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Protective Effect of Docosahexaenoic Acid on Silica Nanoparticles Induced Biochemical Alterations in Rat Testes

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Abstract **Original Research Article**

Human health effects associated with silica exposure have been widely discussed. Environmental and occupational exposure to silica nanoparticles (SiNPs) is inevitable, as nanomaterials have become part of our daily routine. SiNPs were found to have severe toxicity than silica micro-particles in male reproductive systems. It might be due to the factor that smaller the diameter of silica nanoparticles, the greater will be the toxicity. In the present study, 40 and 80 mg SiNPs were exposed to rats for 60 days and a group treated with docosahexaenoic acid (DHA) along with SiNPs. At the end of the exposure, rats were sacrificed by anaesthetic overdose, rat testes were removed for the biochemical investigations namely, lipid peroxide levels (LPO), protein carbonyl content (PC), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione (GSH). Results of the present study demonstrates that SiNPs intoxicated rats were found increased LPO and PC level while the antioxidant level namely SOD, CAT, GPx and GSH were found to be decreased in the testes when compared with the control rats, while DHA acclimation rats were observed insignificant changes as compared with the SiNPs treated groups. On the basis of results it may be concluded that the oxidative stress markers increased in the intoxicated rats and the DHA supplementation might have rescued the SiNPs exposure.

Keywords: Silica nanoparticles, Docosahexaenoic acid, Glutathione peroxidase, Superoxide dismutase, Catalase.

INTRODUCTION

The chemical compound silicon dioxide, also known as silica is the oxide of silicon, which is indicated by chemical formula SiO_2. It is found in many materials, common on construction sites, soil, landscaping materials and it are mostly found in nature as sand or quartz (Maurya *et al*., 2021). It is a principal component of most types of glass and substances such as concrete, rock and granite etc. The dust created by cutting, grinding, drilling or disturbing these materials can contain crystalline silica particles. The toxic effect of silica nanoparticles resulted from its small size that leads to exponential raise the surface area, also its shape has a crucial role in the toxicity of nanoparticles especially when interacting with the biological system (Rahmanand Padavettan, 2012). In addition, it is demonstrated that dermal exposer of silica nanoparticles penetrates in the skin and may be localized in the lymph nodes, further accumulated in the brain and liver (Hirai *et al*., 2012). Excess exposure of SiNPs may cause oxidative injury, inflammatory reaction following cell membrane disruption, genotoxic impact, necrosis and ultimately dead of cells (Gong *et al*., 2012).

As far as the exposure of humans to SiNPs is concerned, they can enter the body through inhalation, ingestion, skin uptake, injection, or implantation (Oberdorster *et al*., 2005: Yah *et al*., 2012). Thus, the wide use of silica nanoparticles has raised concerns about the negative impact of nanoparticles on human health, mainly on the reproductive systems. It is also documented that SiNPs is closely associated with different disorders like pulmonary injury, heapatotoxicity, immuno-nanotoxicity neurotoxicity, renal toxicity, and irreversible testis damage (Chauhan et al., 2013; Chou et al., 2008; Lin et al., 2008; Schipper et al., 2008; Wu et al., 2011; Bartneck et al., 2012; Vance et al., 2015).

Various studies have been documented that the association between silica exposure and health outcomes such as silicosis, lung cancer and non-malignant

respiratory disease. Liver is a main target organ for biotransformation of xenobiotic compounds and kidney is responsible for elevation of these compounds. It may be damages the normal kidney function (Wang *et al*., 2009). SiNPs accumulation, may leads to generation of free radical due to oxidative stress (Anlar *et al*., 2017). The reacting oxygen species further disrupt the biological membrane integrity evident by increased rate of lipid peroxidation in testes (Li *et al*., 2015).

It has been reported that reproductive performance is reduced after the exposure of SiNPs. It may be due to SiNPs exposure, which induced alteration in the pathophysiology of testes (Liu *et al*., 2018). Levine *et al*., observed that SiNPs decreases in testis coefficients, destruction of normal seminiferous tubule structures, disturbance in the balance of sex hormones, and reduction in quantity/quality of sperms. Docosahexaenoic acid (DHA) is a type of ω-3 fatty acid as a long-chain polyunsaturated fatty acid (PUFA). DHA acts as a unique nutrient and it's regularly eaten in the form of fish oil or algal supplements. The DHA supplementation may reduce the oxidative stress which is induced by SiNPs. Thus, the present study is demonstrating the reproductive toxicity induced by the silica nanoparticles along with potential mechanism of Docosahexaenoic Acid (DHA) for male reproductive toxicity.

MATERIALS AND METHODS

Preparation of SiNPs

In this study, the used SiNPs (White Nanopowder) were purchased from (Sigma-Aldrich Solutions), with diameters of 10-30 nm and purity 99%. The different concentrations of silica suspended were prepared by dissolving each of (40 and 80 gm) of SiNPs with 1 litter of distilled water to prepare each of the level doses (40 and 100 mg/kg bw) respectively.

Laboratory animals

Thirty male rats (the body weight in between 120±10gm) were housed to a 12 light /dark cycle at the animal house of the Nims University and environmental condition was maintained at a constant temperature of (22-25^{0}C), water and food in the form of commercial pellet were provided *ad libitum*.

Experimental Design

The present study included an effect of oral administration silica nanoparticles at two different concentrations for 60 days. Thirty male rats were randomly divided into five identical groups, each group contains (06) animals as follow and the doses of SiNPs were given by intra peritoneal (IP) and DHA orally through cannula.

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

Body and Testes weight

A body weight of male rats for each group has been monitored at beginning and the end of the experiment. At the end of the experiment, all rats of each group were sacrificed by anesthetic dose of sodium penta-barbitol and testes were removed and kept directly in formalin (10%) for histological studies and to be used for sperm analysis including (sperm concentration, viability, sperm motility, percentage live and dead sperm).

Sperm Morphology

After the removal of the testes, it's placed in a warm concave watch glass containing 5 ml of normal saline. It was cut by scalpel blade into very small pieces to release the sperm in it to determine the concentration, vitality and motility of sperms. The percentage of motility sperm is calculated using method of Cheng *et al*., 2006, the percentage of viability are estimated by using method of Bambe, 1998. The sperm count was done by using haemocytometer in which slide chamber was installed on the optical microscope and covered with cover slip (Lucio *et al*., 2009), 10 microliters of diluted sample were taken by a pipette and slowly inserted under the coverslip on both sides of the slide chamber and then left about 5-10 minutes to settle the sperm on the slide and was counted five squares by used 40x microscope objective. The concentration of sperm was calculated by multiplying the mean of sperm count calculated in the multiplier factor (10^6). The viability was determined by using Nigrosin - Eosin stain at 40x microscope objectives (Lucio *et al*., 2009).

Biochemical Estimations

The 10% (w/v) homogenate of the testes was ready to prepare and exploitation York's homogenizer fitted with Teflon plunger in 0.1 M phosphate buffer (pH 7.1). The complete homogenate material was centrifuged at 2500 x g for 10 minutes in a refrigerated centrifuge. The pellet consisting of nuclear fraction and cell rubble was discarded. The supernatant was more centrifuged at 11000 x g for 15 minutes and mitochondrial fraction was separated. The clear supernatant was more centrifuged at 10500 x g for 90 minutes and therefore the resultant supernatant was used for the enzyme activities.

The protein estimation was done by the method of (Lowery *et al*., 1995). Lipid peroxide level (LPO- n mole MDA/g tissue) was carried out by using spectrophotometer at 532 nm according the method of Okhawa *et al*., 1979. The activity of superoxide dismutase (SOD) was measured by using spectrophotometer at 560 nm by the method of Mc Cord and Fridovich, 1969. The activity of catalase was done by the method of Aebi *et al*., 1974 by using hydrogen peroxide as substrate. Glutathione (GSH) were done by Ellman *et al*., 1959 and expressed as µ mole /g tissue.

Statistical analysis

Statistical analyses were performed by using one- way ANOVA depending on the Graphpad. Statistical minimum P value significance was defined as P<0.05.

RESULT

After the 60 days of experimental period the terminal body weight was found to be significantly (P<0.001) reduced in group-2 and group -3 by 16% and 27% respectively when it was compared with the Group-1. The DHA treated, group-4 and group-5 were significantly (P<0.001) recovered by 13% and 18% when compared with the group-2 and group-3 respectively. The weight of the testes of the control and experimental rats were observed. Testes weights was found to be significantly (P<0.05) reduced in group-2 and group-3 by 14% and 30% respectively as compared with the Group-1. The DHA treated groups, group-4 and group-5 was significant (P<0.05) increased by 6% and 21% as compared with group-2 and group-3 respectively.

Table-1: The Terminal body weight and Testes weight of control and SiNPs treated groups

	Body Weight (gm)	Testis Weight (gm)
Group-1	128.23	1.45
Group-2	107.57	1.25
Group-3	93.52	1.01
Group-4	121.42	1.33
Group-5	110.23	1.22

The results of the sperm morphology showed that there are statistically significant differences in the concentration, motility, and abnormality of sperm. The mean sperm counts per epididymal volume of rats administered with SiNPs significantly (P<0.001) decreased by 29% in group-2 and 55% in group-3, when compared with the group-1. The DHA administered groups showed significantly (P<0.01) increased by 27% in group-4 and 41% in group-5 when compared with the group-2 and group-3 respectively. Sperm motility was found to be markedly (P<0.001) reduced by 40% in group-2 and 56% in group-3 when these were compared with group-1. Moreover significant (P<0.001) increment was observed in DHA administered group-4 and group-5 by 30% and 44% when compared with the group-2 and group-3 respectively. The sperm viability was found to be significantly reduced by 30% (P<0.001) in group-2 and 57% in group-3 when these were compared with group-1. The DHA administered groups showed significant (P<0.01) recovery by 20% in group-4 and 39% in group-5 when compared with the group-2 and group-3 respectively. The pH of the semen of control and experimental rats was insignificant (p>0.05) change between the groups.

Table 2: Sperm Count, Motility, Viability and pH in the control and SiNPs treated groups

	Group-1	Group-2	Group-3	Group-4	Group-5
Sperm Count (10^6/ ml)	92.15	65.32	41.67	82.78	58.97
Sperm Motility (%)	88.8	53.62	39.22	69.82	76.48
Sperm Viability (%)	92.05	63.85	38.80	76.62	54.00
Seminal pH	7.4	8.2	8.4	7.5	7.7

The present study exhibited that, the concentration of total protein significantly (P<0.01) decreased by 33% in group-2 and 41% in group-3 while there was a significant (P<0.05) increase in the concentration of total protein by 41% in group-4 and 29% in group-5. The PC content was found to be considerably increased by 31% (p<0.05) in group-2 and 54% (p<0.001) in group-3. On the other hand significant reduction was found in DHA administered groups by 16% (group-4) and 18 % (group-5).

Lipid peroxide levels and antioxidant i.e., SOD, CAT, GPx and GSH were estimated in experimented and control rats. Present finding are suggested that a close relationship among silica toxicity, increased oxidative stress and diminished spermatogenesis in experimental animals. The lipid peroxide levels were found significantly increased in SiNPs administered groups i.e. in group-2 and group-3 by 30% (P<0.01) and 63% (P<0.001) respectively. Whereas the DHA treated group exhibited reduction in the level of lipid peroxides by 15% (P<0.05) in group-4 and 25% (P<0.001) in group-5 when compared with group-2 and group-3 respectively. A remarkable (P>0.001) reduction were observed in the activity of superoxide dismutase enzyme in silica treated groups i.e. group-2 and group-3 by 30% and 42% respectively. While silica treatment along with DHA supplementation, the activity of SOD recovers nears to control and observed in group-4 and group-5 by 34% and 38% respectively. The activity of catalase were significantly (P<0.001) reduced in SiNPs administered groups viz. group-2 and group-3 by 37% and 61% respectively. Moreover after the administration of DHA a considerable (P<0.001) recovery was observed in the activity of catalase in group-4 and group-5 by 43% and 75% respectively.

Glutathione peroxidase, a selenium dependent enzyme, metabolizes these peroxides and protects cell membrane from damage. The activities of glutathione peroxidase were significantly (P<0.001) reduced in silica administered groups i.e. in group-2 and group-3 by 20% and 32%. A remarkable recovery was observed in DHA administered group viz. by 16% (P<0.01) in group-4 and 34% (P<0.001) in group-5 as compared with group-2 and group-3 respectively. The glutathione concentration is reduced in SiNPs treated groups i.e. group-2 and group-3 by 23% (P<0.05) and 36% (P<0.001) respectively. While supplementation of DHA exhibited remarkable increment in the level of glutathione by 16 % in group-4 and 30% in group-5, when they were compared with group-2 and group-3 respectively.

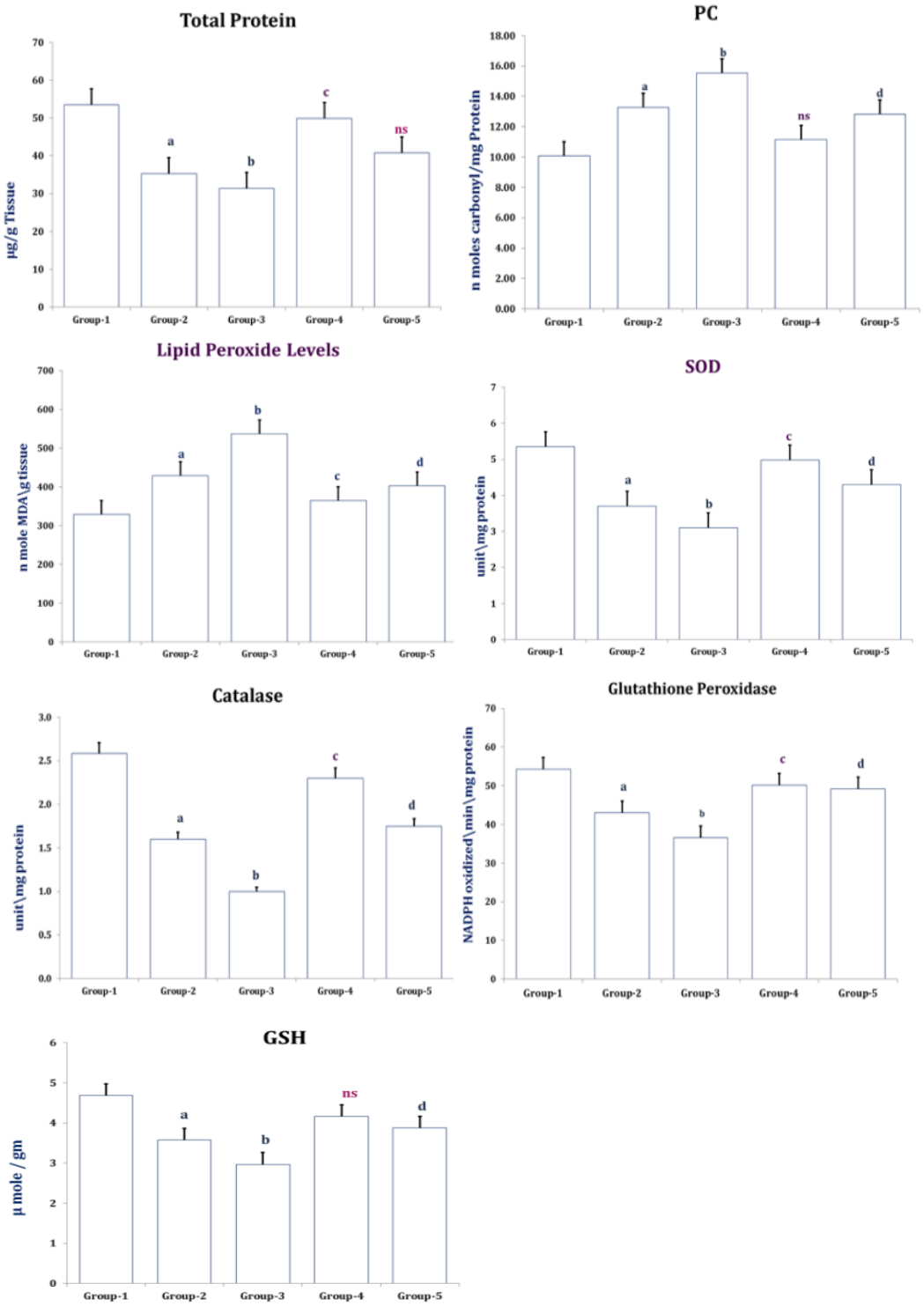

Fig 3: Protein estimation, PC content, Lipid peroxide level, Superoxide dismutase, Catalase, GPx and GSH levels in Testes of control and SiNPs treated group. The results are expressed as Mean ± SEM in six rat of each group (N=6). Superscripts relate significant (p<0.05) comparison with Control and treated group

DISCUSSION

In present study we investigated that SiNPs induced changes in rat testes and modulation by DHA. After the treatment of the SiNPs we observed that terminal body and testes weight of control and experimental rats were significantly reduced. It may be due to the loss of lipid, protein and other biomolecules and deterioration of metabolic activities. Ganrot (1986) reported that the loss in body weight has also been attributed to the increased mobilization of fat deposits, owing to enhanced synthesis of glucose from non-carbohydrate sources.

The results of the present study demonstrated that SiNPs decreased sperm motility and viability after 60 days treatment of both the doses of SiNPs. It is suggestive that, SiNPs alters semen quality and sperm DNA integrity or its associated proteins in the testis by generation of ROS which could intensify testicular dysfunction and further leads to reduced sperm quality. Low levels of ROS produced by spermatozoa are needed for physiological processes involving sperm capacitation and the acrosome reaction. Excessive generation of ROS by SiNPs may leads to reduced mitochondrial membrane potential and is associated with a decreasing energy availability, which may impede sperm motility (Tremellen, 2008). Sperm membrane constituted by high level of unsaturated fatty acids which makes spermatozoa particularly susceptible to oxidative damage (Wang *et al*., 2003). Oxidative stress is known as one of the most vital causes of male infertility (Hussein *et al*., 2016), and is one of the main mechanisms of this deterioration (Tremellen, 2008). Oxidative stress induced by NPs exposure comprises mitochondrial respiration mitochondrial apoptosis, alteration of calcium homeostasis, activation of the NADPH oxidase system, and depletion of antioxidant enzymes (Manke *et al*., 2013).

Nanoparticles have been shown to generate reactive oxygen species (ROS) by pro-oxidant functional groups, active redox cycling and particle cell interactions (Almansour *et al*., 2018). Oxidative stress can cause a variety of biochemical reactions that are potentially harmful for physiological process and function (Kim *et al*., 2014). Reactive oxygen species constitute a pool of oxidative species including superoxide anion, hydroxyl radical, hydrogen peroxide, singlet oxygen. The antioxidant defense mechanism is very important in response to reactive oxygen species toxicity. SOD superoxide dismutase is the first line of the defense mechanism and scavenges superoxide radicals. Excessive production of ROS in mitochondrial is leading cause of cellular damage. Importantly, the fertility of spermatozoa depends upon trans-membrane potential of mitochondria which is regulated through electron-transport chain (Wang *et al*., 2003). Moreover, oxidative damage associated with the impairment of reproductive physiology (Aitken *et al*., 2010) and excessive production of ROS may lead to ATP depletion, DNA damage, lipid peroxide levels and diminished fertility in population. The glutathione is an important antioxidant. GPx is the enzyme which catalyzes the conversion of reduced glutathione in to the oxidized glutathione. The decreased level of the GPx activity is the reflection of oxidative stress. GSH plays an important role in the maturation of sperm and positive correlation between GSH and sperm quality has been established (Bhardwaj *et al*., 2000). In the present study we found that reduced GSH levels in the seminal plasma.

The major outcomes of reactive oxygen species damage to a tissue are lipid peroxidation. In this study, DHA is responsible to reduce lipid peroxide levels in Silica treated rats. These results indicate that DHA is accomplished with anti-peroxidative properties. DHA in recent years, particularly with regards to a promising role for the nutrient in neurodevelopment, neuro-cognition and neurodegenerative disorders. DHA is responsible to stimulate the stress-signaling pathway which is used by cells to induce the synthesis of proteins which is responsible for the counteraction and detoxification of the oxidative stress.In the present study DHA reinstate the antioxidant levels and protect the deterioration of sperm structure and function.

CONCLUSION

On the basis of results it may conclude that SiNPs exposure directly correlates with reduced quality of sperm and increased production of reactive oxygen species which further leads to biochemical alterations in testes. On the other hand, It is found that DHA recover antioxidant potential against the dose dependent SiNPs induced reproductive toxicity along with impairment of spermatogenesis.

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REFERENCES

- Aitken, R. J., & De Iuliis, G. N. (2009). On the possible origins of DNA damage in human spermatozoa. *MHR: Basic science of reproductive medicine*, *16*(1), 3-13.
- Almansour, M., Alarifi, S., & Jarrar, B. (2018). In vivo investigation on the chronic hepatotoxicity induced by intraperitoneal administration of 10-nm silicon dioxide nanoparticles. *International journal of nanomedicine*, *13*, 2685.
- Anlar, H. G., Bacanli, M., İritaş, S., Bal, C., Kurt, T., Tutkun, E., ... & Basaran, N. (2017). Effects of occupational silica exposure on oxidative stress and immune system parameters in ceramic workers in Turkey. *Journal of Toxicology and Environmental Health, Part A*, *80*(13-15), 688-696.

- Axiner, E., Malqvist, M., Linda–Forsberyg, C., & Rodringuez-Mertias, H. (1999). Regginal histology of the duct epididymis in the domestic Cat. *J Reprot Develop*, 45, 151-160.
- Bambe, K. (1998). Evolution of acrosomalintergrity of boar spermatozoa by bright field microscopy using an Eosin – Nigrosin stain. *Theriogenol*, 29, 1245-125.
- Bartneck, M., Ritz, T., Keul, H. A., Wambach, M., Bornemann, J., Gbureck, U., ... & Tacke, F. (2012). Peptide-functionalized gold nanorods increase liver injury in hepatitis. *Acs Nano*, *6*(10), 8767-8777.
- Bhardwaj, A., Verma, A., Majumdar, S., & Khanduja, K. L. (2000). Status of vitamin E and reduced glutathione in semen of oligozoospermic and azoospermic patients. *Asian journal of andrology*, *2*(3), 225-228.
- Cheng, D., Zheng, X. M., Li, S. W., Yang, Z. W., & Hu, L. Q. (2006). Effects of epidermal growth factor on sperm content and motility of rats with surgically induced varicoceles. *Asian journal of andrology*, *8*(6), 713-717.
- Chou, C. C., Hsiao, H. Y., Hong, Q. S., Chen, C. H., Peng, Y. W., Chen, H. W., & Yang, P. C. (2008). Single-walled carbon nanotubes can induce pulmonary injury in mouse model. *Nano letters*, *8*(2), 437-445.
- Chauhan D S, Singh V P, Mishra S, Tripathi S, Tiwari M, Tomar A. (2013). Influence of fluoride exposure on hypothalamic pituitary gonadal axis hormones and semen quality. Asian J. Biol. Life Sci. Sep-Dec 2013 .Vol-2. Issue-3. 201-206.
- Ganrot, P. O. (1986). Metabolism and possible health effects of aluminum. *Environmental health perspectives*, *65*, 363-441.
- Gong, C., Tao, G., Yang, L., Liu, J., He, H., & Zhuang, Z. (2012). The role of reactive oxygen species in silicon dioxide nanoparticle-induced cytotoxicity and DNA damage in HaCaT cells. *Molecular biology reports*, *39*(4), 4915-4925.
- Hirai, T., Yoshikawa, T., Nabeshi, H., Yoshida, T., Akase, T., Yoshioka, Y., ... & Tsutsumi, Y. (2012). Dermal absorption of amorphous nanosilica particles after topical exposure for three days. *Die Pharmazie*, *67*(8), 742-743.
- Hussein, M. M., Ali, H. A., Saadeldin, I. M., & Ahmed, M. M. (2016). Querectin alleviates zinc oxide nanoreprotoxicity in male albino rats. *Journal of biochemical and molecular toxicology*, *30*(10), 489-496.
- Levine, H., Jørgensen, N., Martino-Andrade, A., Mendiola, J., Weksler-Derri, D., Mindlis, I., ... & Swan, S. H. (2017). Temporal trends in sperm count: a systematic review and meta-regression analysis. *Human reproduction update*, *23*(6), 646-659.
- Li, S., Tan, H. Y., Wang, N., Zhang, Z. J., Lao, L., Wong, C. W., & Feng, Y. (2015). The role of oxidative stress and antioxidants in liver diseases. *International journal of molecular sciences*, *16*(11), 26087-26124.
- Lin, P., Chen, J. W., Chang, L. W., Wu, J. P., Redding, L., Chang, H., ... & Yang, R. S. (2008). Computational and ultrastructural toxicology of a nanoparticle, Quantum Dot 705, in mice. *Environmental science & technology*, *42*(16), 6264-6270.
- Liu, J., Yang, M., Jing, L., Ren, L., Wei, J., Zhang, J., ... & Sun, Z. (2018). Silica nanoparticle exposure inducing granulosa cell apoptosis and follicular atresia in female Balb/c mice. *Environmental Science and Pollution Research*, *25*(4), 3423-3434.
- Lucio, R. A., Tlachi, J. L., López, A. A., Zempoalteca, R., & Velázquez-Moctezuma, J. (2009). Analysis of the parameters of the ejaculate in the laboratory Wistar rat: technical description. *Veterinaria México*, *40*(4), 405-415.
- Manke, A., Wang, L., & Rojanasakul, Y. (2013). Mechanisms of nanoparticle-induced oxidative stress and toxicity. *BioMed research international*, *2013*, 942916.
- Maurya, M. K. (2021). Silica Nanoparticles Induced Oxidative Stress in Different Brain Regions of Male Albino Rats. *Sch Acad J Biosci*, *5*, 139-144.
- Oberdörster, G., Oberdörster, E., & Oberdörster, J. (2005). Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. *Environmental health perspectives*, *113*(7), 823-839.
- Rahman, I. A., & Padavettan, V. (2012). Synthesis of silica nanoparticles by sol-gel: size-dependent properties, surface modification, and applications in silica-polymer nanocomposites—a review. *Journal of Nanomaterials*, *2012*.
- Schipper, M. L., Nakayama-Ratchford, N., Davis, C. R., Kam, N. W. S., Chu, P., Liu, Z., ... & Gambhir, S. S. (2008). A pilot toxicology study of single-walled carbon nanotubes in a small sample of mice. *Nature nanotechnology*, *3*(4), 216-221.
- Sies, H. (2015). Oxidative stress: a concept in redox biology and medicine. *Redox biology*, *4*, 180-183.
- Skakkebaek, N. E., Rajpert-De Meyts, E., Buck Louis, G. M., Toppari, J., Andersson, A. M., Eisenberg, M. L., ... & Juul, A. (2016). Male reproductive disorders and fertility trends: influences of environment and genetic susceptibility. *Physiological reviews*, *96*(1), 55-97.
- Tremellen, K. (2008). Oxidative stress and male infertility—a clinical perspective. *Human reproduction update*, *14*(3), 243-258.
- Vance, M. E., Kuiken, T., Vejerano, E. P., McGinnis, S. P., Hochella Jr, M. F., Rejeski, D., & Hull, M. S. (2015). Nanotechnology in the real world: Redeveloping the nanomaterial consumer products inventory. *Beilstein journal of nanotechnology*, *6*(1), 1769-1780.
- Wang, F., Gao, F., Lan, M., Yuan, H., Huang, Y., & Liu, J. (2009). Oxidative stress contributes to silica

nanoparticle-induced cytotoxicity in human embryonic kidney cells. *Toxicology in vitro*, *23*(5), 808-815.

- Wang, X., Sharma, R. K., Gupta, A., George, V., Thomas Jr, A. J., Falcone, T., & Agarwal, A. (2003). Alterations in mitochondria membrane potential and oxidative stress in infertile men: a prospective observational study. *Fertility and sterility*, *80*, 844-850.
- Wu, J., Wang, C., Sun, J., & Xue, Y. (2011). Neurotoxicity of silica nanoparticles: brain localization and dopaminergic neurons damage pathways. *ACS nano*, *5*(6), 4476-4489.
- Yah, C. S., Simate, G. S., & Iyuke, S. E. (2012). Nanoparticles toxicity and their routes of exposures. *Pak J Pharm Sci*, 25, 477-491.