

# **TOXICITY EVALUATION OF EXPOSURE TO NANOPESTICIDES**

**BY**

**SHIMAA GHAREEB MAHMOUD MARZOUK**

A thesis submitted in partial fulfillment

of

the requirements for the degree of

**DOCTOR OF PHILOSOPHY**

in

Agricultural Science

**(Pesticides)**

**Plant Protection Department**

**Faculty of Agriculture**

**Zagazig University**

**2021**

## ABSTRACT

Nanomaterials have been widely used in different fields like industry, medicine, electronics, and agriculture. Nanopesticides may have some effects on the rats, and thus the safety of their use as pesticide needs to be studied. The present study aimed to evaluate the toxicity orally administered of titanium dioxide nanoparticles ( $\text{TiO}_2$  NPs) and titanium silicon oxide nanoparticles ( $\text{TiSiO}_4$  NPs). The expression profile of genes encoding oxidative stress-related enzymes (Gpx, Cu-Zn SOD), protein folding (Hsp70), apoptosis (p53), and metal toxicity (Mt1) was determined in the liver of male rats at 7 and 28 days after a single oral dose (250 mg/kg b.w.) of  $25 \pm 5$  nm  $\text{TiO}_2$  NPs. At 7 days, changes in the expression of all genes were observed. Hepatic effects of  $\text{TiO}_2$  NPs on (Gpx, Cu-Zn SOD, p53, and Hsp70) genes were reversible at 28 days. Except for the Mt1 gene, the gene expression remained high after 28 days. Our study focuses on the potential toxic effect of exposure to  $\text{TiSiO}_4$  in male rats using biochemical markers (Total protein (TP), Albumin, Total cholesterol, Triglycerides, Superoxide dismutase (SOD), glutathione peroxidase (GPx), Immunoglobulin G (IgG), Immunoglobulin M (IgM), acetylcholinesterase (AChE), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Lactate dehydrogenase (LDH), bilirubin, creatinine, urea, Luteinizing hormone (LH), Follicle-stimulating hormone (FSH), Progesterone, Testosterone, and Comet assay). Distribution of Si and Ti in rat tissues and histopathological examination of target organs in  $\text{TiSiO}_4$  NPs exposed rats were also studied. Differences between the  $\text{TiSiO}_4$  NPs groups and the control group in some biochemical markers values and histopathological findings were found. The accumulation of Si in the organs was also noticed.

## CONTENTS

<b>I. INTRODUCTION</b>	1
<b>II. REVIEW OF LITERATURE</b>	3
1. Nanomaterials	3
1.1. Nanomaterials Classification	3
1.2. Nanotechnology	4
1.2.1. Applications of nanotechnology in agriculture	6
1.2.2. Applications of nanotechnology in plant protection	6
1.2.3. Titanium dioxide nanoparticles (TiO <sub>2</sub> NPs)	9
1.2.4. Silica dioxide nanoparticles (SiO <sub>2</sub> NPs)	12
2. Genotoxicity of nanoparticles	14
3. Effect of TiO <sub>2</sub> nanoparticles on gene expression	16
4. Effect of TiO <sub>2</sub> and SiO <sub>2</sub> nanoparticles on some biochemical aspects	18
4.1. TiO <sub>2</sub> nanoparticles	18
4.2. SiO <sub>2</sub> nanoparticles	21
5. Histopathological studies on the effects of TiO <sub>2</sub> and SiO <sub>2</sub> nanoparticles in rats	22
5.1. TiO <sub>2</sub> nanoparticles	22
5.2. SiO <sub>2</sub> nanoparticles	23
6. Distribution of TiO <sub>2</sub> NPs and SiO <sub>2</sub> NPs in tissues	24
6.1. TiO <sub>2</sub> nanoparticles	24
6.2. SiO <sub>2</sub> nanoparticles	25

<b>III. MATERIAL AND METHODS</b>	27
A. Materials	27
I. Test compounds	27
II. Reagents and other Chemicals	27
III. Test animal	28
B. Methods	29
1. Characterization of the tested nanoparticles (NPs)	29
2. Gene expression analysis in male rats liver exposed orally to titanium dioxide nanoparticles	29
2.1. Animal treatment	29
2.2. Preparation of reagents for RNA Extraction	29
2.3. RNA Extraction from liver	30
2.4. Determination of RNA concentration	31
2.5. cDNA synthesis via Reverse Transcription	31
2.6. The real time Polymerase Chain Reaction (R.T. PCR)	32
3. Studying the potential toxic effect of exposure to titanium silicon oxide nanoparticles (TiSiO <sub>4</sub> ) in male rats	34
3.1. Animal treatment	34
3.2. Rats samples	35
3.2.1. Serum preparation	35
3.3. Serum biochemical markers determination	35
3.3.1. Total protein	36

3.3.2. Total albumin	36
3.3.3 Total Cholesterol	37
3.3.4. Triglycerides	38
3.3.5. Oxidative stress markers	40
3.3.5.1 Superoxide dismutase EC 1.15.1.1 (SOD)	40
3.3.5.2. Glutathione peroxidase EC 1.11.1.9 (GPx)	42
3.3.6. Immune system function markers	43
3.3.6.1. Immunoglobulin G and M (IgG and IgM)	43
3.3.7. Neurotoxicity marker	45
3.3.7.1. Acetylcholine esterase EC 3.1.1.7. (AChE)	45
3.3.8. Liver function markers	47
3.3.8.1. Alanine aminotransferase EC2.6.1.2 (ALT)	47
3.3.8.2. Aspartate aminotransferase EC2.6.1.1 (AST)	48
3.3.8.3. Lactate dehydrogenase EC 1.1.1.27 (LDH)	49
3.3.8.4.Total Bilirubin	50
3.3.9. Kidney function markers	51
3.3.9.1. Creatinine	51
3.3.9.2 Urea	52
3.3.10. Reproductive toxicity markers	54
3.3.10.1. Follicle-stimulating hormone (FSH) and Luteinizing hormone (LH)	54
3.3.10.2. Testosterone	56
3.3.10.3. Progesterone	56

3.4. Genotoxicity (DNA damage marker)	57
3.4.1. Comet DNA assay	57
3.5. Histopathological examination of Liver, kidneys, spleen and lung tissues in male rats exposed orally to TiSiO <sub>4</sub>	59
4. Determination of Si and Ti in tissues of male rats exposed orally to TiSiO <sub>4</sub>	59
4.1. Animal treatment	59
4.2. Silicon (Si) and titanium (Ti) determination	60
5. Data analysis	60
<b>IV. RESULTS AND DISCUSSION</b>	61
1. Characterization of titanium dioxide nano particles (TiO <sub>2</sub> NPs) and titanium silicon oxide nanoparticles (TiSiO <sub>4</sub> NPs)	61
1.1. Transmission electron microscopy (TEM)	61
1.2. X-ray diffraction (XRD)	61
2. Gene expression analysis in male rats liver exposed orally to titanium dioxide nanoparticles	63
2.1. Oxidative stress genes (Cu-Zn SOD, Gpx)	63
2.2. Protein folding gene (Hsp70)	67
2.3. Apoptosis gene (p53)	70
2.4. Metal toxicity gene (Mt1)	73
3. The potential toxic effect of oral exposure to TiSiO <sub>4</sub> (250 mg/kg b.w.) in male rats using biochemical markers	78

3.1. Total protein	78
3.2. Albumin	78
3.3. Total cholesterol	80
3.4. Triglycerides	80
3.5. Oxidative stress markers	81
3.5.1. Superoxide dismutase EC 1.15.1.1 (SOD)	81
3.5.2. Glutathione peroxidase EC 1.11.1.9 (GPx)	83
3.6. Immune system function markers	84
3.6.1. Immunoglobulin G (IgG)	84
3.6.2. Immunoglobulin M (IgM)	85
3.7. Neurotoxicity marker	86
3.7.1. Acetylcholine esterase EC 3.1.1.7. (AChE)	86
3.8. Liver function markers	87
3.8.1. Alanine aminotransferase EC 2.6.1.2 (ALT)	87
3.8.2. Aspartate aminotransferase EC 2.6.1.1 (AST)	89
3.8.3. Total bilirubin	89
3.8.4. Lactate dehydrogenase EC 1.1.1.27 (LDH)	90
3.9. Kidney function markers	91
3.9.1. Creatinine	91
3.9.2. Urea	92
3.10. Reproductive toxicity markers	93
3.10.1. Follicle stimulating hormone (FSH):	93
3.10.2. Luteinizing hormone (LH)	93

3.10.3. Testosterone	95
3.10.4. Progesterone	95
4. Genotoxicity (DNA damage marker)	97
4.1. Comet assay:	97
5. Histopathological examination of liver, kidney, spleen and lung tissues in male rats exposed orally to TiSiO <sub>4</sub>	101
5.1. Liver histopathology	101
5.2. Kidney histopathology	103
5.3. Spleen histopathology	108
5.4. Lung histopathology	108
6. Distribution of Si and Ti in tissues of male rats exposed orally to titanium silicon oxide nano particles (TiSiO <sub>4</sub> NPs)	115
<b>V. SUMMARY AND CONCLUSION</b>	122
<b>VI. REFERENCES</b>	129
<b>ARABIC SUMMARY</b>	



## LIST OF TABLES

No.	Title	Page
<b>1</b>	Antimicrobial activity of titanium dioxide (TiO <sub>2</sub> ) NPs against several microorganisms.	10
<b>2</b>	Applications of Silica NPs in agriculture fields and plant protection.	13
<b>3</b>	Primers sequences and PCR program.	33
<b>4</b>	Serum total protein, albumin, total cholesterol, and triglycerides content in male rats exposed orally to 250 mg/kg b. w. TiSiO <sub>4</sub> NPs.	79
<b>5</b>	Oxidative stress (SOD and GPx activity), Immunotoxicity (IgG and IgM), and Neurotoxicity (AChE activity) in serum male rats exposed orally to 250 mg/kg b. w. TiSiO <sub>4</sub> NPs	82
<b>6</b>	Hepatic and renal toxicity in male rats exposed orally to 250 mg TiSiO <sub>4</sub> NPs /kg b. w	88
<b>7</b>	Sex hormones (FSH, LH, testosterone, and progesterone) levels in serum male rats exposed orally to 250 mg TiSiO <sub>4</sub> NPs /kg b.w.	94
<b>8</b>	Comet assay indices in liver male rats exposed orally to 250 mg TiSiO <sub>4</sub> NPs /kg b. w.	99
<b>9</b>	Silica content (mg/kg tissue) in male rat liver, kidney and spleen tissues after 1, 3, and 6 days post oral administration of 250 mg TiSiO <sub>4</sub> NPs /kg b.w.	116
<b>10</b>	Titanium content (mg/kg tissue) in male rat liver, kidney and spleen tissues after 1, 3, and 6 days post oral administration of 250 mg TiSiO <sub>4</sub> NPs /kg b.w.	117

## LIST OF FIGURES

No.	Title	Page
<b>1</b>	Applications of nanotechnology.	5
<b>2</b>	Applications of nanotechnology in agriculture.	7
<b>3</b>	Different NM used in plant protection and fertilizer publications and patents.	7
<b>4</b>	Uses of nanoparticles in plant protection.	8
<b>5</b>	Mechanisms of antimicrobial activity of TiO <sub>2</sub> NPs.	11
<b>6</b>	Transmission electron microscope images of (A) TiO <sub>2</sub> NPs (B) TiSiO <sub>4</sub> NPs.	61
<b>7</b>	X-ray diffraction pattern of TiO <sub>2</sub> NPs.	62
<b>8</b>	X-ray diffraction pattern of TiSiO <sub>4</sub> NPs.	62
<b>9</b>	Effect of TiO <sub>2</sub> NPs on SOD gene expression in male rat liver.	64
<b>10</b>	Effect of TiO <sub>2</sub> NPs on GPx gene expression in male rat liver.	64
<b>11</b>	Amplification curves for the quantitative real-time PCR of SOD and GPx cDNA from both TiO <sub>2</sub> NPs - treated and control groups.	65
<b>12</b>	Melting curves of the PCR products of SOD cDNA amplification of both TiO <sub>2</sub> NPs –treated and control groups.	65
<b>13</b>	Melting curves of the PCR products of GPx cDNA amplification of both TiO <sub>2</sub> NPs –treated and control groups.	66

<b>14</b>	Effect of TiO <sub>2</sub> NPs on Hsp70 gene expression in male rat liver.	68
<b>15</b>	Amplification curves for the quantitative real time PCR of Hsp70 cDNA from both TiO <sub>2</sub> NPs – treated and control groups.	68
<b>16</b>	Melting curves of the PCR products of Hsp70 cDNA amplification of both TiO <sub>2</sub> NPs – treated and control groups.	69
<b>17</b>	Effect of TiO <sub>2</sub> NPs on p53 gene expression in male rat liver.	71
<b>18</b>	Amplification curves for the quantitative real time PCR of p53 cDNA from both TiO <sub>2</sub> NPs – treated and control groups.	71
<b>19</b>	Melting curves of the PCR products of p53 cDNA amplification of both TiO <sub>2</sub> NPs–treated and control groups.	72
<b>20</b>	Effect of TiO <sub>2</sub> NPs on Mt1 gene in male rat liver.	74
<b>21</b>	Amplification curves for the quantitative real time PCR of Mt1 cDNA from both TiO <sub>2</sub> NPs – treated and control groups.	74
<b>22</b>	Melting curves of the PCR products of Mt1 cDNA amplification of both TiO <sub>2</sub> NPs–treated and control groups.	75
<b>23</b>	Amplification curves for the quantitative real time PCR of GAPDH cDNA from both TiO <sub>2</sub> NPs – treated and control groups.	77

<b>24</b>	Melting curves of the PCR products of GAPDH cDNA amplification of both TiO <sub>2</sub> NPs – treated and control groups.	77
<b>25</b>	Fluorescence microscopy image of liver cell comets of male rats exposed to 250 mgTiSiO <sub>4</sub> NPs /kg b.w.; (A) : Control , (B): 7 days post treatment and (C): 28 days post treatment.	100
<b>26</b>	A section of control male rat liver.	102
<b>27</b>	A section of male rat liver, A: after 7 days post oral administration of 250 mg TiSiO <sub>4</sub> / kg b.w., B: 28 days post treatment	104
<b>28</b>	A section of control male rat kidney.	105
<b>29</b>	A section of male rat kidney after, 7 days post oral administration of 250 mg TiSiO <sub>4</sub> / kg b.w.	106
<b>30</b>	A section of male rat kidney after, 28 days post oral administration of 250 mg TiSiO <sub>4</sub> / kg b.w.	107
<b>31</b>	A section of control male rat spleen.	109
<b>32</b>	A section of male rat spleen, after 7 days post oral administration of 250 mg TiSiO <sub>4</sub> / kg b.w.	110
<b>33</b>	A section of male rat spleen after, 28 days post oral administration of 250 mg TiSiO <sub>4</sub> / kg b.w.	111
<b>34</b>	A section of control male rat lung.	112
<b>35</b>	A section of male rat lung, after 7 days post oral administration of 250 mg TiSiO <sub>4</sub> / kg b.w.	113
<b>36</b>	A section of male rat lung, after 28 days post oral administration of 250 mg TiSiO <sub>4</sub> / kg b.w.	114