

Oxidative and Patho-physiological Effects of Vanadium Oxide Nanoparticles (VONPs) in Albino Rats

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Abstract

Nanotechnology enables the development of novel functions, such as antimicrobial applications and drug delivery systems. However, studies on the toxic effect of this new class of particles, especially vanadium oxide nanoparticles (VO-NPs), on living organisms are lacking. The outcomes of this study include the determination of the oxidative, neurological, respiratory, and reproductive toxicities of VO-NPs and their antimicrobial activity against *Salmonella*. The study comprised 26 Albino Wistar rats divided in three groups, the control group received normal saline (G^c), while two other groups received VO-NPs intraperitoneally at 1000 µg/kg/day (G₁) and 1500 µg/kg/day (G₂) for 10 days. Levels of ROS, an index of oxidative stress, as well as TBARS, and the activities of the antioxidant enzymes SOD, CAT, and POD were measured. The antimicrobial activity against *Salmonella* was performed by using the agar well diffusion method. Statistical analysis of the result involved using ANOVA and Tukey's test on the results with a significance level set at $P \leq 0.05$. In the treated rats, ROS and TBARS levels were elevated in the lung, brain, and testes compared to the control group, while CAT, SOD, and POD activities were significantly ($P < 0.05$) reduced. However, in the testes of Group 1 rats, the reductions in CAT, SOD, and POD were statistically non-significant ($P > 0.05$). Lethargy, anxiety, and other locomotory abnormalities were observed in all the treated rats. VO-NPs demonstrated dose-dependent antimicrobial activity against *Salmonella*, as evidenced by an increase in the zone of inhibition from 9 mm at the lowest concentration (5 µL) to 17 mm at the highest (20 µL). In this study, VO-NPs induced oxidative stress and histopathological alterations in the vital organs of test animals, which may be associated with inflammation and reduced antioxidant enzyme activity. These findings highlight the potential toxicological effects of VO-NPs, suggesting that their use in industrial and biomedical applications should be approached with caution.

Introduction

Human dreams and imagination often give rise to new science and technology. Nanotechnology, a 21st-century frontier, was born out of such dreams (Halliwell, 2024).

Nanotechnology is defined as the understanding and control of matter at dimensions between 1 and 100 nm where unique phenomena enable novel applications (Rahman et al.,

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2024). This rapidly growing field allows cross-disciplinary researchers the opportunity to design and develop multifunctional nanoparticles that can target, diagnose, and treat diseases such as cancer. The small size, surface tailorability, improved solubility, and multifunctionality of nanoparticles open many new research avenues for biologists (Moreno & Sipponen, 2024). It has the potential to change our perspectives and expectations and provide us with the capability to resolve global issues (Efunuga et al., 2024; Ikhuoria et al., 2024b).

Vanadium oxide nanoparticles are receiving increasing interest in drug delivery and medical health due to their chemical characteristics and bioactivity. Their high surface area enables a controlled drug release making them an excellent contenders for drug delivery (Javid-Naderi et al., 2023). Their use for cancer treatment has been highlighted (Kostenkova et al., 2024). Vanadium oxide nanoparticles have therapeutic potential through their anti-oxidative effects and the regulation of oxidative stress, which is useful in treating inflammatory and oxidative-associated diseases such as heart disease (Gupta et al., 2024) and other neurodegenerative diseases (He et al., 2024). Vanadium oxide nanoparticles exhibit strong antimicrobial activity against multiple bacterial strains (Uwadia et al., 2024). Scientists have also revealed that vanadium oxide nanoparticles can inhibit the growth of bacteria, and algae on surfaces in contact with water, or offshore platforms (Patel et al., 2024). VO-NPs used for various applications are toxicologically hazardous, especially due to their oxidative stress in different organs (Li et al., 2024). Vanadium oxide NPs are currently being used for coating stainless steel to make it resistant to corrosion (Anani et al., 2024). Thus, there is a very high probability of occupational toxicity due to the inhalation of these NPs by people working in such industries (Mohammed et al., 2024).

In the organs, they can result in respiratory distress, lung inflammation and fibrosis (Parveen et al., 2025), they can easily cross the blood-brain barrier leading to neurotoxicity causing oxidative damage, impaired physical activity and learning ability (Patel et al., 2024). In the testes, and in the reproductive system in general, vanadium oxide nanoparticles have been associated with reduced sperm quantity and quality, including impaired fertility, likely due to oxidative damage to the sperm. (Castellini et al., 2024). VO-NPs affect the blood indices related to RBCs and WBCs, which are instrumental in immune competence and wellness. These alterations in the blood composition can lead to immunosuppression, anemia, and thrombophilia (Musarrat et al., 2024; Nellessen et al., 2024).

Biochemical markers and histopathological indicators are analyzed in this work to determine the neurological, respiratory, and testicular toxicities after VO-NPs exposure. From the published work, minimal information could be

obtained concerning the evaluation and control of oxidative stress indices and antioxidant enzymes (superoxide dismutase, SOD; catalase, CAT; peroxidase, POD) at various dosages of vanadium oxide nanoparticles (Noeman et al., 2011). The threats of vanadium oxide nanoparticles to the environment and the population are observed when the compound is frequently and continually used in its nanoparticle form. Hence, it is important to study the potential mechanisms of injury in exposed animals. The current study eventually opens up possibilities for understanding definite health hazards of vanadium oxide nanoparticles in existence, and their proper usage in numerous fields and professions.

Materials and Methods

Vanadium oxide nanoparticles and experimental plan

Vanadium oxide NPs were prepared by the Institute of Physics, Islamia University of Bahawalpur, Pakistan. All substances and reagents utilized in the current experimental trial were laboratory-grade. Twenty-six adult albino rats (13 male and 13 female) weighing between 130 and 140 gm were obtained from the Animal House at the Islamia University of Bahawalpur. The rats were all healthy and free of disease. The rats were brought to the laboratory for adaptation fifteen days before the study started. The rats received regular feed and clean fresh water on a daily basis during the testing period. The rats were kept in clean wire cages in typical laboratory settings throughout the experiment. They were housed at $25\pm 2^{\circ}\text{C}$, $55\pm 5\%$ relative humidity, with 14 hours of light and 10 hours of darkness. Throughout the trial, the rats were cared for and used in accordance with laboratory animal welfare protocols.

Animal groups and nanoparticle doses

Throughout the trial, rats were randomly assigned to one of three groups (G_c , G_1 , or G_2) and housed in wire cages. Group G_c acted as the control. In Group G_1 , rats were given $1000\mu\text{g/kg/day}$ of vanadium oxide nanoparticles whereas Group G_2 got $1500\mu\text{g/kg/day}$ intraperitoneally for 10 days. Every day, all of the rats were checked for abnormalities.

Autopsy and tissue collection

All the rats, including the untreated control group, were euthanized after being exposed to Vanadium oxide NPs, and autopsies were performed to separate different tissues. The obtained organs/tissues (lungs, brain, and testes) were triturated individually in Petri plates and homogenized with cooled double distilled water. The resulting mixtures were subsequently centrifuged at 3000 rpm for a period of ten minutes. The supernatant after centrifugation was collected and kept at -20°C for subsequent analysis. Approximately 0.1 g of each organ tissue was homogenized and tested. A

number of markers linked to oxidative stress and antioxidant status were evaluated, including reactive oxygen species (ROS), Thiobarbituric acid reactive substances (TBARS) (Can et al., 2022) , and different antioxidants such as catalase (CAT), superoxide dismutase (SOD), and peroxidases POD (Akram et al., 2021; Kakkar et al., 1984) .

Antimicrobial Activity

The antimicrobial activity of vanadium oxide against Gram-negative (*Salmonella*) was examined by the Agar well diffusion method. Previously confirmed non-typhoidal *Salmonella* isolates from salmonellosis cases were used for antimicrobial susceptibility testing (AST). The bacterial suspension was adjusted to 0.5 McFarland standard before testing. The 0.01% vanadium oxide stock solution contained 0.01 g of vanadium oxide in 1 mL of DMSO to ensure even distribution of the drugs. To assess any antimicrobial effects of the solvent, a DMSO control containing only DMSO (without nanoparticles) was included in the agar well diffusion assay. Wells were made of diameter 5mm with the help of a sterile tip of the required size and then the plates were incubated at 37 °C for 24 h for bacterial observation growth and activity of vanadium oxide nanoparticle.

Histopathological Analysis

Rat organs from both treated and untreated groups were excised, rinsed and fixed in 10% formalin for histopathological evaluation. These specimens were processed according to conventional paraffin embedding protocols. After staining, representative sections from each tissue were examined under a light microscope to assess potential histological alterations induced by VO nanoparticles in the treated albino rats.

Statistical analysis

Using the IBM statistical software program (SPSS), the ANOVA test was used to statistically analyze the data on oxidative and antioxidant biomarker enzymes. The means of all investigated measures, including hematological analysis, oxidative biomarkers, and antioxidant enzymes, were compared to the control group using Tukey's test. The significance threshold was set at $P \leq 0.05$.

Results

Behavioral Modifications

High-dose Vanadium oxide NP-treated rats showed behavioral and neurological alterations like depression, weakness, locomotory abnormalities, and weight loss.

Relative weight of organs (lungs, brain, and testes)

The relative weight of organs (lungs, brain, and testis) of rats exposed to varied concentrations of vanadium oxide NPs was measured. After two weeks of intraperitoneal injection with varied doses of vanadium oxide NPs, a significant rise ($P \leq 0.05$) in the relative weight of the brain and testis of both treated groups was noted. While a significant decrease was noted in the relative weight of lungs. These findings showed a considerable increase in the relative weight of the brain and testes along with a reduction in the lung weight suggesting the detrimental effects of vanadium oxide NP (Table 1).

Table 1. Variation in relative weight of organs (lungs, brain, and testes)

Parameters Weight (g)	Male		
	G _c	G ₁ (1000µg/Kg/Day)	G ₂ (1500µg/Kg/Day)
Brain	0.96±0.28	1.17±0.18	1.47±0.01
Lungs	0.87±0.10	0.65±0.02	0.58±0.01
Testes	0.81±0.01	0.93±0.02	0.99±0.01

Antioxidant enzymes and oxidative stress biomarker analysis of Lungs

In the brain, ROS and TBRAS exhibited significantly elevated levels ($P \leq 0.05$) in rats at higher doses of vanadium oxide NPs (1000 µg/kg/day and 1500 µg/kg/day). The levels of antioxidant enzymes CAT, SOD, and POD markedly decreased ($P \leq 0.05$) in albino rats at elevated dosages of nanoparticles, indicating stress relative to the control group.

Table 2. Antioxidant enzymes and oxidative stress biomarkers in the lungs of albino rats treated with high doses of vanadium oxide NPs.

Parameter of lungs	Male		
	G _c	G ₁ (1000µg/Kg/Day)	G ₂ (1500µg/Kg/Day)
CAT	6.84±0.27	6.15±0.18	5.73±0.12
SOD	0.84±0.006	0.61±0.21	0.55±0.006
POD	0.77±0.006	0.52±0.02	0.44±0.002
TBRAS	2.91±0.01	3.39±0.09	3.48±0.14
ROS	0.67±0.01	1.07±0.18	1.45±0.21

Antioxidant enzymes and oxidative stress biomarker analysis of Brain

The levels of ROS significantly raised ($P \leq 0.05$) in the Brains of the treatment group (G_1 and G_2) of albino rats. The TBARS results indicated a significant increase in both treated groups, which were administered 1000 $\mu\text{g/kg/day}$ and 1500 $\mu\text{g/kg/day}$ of vanadium oxide NPs, respectively, compared to the healthy/untreated group. The antioxidant biomarkers (SOD, CAT, and POD) showed a significant reduction ($P \leq 0.05$) in both treated groups.

Table 3. Antioxidant enzymes and oxidative stress biomarkers in the brain of albino rats treated with high doses of vanadium oxide NPs.

Parameter of brain	Male		
	G_c	G_1 (1000 $\mu\text{g/Kg/Day}$)	G_2 (1500 $\mu\text{g/Kg/Day}$)
CAT	1.57 $\pm$ 0.006	1.31 $\pm$ 0.04	1.12 $\pm$ 0.05
SOD	1.50 $\pm$ 0.071	1.34 $\pm$ 0.01	1.19 $\pm$ 0.002
POD	0.58 $\pm$ 0.13	0.26 $\pm$ 0.01	0.19 $\pm$ 0.09
TBRAS	2.52 $\pm$ 0.25	2.61 $\pm$ 0.12	2.73 $\pm$ 0.14
ROS	1.14 $\pm$ 0.004	1.43 $\pm$ 0.05	1.84 $\pm$ 0.16

Antioxidant enzymes and oxidative stress biomarker Analysis of Testes

In the testes, oxidative stress indicators were significantly elevated ($P \leq 0.05$) in rats housed in both groups as treated with 1000 $\mu\text{g/kg/day}$ and 1500 $\mu\text{g/kg/day}$ of vanadium oxide NPs. The level of CAT, SOD, and POD was non-significantly reduced ($P \geq 0.05$) in Group 1 while a significant reduction ($P \leq 0.05$) in Group 2 as compared to the control group (Table 4).

Table 4. Analysis of antioxidant enzymes and oxidative stress biomarkers of the testes of albino rats treated with high doses of vanadium oxide NPs

Parameter of testes	Male		
	G_c	G_1 (1000 $\mu\text{g/Kg/Day}$)	G_2 (1500 $\mu\text{g/Kg/Day}$)
CAT	2.90 $\pm$ 0.06	2.40 $\pm$ 0.07	2.18 $\pm$ 0.12
SOD	0.90 $\pm$ 0.037	0.73 $\pm$ 0.03	0.59 $\pm$ 0.01
POD	1.59 $\pm$ 0.02	1.27 $\pm$ 0.01	1.19 $\pm$ 0.06
TBRAS	2.79 $\pm$ 0.03	2.53 $\pm$ 0.12	2.50 $\pm$ 0.12
ROS	1.53 $\pm$ 0.01	1.72 $\pm$ 0.01	1.92 $\pm$ 0.01

Histopathological studies (Brain)

The untreated group showed normal histological architecture of brain where as rats exposed to VO-NPs exhibited various microscopic alterations. The degree of histopathological damage ranged from mild to severe, characterized by neuronal necrosis, cytoplasmic vacuolization, intracellular edema, neuronal atrophy, and congestion of neural cells (Fig 1).

Table 5. Severity of histopathological lesions in rats brain

Parameter (Brain)	Groups/Treatment		
	G_c (Control)	G_1	G_2
Eccentric nuclei of neuron	-	++	++++
Hypertrophy of cytoplasm of neurons	-	++	++++
Edema	-	+	++
Microgliosis	-	+	++
Atrophy of neuron	-	++	+++
Necrosis of neuron	-	+++	+++
Inflammatory reactions	-	+++	++++
Neuronal degeneration	-	+++	++++
Vacuolation of neuron	-	+	++

Testes

The testicular tissue of VO-NPs exposed rats revealed mild to very severe pathological lesions, including thickened connective tissue, seminiferous tubule degeneration, pyknotic nuclei, spermatogenic arrest, tubular damage, and necrotic spermatocyte infiltration across all exposed groups (Table 6) (Fig 2).

Table 6. Severity of histopathological changes in rat testes

Parameters	Groups/Treatment		
	G_c (control)	G_1	G_2
Increased connective tissues	-	++	+++
Disorganization of spermatogonia and sertoli cells	-	+++	++++
Necrosis of spermatids	-	++	++++
Inflammatory process	-	+++	+++
Degeneration of seminiferous tubules	-	++	++++
Sloughed cells	-	++	++++
Increased cellular debris in lumen of seminiferous tubules	-	++	+++
Arrest of process of spermatogenesis	-	+++	++++
Testicular tubular degeneration	-	+	++
Necrosis of germinal epithelium of seminiferous tubules	-	+++	++++

Figure 1:
Photomicrograph of different sections of brain of albino rats administered different doses of vanadium oxide nanoparticles (VONPs) exhibiting necrosis of neuron, degeneration, enlarged cytoplasm, atrophy of nuclei and eccentric nuclei. 400X; H & E Stain

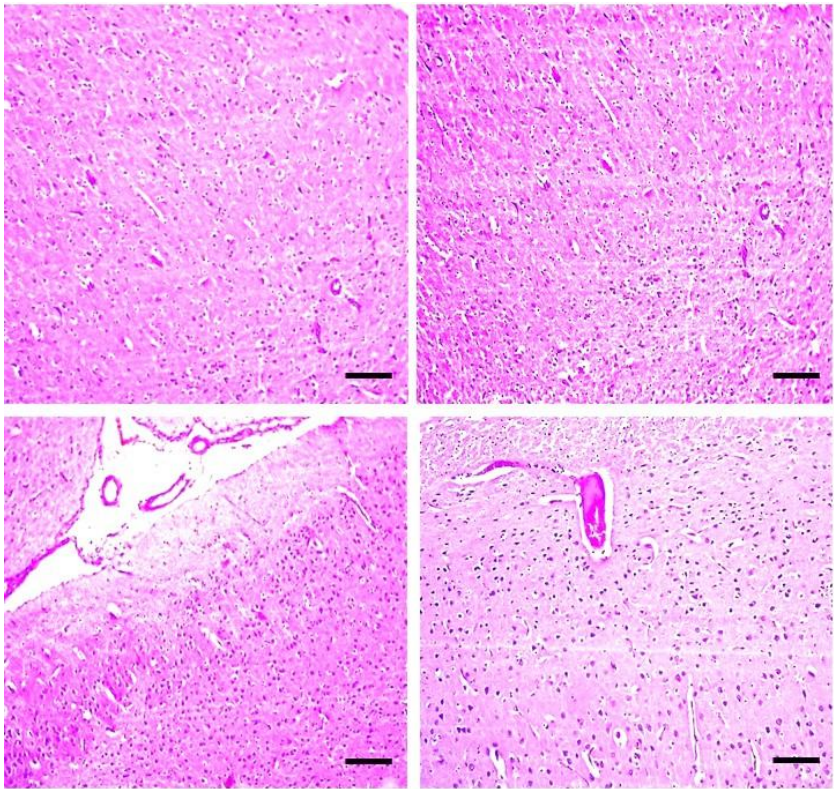
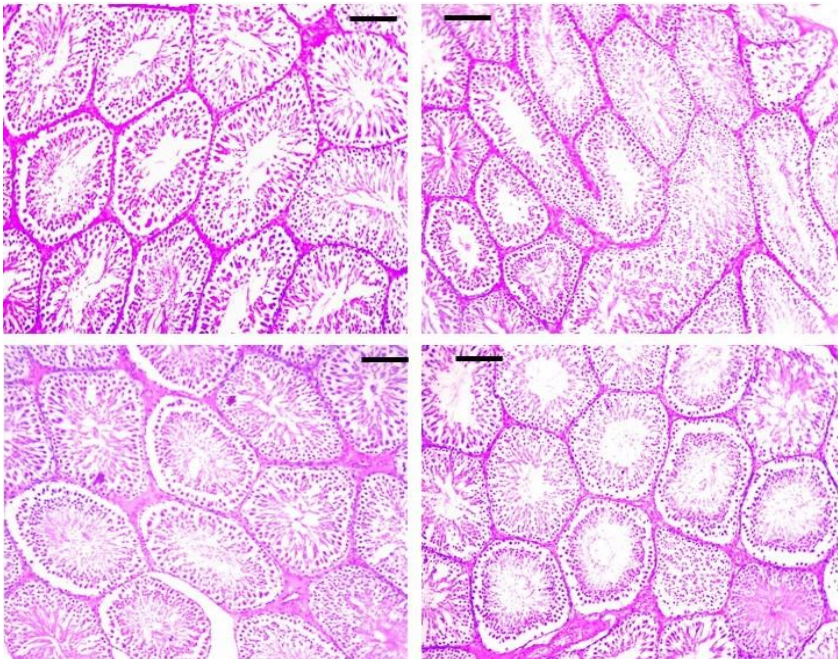


Figure 2:
Photomicrograph of different sections of testes of albino rats administered different doses of vanadium oxide nanoparticles (VONPs) exhibiting necrosis of spermatids, arrest of process of spermatogenesis, sloughed cells, and admixtures of necrotic tissues in center of seminiferous tubules. 400X; H & E Stain



In group G₃ rats, seminiferous tubules containing normal sperm were markedly fewer than those observed in other groups (Table 7).

Table 7. Changes in seminiferous tubule morphology within the testes of rats

Parameter	Gc (control)	G ₁	G ₂
No. of seminiferous tubules with normal spermatogenesis	99%	89%	78%
Diameter of seminiferous tubules	98%	88%	77%

Antimicrobial Activity Evaluation

After the incubation period, the plates were examined for zones of inhibition, which appeared to be clear areas indicative of the antimicrobial activity of vanadium oxide. The results showed vanadium oxide activity against *Salmonella*. Zone of inhibition was observed as 9mm at 5μl, 12mm at 10μl concentration, and 17mm at 20μl concentration of vanadium oxide prepared solution as shown in table.

Table 8. Antimicrobial activity of vanadium oxide at different concentrations against *Salmonella*

Well No	Vanadium oxide concentration (μl)	Zone diameter (mm)
Sal1	5	9
Sal2	10	12
Sal3	20	17

Discussion

Research suggests that estimating histopathological changes, antioxidant enzyme levels, and oxidative stress biomarkers is crucial for monitoring the hazardous effects of substances. Vanadium oxide nanoparticles are widely used in various fields, including medicine, microbiology, agriculture, energy production, and public health management (Ikhuoria et al., 2024a). Exposure to high amounts of vanadium oxide can pose health hazards over time (Assem & Levy, 2009). There is less information available on the toxicological consequences of these nanoparticles on living organisms. It is noted that these NPs induce oxidative stress via interaction with polyunsaturated fatty acids in the cell membrane of various visceral organs including the brain, testes, muscles etc. (Sudhbose et al., 2024). Treatment with VO NPs resulted in behavioral changes such as anxiety, locomotor abnormalities,

distress, myasthenia, and lethargy in rats. The histopathological alterations observed in this research study including, inflammatory materials, necrosis of myocytes, myo-fibrosis can also be related to induction of oxidative stress by nanoparticles causing disruption of mechanisms of oxidative phosphorylation, inhibition of production of ATP, glycolysis and inhibition of acetylcholinesterase in muscles of treated rats (Raghupathy et al., 2010). The clinical changes in albino rats may be attributed to oxidative stress caused by free radical production as reported earlier (Jarrar et al., 2021; Mahmood et al., 2024). The study investigated the toxicity of vanadium oxide NPs at two distinct dosages in albino rats. In this study, rats treated with vanadium oxide NPs showed higher levels of oxidative stress biomarkers and decreased levels of antioxidant enzymes in the lungs, brain, and testes, potentially indicates an inflammation, as explained in the previous studies (Afzal et al., 2024; Zhuo et al., 2024). Zhuo et al (2024) reported that the exposure to NPs activates NLRP3 inflammasomes, resulting in mitochondrial damage, inflammation, and oxidative stress. Reduced antioxidant biomarker levels in rats' visceral organs may be attributed to oxidative stress. Previous research suggests that oxidative stress can cause cell harm and alter physiological functioning, leading to tissue necrosis through apoptosis (Ali et al., 2024). Vanadium oxide NPs may cause oxidative issues in the lungs, brain, and testes possibly due to increased free radical release, depletion of antioxidants, and activation of signaling cascades (Venkatappa et al., 2022). Free radicals interact with lipids, DNA, and proteins in many cells, leading to oxidative stress-related damage. This study found that vanadium oxide NPs-induced pathological alterations in cell membrane integrity led to higher levels of oxidative stress parameters (TBARS and ROS) in several visceral organs of rats, resulting in lower levels of antioxidant content (Raghupathy et al., 2010). ROS cause oxidation of nucleotide bases and break in the strands which can lead to conformational changes and strand crosslinks. These changes as well as suppression of DNA repair mechanism might induce genotoxicity (Li et al., 2018). Such genotoxic effects have been reported for other nanoparticles as well (Attia et al., 2018; Tulinska et al., 2022). Previously, it is reported that induction of oxidative stress in visceral organ enhances the quantity of aniline which damages lipids proteins, and DNA in spleen which can be related to DNA damage by over production of free radicals and initiation of DNA glycosylases expression (APE1, OGG1, NTH1, NEIL1/2 and PNK). in rats. Additionally, pathological alterations in different visceral organs of nanoparticle treated rats could also be related to up-regulation of different inflammatory mediator like cytokines, IL-6, TNF-α and IL- 1 through activation of nuclear factor-kappa B,

redox-sensitive transcription mechanisms and AP-1 as a result of oxidative stress (Makhdoumi et al., 2019).

In the current study, the antimicrobial activity of vanadium oxide was checked against *Salmonella*. Zone inhibition was observed as 9mm at 5µl, 12mm at 10µl concentration, and 17mm at 20µl concentration of vanadium oxide prepared solution, also explained by Kışla et al (2024). Published literature also showed the antimicrobial activity of vanadium oxide nanoparticles at different concentrations (Aliyu et al., 2017; Selvakumar et al., 2022).

Conclusion

This study illustrates the systemic toxicity of vanadium oxide nanotoxicity in rats, as evidenced by oxidative stress, a decrease in antioxidant activity, and genotoxicity, on a variety of organs and tissues, such as the lungs, brain, and testes. Additionally, these nanoparticles demonstrated increased levels of reactive oxygen species (ROS), lipid peroxidation, DNA damage, and a decrease in antioxidant enzyme levels. Consequently, the adverse health effects of VO NPs in vivo in albino rats are suggested to be a time- and dose-dependent response, as they directly or indirectly affect live cells. Future studies should explore the chronic toxicity of VO NPs, assess recovery potential post-exposure, and evaluate molecular mechanisms in greater depth. These findings also highlight the need for stricter regulatory oversight and safe handling protocols for nanomaterials in biomedical and environmental applications.

Author Contributions:

Conceptualization, Ayesha Mohiuddin, Muhammad Faisal Maqsood;

Writing—original draft preparation, Roshan Ara, Shahida Younas, Samra Farrukh, Muhammad Pervaiz, Ayesha Mohiuddin;

Writing—review and editing, Ayesha Mohiuddin, Muhammad Faisal Maqsood;

Supervision, Ayesha Mohiuddin, Muhammad Faisal Maqsood;

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