COMPARATIVE ANTIOXIDANT EFFECTS OF N-ACETYLCYSTEINE AND CURCUMIN ON TITANIUM DIOXIDE NANOPARTICLES INDUCED ORCHIDOTOXICITY IN HEALTHY ADULT ALBINO RATS

Sahar M. Abo El Wafa[1], Mohammed F. Khodary[1], Alaa E. Abd Elkader[1*], Ankita K. Parmar[2] and Noha S. Elnajjar[1]

[1] Forensic Medicine & Clinical Toxicology Department, Faculty of Medicine, Benha University, Egypt

[2] Forensic talents India, Ahmedabad, Gujarat, India

[*] Corresponding author: Alaa E. Ibrahim, Forensic Medicine & Clinical Toxicology Department, Faculty of Medicine, Benha University, Egypt, Alaa.elsayed10@yahoo.com, +201284544426

Submit Date 2022-08-12
Revise Date 2022-12-17
Accept Date 2022-01-03

ABSTRACT

Introduction: With widespread applications of nanoparticles (NPs) including titanium dioxide nanoparticles (TiO_2NPs) in different fields, many adverse effects may threaten both environmental and medical health including the male reproductive system. **Aim of this work**: To examine the ameliorative effect of N-acetyl cysteine (NAC) and curcumin (Cur) against TiO_2NPs induced testis toxicity in adult albino rats**. Materials and Methods**: Sixty-four adult male albino rats were classified into eight groups. Group 1: control received a regular diet, water, and normal saline. Group 2: vehicle, received corn oil. Group 3: gavaged orally with NAC (100 mg/kg). Group 4: orally gavaged with curcumin (200 mg/kg) once a day. Group 5: gavaged orally with TiO_2NPs (100mg/kg) once a day. Group 6: orally gavaged once daily with TiO_2NPs (100 mg/kg) and NAC (100 mg/kg). Group 7: orally received TiO_2NPs (100mg/kg) and curcumin (200mg/kg) once a day. Group 8: gavaged orally TiO_2NPs (100mg/kg) followed by NAC (100mg/kg) and curcumin (200mg/kg). **Results**: The results revealed that TiO_2NPs induced a significant decrease in final body weight, weight body gain, and testis weight testicular tissues, TiO_2NPs increased oxidative stress as evidenced by decreased levels of antioxidants such as superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) and higher levels of the lipid peroxidation marker malondialdehyde (MDA). In addition to harming the testicular histological architecture, TiO_2NPs significantly decreased the levels of the sex hormones testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH). They also significantly decreased sperm motility, viability, cell count, and concentration. In the testicular tissues, TiO_2NPs led to the downregulation of 17-beta hydroxysteroid dehydrogenase 3 (17-HSD) and the overexpression of proapoptotic gene (Bax) transcripts. Conversely, NAC and/or curcumin had a protective effect on testicular tissue. **Conclusion:** We propose that NAC and curcumin may be employed to lessen the toxicity and oxidative damage caused by ingesting TiO_2NPs. TiO_2NP exposure caused oxidative damage and morphological injury in the testis. **Keywords**: Titanium dioxide nanoparticles; N-acetyl cysteine; curcumin; 17-beta hydroxysteroid dehydrogenase 3; Bax gene expression; oxidative stress markers; hormonal analysis.

INTRODUCTION

Nanotechnology plays a big part in our modern daily lives, ranging from the biomedical sector to the energy sector. There are different physicochemical and biological methods to synthesize nanoparticles (NPs) for multiple applications **(Mughal et al., 2021).**

Researchers have begun to pay attention to the health hazards posed by NPs as a result of the rapid expansion in the field of advanced nanotechnologies and all of its far-reaching benefits **(Ema et al., 2010)**. Throughout evolution, humans have been exposed to a variety of airborne NPs, but the intensity of that exposure has considerably grown in recent years due to

the many ways in which NPs are used in items in our daily lives **(Oberdorster et al., 2005)**. The release of NPs into the environment as nanostructure materials, which could hurt the ecosystem, is another risk associated with this increased production rate of NPs **(Cappello et al., 2017).**

The blood-testis barrier, which protects the reproductive tissues, is one biological barrier that NPs can cross because of their nano size **(Rollerova et al., 2015)**. As a result, the spermatogenic effects of NP crossing may be toxic. After exposure, NPs travel through many pathways to the reproductive system, where they primarily target the testicles and epididymis in males **(Zhao et al., 2014).**

There are several ways that titanium dioxide (Tio2) can enter the body, including ingestion, cutaneous penetration, inhalation, and injection **(Jin et al., 2008)**. For NPs, skin contact and inhalation are regarded as being particularly crucial **(Shukla et al., 2011).** However, because TiO2 is utilized as a food additive in toothpaste and capsules, oral ingestion is the possible exposure route for the general population **(Wang et al., 2007*)***.

TiO2NPs are among the most widely utilized nanoparticles in medicine, drug delivery, engineering, agriculture, personal care, cosmetics, toothpaste, sunscreens, clothing, paints, and coverings, as well as as an imaging agent and in food **(Chabanyuk, 2014; Galletti, 2016; Yang et al., 2017).**

The food business uses nano-TiO2 extensively. Coated candies, preserved fruit, chewing gum, fizzy drinks, powdered drinks, milk, dairy products, and other food categories have all been produced using it **(Weir et al., 2012; Chen et al., 2013).**

Deoxyribonucleic acid (DNA) damage, apoptosis, and anomalies in the mitochondria have all been described as being caused by Tio2NPs and oxidative stress **(Kang et al., 2009).**

NAC is a thiol-containing amino acid. It is available as a safe and cheap medication as a mucolytic drug **(Youssef et al., 2006; Larsson et al., 2015).** NAC can reduce oxidative stress directly, inhibit the nuclear factor kappa b (NF-B) inflammation pathway and the release of inflammatory cytokines, and increase the production of GSH. This ability to reduce oxidative stress is characterized by the release of sulfhydryl groups, which in turn reduce Reactive Oxygen Species (ROS) levels **(Xue et al., 2011).** It has a powerful antioxidant activity and it is one of the suggested alternatives for treating diseases associated with the generation of ROS **(Farag et al., 2021).**

The yellow pigment known as Cur, which is included in turmeric's (Curcuma longa) rhizome, offers a variety of health benefits, including anti-inflammatory, anti-carcinogenic, antioxidant, and hypocholesterolemic actions. **(Hewlings and Kalman., 2017).**

AIM OF THE WORK

The main objective of the present work was to study the potential ameliorative effect of both antioxidants NAC and Cur on the effect of TiO2NPs on the testis through histopathological examination of the testis, evaluation of sperm function, hormonal analysis, oxidative stress markers and messenger ribonucleic acid (mRNA) transcripts in healthy adult male albino rats.

MATERIALS AND METHODS

The experimental design study was approved by the Research Ethics Committee at the Faculty of Medicine, Benha University (REC-FOMBU), Egypt with approval number: MS 17.11.2020.

This study was conducted on 64 healthy adult male albino rats, about 3-4 months old; their average main weight was ranging between 180 gm to 200 gm**.**

At the animal bread house in the Anatomy Department of the Benha Faculty of Medicine, all the animals underwent one week of passive preliminaries (taking food and water without any medications) before beginning the experiment, to ascertain their physical well-being, and to exclude any diseased animals. An identical diet was given to all of the animals (Wheat, Bread & Milk). For all animals, medication administration was

scheduled to begin at noon. The animals were anesthetized with ether and killed 24 hours after the final dose was administered.

(II) Chemicals:

- **TiO_2NPs:**

Design: It was purchased from Sigma Chemical Campany, Egypt.

Intervention: Titanium (IV) oxide, a mixture of rutile and anatase nanoparticles.

Parameters assessed: <150 nm particle size, 40 wt. % in H2O, 99.5% trace metals basis.

- **NAC** was purchased commercially available from SEDICO Chemical Company, Egypt.
- **Cur** was purchased from Sigma- Aldrich Company, Egypt. It was in the form of 100 % pure bright yellow to the orange color powder.
- **Corn oil** was obtained from the local market.
- All other compounds were of the best commercially obtainable quality.

(III) Grouping and experimental design:

Rats were randomly and equally divided into 8 groups, each one consisting of 8 rats, and all groups were treated daily for 8 weeks, as follows:

- **Group 1 (Control group):**

Rats were left without intervention to measure the basic parameters, free access to food is allowed, gavaged with distilled water and normal saline 1 ml.

- **Group 2 (Vehicle group):**

Each rat received 1 ml of corn oil.

Group 3 (NAC group):

Each rat was gavaged orally with NAC (100 mg/kg B.W) **(Khayal et al., 2019).**

- **Group 4 (Cur group):**

Each rat was treated with Cur 200 mg/kg B.W **(Aggarwal et al., 2015).**

- **Group 5 (TiO_2NPs):**

It received TiO_2NPs (100 mg/kg B.W) **(Morgan et al., 2017; Jafari et al., 2020; Morgan et al., 2015).**

- **Group 6 (TiO2NPs and NAC group):**

Each rat orally received TiO_2NPs (100 mg/kg B.W) followed by 1 hour by NAC (100 mg/kg B.W).

Group 7 (TiO2NPs and Cur group):

Each rat orally received TiO_2NPs (100 mg/kg B.W) followed by 1 hour by Cur (200 mg/kg B.W).

- **Group 8 (TiO2NPs, NAC, and Cur group):**

Rats were treated with a single dose of TiO_2NPs (100 mg/kg B.W) orally by gavage tube followed by 1 hour by a single dose of NAC (100 mg/kg) orally by gastric tube and Cur (200 mg/kg) by gastric tube.

(IV): Parameters of the study:

1. Body weight and relative weight of testis:

Body weights at the beginning and end were noted. Rats were dissected, their testes were taken out and stripped of fatty tissues and blood vessels, blotted, and their weights were calculated after the experimental period.

2. Biochemical study for hormonal analysis:

Blood samples were taken from their hearts with 5 ml syringes for estimation (FSH, LH, and testosterone) to be measured by routine laboratory tests, I.e., radioimmunoassay (RIA) according to the method reported by **(Picard et al., 2008).**

3. Biochemical study for oxidative stress markers:

- Determination of SOD, MDA, CAT, and GSH levels in testicular tissue

Handling of tissue samples for estimation of oxidative stress parameters:

Using commercially available colorimetric techniques and diagnostic kits provided by Bio Diagnostic Company, Egypt, we measured the levels of MDA, SOD, CAT, and GSH in the testicular tissue while adhering to the manufacturer's recommendations **(Hussein et al., 2018).** The procedure was used to measure the MDA levels in tissue samples described by **El-Akabawy and El-Sherif (2016).** The SOD concentration in tissue samples was estimated using the method described by **Rasyidah et al. (2014).** The CAT activity of testes homogenate was

determined following the method reported by **Aebi (1984)**. The technique was used to measure the GSH level in tissue samples described by **Siervo et al. (2015).**

4. Semen analysis:

a. Sperm motility:

The development of sperm motility was investigated according to the method reported by **Bearden and Fuquay (1984).**

b.Sperm liveability:

This was assessed and the percentage was calculated according to **Olugbenga et al. (2011).**

c.Sperm count and concentration:

This was accomplished using the technique used by **Bearden and Fuquay (1984*)***.

d.Sperm abnormalities:

This was recorded according to **Evans and Maxwell (1987).**

5. Relative quantitative PCR for 17β-HSD and Bax mRNA transcripts (Hussein et al.,2019):

Following the manufacturer's instructions, total RNA was obtained using the total RNA purification kit (Jena Bioscience). Utilizing reverse transcription kits, first-strand complementary DNA (cDNA) synthesis was carried out (enzynomics). The relative expression of the target genes' mRNAs in the testis was assessed using real-time polymerase chain reaction (PCR) with SYBR green and GADPH as an internal control. Using the recommended primers and the HERAPLUS SYBR® Green qPCR Master Mix, the extracted cDNA was amplified by the manufacturer's instructions (Willowfort). Data on expression were examined using the 2ΔΔCt statistical method **(Livak and Schmittgen 2001).**

6. Histopathological study by light microscope:

As indicated by **Lamberg and Rothstein (1978)**, Tissues were fixed for 6–8 hours, then transported to 70% alcohol before being sent to histology at the Pathology Department, Faculty of Medicine, Benha University for robotic drying, paraffin implanting, segmenting, and recoloring.

Statistical analysis:

Using SPSS [Statistical bundle for social science] version 20, the data had been collected, tabulated, and analyzed. For quantitative data, the mean and standard deviation were added; for subjective data, recurrence and dissemination were. The recognized significance level in this investigation started at 0.05. One-way analysis of variance (ANOVA) was used in statistical comparisons to compare mean values between the treatment groups and the control group.

RESULTS

1. Body weight and relative weight of testis:

Body weight gain in the TiO_2NPs group was significantly less than in other groups, but showed insignificant differences between TiO_2NPs + NAC group and TiO_2NPs + Cur group. Body weight gain in TiO_2NPs + NAC + Cur group was significantly more than TiO_2NPs + NAC group and TiO_2NPs + Cur group.

Testis weight in the TiO_2NPs group was significantly less than all other groups except the TiO_2NPs+ Cur group.

Also, there was no statistically significant between TiO_2NPs + NAC group, TiO_2NPs + Cur group, and TiO_2NPs + NAC + Cur group shown in **Table (1).**

2. Hormonal Analysis study results:

As shown in **Table (2)**, FSH, LH, and testosterone levels were a significant decrease in the TiO_2NPs group compared with other experimental groups. With the administration of NAC or Cur, they were significantly more than in the TiO_2NPs group. LH and testosterone in combined administration of TiO_2NPs + NAC + Cur group were significantly more than TiO_2NPs + NAC group and TiO_2NPs + Cur group. FSH level was insignificantly different between TiO_2NPs + NAC + Cur group and TiO_2NPs + Cur group and TiO_2NPs + NAC group.

3. Oxidative Stress Markers results:

The level of MDA was significantly increased in the TiO_2NPs group compared with the other experimental51 groups. Administration of NAC and/or Cur

significantly reduced the concentration of MDA. In contrast to the other experimental groups, the TiO_2NPs group had significantly lower SOD and CAT activity as well as GSH levels.

Similar to how NAC or Cur administration reduced the effects of TiO_2NPs, the combination had better results than either individual therapy (TiO_2NPs+ NAC + Cur group were highly significant than TiO_2NPs + NAC group and TiO_2NPs + Cur group but the last significant lower than TiO_2NPs + NAC group) described in **Table (3).**

4. Semen analysis:

As shown in **Table (4)**, sperm motility, livability, cell count, and concentration were significantly reduced in the TiO_2NPs group compared with the other experimental groups. However, NAC and Cur, both individually or in combination, showed a reversal of this decrease but, TiO_2NPs + NAC group was insignificantly different from the TiO_2NPs + Cur group. Sperm livability in TiO_2NPs + NAC + Cur group was significantly higher than TiO_2NPs + NAC group and TiO_2NPs + Cur group. Sperm concentration in TiO_2NPs + NAC + Cur group was significantly higher than TiO_2NPs + NAC group and TiO_2NPs + Cur group. Conversely, sperm abnormalities were significantly increased in the TiO_2NPs group compared with the other groups.

5. Relative quantitative PCR for 17β-HSD and Bax mRNA transcripts:

As shown in **(Fig. 1)** that downregulation in HSD17B3 gene expression was found in testicular tissues following TiO_2NPs treatment, compared with the control group, vehicle group, NAC group, and Cur group by 0.011, 1, 1.089, 2.301, and 2.107 fold changes respectively.

However, this level was upregulated in TiO_2NPs + NAC group, TiO_2NPs + Cur group and TiO_2NPs + NAC + Cur group compared with the TiO_2NPs group by 68.663, 25.624, 213.236, and 1 fold changes respectively.

Upregulation in BAX gene expression was found in testicular tissues following TiO_2NPs treatment, compared with the control group, vehicle group, NAC group, and Cur group by 2.234, 1, 1.069, 0.804, and 1.074 fold changes respectively.

However, this level was downregulated in TiO_2NPs + NAC group, TiO_2NPs + Cur group and TiO_2NPs + NAC + Cur group compared with the TiO_2NPs group by 0.569, 0.649, 0.498, and 1 fold changes respectively.

6. Histopathological result by light microscope:

As regarding histopathological study, the testis of control group and vehicle group showed normal architecture with well-arranged closely matted seminiferous tubules and normal interstitial tissues **(Fig. 2a)** and **(Fig. 2b)**. The testis of NAC group showed seminiferous tubuless contains sertoli cells, spermatogonia, spermatocyte and sperms **(Fig. 2c).** The testis of Cur group showed normal architecture with well-arranged closely matted seminiferous tubules, normal Blood vessles and normal interstitial tissues **(Fig. 2d).** The testis of TiO_2NPs group showed marked loss of normal architecture of seminiferous tubules, atrophy and detachment of basement membrane **(Fig. 2e)** and **(Fig. 2f)**. Also showed degeneration of spermatogonia and spermatocyte and decrease in sperm count **(Fig. 2g)**. The testis of TiO_2NPs + NAC group showed mild loss of normal architecture of seminiferous tubule, mild detachment and mild vaculation **(Fig. 2h).** The testis of TiO_2NPs + Cur group showed very mild restore to architecture of seminiferous tubules with intercellular vacuoles, improvement of numbers of spermatocytes and sperms filling lumen and less atrophy **(Fig. 2i).** The testis of TiO_2NPs + NAC + Cur group showed restore of normal architecture of seminiferous tubules, near normal spermatogenesis and normal interstitial tissues **(Fig. 2j).**

Table (1): Body weight of rats and relative weight of testis before and after 8 weeks of treatment in the studied groups using ANOVA

	Initial body weight (gm)	**Final body weight (gm)**	**Weight gain (gm)**	**Testis weight (gm)**
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**
Control group	192 ± 3.46	231.25 ± 6.71 (efgh)*	39.25 ± 5.06 (efgh)*	1.63 ± 0.13 (efg)*
Vehicle group	191.87 ± 3.91	236.75 ± 6.63(fgh)*	44.75 ± 9.65 (efgh)*	1.64 ± 0.11 (efg)*
NAC group	187.87± 20.15	232 ± 6.28 (efgh)*	37.37 ± 8.45 (efgh)*	1.72 ± 0.06 (efgh)*
Cur group	193.62 ± 4.72	236.25 ± 3.61 (efgh)*	42.87 ± 3.36 (efgh)*	1.65 ± 0.043 (efg)*
TiO_2NPs group	187.5 ± 3.58	169.375 ± 5.85(acdfgh)*	-18 ± 5.45(abcdfgh)*	1.31 ± 0.04 (abcdfh)*
TiO_2NPs and NAC group	190.5 ± 4.93	194.25 ± 4.89(abcdeh)*	4.25 ± 8.08 (abcdeh)*	1.52 ± 0.02 (abcde)*
TiO_2NPs and Cur group	189.75 ± 3.45	187.625 ± 5.04(abcdeh)*	-2.125 ± 6.22 (abcdeh)*	1.42 ± 0.03 (abcdh)*
TiO_2NPs, NAC, and Cur group	189.25 ± 6.63	212.75 ± 4.95 (abcdefg)*	23.5 ± 8.83 (abcdefg)*	1.57 ± 0.03(ceg)*

*****Statistically significant, **a**: significant with the control group, **b**: significant with the vehicle group, **c**: significant with NAC group, **d**: significant with curcumin group, **e**: significant with TiO_2NPs group, **f**: significant with TiO_2NPs and NAC group, **g**: significant with TiO_2NPs and curcumin group, **h**: significant with NAC, TiO_2NPs and Cur group, **TiO_2NPs**: Titanium dioxide nanoparticles, **NAC**: N-acetyl cysteine, **SD**: ± standard deviation.

Table (2): Hormonal Analysis in the studied groups after 8 weeks of treatment in the studied groups using ANOVA

	FSH hormone (U/mL) Mean ± SD	**LH hormone (U/mL) Mean ± SD**	**Testosterone hormone (ng/dL) Mean ± SD**
Control group	0.83 ± 0.07 (cdefgh)*	2.49 ± 0.18 (cdefgh)*	5.26 ± 0.03 (cdefgh)*
Vehicle group	0.86 ± 0.06 (efgh)*	2.41 ± 0.22 (cdefgh)*	4.98 ± 0.03 (cdefgh)*
NAC group	0.92 ± 0.05 (aefgh)*	2.76 ± 0.06 (abefgh)*	5.70 ± 0.43 (abefgh)*
Cur group	0.92 ± 0.04 (aefgh)*	2.69 ± 0.06 (abefgh)*	5.68 ± 0.23 (abefgh)*
TiO_2NPs group	0.21 ± 0.02 (abcdfgh)*	1.51 ± 0.3 (abcdfgh)*	1.91 ± 0.07 (abcdfgh)*
TiO_2NPs and NAC group	0.68 ± 0.03 (abcde)*	1.92 ± 0.02 (abcdeh)*	3.68 ± 0.09 (abcdegh)*
TiO_2NPs and Cur group	0.64 ± 0.04 (abcde)*	1.88 ± 0.04 (abcdeh)*	3.18 ± 0.11 (abcdefh)*
TiO_2NPs, NAC, and Cur group	0.69 ± 0.04 (abcde)*	2.23 ± 0.03 (abcdefg)*	4.19 ± 0.07 (abcdefg)*

*****Statistically significant, **a**: significant with the control group, **b**: significant with the vehicle group, **c**: significant with NAC group, **d**: significant with Cur group, **e**: significant with TiO_2NPs group, **f**: significant with TiO_2NPs and NAC group, **g**: significant with TiO_2NPs and Cur group, **h**: significant with NAC, TiO_2NPs and Cur group, **TiO_2NPs**: Titanium dioxide nanoparticles, **NAC**: N-acetyl cysteine, **FSH**: Follicle-stimulating hormone, **LH**: Luteinizing Hormone, **SD**: ± standard deviation.

Table (3): Oxidative stress markers in the studied groups after 8 weeks of treatment in the studied groups using ANOVA

	SOD Antioxidants (u/mg) Mean ± SD	**MDA Antioxidants (u/mg) Mean ± SD**	**Catalase Antioxidants (u/mg) Mean ± SD**	**GSH Antioxidants (u/mg) Mean ± SD**
Control group	984.64 ± 1.45 (cdefgh)*	60.24 ± 1.36 (cdefgh)*	919.79 ± 2.91 (cdefgh)*	724.82 ± 1.07 (bcdefgh)*
Vehicle group	982.26 ± 1.27 (cdefgh)*	60.99 ± 1.09 (defgh)*	917.25 ± 3.35 (cdefgh)*	720.26 ± 1.13 (acdefgh)*
NAC group	995.16 ± 1.24(abefgh)*	65.63 ± 1.63 (adefgh)*	960 ± 1.98 (abefgh)*	795.51 ± 1.19 (abdefgh)*
Cur group	993.6 ± 1.65 (abefgh)*	67.515 ± 0.77 (abcefgh)*	956.98 ± 3.11 (abefgh)*	790.39 ± 1.62 (abcefgh)*
TiO_2NPs group	385.23 ± 1.98 (abcdfgh)*	178.12 ± 1.29 (abcdfgh)*	279.87 ± 1.34 (abcdfgh)*	231.98 ± 1.15 (abcdfgh)*
TiO_2NPs and NAC group	698.74 ± 1.75 (abcdegh)*	119.18 ± 1.13 (abcdeh)*	640.11 ± 1.38 (abcdegh)*	430.17 ± 1.53 (abcdegh)*
TiO_2NPs and Cur group	688.35 ± 2.23 (abcdefh)*	120.31 ± 1.05 (abcdeh)*	631.08 ± 1.31 (abcdefh)*	425.42 ± 1.18 (abcdefh)*
TiO_2NPs, NAC, and Cur group	705.04 ± 2.89 (abcdefg)*	100.41 ± 1.05 (abcdeg)*	699.18 ± 1.29 (abcdefg)*	502.13 ± 1.16 (abcdefg)*

*Statistically significant, **a**: significant with the control group, **b**: significant with the vehicle group, **c**: significant with NAC group, **d**: significant with Cur group, **e**: significant with TiO_2NPs group, **f**: significant with TiO_2NPs and NAC group, **g**: significant with TiO_2NPs and Cur group, **h**: significant with NAC, TiO_2NPs and Cur group, **TiO_2NPs**: Titanium dioxide nanoparticles, **NAC**: N-acetyl cysteine, **SOD**: Superoxide dismutase, **MDA**: Malondialdehyde, **GSH**: glutathione, **SD**: ± standard deviation.

Table (4): Sperm motility, morphology, and concentration in the studied groups after 8 weeks of treatment in the studied groups using ANOVA

	Sperm motility (%) Mean ± SD	**Sperm livability (%) Mean ± SD**	**Sperm count (million) Mean ± SD**	**Sperm concentration (million/ml) Mean ± SD**	**Sperm abnormality (%) Mean ± SD**
Control group	70 ± 8.02 (efgh)*	60.5 ± 3.89 (befgh)*	256.12 ± 14.76 (efgh)*	1.86 ± 0.08 (befgh)*	25.12 ± 3.31 (bcefgh)*
Vehicle group	61.87 ± 9.4 (fgh)*	53.37 ± 3.66 (acdefg)*	246.25 ± 5.17 (defgh)*	1.63 ± 0.05 (acdefgh)*	32 ± 2.39 (acdefgh)*
NAC group	66.87 ± 10.57 (efgh)*	65 ± 4.2 (befgh)*	261.62 ± 6.37(efgh)*	1.94 ± 0.05 (befgh)*	20.5 ± 1.41 (abefgh)*
Cur group	63.12 ± 8.42 (efg)*	62.37 ± 3.58 (befgh)*	264.5 ± 8.96 (befgh)*	1.90 ± 0.05 (befgh)*	21.37 ± 1.4 (befgh)*
TiO_2NPs group	25 ± 6.55 (acdfh)*	16.87 ± 5.3 (abcdfgh)*	73.5 ± 8.58 (abcdfgh)*	0.74 ± 0.08 (abcdfgh)*	85.37 ± 4.59 (abcdfghdf)*
TiO_2NPs and NAC group	43.12 ± 7.04 (abcde)*	43.12 ± 3.6 (abcdeh)*	214 ± 13.33 (abcde)*	1.01 ± 0.05 (abcdeh)*	51.62 ± 2.13 (abcdeh)*
TiO_2NPs and Cur group	37.5 ± 6.54 (abcdh)*	43.25 ± 3.37 (abcdeh)*	209.37 ± 11.41 (abcde)*	1.00 ± 0.05 (abcdeh)*	50.25 ± 2.37 (abcdeh)*
TiO_2NPs, NAC, and Cur group	53.75 ± 7.44 (abceg)*	50 ± 2.39 (acdefg)*	221.87 ± 11.93 (abcde)*	1.18 ± 0.07 (abcdefg)*	40 ± 2.82 (abcdefg)*

*Statistically significant, **a**: significant with the control group, **b**: significant with the vehicle group, **c**: significant with NAC group, **d**: significant with Cur group, **e**: significant with TiO_2NPs group, **f**: significant with TiO_2NPs and NAC group, **g**: significant with TiO_2NPs and Cur group, **h**: significant with NAC, TiO_2NPs and Cur group, **TiO_2NPs**: Titanium dioxide nanoparticles, **NAC**: N-acetyl cysteine, **SD**: ± standard deviation.

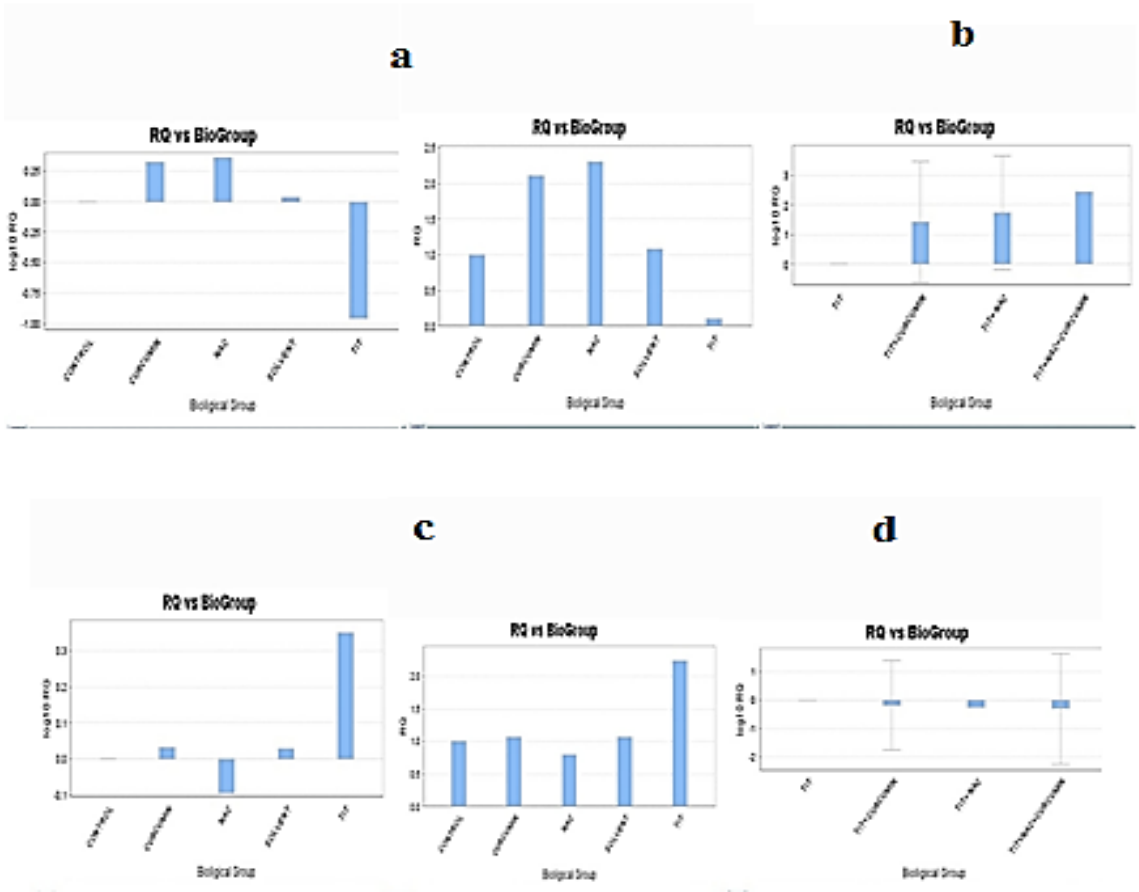

Figure 1: (a) fold changes of HSD17B3 between the control group (as calibrator) and vehicle, NAC, Cur, and TiO_2NPs group, (b) fold changes of HSD17B3 between TiO_2NPs group (as calibrator) and TiO_2NPs + NAC group, TiO_2NPs + Cur group and TiO_2NPs + NAC + Cur group, (c) fold changes of BAX between control group (as calibrator) and vehicle, NAC, Cur and TiO_2NPs group, (d) Fold changes of BAX between TiO_2NPs group (as calibrator) and TiO_2NPs + NAC group, TiO_2NPs + Cur group and TiO_2NPs + NAC + Cur group.

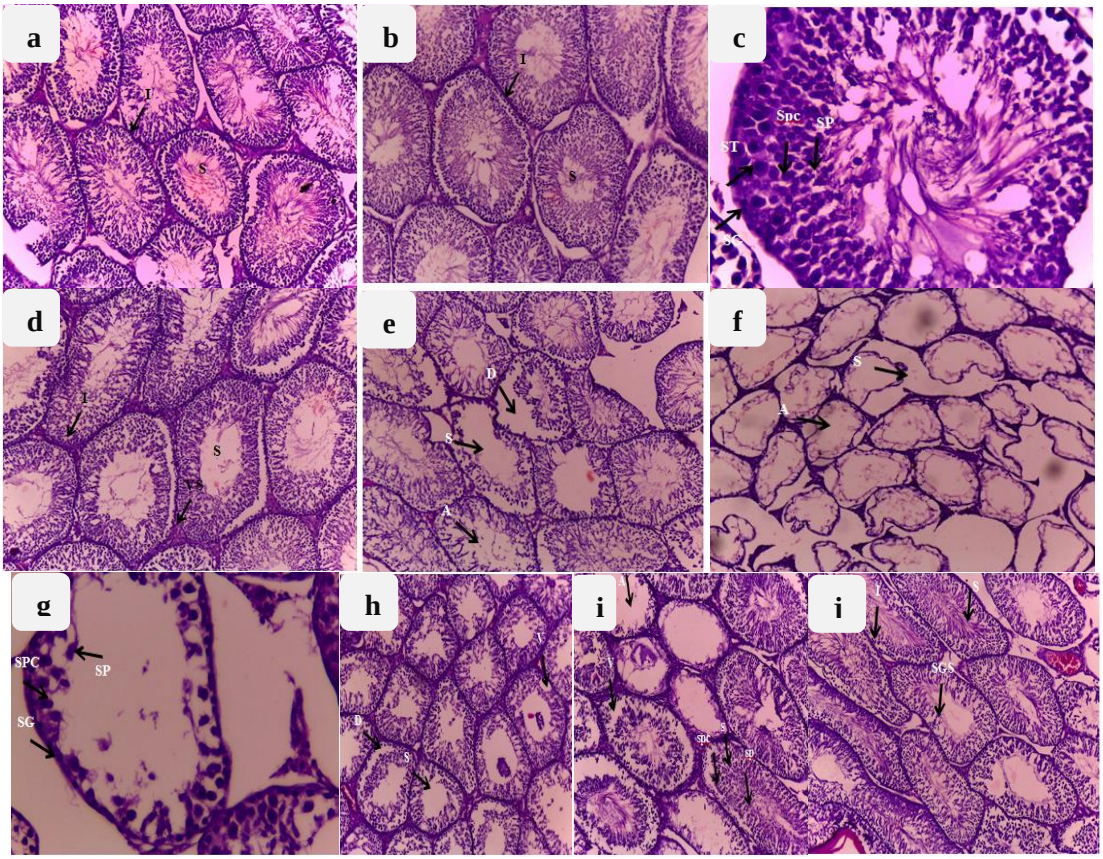

Figure 2 : **(a):** Photomicrograph segment of a rat testis from a control group (1) demonstrates normal architecture, including well-organized, closely matted seminiferous tubules (S) and normal interstitial tissues (I) **(Hx & E x 100)**, **(b):** A photomicrograph segment of rat testis made from a vehicle group (2) demonstrates typical architecture, including well-organized, densely matted seminiferous tubules (S) and normal interstitial tissues (I) **(Hx & E x 100)**, **(c):** Photomicrograph section in rat's testis prepared from a NAC group (3) showing the seminiferous tubules contains sertoli cells (ST), spermatogonia (SG), spermatocyte (SPC),and sperms (SP) **(Hx &Ex 400)**, **(d):** Photomicrograph section in rat's Testis from the Cur-treated group (4) displaying normal architecture and densely matted, well-arranged seminiferous tubules (S), interstitial tissues and blood vesicles in a normal state (I) **(Hx & E x 100)**, **(e) and (f):** Photomicrograph section in rat's testis obtained from the TiO_2NPs -treated group (5) demonstrating a substantial loss of the seminiferous tubules' typical architecture (S), atrophy (A), and basement membrane separation (D) **(Hx & E x 100)**, **(g):** Photomicrograph section in rat's testis taken from the TiO_2NPs -treated group (5) demonstrating spermatogonia (SG) and spermatocyte (SPC) degradation and a decrease in the number of sperm (SP) **(Hx & E x 400)**, **(h):** Photomicrograph section in rat's testis obtained from the TiO_2NPs + NAC-treated group (6) demonstrating mild detachment (D), mild vaculation, and mild disruption of the typical architecture of the seminiferous tubule (S) (v) **(Hx & E x 100)**, **(i):** Photomicrograph section in rat's testis obtained from TiO_2NPs + Cur treated group (7) demonstrating very moderate restoration of seminiferous tubule architecture (s) with intercellular vacuoles (V), improvement of the quantity of spermatocytes (spc) and sperms (sp) filling lumen, and reduced atrophy (A) **(Hx & E x 100)**, **(j):** Photomicrograph section in rat's testis produced from the TiO_2NPs + NAC, + Cur treated group (8) demonstrating the restoration of normal seminiferous tubule architecture (S), nearly normal spermatogenesis (SGS), and normal interstitial tissues (I) **(Hx & E x 100)**.

DISCUSSION

In the current study, final body weight, weight gain, and testis weight in the TiO_2NPs group were highly statistically significantly less than other experimental groups. Final body weight, weight gain, and testis weight in TiO_2NPs + NAC + Cur group was highly

statistically significantly more than TiO_2NPs + NAC group and TiO_2NPs + Cur group. Weight gain and final body weight were insignificantly different between TiO_2NPs + NAC group and the TiO_2NPs + Cur group.

This was in agreement with **Khayal et al. (2019),** who reported that TiO2NPs treated rats revealed a significant reduction in body weight in comparison with other groups. The addition of NAC to TiO_2NPs provided significant protection against body weight loss caused by TiO_2NPs.

Cur induced a significant increase in the weight of mouse testis when used as a protective agent against TiO_2NPs testicular toxicity in **Karimi et al. (2019)** who suggested that the role of Cur in enhancing testicular weights may be due to its ability to prevent spermatogenesis defects and prevention of germ cell death in the seminiferous tubules increasing all stages of spermatogenic cells.

In the current study, FSH, LH, and testosterone hormone levels in the TiO_2NPs group were highly statistical and significantly less than in other experimental groups. LH and Testosterone levels in TiO_2NPs + NAC + Cur group were highly statistical and significantly more than TiO_2NPs + NAC group and TiO_2NPs + Cur group, while FSH level was insignificantly different between these groups.

The current work was in agreement with **Said et al. (2022),** who reported that in the rats exposed to TiO_2NPs, levels of FSH, LH, and Testosterone decreased compared to the control group. In mammals, LH hormone, which is released by the pituitary gland in response to gonadotropin-releasing hormone (GnRH) from the hypothalamus, stimulates the Leydig cells in the testis to produce testosterone. As a result, a decrease in LH level and Leydig cell oxidative damage brought on by ROS formation and the depletion of antioxidant reserves are both expected outcomes of a decrease in testosterone levels **(Elewa et al., 2019).**

Mohammadi Fartkhooni et al. (2013), reported that compared to the control and placebo groups, the TiO2-treated group's LH level dramatically increased and its testosterone level fell.

While, there is no discernible difference between the FSH hormone level and the control or placebo.

Numerous studies have demonstrated that NAC can control the levels of testosterone, follicular stimulating hormone, and luteinizing hormone in response to a variety of contaminants **(Shahrzad et al., 2020; Verdi et al., 2019).** NAC can stop testosterone loss because oxidative stress in Leydig cells directly affects this process **(Malmir et al., 2018).**

Regarding Cur; **Mohamadpour et al. (2017),** it has been shown that curcumin can raise the levels of ovarian hormones (testosterone, FSH, and LH) in chronically stressed rats.

This might be a result of Cur's protection of Leydig cells.

However, the present results weren't matched with **Miura et al., (2017)**, who didn't observe any significant difference in levels of LH, FSH, and GnRH after treatment with TiO_2NPs.

In the current study, SOD, Catalase and GSH antioxidants in the TiO_2NPs group were highly statistically significantly less than other groups. In TiO_2NPs + NAC + Cur group these levels were highly statistically significantly more than in TiO_2NPs + NAC group and TiO_2NPs + Cur group. While MDA antioxidants in the TiO_2NPs group were highly statistically significantly more than all groups. MDA antioxidants in TiO_2NPs + NAC + Cur group were highly statistically significantly less than other experimental groups.

The current results were in agreement with **Elnagar et al. (2018),** They claimed that when compared to the control and NAC groups, the GSH level of the TiO_2NPs group significantly decreased. In addition, GSH levels in the TiO_2NPs + NAC groups were significantly higher than in the control and NAC groups. Additionally, the MDA level of the TiO_2NPs group significantly increased in comparison to the control and NAC groups. In contrast, the MDA level of the TiO_2NPs + NAC group was significantly

lower than that of the TiO_2NPs group. Significant variations in MDA levels imply that the oxidative stress induced by the discharged nanoparticles is likely the mediating factor in the genesis of degenerative lesions.

El-Kirdasy et al. (2014) attributed the antioxidant activity of NAC to guard against oxidative stresses.

The current results were matched with **Haroun et al. (2020),** who reported that Cur significantly decreased MDA levels while GSH levels increased. The role of Cur in reducing MDA was reported by **Alizadeh and Kheirouri (2019)** as Cur can prevent the synthesis of MDA and function as a free radical scavenger.

In the current study, sperm motility, viability, count, and concentration were in the TiO_2NPs group highly statistically significantly less than other groups. Sperm abnormality in the TiO_2NPs group was highly statistically significantly more than in other experimental groups. In all measured sperm parameters, there was an insignificant difference between TiO_2NPs + NAC group and TiO_2NPs + Cur group.

The current results were in agreement with **Said et al. (2022),** who stated that as compared to the control group, the TiO_2NPs treated group showed a substantial decrease in sperm motility and concentration. However, The percentage of sperm abnormalities significantly increased in the TiO_2NP animals when compared to the control group.

The data collected indicate that there is a good chance that NAC will increase the count **(Verdi et al., 2019),** motility **(Kheradmandi et al., 2019),** viability **(Malmir et al., 2018),** and normal morphology of spermatozoa **(Kheradmandi et al., 2019)** via strengthening the antioxidant enzyme system and having an antioxidant effect. NAC enhances spermatogenesis in several ways, such as by lowering ROS levels to preserve membrane integrity and avoid lipid peroxidation, which is crucial for sperm shape **(Malmir et al., 2018).**

Soleimanzadeh and Saberivand (2013) and **Karimi et al. (2019)** reported that; Cur may have protective effects on the morphology, quantity, movement, and viability of sperm in adult male Wister rats by acting as excellent antioxidants by scavenging free radicals. Through its phenolic, β-diketone, and methoxy functional groups, Cur has been demonstrated to have high antioxidant activity and reduce oxidative stress **(Aparnak and Saberivand, 2019).**

In the current study downregulation in HSD17B3 gene expression was found in testicular tissues following TiO2NPs treatment. However, this level was upregulated in TiO_2NPs + NAC group, TiO_2NPs + Cur group, and TiO_2NPs + NAC + Cur group compared with the TiO_2NPs group.

The present results were matched with **Said et al. (2022)** and **Hussein et al. (2019),** who reported that 17BHSD significantly downregulated the steroidogenesis-related genes in testicular tissue after exposure to TiO_2NPs compared to the control group.

The current result is in agreement with **El-Kirdasy et al. (2014),** who reported that TiO2 buildup in the testes decreased the activity of 17-HSD and other genes involved in steroidogenesis and cholesterol transport. Rats exposed to TiO2 for three months had considerably lower levels of 17-HSD expression, which was again upregulated in the presence of NAC and TiO_2NPs.

In the present study, upregulation in BAX gene expression was found in testicular tissues following TiO_2NPs treatment, compared with the control group, vehicle group, NAC group, and Cur group. However, this level was downregulated in TiO2NPs + NAC group, TiO_2NPs + Cur group, and TiO_2NPs + NAC + Cur group compared with the TiO_2NPs group.

The current results agreed with **Orazizadeh et al. (2020)** and **Zhang et al. (2018),** who reported that Bax expression was significantly reduced in the control group in comparison with the rats in the TiO_2NPs group. In the same way, **Hussein et al. (2019),** reported that testicular tissues from the TiO_2NPs group showed a substantial increase in the Bax gene expression. The cause of testicular injury, the peroxidation of membrane

phospholipids by lipid hydro-peroxides, is related to the accumulation of hydro-peroxides and can result in cytotoxicity. Our biochemical data, which demonstrated an elevated amount of lipid peroxidation and Bax expression, are compatible with the necrotic circumstances described **(Thakur et al., 2014).**

The present results matched with **Zhao et al. (2015),** who reported that treatment with Cur markedly and simultaneously reduced Bax expression while increasing Bcl-2 expression, improving the Bcl-2/Bax ratio.

In the current study, the Testis of the rat group treated with TiO_2NPs alone (group 5) showed disturbed normal anatomy. While the rat group received NAC concomitant with TiO_2NPs (group 6), mild improvement in histopathological findings was detected.

However, in the rat group that received Curcumin concomitant with TiO_2NPs (group 7), the improvement in histopathological findings was less than that was seen in TiO_2NPs + NAC treated group, While in the rat group that received NAC and Cur concomitant with TiO_2NPs (group 8), marked improvement in histopathological findings was detected.

The current results were matched with **Hussein et al. (2019)**, who reported that the seminiferous tubules of the TiO_2NPs -treated rats showed evident signs of degeneration, loss of normal architecture, and a decrease in spermatogenic cells. Additionally, there was a decline in the number of Leydig cells, which may have been caused by free radicals and ensuing lipid peroxidation, as well as a decrease in sperm counts in comparison to testis sections from the control group.

The present results matched with **Elnagar et al. (2018),** who reported that revealed testicular tissue showed some improvement when NAC and TiO2NPs were combined, as shown by histological analysis.

Also, the current results agree with **Karimi et al. (2019)** both the Cur and control groups' testicular parts appeared normal. The histologic criteria were dramatically raised in the TiO_2NPs group due to the disarray of germ cell layers, separation, sloughing, and atrophy that was observed. Curcumin can either support the regeneration of damaged cells or maintain the integrity of cellular membranes, preventing cellular harm **(El-Maddawy and El-Sayed, 2018 & Mohammed, 2019).**

Free radicals can cause membrane-bound PUFA acids of mammalian testes and other biomembranes to oxidize, which impairs membrane integrity and causes seminiferous tubule degeneration, which may explain the histopathological changes in testicular tissue. As a result, tissue deterioration and testicular atrophy take place **(Mandal and Das, 2011 & Mitra et al., 2013).**

CONCLUSION

TiO_2NPs administered orally caused DNA damage and toxic consequences in the testes, and these negative effects may be related to the development of oxidative stress and changed oxidative stress indicators which led to downregulation in the HSD17B3 gene and upregulation of the BAX gene. Also, TiO_2NPs altered semen parameters, altered hormonal levels and caused histopathological abnormality in the testis.

Administration of NAC and Cur along with TiO_2NPs, protected against TiO_2 damaging effect.

RECOMMENDATIONS

- Depending on the results of this study, the following guidelines are recommended:
  - Promoting widespread public awareness of the risks to health posed by chronic exposure to TiO_2NPs and other nanoparticles, with a focus on the impact of these substances on the reproductive system, especially if no medical advice is sought.
  - In patients exposed to TiO_2NPs, regular examination of testicular functions such as testosterone level and semen analysis is strongly advised.
  - The use of TiO_2NPs should be prevented or decreased due to their harmful effect.

- Further studies should be conducted to find a safe alternative to TiO_2NPs.
- Administration of NAC and Cur along with TiO_2NPs, protected against TiO2 damaging effect. So, if TiO2 is necessary any of them or both should accompany it.

Conflict of interest

The authors declare that they have no competing interests.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sector.

REFERENCE

Aebi H (1984): Catalase in vitro. Methods in Enzymology, 105:121-126.

Alizadeh, M and Kheirouri, S (2019). Curcumin reduces malondialdehyde and improves antioxidants in humans with diseased conditions: a comprehensive meta-analysis of randomized controlled trials. BioMedicine, 9(4): 10-22.

Aparnak P and Saberivand A (2019): Effects of curcumin on canine semen parameters and expression of NOX5 gene in cryopreserved spermatozoa. Vet Res Forum, 10 (3): 221-226.

Bearden JH and Fuquay JW (1984): Applied animal reproduction. Reston publishing company inc. A prentice Halls Company Virginia. 341-345.

Cappello, T., Vitale, V., Oliva, S., Villari, V., Mauceri, A., Fasulo, S., and Maisano, M. (2017): Alteration of neurotransmission and skeletogenesis in sea urchin Arbacia lixula embryos exposed to copper oxide nanoparticles. *Comparative biochemistry and physiology*. 199: 20–27.

Chabanyuk Y (2014): Assessing Toxicity of Titanium Dioxide (TiO2) Nanoparticles on Pseudomonas Species Biofilms. Thesis. Toronto, Ryerson University. https: // rshare.library.ryerson.ca/ articles/thesis/Assessing_ toxicity_ of_titanium_dioxide_ TiO2_ nanoparticles_on_Pseudomonas_species_biofilms/14663781/files/28149624.

Chen, X. X., Cheng, B., Yang, Y. X., Cao, A., Liu, J. H., Du, L. J., Liu, Y., Zhao, Y., and Wang, H. (2013): Characterization and preliminary toxicity assay of nano-titanium dioxide additive in sugar-coated chewing gum. Small *(Weinheim an der Bergstrasse, Germany)*. 9: 1765–1774.

El-Akabawy G and El-Sherif NM (2016): Protective role of garlic oil against oxidative damage induced by furan exposure from weaning through adulthood in adult rat testis. Acta histochemical, 118(5): 456-463.

Elewa, Y. H., Mohamed, A. A. R., Galal, A. A., El-Naseery, N. I., Ichii, O., and Kon, Y. (2019): Food Yellow4 reprotoxicity concerning the localization of DMC1 and apoptosis in rat testes: Roles of royal jelly and cod liver oil. Ecotoxicol. Environ. Saf. 169: 696–706.

El-Kirdasy, A. F., Nassan, M. A., Baiomy, A. A. A., Ismail, T. A., Soliman, M. M., and Attia, H. F. (2014): Potential ameliorative role of n-acetylcysteine against testicular dysfunction induced by titanium dioxide in male albino rats. American Journal of Pharmacology and Toxicology, 9(1): 29-39.

El-Maddawy ZK and El-Sayed YS (2018): Comparative analysis of the protective effects of curcumin and N-acetyl cysteine against paracetamol-induced hepatic, renal, and testicular toxicity in Wistar rats. Environ Sci Pollut Res, 25(4): 3468-3479.

Elnagar AMB, Ibrahim A and Soliman AM (2018): Histopathological Effects of Titanium Dioxide Nanoparticles and The Possible Protective Role of N-Acetylcysteine on The Testes of Male Albino Rats. *International Journal of Fertility and Sterility***,**12: 249-256.

Ema, M., Kobayashi, N., Naya, M., Hanai, S., and Nakanishi, J. (2010): Reproductive and developmental toxicity studies of manufactured nanomaterials. *Reproductive toxicology*. 30: 343–352.

Evans G and Maxwell WMC(1987): Handling and examination of semen. In: Maxwell WMC, (eds). Salamon's artificial insemination of sheep and goats. Sydney, Australia: Butterworths, 93-106.

Farag, A., FARUK, E., KHARBOUSH, T., and Abu-Raia, N. H. (2021): Protective effect of N-acetylcysteine (NAC) against Di-Ethylhexyl phthalate (DEHP) induced pulmonary toxicity in male albino rats (histological and immunohistochemical study). The Egyptian Journal of Forensic Sciences and Applied Toxicology, 21(2), 89-109.

Galletti A (2016): Toxicity Evaluation of TiO2 Nanoparticles Embedded in Consumer Products. Thesis. Miami, University of Miami. https://scholarship.miami.edu/esploro/outputs/graduate/Toxicity-Evaluation-of-TiO2-Nanoparticles.

Haroun, M. R., Eldin, A. A., EL-Khouly, S. M., and Mostafa, I. A. (2020): The Protective Effect of Ascorbic Acid and Curcumin on the Chronic Toxicity of Meloxicam on the Testis of Adult Albino Rats. Benha Journal of Applied Sciences, 5 (2): 189-207.

Hewlings SJ and Kalman DS (2017): Curcumin: A Review of Its Effects on Human Health. Foods, 6(10): 92-102.

Hussein, M., Gad, E., Ahmed, M. M., Arisha, A. H., Mahdy, H. F., Swelum, A. A. A. et al., (2019): Amelioration of titanium dioxide nanoparticle reprotoxicity by the antioxidants morin and rutin. Environ Sci Pollut Res Int, 26:29074–29084.

Hussein, S. A., Khalaf-Allah, S. S., Tag El-Din, H. A., Amin, A., and Khallaf, R. M. (2018): Potential Role of Thymoquinone in Imidaclopride Induced Testicular Toxicity in Male Albino Rats. *Benha Veterinary Medical Journal*, 34(3): 64-82.

Jin, C. Y., Zhu, B. S., Wang, X. F., and Lu, Q. H. (2008): Cytotoxicity of titanium dioxide nanoparticles in mouse fibroblast cells, *Chemical research in toxicology*. 21(9): 1871–1877.

Kang, S. J., Kim, B. M., Lee, Y. J., Hong, S. H., and Chung, H. W. (2009): Titanium Dioxide Nanoparticles Induce Apoptosis Through the JNK/p38-Caspase-8-Bid Pathway in Phytohemagglutinin Stimulated Human Lymphocytes. *Biochemical and biophysical research communications*. 386(4): 682-687.

Karimi S, Khorsandi L and Nejaddehbashi F (2019): Protective effects of Curcumin on testicular toxicity induced by titanium dioxide nanoparticles in mice. JBRA Assisted Reproduction, 23 (4): 344-351.

Khayal E, Amin D and Shabana M (2019): The Possible Protective Role of N-Acetyl Cysteine against Titanium Dioxide Nanoparticles Intestinal Toxicity in Adult Male Albino Rats. Ain Shams Journal of Forensic Medicine and Clinical Toxicology, 33(2): 59–69.

Kheradmandi, R., Jorsaraei, S. G. A., Feizi, F., Moghadamnia, A. A., and Neamati, N. (2019): Protective effect of N-acetyl cysteine on chlorpyrifos-induced testicular toxicity in mice. International Journal of Fertility & Sterility, 13(1): 51-66.

Lamberg S and Rothstein R (1978): Laboratory Manual of Histology and Cytology. (2nd ed.), A.V.I. Publishing Company, West Port Connecticut, 137-140.

Larsson, S.C., Håkansson, N., and Wolk, A., (2015): Dietary cysteine and other amino acids and stroke incidence in women. Stroke. 46(4): 922–926.

Livak KJ and Schmittgen TD (2001): Analysis of relative gene expression data using real-time quantitative PCR

and the 2− ΔΔCT method. methods, 25(4), 402-408.

Malmir, M., Soleimani Mehranjani, M., Naderi Noreini, S., and Faraji, T. (2018): Protective antioxidant effects of N-acetylcysteine against impairment of spermatogenesis caused by paranonylphenol. Andrologia, 50(10): 13114-13124.

Mandal TK and Das NS (2011): Correlation of testicular toxicity and oxidative stress induced by chlorpyrifos in rats. Hum Exp Toxicol., 30(10): 1529-1539.

Mitra S, Srivastava A and Khandelwal S (2013): Tributyltin chloride induced testicular toxicity by JNK and p38 activation, redox imbalance and cell death in Sertoli-germ cell co-culture. Toxicology, 314(1): 39–50.

Miura, N., Ohtani, K., Hasegawa, T., Yoshioka, H., and Hwang, G. W. (2017): High sensitivity of testicular function to titanium nanoparticles. J. Toxicol. Sci. The Japanese Society of Toxicology;42(3):359–66.

Mohamadpour, M., Noorafshan, A., Karbalay-Doust, S., Talaei-Khozani, T., and Aliabadi, E. (2017): Protective effects of curcumin co-treatment in rats with establishing chronic variable stress on testis and reproductive hormones. International Journal of Reproductive BioMedicine,15(7): 447-452.

Mohammadi Fartkhooni, F., Noori, A., Momayez, M., Sadeghi, L., Shirani, K., and Yousefi Babadi, V. (2013): The effects of nano titanium dioxide (TiO2) in spermatogenesis in wistar rat. Euro J Exp Bio, 3(4): 145–149.

Mohammed HO (2019): The Possible Role of Curcumin against Changes Caused by Paracetamol in Testis of Adult Albino Rat (Histological, Immunohistochemical, and Biochemical Study). Egypt. J. Histol., 2019: 1189-1201.

Mughal, B.; Zaidi, S.Z.J.; Zhang, X.; and Hassan, S.U. (2021): Biogenic nanoparticles: Synthesis, characterization, and applications. Applied Sciences. 11(6): 2598-2616.

Oberdorster, G.; Oberdorster, E; and Oberdorster, J. (2005): Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environment Health Perspective. 113: 823–839.

Olugbenga, O. M., Olukole, S. G., Adeoye, A. T., and Adejoke, A. D. (2011): Semen characteristics and sperm morphological studies of the West African Dwarf Buck treated with Aloe vera gel extract. *Iranian Journal of Reproductive Medicine*, *9*(2), 83-88.

Orazizadeh, M., Khorsandi, L., Mansouri, E., and Fakhredini, F. (2020): The effect of glycyrrhizin acid on Bax and Bcl2 expression in hepatotoxicity induced by Titanium dioxide nanoparticles in rats. Gastroenterology and Hepatology From Bed to Bench, 13(2): 168-176.

Picard, M., Rossier, C., Papasouliotis, O., and Lugan, I. (2008): Bioequivalence of recombinant human FSH and recombinant human LH in a fixed 2:1combination: two phases I, randomized, crossover studies. *Current* Medical *Research* and *Opinion*, 24(4): 1199-1208.

Rasyidah, T., Suhana, S., Nur-Hidayah, H., Kaswandi, M., and Noah, R. (2014): Evaluation of the antioxidant activity of Zingiber officinale (ginger) on formalin-induced testicular toxicity in rats. *Journal of Medical* and *Biological Engineering*, 3(3): 149-153.

Rollerova, E., Jurcovicova, J., Mlynarcikova, A., Sadlonova, I., Bilanicova, D., Wsolova, L., Kiss, A., Kovriznych, J., Kronek, J., Ciampor, F., Vavra, I., and Scsukova, S. (2015): Delayed adverse effects of neonatal exposure to polymeric nanoparticle poly (ethylene glycol)-block-polylactide methyl ether on hypothalamic–pituitary–ovarian axis development and function in Wistar rats. *Reproductive toxicology*. 57: 165–175.

Said, A. A., Nasr, Y., Galal, A. A., Abdelhamid, A. E., Mohamed, H. A., Metwally, M. M. et al., (2022): Concerns with Male Infertility Induced by Exposure to Titanium Nanoparticles and the Supporting Impact of Pelargonium graveolens Essential Oil: Morphometric Records in Male-Wistar Rats. Life. MDPI;12(5):639.

Shahrzad, E., Shariati, M., Naimi, S., and Edalatmanesh, M. A. (2020): Protective effect of N-acetylcysteine on changes in serum levels of Pituitary–Gonadal axis hormones and testicular tissue in acrylamide-treated adult rats. Advances in Human Biology, 10(1): 16-21.

Shukla, R. K., Sharma, V., Pandey, A. K., Singh, S., Sultana, S., and Dhawan, A. (2011): ROS-mediated genotoxicity induced by titanium dioxide nanoparticles in human epidermal cells. Toxicology In Vitro, 25: 231–241.

Siervo, G. E., Vieira, H. R., Ogo, F. M., Fernandez, C. D., Gonçalves, G. D., Mesquita, S. F., et al., (2015): Spermatic and testicular damages in rats exposed to ethanol: influence of lipid peroxidation but not testosterone. Toxicology, 1(33): 1 - 8.

Soleimanzadeh A and Saberivand A (2013): Effect of curcumin on rat sperm morphology after the freeze-thawing process. In Veterinary Research Forum. 4(3): 185-189.

Thakur, B. K., Zhang, H., Becker, A., Matei, I., Huang, Y., Costa-Silva, B., et al., (2014): Double-stranded DNA in exosomes: a novel biomarker in cancer detection. *Cell Research*, *24*(6), 766-769.

Verdi, A., Sahraei, S. S., Asa, E., Jannatifar, R., and Masaeimanesh, M. B. (2019): The effect of rhFSH on Sperm DNA Fragmentation and sperm parameters in Oligozoospermia Infertile Men. Research in Molecular Medicine, 8(2), 55-62.

Wang, J., Zhou, G., Chen, C., Yu, H., Wang, T., Ma, Y., et al., (2007): Acute toxicity and biodistribution of different sized titanium dioxide particles in mice after oral administration. Toxicology Letters, 168: 176–185.

Weir, A., Westerhoff, P., Fabricius, L., Hristovski, K., and von Goetz, N. (2012): Titanium dioxide nanoparticles in food and personal care products. *Environmental science & technology*. 46: 2242–2250.

Xue, C., Liu, W., Wu, J., Yang, X., and Xu, H. (2011): Chemoprotective effect of N-acetylcysteine (NAC) on cellular oxidative damages and apoptosis induced by nano titanium dioxide under UVA irradiation. *oxicology in vitro: an international journal published in association with BIBRA.* 25(1): 110-116.

Yang, Y., Qin, Z., Zeng, W., Yang, T., Cao, Y., Mei, C., and Kuang, Y. (2017): Toxicity assessment of nanoparticles in various systems and organs. Nanotechnology Reviews. 6(3): 279-289.

Youssef, G., Makin, B., Ali, A. M., Waly, M., Alaa, N., & Abou-Setta, A. (2006): N- acetyl-cysteine in anovulatory women: the impact of the postcoital test. *Middle East Fertility Society Journal*. 11: 109–112.

Zhang, L., Xie, X., Zhou, Y., Yu, D., Deng, Y., Ouyang, J., et al., (2018): Gestational exposure to titanium dioxide nanoparticles impairs the placentation through dysregulation of vascularization, proliferation, and apoptosis in mice. International Journal of Nanomedicine, 13: 777-789.

Zhao, H., Gu, W., Ye, L., and Yang, H. (2014): Biodistribution of PAMAM dendrimer conjugated magnetic nanoparticles in mice. j*ournal of materials science. Materials in medicine*, 25: 769–776.

Zhao, L., Gu, Q., Xiang, L., Dong, X., Li, H., Ni, J., et al., (2015): Curcumin inhibits apoptosis by modulating Bax/Bcl-2 expression and alleviates oxidative stress in testes of streptozotocin-induced diabetic rats. Therapeutics and Clinical Risk Management, 13: 1099-1105.

الملخص العربي

مقارنة التأثيرات المضادة للأكسدة للاسيتيل سيستين والكوركومين علي التسمم الناتج من الجسيمات النانوية لثاني أكسيد التيتانيوم علي خصية الجرذان البيضاء البالغة

سحر محمد أبو الوفا[١] , محمد فريد خضيري[١], آلاء السيد إبراهيم عبد القادر[١*]، انكيتا كيرت بارمار[٢] و نهى سليمان النجار[١]

[١]: قسم الطب الشرعي والسموم الإكلينيكية كلية الطب البشري – جامعة بنها – مصر.

[٢]: كلية العلوم بجامعة جوجارات أحمد أباد، غوجارات، الهند.

المقدمة: مع التطبيقات واسعة النطاق للجسيمات النانوية، بما في ذلك الجسيمات النانوية لثاني أكسيد التيتانيوم في مجالات مختلفة، أدت إلي العديد من الآثار الضارة للصحة و البيئة.

هدف البحث: معرفه تأثير المحسن للاسيتيل سيستين والكركمين ضد تسمم الخصية الناتج من الجسيمات النانوية لثاني أكسيد التيتانيوم في الجرذان البيضاء البالغة.

خطة البحث: تم تقسيم أربعة وستين ذكر من الجرذان البيضاء البالغة الي ثماني مجموعات. المجموعة (١) المراقبة السلبية: تعطي ماء ونظام غذائي فقط. المجموعة (٢)المواد المذيبة :٤ فئران تعالج بمحلول ملح عادي و٤ آخرون بزيت ذرة بالفم. المجموعة (٣): تم معالجتهم بالاسيتيل سيستين ١٠٠ملجم/كجم. المجموعة (٤): تم معالجتهم ب كركمين ٢٠٠ملجم/كجم بالفم مرة واحدة يوميًا. المجموعة (٥): تم معالجتهم بالجسيمات النانوية لثاني أكسيد التيتانيوم ١٠٠ ملجم/كجم عن طريق الفم مرة يوميًا. المجموعة (٦): تم معالجتهم باسيتيل سيستين ١٠٠ملجم/كجم والجسيمات النانوية لثاني أكسيد التيتانيوم ١٠٠ملجم/كجم مرة يوميًا. المجموعة (٧): تم معالجتهم بالكركمين ٢٠٠ملجم/كجم و الجسيمات النانوية لثاني أكسيد التيتانيوم ١٠٠ ملجم/كجم مرة يوميًا. المجموعه (٨): تم معالجتهم بالاسيتيل سيستين ١٠٠ملجم/كجم و كركمين ٢٠٠ملجم/كجم مع الجسيمات النانوية لثاني أكسيد التيتانيوم ١٠٠ملجم/كجم مرة واحدة يوميًا.

نتائج الدراسة: أوضحت النتائج ان الجسيمات النانوية لثاني أكسيد التيتانيوم تسببت في انخفاض في وزن الجسم ووزن الخصية . الجسيمات النانوية لثاني أكسيد التيتانيوم تعزز الإجهاد التأكسدي، ويشار إليه من خلال انخفاض مستويات مضادات الاكسدة مثل (SOD, CAT, GSH) وزيادة مستويات MDA في انسجة الخصية. تقلل الجسيمات النانوية لثاني أكسيد التيتانيوم بشكل كبير من مستويات الهرمونات الجنسية (هرمون التستوستيرون, LH, FSH). كما يقلل من حركة الحيوانات المنوية، وحيويتها، وعددها، وتركيزها، ويزيد من تشوهات الحيوانات المنوية، بالإضافة إلى إتلاف البنية النسيجية في أنسجة الخصية. كما نتج عن استخدام الجسيمات النانوية لثاني أكسيد التيتانيوم تقليل التعبير الجيني لجين 17b-HSDوزيادة التعبير الجيني لجين Baxفي أنسجة الخصية. وعلى النقيض من ذلك، كان للاسيتيل سيستايين و / أو الكركمين تأثير وقائي على أنسجة الخصية.

التوصيات: يتسبب التعرض للجسيمات النانوية لثاني أكسيد التيتانيوم في أضرار مأكسدة وإصابة في الخصية. ومن المقترح أنه يمكن استخدام الاسيتيل سيستين والكركمين للتخفيف من السمية والاضرار التأكسدية المرتبطة بتناول الجسيمات النانوية لثاني أكسيد التيتانيوم.