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

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ABSTRACT

**Introduction**: With widespread applications of nanoparticles including titanium dioxide nanoparticles (TiO2NPs) in different fields, many adverse effects may threat both environmental and medical health**. Aim of this work**: To examine ameliorative effect of N-acetyl cysteine (NAC) and curcumin against TiO2NPs induced testis toxicity in adult albino rats**. Material and methods**: Sixty four adult male albino rats were classified into eight groups. Group 1: control received regular diet and water. Group 2: solvent control, 4 rats administered oral dose of corn oil and 4 rats received oral dose of normal saline. 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 TiO2NPs (100mg/kg) once a day.Group 6: orally gavaged once daily with TiO2NPs (100 mg/kg) and NAC (100 mg/kg). Group 7: orally received TiO2NPs (100mg/kg) and curcumin (200mg/kg) once a day. Group 8: gavaged orally TiO2NPs (100mg/kg) followed by NAC (100mg/kg) and curcumin (200mg/kg). **Results**: TiO2 NPs caused a considerable reduction in ultimate body weight, weight gain, and testis weight, according to the findings. Reduced levels of antioxidants such as superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) in testicular tissues, as well as elevated levels of the lipid peroxidation marker malondialdehyde (MDA), suggested that TiO2NPs increased oxidative stress. TiO2NPs significantly lowered sex hormone levels (FSH, LH and testosterone), sperm motility, viability, sperm cell count and concentration, and sperm abnormalities, as well as causing damage to the testicular histological architecture in testicular tissues, TiO2NPs resulted in the downregulation of 17-HSD and the activation of proapoptotic gene (Bax) transcripts. NAC and/or curcumin, on the other hand, exhibited a protective impact on testicular tissue. **Conclusion**: TiO2NPs exposure causes induced oxidative damage and morphological injury in the testis and we recommend that NAC and curcumin could be used to mitigate the toxicity and oxidative damage related to TiO2NPs intake.

**Keywords**: Titanium dioxide nanoparticles; N-acetyl cysteine; curcumin;17-beta hydroxysteroid dehydrogenase 3; bax gene expression ; oxidative stress markers ; hormonal analysis.

**INTRODUCTION**

Nanotechnology is an exciting new topic of study with applications in residential, industrial, and biomedical settings. Nanoparticles have unique features due to their small size and surface area **(Esquivel et al., 2015).**

The enormous expansion in the sophisticated field of nanotechnologies, with all of its far-reaching benefits, has called researchers' attention to the health hazards posed by nanoparticles. Humans have been exposed to numerous airborne NPs throughout evolution, but the degree of exposure has recently greatly risen due to the widespread usage of nanoparticles in everyday items. This increased rate of NP creation also raises hazards due to their release into the environment as nanostructural materials, which may have a harmful effect on the ecosystem **(Sajid et al., 2015).**

Nanoparticles have the capacity to pass biological barriers such as the BTB, which protects reproductive tissues, due to their nano size. As a result, NP crossing has a negative impact on spermatogenesis. Following NP exposure, NPs enter the reproductive system via many routes, with the epididymis and testis being the primary targets in males **(Iftikhar et al., 2021).**

Inhalation, ingestion, cutaneous penetration, and injection are all ways for Tio2 to enter the human body. Nanoparticles are most dangerous when inhaled or come into touch with the skin ***(Shukla et al., 2021).*** Oral consumption, however, is a potential route of exposure for the general public because TiO2 is utilised as a food additive in toothpaste and capsules ***(Wang et al., 2017)***.

 Tio2NPs are widely employed in therapy, drug delivery, engineering, agriculture, personal care goods, cosmetics, sunscreens, toothpaste, electronics, clothing, paints, and coverings, as well as as an imaging agent and consumables **( Al-Doaiss et al., 2019).**

In the food business, nano-TiO2 is commonly used. Coated sweets, preserved fruits, chewing gum, carbonated drinks, powdered drinks, milk and dairy products, and other food categories have all employed it **(Jia et al., 2017).**

Tio2NPs have been linked to a number of negative biological consequences, including DNA damage, apoptosis, and mitochondrial dysfunction ***(Wiesenthal et al., 2021)*** and oxidative stress (***Montazer et al., 2021).***

 Since the last decade, nearly 6 million tonnes of titanium dioxide (TiO2) have been produced as a pigment, with nanoscale titanium dioxide (nano-TiO2) accounting for close to 5% of total output. According to the same estimate, up to 50% TiO2 might be generated in nanoform by 2023 **(Valentini et al., 2019)**.

N-acetylcysteine (NAC) is a thiol containing amino acid. It is available as a safe and cheap medication as a mucolytic drug **(Khayal et al., 2019).** NAC is known for its antioxidant properties, which are achieved by releasing sulfhydryl groups, which reduce Reactive Oxygen Species (ROS) levels. It also has the ability to reduce oxidative stress, suppress the nuclear factor kappa b (NF-B) inflammation pathway and inflammatory cytokines secretion, and boost GSH production **(Elnagar et al., 2018).**

 Curcumin (Cur), a yellow pigment found in the rhizome of the turmeric plant (Curcuma longa), possesses anti-inflammatory, anti-carcinogenic, antioxidant, and hypocholesterolemic effects **(Hewlings and Kalman., 2017).**

**AIM OF THE WORK**

This current experimental study was conducted as a result of the widespread of nanotechnology especially titanium dioxide nanoparticles in our daily lives and to know it's effect on the testis and the ameliorative effect of both antioxidants NAC and curcumin through histopathological examination of testis, evaluation of sperm function, hormonal analysis, oxidative stress markers and mRNA transcripts in healthy adult male albino rats.

**MATERIAL AND METHODS**

This study was conducted on 64 adult male albino rats, about 90 days old; their main weight was ranging from 180 gm to 200 gm**.**

Before beginning the experiment, all of the animals were given a week of passive preliminaries (taking food and water without any medications) to help them adjust to their new environment at the animal bread house in the Anatomy Department of the Benha Faculty of Medicine, as well as to ensure their physical well-being and rule out any diseased animals. The identical diet was given to all of the animals (Wheat, Bread & Milk). The time of drug delivery was set at 12 p.m. for all animals.

The animals were sedated with ether and slaughtered twenty-four hours following the final dosage of treatment.

**(II) Chemicals:**

* **Titanium dioxide nanoparticles:**

Titanium dioxide nanoparticles was purchased from Sigma Chemical Campany, Egypt.

Titanium (IV) oxide, mixture of rutile and anatase nanoparticles, <150 nm particle size, 40 wt. % in H2O, 99.5% trace metals basis.

* **N acetyl cysteine**: NAC was purchased commercially available from SEDICO Chemical Company, Egypt.
* **Curcumin:** Curcumin was purchased from Sigma- Aldrich Company, Egypt. It was in the form of 100 % pure bright yellow to orange color powder.
* **Corn oil:** Corn oil was obtained from the local market.

**(III) Grouping and experimental design:**

At the beginning of the experiment, rats will be randomly divided into 8 groups, as follows:

* **Group 1 (control group) 8 rats:**

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

* **Group 2 (Solvent control group) 8 rats:**

4 rats were received oral dose of corn oil, 4 rats were received oral dose of normal saline.

* **Group 3 (NAC) 8 rats:**

 Each rat gavaged orally with N-acetyl cysteine (100 mg/kg) or 10 mg/100 gm (1 ml/100 g) body weight once daily.

* **Group 4 (Curcumin group) 8 rats:**

Each rat was treated with Cur (200 mg/kg) or 20 mg/100 g (2 ml/100 g) body weight once daily.

* **Group 5 (Titanium dioxide nanoparticles group “TiO2NPs”) 8 rats:**

 It received TiO2NPs (100 mg/kg) or 10 mg/100 gm (1 ml/100 g) body weight once daily.

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

Each rat orally received NAC (100 mg/kg B.W) followed by 1 hour by TiO2NPs (100 mg/kg B.W) once a day.

* **Group 7 (TiO2NPs and Curcumin group):**

Each rat orally received Curcumin (200 mg/kg B.W) followed by 1 hour by TiO2NPs (100 mg/kg B.W) once a day.

* **Group 8 (TiO2NPs, NAC and Curcumin group)**

Rats was treated with a single dose of titanium dioxide nanoparticles (100 mg/kg B.W) orally by gavage tubeorally followed by 1 hour by a single dose of NAC (100 mg/kg) orally by gastric tube and Curcumin (200 mg/kg) orally by gastric tube daily.

All groups were treated daily, for 8 weeks.

**(IV) : Parameters of the study:**

**1.Body weight and relative weight of testis:**

Body weights were taken at the start and at the end. Rats were dissected after the experiment, with testes removed and stripped of fatty tissues and blood vessels, blotted, and their weights determined. **2.Biochemical study for hormonal analysis:**

Blood samples were taken 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 level in testicular tissue

**Handling of tissue samples for estimation of oxidative stress parameters:**

Following the manufacturer's instructions, MDA, SOD, CAT, and GSH levels in testicular tissue were determined using commercially available colorimetric methods (diagnostic kits supplied by Bio Diagnostic Company, Egypt) **(Hussein et al., 2018).** The concentration of MDA in tissue samples was measured using the method reported by **El-Akabawy and El-Sherif (2016).** The concentration of SOD in tissue samples was measured according to the method given by **Rasyidah et al. (2014).** The CAT activity of the homogenate of testes was measured using the technique described by **Aebi (1984)**. The concentration of GSH in tissue samples was measured using the method reported by **Siervo et al. (2015).**

**4Semen analysis:**

**a.Sperm motility:**

 The increasing motility of sperms was investigated using a method described by **Bearden and Fuquay (1984).**

**b.Sperm liveability:**

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

**c.Sperm count and concentration:**

This was determined according to the technique reported by **Bearden and Fuquay (1984*)***.

**d.Sperm abnormalities**:

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

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

The total RNA purification kit (Jena Bioscience) was used to isolate total RNA according to the manufacturer's instructions. Reverse transcription kits were used to make first-strand complementary DNA (cDNA) (enzynomics). The relative expression of the mRNAs of target genes in the testis was measured using real-time PCR with SYBR green and GADPH as an internal reference. The isolated cDNA was amplified using HERAPLUS SYBR® Green qPCR Master Mix and the specified primers, as directed by the manufacturer (Willowfort). The 2ΔΔCt method was used to assess the expression data **(Livak and Schmittgen 2001).**

**6.Histopathological study by light microscope**:

 According to **Lamberg and Rothstein (1978)**, testicles absorbed bouin's solution, which is used to fix organs that require extensive morphologic examination, such as testis. Tissue samples were fixed for 6-8 hours and then shifted to 70% liquor before being sent to the Pathology Department, Faculty of Medicine, Benha University, for robotized drying, paraffin implanting, segmenting, and recoloring.

**Statistical examination:**
The data was gathered, organised into tables, and then broken down using SPSS [Statistical Package for Social Science] version 20. For quantitative information, mean and standard deviation were developed; for subjective information, recurrence and dissemination were introduced. In this study, the acknowledged degree of significance was set at 0.05 [P ≤0.05 was regarded significant, while P ≤0.01 was considered very significant].

**RESULTS**

**1.Body weight and relative weight of testis:**

Body weight gain and testis weight in TiO2NPs group showed highly significant less than other groups. In addition of NAC and/or curcumin were highly significant more than TiO2NPs group. However body weight gain and testis weight showed insignificantly different between TiO2NPs and NAC group and TiO2NPs and curcumin group. Body weight gain in TiO2NPs, NAC and curcumin group was highly significant more than TiO2NPs and NAC group and TiO2NPs and curcumin group as shown in **Table (1).**

**2.Hormonal Analysis study results:**

As shown in **Table (2)** , FSH, LH and testosterone levels showed a significant decrease in TiO2NPs group compared with other experimental groups. The administration of NAC or curcumin were highly significant more than TiO2NPs group. LH and testosterone in combined administration of NAC and curcumin was highly significant more than TiO2NPs and NAC group and TiO2NPs and curcumin group. FSH level was insignificantly different between TiO2NPs, NAC and curcumin group and TiO2NPs and curcumin group and TiO2NPs and NAC group.

**3.Oxidative Stress Markers results:**

The level of MDA significantly increased in TiO2NPs group compared with the other experimental groups. Administration of NAC and/or curcumin reduced concentration of MDA. In TiO2NPs, NAC and curcumin group (119.18 ± 1.13) were highly significant less than TiO2NPs and NAC group and TiO2NPs and Curcumin group (p <0.001). MDA antioxidants were insignificantly different between TiO2NPs and NAC group and TiO2NPs Curcumin group (P= 0.560).

Meanwhile, in the TiO2NPs group, SOD and CAT activity, as well as GSH levels, were significantly lower than in the other experimental groups. Similarly, NAC or curcumin administration reduced the effects of TiO2NPs, with the combination treatment outperforming the individual treatment (TiO2NPs, NAC, and curcumin group was highly significant more than TiO2NPs and NAC group and TiO2NPs and curcumin group, but the last was highly significant less than TiO2NPs and NAC group) as described in **Table (3).**

**4.Semen analysis:**

As indicated in **Table (4)**, the TiO2NPs group had considerably lower sperm motility, viability, cell count, and concentration than the other experimental groups. NAC and curcumin, either alone or in combination, were able to reverse this decline. but, TiO2NPs and NAC group was insignificantly different with TiO2NPs and curcumin group. TiO2NPs, NAC and curcumin group were significantly higher than TiO2NPs and NAC group and TiO2NPs and curcumin group in sperm livability. TiO2NPs, NAC and curcumin group were highly significant more than TiO2NPs and NAC group and TiO2NPs and curcumin group in sperm concentration. Conversely, sperm abnormalities were highly significant increased in the TiO2NPs group compared with the other groups.

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

 **(Fig. 1)** show that downregulation in HSD17B3 gene expression was found in testicular tissues following TiO2NPs treatment ,compared with the control group, solvent group, NAC group and curcumin group by 0.011 , 1 , 1.089 , 2.301 and 2.107 fold changes respectively.

However, this level was upregulated in TiO2NPs and NAC group , TiO2NPs and curcumin group and TiO2NPs, NAC and curcumin group compared with TiO2NPs group by 68.663 , 25.624 , 213.236 , 1 fold changes respectively.

Upregulation in BAX gene expression was found in testicular tissues following TiO2NPs treatment, compared with the control group, solvent group, NAC group and curcumin group by 2.234 , 1 , 1.069 , 0.804 and 1.074 fold changes respectively.

However, this level was downregulated in TiO2NPs and NAC group , TiO2NPs and curcumin group and TiO2NPs, NAC and curcumin group compared with TiO2NPs group by 0.569 , 0.649 , 0.498 , 1 fold changes respectively.

**6.Histopathological result by Light microscope:**

The typical architecture of seminiferous tubules and interstitial tissues was seen in the control, solvent, NAC, and curcumin groups (Leydig cells). The TiO2NPs group, on the other hand, showed clear signs of loss of normal architecture, seminiferous tubule degeneration, and a decrease in the number of spermatogenic cells. Furthermore, compared to the testis sections in the control group, a decrease in the number of Leydig cells, intercellular vaculaization, and eventual atrophy with a lower sperm count, there was a decrease in the number of Leydig cells, intercellular vaculaization, and eventual atrophy with a lower sperm count. In comparison to the TiO2NPs group, the TiO2NPs and NAC group showed a moderate improvement, with a normal regular distribution in spermatogenesis cycles and improved sperm counts and Leydig cells. Curcumin with TiO2NPs co-treatment resulted in modest hyperemia and a moderate improvement in spermatogenesis cycles, as well as an increase in sperm count. As demonstrated in, these modifications were partially increased following NAC or curcumin treatments and nearly totally recovered when both NAC and curcumin were used together **(Fig. 2).**



**Fig. 1:** (a) fold changes of HSD17B3 between control group ( as calibrator ) and solvent , NAC, curcumin and TiO2NPs group, (b) fold changes of HSD17B3 between TiO2NPs group ( as calibrator ) and TiO2NPs and NAC group, TiO2NPs and curcumin group and TiO2NPs, NAC and curcumin group, (c) fold changes of BAX between control group ( as calibrator ) and solvent , NAC, curcumin and TiO2NPs group, (d) Fold changes of BAX between TiO2NPs group ( as calibrator ) and TiO2NPs and NAC group, TiO2NPs and curcumin group and TiO2NPs, NAC and curcumin group.



**Fig. 2** : **(a):** Photomicrograph of a slice of rat testis taken from a control group (1), demonstrating normal architecture with well-organized densely matted seminiferous tubules (S) and normal interstitial tissues (I) (Hx & E x 100), **(b):** Photomicrograph of a segment of rat testis produced from a solvent group (2), revealing typical architecture with densely matted seminiferous tubules (S) and normal interstitial tissues (I) (Hx & E x 100), **(c):** Photomicrograph of a piece of rat testis taken from a NAC group (3), revealing sertoli cells (ST), spermatogonia (SG), spermatocyte (SPC), and sperms (SP) in the seminiferous tubules (Hx &Ex 400)**, (d):** Photomicrograph of a section of rat's testis prepared from curcumin-treated group (4), showing normal architecture with well-arranged closely matted seminiferous tubules (S), normal blood vessles (vs), and normal interstitial tissues (I) (Hx & E x 100), **(e) and (f):** Photomicrograph of a slice of rat testis from the TiO2NPs-treated group (5), demonstrating severe loss of normal architecture of seminiferous tubules (S), atrophy (A), and basement membrane separation (D) (Hx & E x 100), **(g):** Photomicrograph of a portion of rat testis from the TiO2NPs-treated group (5), exhibiting spermatogonia (SG) and spermatocyte (SPC) degradation as well as a decrease in sperm (SP) count (Hx & E x 400), **(h):** Photomicrograph of a portion of rat testis generated from TiO2NPs and NAC treated group (6), demonstrating slight loss of normal seminiferous tubule architecture (S), mild detachment (D), and mild vaculation (v) (Hx & E x 100), (**i**): Photomicrograph of a segment of rat testis taken from TiO2NPs and curcumin-treated group (7), demonstrating very minor restoration of seminiferous tubule architecture (s) with intercellular vacuoles (V), increased numbers of spermatocytes (spc) and sperms (sp) filling lumen, and less atrophy (A) (Hx & E x 100), **(j):** Photomicrograph of a portion of rat testis produced from TiO2NPs, NAC, and curcumin treated group (8), demonstrating the restoration of normal architecture of seminiferous tubules (S), near normal spermatogenesis (SGS), and normal interstitial tissues (I) (Hx & E x 100).

**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 | 39.25 ± 5.06 | 1.63 ± 0.13 |
| Solvent control group | 191.87 ± 3.91 | 236.75 ± 6.63 | 44.75 ± 9.65 | 1.64 ± 0.11 |
| NAC group | 187.87± 20.15 | 232 ± 6.28 | 37.37 ± 8.45 | 1.72 ± 0.06 |
| Curcumin group | 193.62 ± 4.72 | 236.25 ± 3.61 | 42.87 ± 3.36  | 1.65 ± 0.043 |
| TiO2NPs group | 187.5 ± 3.58 | 169.375 ± 5.85(acd)\*\* | -18 ± 5.45(abcd)\*\* | 1.31 ± 0.04 (abcd)\*\* |
| TiO2NPs and NAC group | 190.5 ± 4.93 | 194.25 ± 4.89(abcde)\*\* | 4.25 ± 8.08 (abcde)\*\* | 1.52 ± 0.02 (ab)\*(cde)\*\* |
| TiO2NPs and Curcumin group | 189.75 ± 3.45 | 187.625 ± 5.04(abcde)\*\* | -2.125 ± 6.22 (abcde)\*\* | 1.42 ± 0.03 (abcd)\*\* |
| TiO2NPs, NAC, and Curcumin group | 189.25 ± 6.63 | 212.75 ± 4.95 (abcdefg)\*\* | 23.5 ± 8.83 (abcdefg)\*\* | 1.57 ± 0.03(ceg)\*\* |

\*Statistically significant as p ≤0.05, \*\*Statistically highly significant as p ≤0.01, a: significant with control group, b: significant with solvent group, c: significant with NAC group, d: significant with curcumin group, e: significant with TiO2NPs group, f: significant with TiO2NPs and NAC group, g: significant with TiO2NPs and curcumin group, TiO2NPs: 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 | 2.49 ± 0.18 | 5.26 ± 0.03 |
| Solvent control group | 0.86 ± 0.06 | 2.41 ± 0.22 | 4.98 ± 0.03 |
| NAC group | 0.92 ± 0.05a\*\* | 2.76 ± 0.06 (ab)\*\* | 5.70 ± 0.43 (ab)\*\* |
| Curcumin group | 0.92 ± 0.04 a\*\* | 2.69 ± 0.06 (ab)\*\* | 5.68 ± 0.23 (ab)\*\* |
| TiO2NPs group | 0.21 ± 0.02 (abcd)\*\* | 1.51 ± 0.3 (abcd)\*\* | 1.91 ± 0.07 (abcd)\*\* |
| TiO2NPs and NAC group | 0.68 ± 0.03 (abcde)\*\* | 1.92 ± 0.02 (abcde)\*\* | 3.68 ± 0.09 (abcde)\*\* |
| TiO2NPs and Curcumin group | 0.64 ± 0.04 (abcde)\*\* | 1.88 ± 0.04 (abcde)\*\* | 3.18 ± 0.11 (abcdef)\*\* |
| TiO2NPs, NAC, and Curcumin group | 0.69 ± 0.04 (abcde)\*\* | 2.23 ± 0.03 b\*(acdefg)\*\* | 4.19 ± 0.07 (abcdefg)\*\* |

\*Statistically significant as p ≤0.05, \*\*Statistically highly significant as p ≤0.01, a: significant with control group, b: significant with solvent group, c: significant with NAC group, d: significant with curcumin group, e: significant with TiO2NPs group, f: significant with TiO2NPs and NAC group, g: significant with TiO2NPs and curcumin group, TiO2NPs: 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 | 60.24 ± 1.36 | 919.79 ± 2.91 | 724.82 ± 1.07 |
| Solvent control group | 982.26 ± 1.27 | 60.99 ± 1.09 | 917.25 ± 3.35 | 720.26 ± 1.13 a |
| NAC group | 995.16 ± 1.24(ab)\*\* | 65.63 ± 1.63 a\*\* | 960 ± 1.98 (ab)\*\* | 795.51 ± 1.19 (ab)\*\* |
| Curcumin group | 993.6 ± 1.65 (ab)\*\* | 67.515 ± 0.77 (ab)\*\* | 956.98 ± 3.11 (ab)\*\* | 790.39 ± 1.62 (abc)\*\* |
| TiO2NPs group | 385.23 ± 1.98 (abcd)\*\* | 178.12 ± 1.29 (abcd)\*\* | 279.87 ± 1.34 (abcd)\*\* | 231.98 ± 1.15 (abcd)\*\* |
| TiO2NPs and NAC group | 698.74 ± 1.75 (abcde)\*\* | 119.18 ± 1.13 (abcde)\*\* | 640.11 ± 1.38 (abcde)\*\* | 430.17 ± 1.53 (abcde)\*\* |
| TiO2NPs and Curcumin group | 688.35 ± 2.23 (abcdef)\*\* | 120.31 ± 1.05 (abcdef)\*\* | 631.08 ± 1.31 (abcdef)\*\* | 425.42 ± 1.18 (abcdef)\*\* |
| TiO2NPs, NAC, and Curcumin group | 705.04 ± 2.89 (abcdefg)\*\* | 100.41 ± 1.05 (abcdeg)\*\* | 699.18 ± 1.29 (abcdefg)\*\* | 502.13 ± 1.16 (abcdefg)\*\* |

\*Statistically significant as p ≤0.05, \*\*Statistically significant as p ≤0.01, a: significant with control group, b: significant with solvent group, c: significant with NAC group, d: significant with curcumin group, e: significant with TiO2NPs group, f: significant with TiO2NPs and NAC group, g: significant with TiO2NPs and curcumin group, TiO2NPs: 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 | 60.5 ± 3.89 | 256.12 ± 14.76 | 1.86 ± 0.08 | 25.12 ± 3.31 |
| Solvent control group | 61.87 ± 9.4 | 53.37 ± 3.66 a\*\* | 246.25 ± 5.17 | 1.63 ± 0.05 a\*\* | 32 ± 2.39 a\*\* |
| NAC group | 66.87 ± 10.57 | 65 ± 4.2 b\*\* | 261.62 ± 6.37 | 1.94 ± 0.05 b\*\* | 20.5 ± 1.41 a\*b\*\* |
| Curcumin group | 63.12 ± 8.42 | 62.37 ± 3.58 b\*\* | 264.5 ± 8.96 b\*\* | 1.90 ± 0.05 b\*\* | 21.37 ± 1.4 b\*\* |
| TiO2NPs group | 25 ± 6.55 (abd)\*\* | 16.87 ± 5.3 (abcd)\*\* | 73.5 ± 8.58 (abcd)\*\* | 0.74 ± 0.08 (abcd)\*\* | 85.37 ± 4.59 (abcd)\*\* |
| TiO2NPs and NAC group | 43.12 ± 7.04 (abcde)\*\* | 43.12 ± 3.6 (abcde)\*\* | 214 ± 13.33 (abcde)\*\* | 1.01 ± 0.05 (abcde)\*\* | 51.62 ± 2.13 (abcde)\*\* |
| TiO2NPs and Curcumin group | 37.5 ± 6.54 (abcd)\*\* | 43.25 ± 3.37 (abcde)\*\* | 209.37 ± 11.41 (abcde)\*\* | 1.00 ± 0.05 (abcde)\*\* | 50.25 ± 2.37 (abcde)\*\* |
| TiO2NPs, NAC, and Curcumin group | 53.75 ± 7.44 (abeg)\*\*c\* | 50 ± 2.39 (acde)\*\*(fg)\* | 221.87 ± 11.93 (abcde)\*\* | 1.18 ± 0.07 (abcdefg)\*\* | 40 ± 2.82 (abcdefg)\*\* |

\*Statistically significant as p ≤0.05, \*\*Statistically highly significant as p ≤0.01, a: significant with control group, b: significant with solvent group, c: significant with NAC group, d: significant with curcumin group, e: significant with TiO2NPs group, f: significant with TiO2NPs and NAC group, g: significant with TiO2NPs and curcumin group, TiO2NPs: Titanium dioxide nanoparticles, NAC: N-acetyl cysteine, SD: ± standard deviation.

**DISCUSSION**

In the current study, the TiO2NPs group had significantly lower final weight, weight increase, and testis weight than the other experimental groups. Final weight, weight gain and testis weight in TiO2NPs, NAC and Curcumin group was highly significant more than TiO2NPs and NAC group and TiO2NPs and Curcumin group. Weight gain and final body weight were insignificantly different between TiO2NPs and NAC group and TiO2NPs and Curcumin group.

This was in agreement with **Khayal et al. (2019)**, who found that TiO2NPs-treated rats had a significantly lower body weight than the control group (P<0.001). Addition of NAC to TiO2NPs provided significant protection against body weight loss caused by TiO2 NPs (P<0.001). In addition, **Silva et al. (2017)** found that TiO2 NPs reduced weight growth and splenomegaly in mice. Hematological indicators were also altered in TiO2NP-treated animals, as were substantial liver damage, histological, and biochemical abnormalities.

Moreover, our results were in line with those observed by **ELSharkawy et al. (2010); Abu-Dief et al. (2015) and Shakeel et al. (2018).** They found that TiO2NPs decreased body weight in the experimental animals. And **Gao et al. (2013),** who found that the TiO2NPs group had a significant reduction in body weight and testis weight. In the study by **ELSharkawy et al. (2010);** anorexia and disturbance in different metabolic systems were observed in TiO2 NPs exposed animals which could be the cause of weight loss.

When given as a preventative treatment against dexamethasone-induced testicular injury, curcumin caused a considerable rise in the weight of mice testis (**Khorsandi et al., 2013)**, and against TiO2NPs testicular toxicity in **Karimi et al. (2019)** who is suggested that the role of curcumin 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 TiO2NPs group were highly significant less than other experimental groups. LH and Testosterone level in TiO2NPs, NAC, and Curcumin group were highly significant more than TiO2NPs and NAC group and TiO2NPs and curcumin group (p <0.001), while in FSH was insignificant difference between this groups.

Our findings were consistent with those of **Said et al. (2022),** who found that rats exposed to TiO2NPs had lower levels of FSH, LH, and Testosterone than the control group. LH hormone, which is secreted by the pituitary gland in response to gonadotropin-releasing hormone (GnRH) from the hypothalamus, stimulates leydig cells in the mammalian testis, causing testosterone synthesis. As a result, a decrease in testosterone is a natural result of a decrease in LH and oxidative damage to Leydig cells produced by ROS production and antioxidant depletion **(Elewa et al., 2019).**

According to **Mohammadi Fartkhooni et al. (2013)**, the TiO2 treated group had significantly higher LH levels and lower Testosterone levels than the control and placebo groups (P<0.001). While the amount of FSH hormone does not differ significantly from control and placebo (P=0.05), Negative feedback can cause an increase in LH levels as a result of decreasing testosterone. The main outcome of LH increase is a spike in GnRH release from the hypothalamus when testosterone is reduced **(Lansdown, 2007).**

NAC has been shown in numerous studies to modulate the amounts of luteinising hormone (LH), follicle-stimulating hormone (FSH), and testosterone in the face of various contaminants **(Shahrzad et al., 2020; Verdi et al., 2019)**. In Leydig cells, oxidative stress can cause testosterone loss, and NAC can help prevent this **(Malmir et al., 2018).**

In the case of curcumin, **Mohamadpour et al. (2017)** found that it can improve the levels of reproductive hormones (testosterone, FSH, and LH) in chronic variable stress rats. This could be due to curcumin's protective action on Leydig cells.

However, our findings differed with those of **Miura et al., (2017)**, who found no significant differences in LH, FSH, or GnRH levels after treatment with TiO2NPs.

In the current study, SOD, Catalase and GSH antioxidants in TiO2NPs group were highly significant less than other groups. TiO2NPs, NAC, and Curcumin group were highly significant more than TiO2NPs and NAC group and TiO2NPs and Curcumin group (p <0.001) While MDAantioxidants in TiO2NPs group were highly significant more than all groups. MDA antioxidants in TiO2NPs, NAC and curcumin group were highly significant less than other experimental groups.

Our findings were consistent with those of **Elnagar et al. (2018)**, who found a substantial reduction in GSH levels in the TiO2NPs group when compared to the control and NAC groups. Furthermore, when comparing the TiO2NPs and NAC groups to the control and NAC groups, there was a substantial rise in GSH levels in the TiO2NPs and NAC groups. In addition, the MDA level in the TiO2NPs group was significantly higher than in the control and NAC groups. MDA levels in the TiO2NPs and NAC groups, on the other hand, were significantly lower (P0.05) than in the TiO2NPs group. Significant variations in MDA levels indicate that pathological lesions are most likely induced by oxidative stress exacerbated by the discharged nanoparticles.

**Long et al. (2007)** showed GSH exhaustion and an increase in lipid peroxidation levels after exposure to TiO2 nanoparticles and reported similar findings.Furthermore, **Gurr et al. (2005)** discovered an exponential increase in MDA formation due to TiO2, which they attributed to increased ROS generation.

NAC's antioxidant actions, when combined, can help protect against oxidative stress. These findings are similar to those reported in the study by **El-Kirdasy et al. (2014).**

 Our results were matched with **Haroun et al. (2020),** who reported that curcumin was significantly decreased MDA level while GSH level were increased. The role of curcumin in reducing MDA was reported by **Alizadeh and Kheirouri (2019)** as curcumin can serve as a free radical scavenger as well as an MDA inhibitor. Curcumin is thought to work as an activator of the intracellular regulatory proteins sirtuins (SIRT1 and SIRT3). SIRT1 and SIRT3 are thought to block OS in cells that reduce MDA, according to various studies.

Curcumin's ability to scavenge free radicals, interact with the oxidative cascade, quench oxygen radicals, inhibit oxidative enzymes, and chelate metal ions, according to **El-Agamy (2010)**, prevents lipid peroxidation and restores antioxidant state. Curcumin has also been demonstrated to promote numerous enzymatic antioxidants, including SOD and catalase, as well as de novo GSH formation.

Similarly, **Said et al. (2022)** found a substantial (p 0.05) rise in the detected amount of MDA in the TiO2NPs group's testicular tissues when compared to the control group. Nonetheless, the TiO2 NPs group showed higher depletion of enzymatic antioxidants (CAT and SOD) and non-enzymatic antioxidants (GSH) than the control group.

In the current study, Sperm motility, viability, count and concentration were in TiO2NPs group highly significant less than other groups. Sperm abnormality in TiO2NPs group was highly significant more than other experimental groups. In all measured sperm parameters, there was insignificant different between TiO2NPs and NAC group and TiO2NPs and curcumin group. And observed when treated with both NAC and curcumin result approximately near to control group.

Our findings were consistent with those of **Said et al. (2022)**, who found a significant decrease (p 0.05) in sperm motility and concentration in the TiO2NPs-treated group compared to the control group. However, when the TiO2 NPs rats were compared to the control group, the percentage of sperm abnormalities increased significantly (p 0.05).

**Takeda et al. (2009)** found many functional and pathologic abnormalities in the progeny of TiO2-injected mice, including reduced daily sperm production. **Guo et al. (2009)** also found that low-dose nanosized TiO2 has no discernible effect on male mice, but that high-dose TiO2 can drastically impair sperm count and function, as well as promote germ cell apoptosis.

Similarly, numerous studies have shown that NAC can improve sperm quality and quantity while also increasing spermatogenesis. With its antioxidant action and enhancing the antioxidant enzyme system, there is a significant likelihood that NAC enhances the count **(Verdi et al., 2019)**, motility **(Kheradmandi et al., 2019),** viability **(Malmir et al., 2018)**, and normal shape of spermatozoa **(Kheradmandi et al., 2019)**. NAC helps spermatogenesis in a variety of ways, including by lowering ROS levels, which helps to maintain membrane integrity and avoid lipid peroxidation, which is critical for sperm shape **(Malmir et al., 2018).**

Curcumin's beneficial effects on sperm parameters are consistent with earlier research. One of the putative protective mechanisms of curcumin on sperm morphology, sperm count, sperm motility, and sperm viability in adult male Wister rats, according to **Soleimanzadeh and Saberivand (2013)** and **Karimi et al. (2019)**, is to scavenge free radicals and so behave as good antioxidants. Curcumin's phenolic, β-diketone, and methoxy functional groups have been proven to have high antioxidant action 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 TiO2NPs and NAC group, TiO2NPs and curcumin group and TiO2NPs, NAC and curcumin group compared with TiO2NPs group.

Our findings were consistent with those of **Said et al. (2022)** and **Hussein et al. (2019),** who found that TiO2NPs exposure resulted in significant (p 0.05) downregulation of steroidogenesis-related genes in testicular tissue in 17BHSD when compared to the control group.

Our findings are consistent with those of **El-Kirdasy et al. (2014)**, who found that accumulating TiO2 in the testes decreased the expression of genes involved in cholesterol transport and steroidogenesis, including 17-HSD. After 3 months of exposure to TiO2, rats' expression of 17-HSD was dramatically reduced, although there was an upregulation of 17-HSD in the NAC and TiO2NPs groups.

HSD17B3 is expressed in testicular Sertoli cells during mouse development, where it produces testosterone from androstenedione obtained from foetal Leydig cells. In foetal life, HSD17B3 expression changes from Sertoli cells to the adult Leydig cell population, which generates testosterone directly in prepubertal life **(Shima et al., 2013).**

Sex development abnormalities are linked to mutations in the HSD17B3 gene **(Mendonca et al., 2017)**.

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

Our findings corroborated those of **Orazizadeh et al. (2020)** and **Zhang et al. (2018)**, who found that Bax expression was considerably lower in the control group than in the TiO2NPs group (P0.001). Similarly, **Hussein et al. (2019)** discovered a substantial increase of Bax gene expression in testicular tissues in the Tio2NPs group. The accumulation of hydro-peroxides can cause cytotoxicity, which is caused by lipid hydro-peroxides peroxiding membrane phospholipids, which is the cause of testicular injury. Our biochemical findings of enhanced lipid peroxidation and Bax expression are compatible with the necrotic conditions discovered **(Thakur et al., 2014).**

Our findings matched those of **Zhao et al. (2015)**, who found that curcumin administration decreased Bax expression while simultaneously increasing Bcl-2 expression, hence increasing the Bcl-2/Bax ratio.

In the current study, Testis of rat group treated with TiO2NPs alone (group 5) showed seminiferous tubules with some spermatogonia arranged adjacent to the basement membrane but not in order as in control rats. They appear scattered and only partially surrounding the whole seminiferous tubule. Many seminiferous tubules showed evident hypocellularity of all stages with decreased spermatogenesis, tubular degeneration and intercellular vaculaization. While in rat group received NAC concomitant with Tio2NPs (group 6), mild improvement in histopathological findings was detected as there was mild restore to architecture of seminiferous tubules and showed different stages of maturation, with much less atrophy and vaculation.

However, in rat group received Curcumin concomitant with TiO2NPs (group 7), the improvement in histopathological findings was less than that was seen in TiO2NPs and NAC treated group, While in rat group received NAC and Curcumin concomitant with Tio2NPs (group 8), marked improvement in histopathological findings was detected as there was marked restore to architecture of seminiferous tubules; with no vacuolization, normal non congested blood vessels and no interstitial edema.

Our findings resembled those of **Hussein et al. (2019)**, who found that TiO2NP-treated rats had lost normal architecture, seminiferous tubule degeneration, and a reduction in the number of spermatogenic cells. In addition, many syncytial cells were found in the seminiferous tubules, as well as a decrease in the number of Leydig cells with a decreased sperm count when compared to the control group's testis sections, which could be due to free reactive radicals and subsequent lipid peroxidation.

Our findings were consistent with those of **Elnagar et al. (2018)**, who observed that administering NAC coupled with TiO2NPs resulted in a partial improvement in testicular tissue, as determined by histological inspection. With modest collagen fibre deposition in the basement membrane, however, cytoplasmic vacuolation was still seen.

In addition, our findings match with those of **Karimi et al. (2019),** who found that testicular portions in both the control and Cur groups appeared normal. Disorganization of germ cell layers, detachment, sloughing, and atrophy were observed in the TiO2NPs group, and the histologic criteria were significantly elevated (p0.01). When compared to TiO2NPs-intoxicated animals, Cur treatment could reduce the criterion. Curcumin has the capacity to prevent cellular harm by stabilising the integrity of cellular membranes or promoting the regeneration of injured cells **(El-Maddawy and El-Sayed, 2018 & Mohammed, 2019 ).**

Free radicals produce lipid peroxidation of membrane bound polyunsaturated acids in mammalian testes and other biomembranes, resulting in membrane integrity deterioration and seminiferous tubule degeneration, which may explain histopathological abnormalities in testicular tissue. Testicular atrophy and tissue degradation occur as a result **(Mandal and Das, 2011 & Mitra et al., 2013).**

**CONCLUSION**

Oral treatment of TiO2NPs caused toxic effects and DNA damage in the testes, which might be related to oxidative stress induction and changed oxidative stress indicators, which resulted in HSD17B3 gene downregulation and BAX gene upregulation.

TiO2NPs also changed the properties of the sperm, altered hormone levels, and induced histological abnormalities in the testis.

The use of NAC and curcumin in combination with TiO2 nanoparticles protected against the harmful effects of TiO2.

**RECOMMENDATIONS**

Depending on the results of this study, the following guidelines are
recommended:
• Creating extensive public awareness of the health risks of chronic TiO2NPs and other nanoparticles consumption, with a particular focus on the reproductive harmful effects, especially without physician consultation.
• Regular evaluation of testicular functions as testosterone level, semen
analysis is highly recommended in patients exposure to TiO2NPs.

•The use of TiO2NPs should be prevented or decreased due to its harmful effect.

•Further studies should be conducted to find a safe alternate to TiO2NPs.

• The combination of NAC and curcumin, as well as TiO2NPs, protected against the harmful effects of TiO2. So, if TiO2 is required, one or both of them, or both, should be present.

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

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**الملخص العربي**

مع التطبيقات واسعه النطاق للجسيمات النانوية بما في ذلك الجسيمات النانوية لثاني أكسيد التيتانيوم في مجالات مختلفة، قد تهدد العديد من الآثار الضارة للصحة البيئية والطبية. الهدف من هذه الدراسة هي معرفه التاأثير المحسن للاسيتيل سيستين والكركمين ضد تسمم الخصية الناتج من الجسيمات النانوية لثاني أكسيد التيتانيوم في الجرذان البيضاء البالغه . تم تقسيم أربعة وستين ذكور الجرذان البيضاء البالغه الي ثماني مجموعات. المجموعه (١) المراقبه السلبيه: تعطي ماء ونظام غذائي فقط. المجموعه (٢)المواد المذيبة :٤ فئران تعالج بمحلول ملح عادي و٤ اخرون بزيت ذرة بالفم. المجموعة (٣): تم معالجتهم بالاسيتيل سيستين ١٠٠مللجم/كجم. المجموعة (٤): تم معالجتهم ب كركمين ٢٠٠مللجم/كجم بالفم مره واحده يوميا. المجموعه (٥): تم معالجتهم ب الجسيمات النانوية لثاني أكسيد التيتانيوم ١٠٠ مللجم/كجم عن طريق الفم مره يوميا. المجموعة (٦): تم معالجتهم باسيتيل سيستين ١٠٠مللجم/كجم و الجسيمات النانوية لثاني أكسيد التيتانيوم ١٠٠مللجم/كجم مره يوميا. المجموعه (٧): تم معالجتهم بالكركمين ٢٠٠مللجم/كجم و الجسيمات النانوية لثاني أكسيد التيتانيوم ١٠٠ مللجم/كجم مره يوميا. المجموعه (٨): تم معالجتهم بالاسيتيل سيستين ١٠٠مللجم/كجم و كركمين ٢٠٠مللجم/كجم مع الجسيمات النانوية لثاني أكسيد التيتانيوم ١٠٠مللجم/كجم مره واحده يوميا. أوضحت النتائج ان الجسيمات النانوية لثاني أكسيد التيتانيوم تسبب في انخفاض في وزن الجسم و وزن الخصية . الجسيمات النانوية لثاني أكسيد التيتانيوم تعزز الاجهاد التأكسدي ويشار اليه من خلال انخفاض مستويات مضادات الاكسدة مثل (SOD, CAT, GSH) في انسجة الخصية . وزيادة مستويات MDA . وتقلل الجسيمات النانوية لثاني أكسيد التيتانيوم بشكل كبير من مستويات الهرمونات الجنسية ( هرمون التستوستيرون, FSH ,LH ) ويقلل من حركه الحيوانات المنويه وحيويتها وعدد خلايا الحيوانات المنويه وتركيزها وزياده تشويهات الحيوانات المنوية بالاضافه الي اتلاف البنية النسيجية للخصية. ونتج من الجسيمات النانوية لثاني أكسيد التيتانيوم من تقليل 17b-HSD وزيادة Bax في انسجه الخصية. علي العكس من ذلك كان للاسيتيل سيستايين و / او الكركمين تأثير وقائي علي انسجه الخصية. يتسبب التعرض للجسيمات النانوية لثاني أكسيد التيتانيوم في اضرار مأكسدة واصابة في الخصية ونقترح انه يمكن استخدام الاسيتيل سيستين والكركمين للتخفيف من السمية والاضرار التأكسدية المرتبطة بتناول الجسيمات النانوية لثاني أكسيد التيتانيوم.