changes induced by SiNPs in rats and its correlates with the dose dependent association of biochemical findings in rat testes.

The negative influence of toxic compounds, xenobiotic on the body weight of the laboratory animal species is recognized and well-documented in published pieces of literature. Previous studies have presented a contradictory report on the effect of nanoparticles on body weight. The decreases in Body weight of rats observed in this study could be attributed to altered physiological process which probably affects the animal's appetite and feeds consumption with consequent effects on the body weight.

In the present study a complete blood snapshot can provide a clear image of the pathophysiology of the SiNPs induced alterations in the living system and further these were compared with the control. The hematological study revealed that alteration in the composition of peripheral blood is a reflection of deteriorated haematopoietic process. The reduced haemoglobin levels in the group-2 and group-3 is suggestive that decreased number of RBCs count which is reflection of rapidly destroyed than they are created or blood loss. The results of the present study suggested that silica may intricate erythropoiesis through cellular metabolism in erythrocytes. In the case of PCV, PTT, the silica administered groups exhibited much difference when compared to the controls. The ESR (erythrocyte sedimentation rate) is remarkedly elevated in silica treated rats and its recovered in DHA treated rats. It is suggestive that the imbalance between the rate of production and destruction of the blood corpuscles (Erythropoiesis).

In the present study, FSH, TT and LH levels were significantly reduced in silica treated groups and it was gradually recovered in DHA administered groups. It is suggestive that in the present study, testosterone levels were found to be gradually reduced as the increased exposure of silica nanoparticles. We also observed that increased level of prolactin which reflects the over production of reproductive hormones. The role of serum prolactin in male infertility is still unclear but the elevated prolactin levels in men are usually the result of overactive prolactin cells in the pituitary gland. Elevated prolactin called Hyperprolactinemia, which inhibits the secretion of the gonadotrophins releasing hormone (GnRH) results it may cause decreased pulsatile release of FSH, LH and Testosterone further it may lead to spermatogenic arrest and altered sperm quality (Masud *et al*., 2007).

CONCLUSION

In this study, we demonstrated that SiNPs causes decrement of quantity and quality of sperms in male rat, in addition to disorganization of testis and epididymis with impairment of spermatogenesis, this suggest that reproductive system consider as target organs to SiNPs and dysfunction of reproductive efficiency. A 100mg / kg bw of DHA supplementation ameliorates SiNPs induced toxicity. DHA supplementation may be recommended for the SiNPs exposed occupational workers.

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