Effect of Silica Nanoparticles on level Cyp19a1 and Cyp17a1 genes in Male Rats

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Abstract. The current study has been designed to evaluate the effect of silica Oxide nanoparticles (SiO2NPs) exposure on the reproductive performance of male rats. Forty adult Wistar albino male rats (aged 60-70 days with body weight 130-140 g), were randomly divided into four equal groups, 10 rats per group: control group were received physiological saline, and three treated groups were administered by gavage at dose (1 mg, 10 mg and 100 mg/kg Body weight) of SiO2NPs suspension daily for 22 days, to study the effect of treated groups with silica nanoparticles on the expression of mRNA for Cyp19a1 and Cyp17a1 genes in testis by using qRT-PCR technique. Experimental results obtained from SiO2NPs treated male rats at the molecular level, the results appear that there is a significant (p<0.05) decrease in expression level of Cyp19a1 and Cyp17a1 genes in testis tissue for groups treated compared to control group.

Keywords: SiO2NPs, Rats, reproductive, genes, Cyp19a1, Cyp17a1

Introduction
Silica nanoparticles (SNPs) are among the most natural components that exist in crust of the earth (Arts et al., 2007). There are several applications for silica nanoparticles such as in biomedicine, foodstuffs, consumer goods and industry. Also silica nanoparticale are used as carriers in delivery of drug (Frohlich and Roblegg, 2012; Wang et al., 2013). Due to high exposure of human body to nanomaterials, Concerns about the safety of nanoparticles have increased (Cho et al., 2013). So there is an urgent need to assess the toxicity of nanomaterials. Cyp17a1 (Cytochrome p450, Family17, Subfamily a1) is the main gene responsible for encoding the Cyp17a1 enzyme, a single gene of 6 kb in length and containing eight exons with the location of intronexon boundaries conserved among species (Youngblood and Payne, 1992). The gene site in humans is 10q24.3 (Fan et al., 1992), whereas in mice it is located on chromosome 19 (Youngblood et al., 1991). Cyp17a1 gene is the main responsible for the generation of hormonal steroids that produce progestin, androgen and estrogen as Raucci and et al.,(2014) note that the Biosynthesis of the testosterone hormone depends on the expression of the steroidal gene origin that includes Cyp17a1, is expressed in all steroid-producing tissues and in the testicle specifically in leydig cells (Pelletier et al., 2001). is the encoded (Cytochrome p450,Family19, Subfamily a1)While Cyp19a1 gene of the aromatic enzyme p450 Aromatase, a monoclonal gene containing 10 exons (Simpson et al., 2002). Its chromosomal location in humans is 15q 21.1 (Chen et al., 1988), whereas in rats it is located on chromosome 9 (Youngblood et
al., 1989). Cyp19a1 is responsible for the synthesis of estrogen by converting the androgen (testosterone, dihydrotestosterone, and androstenedione) into estrogen (Al-Bader and Kilarkaje, 2015), which has a paracrine, endocrine, and autocrine effect that is important not only in regulating male and female reproductive activity but also in growth (Simpson et al., 2000).

Biosynthesis of the testosterone hormone requires the presence of steroid proteins, as steroids act as a regulated protein, and the genes Cyp17a1 and Cyp19a1 act on the production of the side-chain cholesterol enzyme (Sundaram & Kumar, 1996). Steroids are also necessary to transport free cholesterol from outside into the mitochondrial membrane of Leydig cells. This reaction is stimulated by an enzyme encoded by the genes Cyp17a1 and Cyp19a1.

Materials and Methods
Preparation of SiO2NPs

In this study, used SiO2NPs (White nanopowder) were purchased from (SkySpring Nanomaterials, INC), with diameters of 10-30 nm and purity 99%. Treated with Silane coupling Agent. The different concentrations of silica suspended were prepared by dissolving each of (0.1, 1, 10 gm) of SiO2NPs with 1 litter of distal water to prepare each of the level doses (1, 10, 100 mg/kg) respectively. When the silica nanopowder suspended in distal water, sonicated for 5 min and then mixed by vortex for 1 min before using it. Optical properties of silica nanoparticle were measured by spectrophotometer in (Environment Research Unit affiliated to College of Science, University of Al-Qadisiyah).

Experiment animals

The current study has been conducted at the animal house of science college, University of Al-Qadisiyah. Wistar albino males Rats were used in this study as atypical sample for mammals. The period of the administration is 22 days and they were bred under suitable laboratory conditions, at temperature between (20 - 25° C) and with average 14 hours of light to 10 hours of darkness during the study period. The animals were fed on a standard laboratory food (19% protein and 3000 calories) and water ad libidum.

Design Experimental

In this experiment, 40 adult males aged 60 - 70 were used, and their weights ranged from 130 to 140 g. Rats were randomly divided into four groups. Each group consists of 10 rats. The rats of first group control (C) daily received 1 ml of normal saline orally for 22 days and rats of three treated groups were administered orally with silica nanoparticles at levels of doses (1, 10, 100 mg / kg body weight), respectively, daily for 22 days. at the end of the experiment, all rats of each groups were sacrificed by anesthetized by injecting ip a mix of 0.3 ml ketamine and 0.1 ml Xylazine per kg of body weight, testes was dissected to study some molecular indicators.

Molecular analysis

(A)-Materials used in the study: The real-time measurement of polymerase chain reaction was used for quantitative reverse transcription from Bioneer in South Korea using the following primers for Cyp19a1 and Cyp17a1 genes.

| Table (1):show Primers that used in qRT-PCR reaction for detection gene expression |
|-----------------------------------------------|
| Primer   | Sequence   | Amplicon |
| Cyp19a1   | AGCCAAAACCTAACGGCTTTGCG | 118bp   |
|           | TGCATGGGCTTCAGCATTTC |         |
| Cyp17a1   | AACGTTGACTACAGCATTGC | 86bp    |
|           | TCCATCGTGATGCAGTGC |         |
| GAPDH     | ATGCCCATGTTTGTGATG   | 136bp   |
|           | TCCACGATGCAAGTTC |         |
(B) - Examination of polymerase chain reaction for real-time reverse transcription (QRT-PCR)

The polymerase chain reaction was investigated for real-time quantitative reverse cloning to measure the quantitative levels of mRNA, which was transcribed from the testis to denote the amount of gene expression for Cyp19a1 and Cyp17a1 genes, also GaPdH gene was used as a Standard Housekeeping gene to calculate gene expression. This examination was carried out according to the following steps:

1. **Extraction of total RNA**: Total RNA was extracted using the Trizol kit, which was supplied by Korean Pioneer Company. This equipment was prepared according to the manufacturer's instructions. The organs were ejected immediately after being removed with liquid nitrogen and then placed in ependrof tubes which is contained 0.5 mL of DEPC water and then centrifuged at a speed of 12000 cycles / minute and for two minutes, then it was pulled DEPC water and remains only the tissue of the testis then add 1 mL of the Trizol and then crush the tissue of testes were crushed with micropistells, and after downstream milling and homogeneity well. then added 200 microliters of chloroform and the tubes were requested with a vortex and then preserved in the freezer for 10 minutes. Then the tubes were put in a centrifuge for 10 minutes and then the clear transfers in a new ampendorf with the addition of 500 microleter of isopropanol and the tubes were requested with a vortex and then preserved in freeze the refrigerator for 10 minutes after which the tubes are placed in the centrifuge for 10 minutes at a speed of 12000 cycles / min. Then remove the pellet to keep only the deposition pellet and add 1 mL of ethanol alcohol to the precipitate concentration of 80% and requested continuously with a vortex and then put the mixture in a centrifuge speed of 12000 cycle / minute for 5 minutes and was removed from the floating and took the sediment pellet and then dehydrate deposited well by turning aependorf on paper towel and leave it at room temperature for 10 minutes. After that, Free nuclease water added, then the tubes were placed in water bath with 60 c for 10 minutes, then keep the extracted RNA at 70 ° C.

2. **Measuring the concentration and purity of the ribosome DNA**: RNA yield and quality: DNA samples were detected from the samples and RNA concentration (ng/ l) was determined using the Nanodrop spectrophotometer and the DNA purity was measured by reading absorbance at 260 and 280.

3. **Treatment with enzyme DNase1**: The DNA extract was treated using Dnase I treatment to dispose of the DNA residue in the extraction process based on how the enzyme worked as described in Promega company's instructions in the United States of America.

4. **cDNA synthesis**: The cDNA process was used to synthesize complementay DNA (cDNA) from RNA samples to be used to amplify gene of genes expression for target genes and the conservative gene by Real-Time PCR, where the Accupower Rockscript RT Premix kit was used by Korean bioneer. This process was carried out in accordance with the company’s instructions. The concentrations of all RNA samples treated with DNase was standardized to the same concentration as the nanodrop measured by DEPC. then RNA was converted to cDNA by preparation of the PreMix reaction for real-time reverse quantitative.

5. **Quantitative Real-Time PCR (qRT-PCR)**: The qPCR test of the cDNA samples was performed using the Accupower Green Star Real-Time PCR kit and Exicycler ™ 96 Real-Time Quantitative Thermal Block processors from the Korean bioneer company by way Cheon et al. (1999). This test is based on the green Syber dye in kit detection qRT-PCR PreMix which was designed to amplify the PCR of cDNA target genes Cyp17a1 and Cyp19a1 by using primers and GapdH gene as a standard gene (Housekeeping gene) to estimate the amount of the number of copies resulting from the PCR compared to the number of copies for standard curve output of the genome of qPCR. The green Syber dye found in the kit is associated with the new copies of the amplified parts of the target gene and the conservative gene and then recorded fluorescent signals in the thermocycler Real Time PCR. The Genomic standard curve of the GapdH gene for rat Rattus norvegicus (27.9Mbp) which was obtained from Gene Bank NCBI-Gene Bank approximately (~ 1 × 107) and were used as a standard curve for genomic DNA.
6- Real-Time PCR analysis of data analysis: The resulting data from the real-time polymerase chain reaction were analyzed using the Livak and Schmittgen method (2001).

- Statistical Analysis Data of the results of current study were analyzed by using completely randomized design depending on Statistical Analysis System (SAS, 2012). Duncane Multiple range test is used to estimate the significant differences among means of treatments (Duncane, 1955).

-Results
  - Concentration and purity of total RNA

The concentrations and purity of total RNA in the tissues of the testis tissues figure (1) showed high concentrations and sufficient quantities to begin the polymerase chain reaction. Also, the results showed that the ratio between the optical density at 260 and 280 nm were ranging between 1.81 and 2.003. In the tissues of the testis . This is evidence of the purity of the total RNA of the tissue samples included in the study. polymerase chain reaction for real –time quantitative multiplication (qPCR).

![Figure (1) shows the total RNA concentration (ng / l) in the testis tissue of male white rats](image)

- Polymerase chain reaction for real-time quantitative multiplication (qPCR)

The analysis of the data on the examination of qPCR which depend on reaction of SYBR ® green dye is divided into two parts, the primer efficiency estimation and the reaction quantity of gene expression of genes Cyp19a1 and Cyp17a1 corrected by gene expression of the conservative gene (GpdH)

1-The Estimate of primer efficiency

The results of the threshold cycle (Ct) data were calculated from the amplification plot in the qPCR reaction probe during accelerated .Exponential phase for that were included in the study fluorescent signals (flash) for the green Syber dye primer which united with genes primer that were include in the study Cyp19a1and Cyp17a1 which they are reactive with cDNA for mRNA of testis tissues for male rats. from the Ct threshold, the linear regression was calculated using data points and the initiatory efficiency of liner slope was calculated.

2- Relative quantity of target gene expression

In the current study the target gene expression of Cyp19a1and Cyp17a1 genes was calculated using the \( (2^\Delta C_T) \) equation which was known as the Levak and Schmittgen method (2001), by correcting the expression of the target gene with the expression of the conservative gene as a corrected gene. The gene expression of the
control group is considered as a gene for control or standard for both the target genes and (GapdH) gene (house keeping gene) in the study. The first step was the Levak and Schmittgen equation to correct the number of threshold cycles for the target genes by the correction gene, including the standard sample or control group. The second step was to correct the value of Ct for the groups (T1, T2, T3) to the Ct value of the standard sample then the percent of gene expression which expressed by a symbiotic change (fold change).

-Relative Quantities of Gene expression Cyp19a1 and Cyp17a1 in testis tissues:
The results of Cyp17a1 gene levels in the testicular tissues curve(2), Figure (3) showed a significant (P <0.05) decrease in Cyp17a1 gene expression which were levels 2.127, 1.807 and 1.003 a symbiotic change (fold change). a symbiotic change (fold change) for the first, second, and third treated groups with silica nanoparticles compared to the control group, which reached a double change (fold change) for the first, second, and third treated groups with silica nanoparticles which were 3.100, 1.700, 1.010 times respectively for the first, second and third treated groups when compared to the control group, which reached (5.537) a double change.

Figure (2) Correlative variation curves of CYP17A1 and CYP19A1, genes in testicular tissue of male white rats. Yellow curve: C control group, red curve: T1 group, blue curve: T2 group, green curve: T3 group.

Figure (3) shows the effect of silica nanoparticles on the fold change of Cyp17a1 gene in the testis tissue of male rats.
Figure (4) shows the effect of silica nanoparticles on the fold change of Cyp19a1 gene in the testis tissue of male rats.

The correlation coefficients among gene expression of genes CYP19a1 and CYP17a1 and sperm characteristics. Table (2) shows that there is a positive and significant (p < 0.05 or p < 0.01) correlation among gene expression of genes CYP19a1 and CYP17a1 and sperm characteristics (sperm concentration, abnormalities and live sperm) where these traits were increased by increasing genetic expression and were decreased by the lack of genetic expression. A negative and significant correlation was also found between the genetic expression of the different genes with the abnormalities, which increases the abnormalities with the lack of gene expression as shown in the table below.

Table (2): Correlation coefficients between gene expression of different genes and sperm characteristics.

| Traits | Motility | Genes     | Abnormalities | Live sperm | Sperm concentration |
|--------|----------|-----------|---------------|------------|---------------------|
| Testes | CYP19a1  | -0.788**  | 0.794***      | 0.673*     | 0.768*              |
|        | CYP17a1  | -0.880**  | 0.859***      | 0.834**    | 0.888*              |

* Indicates a significant level (P < 0.05).
** Indicates a significant level (P < 0.01).

Discussion

Molecular study

The results showed that there are a significant decrease (P < 0.05) in gene expression Cyp19a1 and Cyp17a1 genes in testis tissues compared with the control group. The low level of genes indicates that silica nanoparticles prevented the synthesis of steroidal hormones that lead to apoptosis of male germ cells during the process of spermatogenesis where the genes Cyp17a1 and Cyp19a1 in the testes are responsible for the production of steroid hormones and also, the biological construction of testosterone depends on the expression of these genes (Raucci et al., 2014; Al-Bader and Kilarkaje, 2015). Therefore, when the level of Cyp19a1 and Cyp17a1 decreases, the level of the testosterone, which affects the process of spermatogenesis, is affected. The testosterone affects the process of converting the spermatid to sperm (Cheng, 2009).
Low levels of genes in exposure to silica nanoparticles may be due to oxidative stress, resulting in an imbalance between reactive oxygen oxides (ROS) and antioxidants, and a decrease in the effectiveness of antioxidant enzymes, including superoxide dismutase (SOD) and high levels of MDA Malondialdehyde, where oxidative stress reduces the number leydig cells responsible for producing testosterone (Gonzalez et al., 2015). These results were agreement with those finding of Zhang et al., (2015), where a decrease a low level of Cyp19a1 was observed when the rats were injected with 5 mg / kg Of silver nanoparticles where effective oxygen classes work on oxidation of the biological compounds such as DNA, proteins and fats, which lead to cell damage and causing genomic instability. The decrease in the level of genes may be attributed to the lower level of testosterone, Garcia et al. (2014) and Clewell et al. (2010) report that exposure to chemicals leads to decrease the level of testosterone in cells leydig through the chemical effect on cyp17a1 expression and testicular enzymes these finding are similar to those results of Zhang et al., (2015) who a found decrease in the level of expression of Cyp17a1 gene when rats were exposed to silver nanoparticles of size 10 and 20 nm.

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