



Ain shams university  
Faculty of Science  
Zoology Department

# **Biological Studies on the Effect of Some Nano-Materials Used in Processing Military Protection Wears on the Skin**

A THESIS SUBMITTED FOR  
THE AWARD OF THE MASTER DEGREE OF SCIENCE  
(IN ZOOLOGY)

**By**

**Sameh Mohamed Mohamed Abouzead**

(B.Sc.)

**Supervised by**

**Dr. Nagui Hassan Fares**  
Professor of Cell Biology and  
Histology - Zoology Department  
Faculty of Science  
Ain Shams University

**Dr. Mohamed Abdelmordy Mohamed**  
Professor of Genetics and Molecular  
Biology - Zoology Department  
Faculty of Science  
Ain Shams University

**Dr. Yomna Ibrahim Mahmoud**  
Assistant Professor of Histology and Cell Biology  
Zoology Department-Faculty of Science  
Ain Shams University

Ain shams university  
Faculty of Science  
Zoology Department

**2015**

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ  
دَرَجَاتٍ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ

صَدَقَ اللَّهُ الْعَظِيمَ

للمجاردة: 11

## DEDICATION

*I dedicate this work to all my family specially to the soul of my father, to my mother, my wife and my children for their support, quiet patience and unwavering love .*

*Thank for you all*

## ACKNOWLEDGEMENTS

*Throughout this work I have required the guidance and technical assistance from a great number of people on frequent occasions, and I have never been met with anything but politeness and enthusiasm. First and foremost, I would like to record my great debts of gratitude to the supervisors of my thesis: **Prof. Dr. Nagui Hassan Fares** (Professor of Cell biology and Histology), **Prof. Dr. Mohamed Abdelmordy Mohamed** (Professor of Genetics and Molecular biology), and **Dr. Yomna Ibrahim Mahmoud** (Assistant Professor of Histology and Cell Biology). This experience would certainly not have been as valuable without their guidance, support, and inspiration. I have no words to express my appreciation for their valuable suggestions, unfailing help, intellectual criticisms and moral support throughout the study. They had confidence in me when I doubted myself, and encouraged me and brought out the good ideas in me. I am sure that they will be remembered in all my future endeavors. I feel really blessed to work under their guidance and I am truly rewarded.*

*Sameh Abouzead*



## CONTENTS

<b>CONTENTS</b>	<b>i</b>
<b>List of Abbreviations</b>	<b>iv</b>
<b>List of Tables</b>	<b>vii</b>
<b>List of Figures</b>	<b>viii</b>
<b>ABSTRACT</b>	<b>xxii</b>
<b>INTRODUCTION</b>	<b>1</b>
<b>AIM OF THE WORK</b>	<b>4</b>
<b>LITERATURE REVIEW</b>	<b>5</b>
1. Zinc Oxide Nanoparticles (ZnO-NPs)	7
1.1. Military Wears Applications	7
1.2. Toxicity and Hazardous Health Effects	9
2. Titanium Dioxide Nanoparticles (TiO <sub>2</sub> -NPs)	16
2.1. Military Wears Applications	16
2.2. Toxicity and Hazardous Health Effects	18
3. Carbon black Nanoparticles (CB-NPs)	26
3.1. Military Wears Applications	26
3.2. Toxicity and Hazardous Health Effects	27
<b>MATERIALS AND METHODS</b>	<b>33</b>
<b>I- MATERIALS</b>	<b>33</b>
1. Chemicals and Reagents	33
1.1. Nanoparticles (NPs)	33
1.2. Chemicals and Reagents	33
2. Animals	33
<b>II- METHODS</b>	<b>34</b>
1. Nanoparticles (NPs) Characterization	34
1.1. Scanning Electron Microscope Analysis	34
1.2. X-ray Fluorescence Analysis	35
2. Animals Preparation and Treatment	37

<b>3. The Histological Studies</b>	<b>39</b>
<b>3.1. Macroscopic Manifestations</b>	<b>39</b>
<b>3.2. Light Microscopy</b>	<b>40</b>
<b>3.3. Electron Microscopy</b>	<b>44</b>
<b>3.4. Morphometric and Statistical Analysis</b>	<b>48</b>
<b>4. Molecular and Biochemical Genetic Studies</b>	<b>49</b>
<b>4.1. Glutathione Enzymes</b>	<b>49</b>
<b>4.1.1. Tissue homogenate preparation</b>	<b>49</b>
<b>4.1.2. Glutathione- S-Transferase (GST) activity</b>	<b>49</b>
<b>4.1.3. Glutathione Reduced (GSH) Level</b>	<b>50</b>
<b>4.1.4. Glutathione Reductase (GR) activity</b>	<b>51</b>
<b>4.1.5. Statistical Analysis</b>	<b>52</b>
<b>4.2. Nonspecific Esterases (Est.) Electrophoresis</b>	<b>52</b>
<b>4.3. General Proteins Electrophoresis</b>	<b>58</b>
<b>RESULTS AND OBSERVATIONS</b>	<b>63</b>
<b>I- Nanoparticles (NPs) Characterization</b>	<b>63</b>
<b>1. Scanning Electron Microscope (SEM)</b>	<b>63</b>
<b>2. X-Ray Fluorescence (XRF)</b>	<b>64</b>
<b>II- Histological and Ultrastructural Studies</b>	<b>69</b>
<b>1. The Control</b>	<b>69</b>
<b>2. Group I (ZnO-NPs treatment)</b>	<b>86</b>
<b>3. Group II (TiO<sub>2</sub> NPs treatment)</b>	<b>116</b>
<b>4. Group III (CB-NPs treatment)</b>	<b>150</b>
<b>III- Morphometric and Statistical Results</b>	<b>206</b>
<b>1. Group I (ZnO-NPs treatment)</b>	<b>206</b>
<b>2. Group II (TiO<sub>2</sub>-NPs treatment)</b>	<b>208</b>
<b>3. Group III (CB-NPs treatment)</b>	<b>210</b>
<b>IV- Molecular and Biochemical Genetics Results</b>	<b>212</b>
<b>1. Glutathione Enzymes</b>	<b>212</b>
<b>1.1. Group I (ZnO-NPs treatment)</b>	<b>212</b>
<b>1.2. Group II (TiO<sub>2</sub>-NPs treatment)</b>	<b>213</b>

1.3. Group III (CB-NPs treatment)	214
2. Nonspecific Esterases (Est.) Electrophoresis	222
2.1. Group I (ZnO-NPs treatment)	222
2.2. Group II (TiO <sub>2</sub> -NPs treatment)	227
2.3. Group III (CB-NPs treatment)	233
3. General Proteins Electrophoresis	239
3.1. Group I (ZnO-NPs treatment)	239
3.2. Group II (TiO <sub>2</sub> -NPs treatment)	242
3.3. Group III (CB-NPs treatment)	246
<b>DISCUSSION</b>	<b>251</b>
<b>SUMMARY</b>	<b>277</b>
<b>REFERENCES</b>	<b>283</b>
<b>Arabic Summary</b>	<b>313</b>

## List of Abbreviations

<b>A549</b>	Human alveolar epithelial cell line
<b>ANOVA</b>	Analysis of Variance
<b>BAL</b>	Broncho-Alveolar Lavage cell line
<b>BEAS-2B</b>	Human bronchial epithelium cell line
<b>Caco-2</b>	Human Colorectal adeno-carcinoma cell line
<b>CB-NPs</b>	Carbon Black Nanoparticles
<b>CDNB</b>	2,4-Dinitrochlorobenzene
<b>DMEM</b>	Dulbecco's Modified Eagle's medium
<b>DTNB</b>	1-Chloro-2,4-Dinitrobenzene
<b><i>E. coli</i></b>	<i>Escherichia coli</i>
<b>EDTA</b>	Ethane-1,2-Diylldinitrilo Tetra-Acetic acid
<b>EDX</b>	Energy Diffraction X-ray spectrum
<b>ELISA</b>	Enzyme Linked Immune Sorbent Assay
<b>ESTs</b>	Esterases
<b>FTIR</b>	Fourier Transform Infra-Red spectroscopy
<b>GR</b>	Glutathione Reductase
<b>GSH</b>	Reduced Glutathione
<b>GSSG</b>	Oxidized Glutathione
<b>GST</b>	Glutathione-S-Transferase
<b>HaCaT</b>	Human keratinocytes cell line
<b>HDF</b>	Human Dermal Fibroblasts cell line

<b>HUVECs</b>	Human umbilical Vein Endothelial cells
<b>ICP-mass</b>	Inductively Coupled Plasma mass spectroscopy
<b>IR</b>	Infrared
<b><i>K. pneumonia</i></b>	<i>Klebsiella pneumonia</i>
<b>KDa</b>	kilodalton
<b>L-132</b>	Human lung epithelial cells
<b>LDH</b>	Lactate Dehydrogenase
<b>LM</b>	Light Microscope
<b>MDA</b>	Malondialdehyde
<b>MMPs</b>	Matrix Metalloproteinase
<b>MTS</b>	Methylthiazol Tetrazolium Salt
<b>MTT</b>	Mitochondria cell viability assay
<b>NADPH</b>	Nicotinamide Adenine Dinucleotide Phosphate-oxidase
<b>NMs</b>	Nanomaterials
<b>N-PAGE</b>	Native Polyacrylamide Gel Electrophoresis
<b>NPs</b>	Nanoparticles
<b>NTMPW</b>	Nano-Treated Military Protection Wears
<b>PBS</b>	Phosphate Buffered Saline
<b>PCR</b>	Polymerase Chain Reaction
<b>RAW264.7</b>	Mouse monocyte macrophage cell line
<b>REF-3</b>	Rat Embryo Fibroblast cell line
<b>Rm</b>	Relative mobility
<b>ROS</b>	Reactive Oxygen Species

<b>RT-PCR</b>	Real-Time quantitative PCR
<b><i>S. aureus</i></b>	<i>Staphylococcus aureus</i>
<b><i>S. epidermidi</i></b>	<i>Staphylococcus epidermidis</i>
<b>SC</b>	Stratum Corneum
<b>SD</b>	Standard Deviation
<b>SDS</b>	Sodium Dodecyl Sulfate
<b>SDS-PAGE</b>	Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis
<b>SEM</b>	Scanning Electron Microscope
<b>SG</b>	Stratum Granulosum
<b>SOD</b>	Super Oxide Dismutase
<b>SS</b>	Stratum Spinosum
<b>TEM</b>	Transmission Electron Microscopy
<b>TEMED</b>	Tetramethylethylenediamine
<b>TiO<sub>2</sub>-NPs</b>	Titanium dioxide Nanoparticles
<b>TOF-SIMS</b>	Time of Flight Secondary Ion Mass Spectrometry
<b>UV</b>	Ultraviolet
<b>UV-VIS</b>	Ultraviolet–Visible Spectroscopy
<b>X</b>	Mean
<b>XRD</b>	X-Ray Diffraction
<b>XRF</b>	X-ray Fluorescence
<b>ZnO-NPs</b>	Zinc Oxide Nanoparticles

## List of Tables

<b>Table.1:</b> Characteristics of the experimental NPs.....	<b>33</b>
<b>Table.2:</b> The experimental groups.....	<b>38</b>
<b>Table.3:</b> The result of XRF analysis of ZnO-NPs.....	<b>68</b>
<b>Table.4:</b> The result of XRF analysis of TiO <sub>2</sub> -NPs.....	<b>68</b>
<b>Table.5:</b> The result of XRF analysis of CB-NPs.....	<b>68</b>
<b>Table.6:</b> Summary of the mean thickness of nucleated epidermal keratinocytes in the skin of rabbits treated with ZnO-NPs (Group I) and their relations.....	<b>207</b>
<b>Table.7:</b> Summary of the mean thickness of nucleated epidermal keratinocytes in the skin of rabbits treated with TiO <sub>2</sub> -NPs (Group II) and their relations.....	<b>209</b>
<b>Table.8:</b> Summary of the mean thickness of nucleated epidermal keratinocytes in the skin of rabbits treated with CB-NPs (Group III) and their relations.....	<b>211</b>
<b>Table.9:</b> Activities of Glutathione- S-Transferase (GST) and Glutathione Reductase (GR), and Glutathione Reduced (GSH) level in the skin of rabbits after exposure to different treatments of ZnO-NPs.....	<b>216</b>
<b>Table.10:</b> The activities of Glutathione- S-Transferase (GST) and Glutathione Reductase (GR), and Glutathione Reduced (GSH) level in the skin of rabbits after exposure to different treatments of TiO <sub>2</sub> -NPs.....	<b>218</b>
<b>Table.11:</b> The activities of Glutathione- S-Transferase (GST) and Glutathione Reductase (GR), and Glutathione Reduced (GSH) level in the skin of rabbits after exposure to different treatments of CB-NPs.....	<b>220</b>
<b>Table.12:</b> The relative activities of esterase bands of ZnO-NPs treated skin utilizing $\alpha$ -naphthyl acetate.....	<b>226</b>
<b>Table.13:</b> The relative activities of esterase bands of TiO <sub>2</sub> -NPs treated skin utilizing $\alpha$ -naphthyl acetate.....	<b>232</b>
<b>Table.14:</b> The relative activities of esterase bands of CB-NPs treated skin utilizing $\alpha$ -naphthyl acetate.....	<b>238</b>

## List of Figures

<b>Figure.1:</b> SEM micrograph displaying the surface morphology, particle size distribution and the aggregation status of ZnO-NPs.....	<b>65</b>
<b>Figure.2:</b> The EDX spectrum of ZnO-NPs showing the elemental composition.....	<b>65</b>
<b>Figure.3:</b> SEM micrograph displaying the surface morphology, particle size distribution and the aggregation status of TiO <sub>2</sub> -NPs.....	<b>66</b>
<b>Figure.4:</b> The EDX spectrum of TiO <sub>2</sub> -NPs showing the elemental composition.....	<b>66</b>
<b>Figure.5:</b> SEM micrograph displaying the surface morphology, particle size distribution and the aggregation status of CB-NPs.....	<b>67</b>
<b>Figure.6:</b> The EDX spectrum of CB-NPs showing the elemental composition.....	<b>67</b>
<b>Figure.7:</b> (a) Photograph of a control rabbit showing healthy animal with normal activity and behavior with no signs of spasm. (b) Dorsal view of a control rabbit showing a shaved skin area with no lesions, erythema or edemas.....	<b>72</b>
<b>Figure.8:</b> Photomicrograph of section of skin of the control group revealing the epidermis organized into four layers, H&E.....	<b>74</b>
<b>Figure.9:</b> Photomicrograph of semithin section of skin of the control group showing the epidermal layers.....	<b>74</b>
<b>Figure.10:</b> Photomicrograph section of the dermis of a control skin, H&E.....	<b>76</b>



<b>Figure.11:</b> Electron micrograph of the upper portion of the epidermis of a control skin.....	<b>78</b>
<b>Figure.12:</b> Electron micrograph of upper epidermis of a control skin.....	<b>78</b>
<b>Figure.13:</b> Electron micrograph showing a part of the stratum spinosum layer of a control skin.....	<b>80</b>
<b>Figure.14:</b> High magnification electron micrograph of a part of stratum spinosum layer of a control skin.....	<b>80</b>
<b>Figure.15:</b> Electron micrograph showing a part of lower epidermis of a control skin.....	<b>82</b>
<b>Figure.16:</b> High magnification electron micrograph of a part of the basal layer cell of a control skin.....	<b>82</b>
<b>Figure.17:</b> Electron micrograph of the dermis of a control skin.	<b>83</b>
<b>Figure.18:</b> Photograph of a IB sub-group rabbits after treatments with 0.5 % ZnO-NPs for 24, 48 and 72 time periods showing normal skin appearance with no erythema, edema or scars formation.....	<b>92</b>
<b>Figure.19:</b> Photograph of a IC sub-group rabbits after treatments with 1.0 % ZnO-NPs for 24, 48 and 72 time periods showing normal skin appearance with no erythema, edema or scars formation.....	<b>94</b>
<b>Figure.20:</b> Photograph of a ID sub-group rabbit after the topical application of 2.0 % ZnO-NPs for 24hrs showing a healthy skin with no lesions, erythema or edemas.....	<b>96</b>
<b>Figure.21:</b> Photograph of a ID sub-group rabbits after the topical application of 2.0 % ZnO-NPs for 48hrs showing definite erythema ranged from slight to moderate, and a slight edema with defined edges.....	<b>96</b>

<b>Figure.22:</b> Photomicrograph of section of a ID sub-group treated skin (ZnO-NPs 2.0 % / 48hrs), H&E.....	<b>98</b>
<b>Figure.23:</b> Photomicrograph of semi-thin section from a ID sub-group treated skin (ZnO-NPs 2.0 % / 48hrs).....	<b>98</b>
<b>Figure.24:</b> Electron micrograph of the upper epidermis of a ID sub-group treated skin (ZnO-NPs 2.0 % / 48hrs) revealing the abnormal thickening of stratum corneum layer.....	<b>100</b>
<b>Figure.25:</b> Electron micrograph of the upper epidermis of a ID sub-group treated skin (ZnO-NPs 2.0 % / 48hrs) showing a part of stratum granular layer cell.....	<b>100</b>
<b>Figure.26:</b> Electron micrograph of a part of stratum spinosum layer of the epidermis of ID sub-group treated skin (ZnO-NPs 2.0 % / 48hrs).....	<b>102</b>
<b>Figure.27:</b> High magnification electron micrograph of a part of stratum spinosum layer of the epidermis of ID sub-group treated skin (ZnO-NPs 2.0 % / 48hrs).....	<b>102</b>
<b>Figure.28:</b> Electron micrograph of the lower epidermis of a ID sub-group treated skin (ZnO-NPs 2.0 % / 48hrs) showing part of a basal layer cell with normal structure.....	<b>104</b>
<b>Figure.29:</b> Electron micrograph of a part of hair follicle (HF) of a ID sub-group treated skin (ZnO-NPs 2.0 % / 48hrs).....	<b>104</b>
<b>Figure.30:</b> Photograph of a ID sub-group rabbit after the topical application of 2.0 % ZnO-NPs for 72hrs showing well defined red patches which could be regarded as sever erythema. Scattered edemas formation throughout the test area was also noted, however, no gross lesions were present.....	<b>106</b>

<b>Figure.31:</b> Photomicrograph of section from a ID sub-group treated skin (ZnO-NPs 2.0 % / 72hrs), H&E.....	<b>108</b>
<b>Figure.32:</b> Photomicrograph of semithin section from a ID sub-group treated skin (ZnO-NPs 2.0 % / 72hrs).....	<b>108</b>
<b>Figure.33:</b> Electron micrograph of a part of the upper epidermis of a ID sub-group treated skin (ZnO-NPs 2.0 % / 72hrs) revealing the hyperkeratinization of stratum corneum layer.....	<b>110</b>
<b>Figure.34:</b> Electron micrograph of the upper epidermis of a ID sub-group treated skin (ZnO-NPs 2.0%/72hr) revealing the thinning of stratum granular layer.....	<b>110</b>
<b>Figure.35:</b> Electron micrograph of part of lower epidermis of a ID sub-group treated skin (ZnO-NPs 2.0%/72hr) revealing markedly keratinocytes disorganization and a stratum spinosum cell with signs of necrosis....	<b>112</b>
<b>Figure.36:</b> High magnification electron micrograph of a part of stratum spinosum cell of the epidermis of a ID sub-group treated skin (ZnO-NPs 2.0 % / 72hrs).....	<b>112</b>
<b>Figure.37:</b> Electron micrograph of a part of stratum basal cell of the epidermis of a ID sub-group treated skin (ZnO-NPs 2.0 % /72hrs).....	<b>114</b>
<b>Figure.38:</b> Electron micrograph of a part of hair follicle (HF) of a ID sub-group treated skin (ZnO-NPs 2.0 % / 72 hr).....	<b>114</b>
<b>Figure.39:</b> Photograph of a IIB sub-group rabbit after treatments with 0.5 % TiO <sub>2</sub> -NPs for 24, 48 and 72 time periods showing normal skin appearance with no erythema, edema or scars formation.....	<b>124</b>