TÜRKİYE CUMHURİYETİ
ADANA ALPARSLAN TÜRKEŞ SCIENCE AND TECHNOLOGY UNIVERSITY

GRADUATE SCHOOL
BIOENGINEERING DEPARTMENT

INVESTIGATION OF THE EFFECTS OF SILICON DIOXIDE NANOPARTICLES AND ENVIRONMENTAL CONTAMINANTS ON IMMUNOCYTOTOXIC AND ANTIOXIDANT DEFENCE SYSTEMS IN MODEL ORGANISM *Galleria mellonella*

MURAT İDİKUT

M.Sc.

ADANA 2024

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DECLARATION OF CONFORMITY

In this thesis study, which was prepared following the thesis writing rules of Adana Alparslan Türkeş Science and Technology University Institute of Graduate School, I declare that I provide all the information, documents, evaluations and results in accordance with scientific ethics and moral codes without resorting to any means or assistance that would be contrary to scientific ethics and traditions. I also declare that I refer to all of the articles I used in this study with appropriate references and accept all moral and legal consequences if a situation is found contrary to my statement regarding my work.

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Murat İDİKUT

ÖZET

MODEL ORGANIZMA *Galleria mellonella*'da SİLİKON DİOKSİT NANOPARTİKÜLLERİ VE ÇEVRESEL KİRLETİCİLERİN İMMÜNOSİTOTOKSİK VE ANTİOKSİDAN SAVUNMA SİSTEMLERİ ÜZERİNDEKİ ETKİLERİNİN ARAŞTIRILMASI

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Bu çalışmada, *Galleria mellonella* son evre larvalarına SiO_2 NP' lerinin LD_{50} değeri ile abamektin ve kadmiyum sülfatın ($CdSO_4$) çevresel derişimlerinin tek başına ve karışım halinde uygulanması sonucunda hemolenf, orta bağırsak ve yağ dokuda superoksit dismutaz (SOD), katalaz (CAT), glutatyon peroksidaz (GPx), glutatyon-s-transferaz (GST), sitokrom P450 (Cyt P450), asetilkolinesteraz (AChE) enzim aktiviteleri ile diferansiyel ve total hemosit sayıları ve apoptotik indeks üzerine etkileri incelenmiştir. SiO_2 NP' lerinin LD_{50} değeri, $CdSO_4$ ve Abamektin ile tek başına ve karışım halinde uygulanması sonucunda, larvaların orta bağırsak ve yağ dokusunda antioksidan ve detoksifikasyon enzim aktivitelerinde doku farklılığına bağlı olarak değişiklikler meydana geldiği gözlemlenmiştir. Total hemosit sayısında, tek başına SiO_2 NP, $CdSO_4$ ve abamektin uygulaması sonucunda kontrol grubuna göre azalma meydana gelirken, SiO_2 NP' lerinin $CdSO_4$ ile karışım halinde uygulandığında artış, abamektin ile birlikte uygulandığında ise azalma meydana geldiği tespit edilmiştir. Diferansiyel hemosit sayıları üzerine etkilerinde ise, prohemosit, plazmatosit, sferülosit, granülosit ve önositoid sayılarında SiO_2 NPs, $CdSO_4$ ve Abamektinim tek başına ve karışım halinde uygulanan gruplarda artış ve azalışlar meydana geldiği gözlemlenmiştir. Bu çalışma sonucunda, SiO_2 NPs, $CdSO_4$ ve Abamektinin tek başına ve karışım halinde uygulanması sonucunda *G. mellonella* larvalarında toksik etkilere neden olduğu ve karışım uygulamaları sonucunda doku farklılıklarına bağlı olarak antioksidan savunma ve immun sistem üzerinde SiO_2 NP'lerinin çevresel kirleticilerin toksik etkilerini artırabileceği belirlenmiştir.

Keywords: *G. mellonella,* Silikon dioksit nanopartikülü (SiO_2), kadmiyum sülfat ($CdSO_4$) ve Abamektin

ABSTRACT

INVESTIGATION OF THE EFFECTS OF SILICON DIOXIDE NANOPARTICLES AND ENVIRONMENTAL CONTAMINANTS ON IMMUNOCYTOTOXIC AND ANTIOXIDANT DEFENCE SYSTEMS IN MODEL ORGANISM *Galleria mellonella*

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In the study, enzyme activities of superoxide dismustase (SOD), catalase (CAT), glutathione peroxidase (GPx), cytochorem P450 (Cyt P450), glutathione-s-transferase (GST), acetylcholinesterase (AChE) and total, differential haemocyte counts and apoptotic index were investigated in haemolymph, midgut and fat body of *Galleria mellonella* exposed to LD_{50} value of SiO_2 NP, environmental concentration of abamectin and cadmium sulphate ($CdSO_4$) singly and in mixture. Alterations in antioxidant and detoxification enzyme activities were observed in the midgut and fat body of *G. mellonella* larvae when exposed to SiO_2 NP, abamectin and $CdSO_4$ singly and in mixture.The total hemocyte count decreased in the SiO_2 and $CdSO_4$ singly applied groups however an increased were observed in the SiO_2 NPs + $CdSO_4$ mixture and a decreased were observed in the SiO2 NPs+abamectin group compared with the control. As for differential hemocyte counts, prohemocytes, plasmatocytes, spherulocytes, granulocytes, and oenocytoids was altered following treatment with SiO_2 NPs, $CdSO_4$, and abamectin sinlgly and in mixture. As a result of this study, it was determined that SiO_2 NPs, $CdSO_4$ and Abamectin lead to toxic effects in *G. mellonella* larvae as a result of single and mixture applications and it was also observed that SiO_2 NPs may increase the toxic effects of environmental pollutants on antioxidant defence and immune system depending on tissue differences as a result of mixture applications.

Keywords: *G. mellonella,* Silicon dioxide nanoparticle (SiO_2 NPs), cadmium sulfate ($CdSO_4$) and Abamectin

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LIST OF ABBREVIATIONS

NPs	: Nanoparticles
Nm	: Nanometer
SiO_2	: silicon dioxide
Cd	: Cadmium
$CdSO_4$	: Cadmium sülfade
ROS	: reactive oxygen species
SOD	: Superoxide dismutase
CAT	: Catalase
GPx	: Glutathione Peroxidase
GST	: Glutathione S-transferase
P450	: Cytochrome P450 Monooxygenase
AChE	: Acetylcholinesterase
PO	: Phenoloxidase
L	: Liter
gr	: gram
ml	: milliliter
O	: Liter
Cu	: Copper
Pb	: Lead
Zn	: Zinc
Al_2	: Aluminium
Cr	: Chromium
Ag	: Silver
Ca^{+2}	: Calsium
ppm	: one part per million
LD_{50}	: Lethal Dose 50
DNA	: Deoxyribonucleic acid
Fe	: Iron
Hg	: Mercury
NaOH	: Sodium hydroxide

NaCI : Potassium chloride

mm : Milliliter

Se : Selenium

sec : Second

NaOH : Sodium hydroxide

NaCI : Sodium chloride

Ag : Silver

Mg : Milligram

µg : Microgram

min : Minutes

µl : Microliter

Cm : Centimeter

µm : Micrometer

$OH^{\cdot}$: Hydroxyl radical

$O_2^{\cdot -}$: Superoxide radical

PBS : Phosphate buffer

SPSS : Statistical Package for the Social Sciences

THC : Total hemocyte count

Al_2O_3 : Aluminium oxide

LPO :Lipid peroxidation

H_2O : Water

mg/L :Milligram / liter

H_2O_2 : Hydrogen Peroxide

KCl :Potasium chloride

KH_2PO_4 :Potassium dihydrogen phosphate

K_2HPO_4 :Dipotassium phosphate

MDA :Methylene dioxyamphetamine

NADPH :Nicotinamide adenine dinucleotide phosphate hydrogen

HP : Haematoporphyrin

NaH_2PO_4 :Sodium dihydrogen phosphate

MDA : Malondialdehyde

Na_2HPO_4 : Disodium hydrogen phosphate

Na_2EDTA : Di sodium ethylene diamine tetra acetic acid

DTT	: Dithiothreitol
mg/l	:Milligram / liter
RCF	: Relative Centrifuge Force
DTNB	: 5,5′-Dithiobis (2-nitrobenzoic acid)
EDTA	: Ethylenediaminetetraacetic acid
µg/mL	:Microgram / milliliter
GR	: Glutathione reductase
AA	: Ascorbic acid
DNPH	: 2,4- dinitrophenylhydrazine
rpm	: Revolutions per minute

LIST OF SYMBOLS

°C : Degrees centigrade

ε : Molar extinction coefficient

↓ : Decrease

↑ : Increase

1. INTRODUCTION

In recent years, nanotechnological innovations have increased the quality of life, economic development and competition in the industry. Nanotechnology is an emerging field of science and technology that provides high-performance materials, intelligent systems and new product development methods. By reason of the development of nanotechnology, nanoparticles of different diameters and sizes are widely used in industrial and commercial fields (Tang et al., 2012; Amelia et al., 2012). Today, nanotechnology has led to significant advances in many fields such as materials, electronic devices, medical applications and energy production. Research and development efforts for the industrial production of nanomaterials are increasing worldwide (Chiang et al., 2012). The properties of materials produced at the nanoscale are dissimilar from traditional materials and enable more diverse applications. In terms of future perspectives, nanotechnology is poised to revolutionize especially the health, environment and energy sectors. Nanoscale materials play a crucial role in medical applications such as drug carriers, biosensors and cancer therapy. The most important structures among nanomaterials are nanoparticles (NPs), which have properties such as large surface areas and high reaction activities. A growing count of studies have found that different forms of NPs are hazardous to numerous tissues, including the kidney, liver, lung and spleen (Xie et al., 2010; Wang et al., 2008; Esmaeillou et al., 2013).

NPs are extremely small materials with sizes ranging from 1 to 100 nm with a variety of shapes (Khan et al., 2019). The physicochemical features of NPs and nanomaterials, such as shape, physicochemical stability, chemical composition, size, surface energy surface area, crystal structure and surface, all have an effect on their toxicity indicators. (Gatoo et al., 2014). Furthermore, although people are becoming more aware of the potential effects of nanoparticles on human health and the environment, their commercial use is

increasing and concerns about their potential harmful effects are growing. Recently, several research have focused on the toxicity impacts of diverse nanoparticles in order to discover different elements of nanotoxicity. Humans are continually exposed to a various of pollutants, including NPs, which are becoming more prevalent as a result of the rapid advancement of nanotechnologies and their unintentional or intentional discharge into the environment. Although these nanotechnological advances have enabled progress, their long-term effects on the environment and non target organisms remain unclear. Moreover, their excessive use can lead to uncontrolled and excessive transfers to upper trophic levels (Cazenave et al., 2019). Due to their special properties at the nanoscale, nanoparticles can interact physicochemically with organic chemicals or metals in the environment, changing their bioavailability and also causing other different reactions, including synergistic, antagonistic and potentiating effects (Liu et al., 2019).

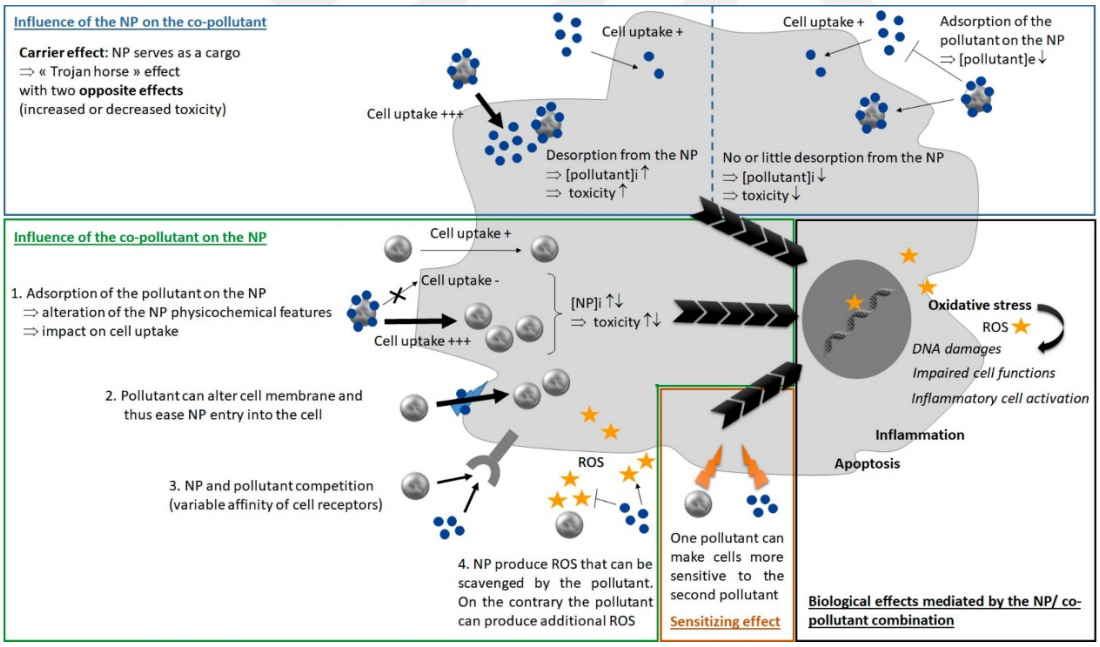

Figure 1.1. A summary of the putative mechanisms causing the combined toxicity of nanoparticles and co-pollutants. ROS = reactive oxygen species, ↓ = decrease, ↑ = increase (Forest, 2021).

Several studies on the effects of nanoparticles on human health and the environment have been carried out recently, in parallel with the increasing use of nanoparticles in daily life (Sun et al., 2011). The ability of nanoparticles to adsorb an environmental pollutant is critical to the toxicity of the NP or pollutant, as it facilitates the entry of the pollutant into the body, particularly through the absorption of nanoparticle-pollutant complexes. Complexed pollutants can be released into the body after entering the cell, raising their concentration and, as a result, bioavailability and toxicity. This process is known as the Trojan Horse effect (Deng et al., 2017; Liu et al., 2018) (Figure 1.1). This idea encourages the entrance of hazardous substances adsorbed on NPs, causing an increase in intracellular pollutant concentration. In other cases, the complexes are effectively swallowed, and toxicity can be reduced if pollutants desorb from nanoparticle pollutant complexes in limited or partial amounts (Deng et al., 2017).

Silica is a form of silicon dioxide (SiO_2), the world's most common mineral. The term "nano silica," which was used in the study, refers to silica particles that are organized at the nanoscale. (Bitar et al. 2012). This arrangement alters the physical characteristics of silica nanoparticles by increasing surface area and showing novel quantum effects and surface (Croissant and Durand, 2018). Silica nanoparticles (SiO_2 NPs) are considered an appropriate material for biomedical applications and are utilized in biosensors, biomarkers, cancer therapy, and DNA/drug delivery (Zhang et al., 2004; Santra et al., 2001; Hirsch et al., 2003; Bharali et al., 2005; Slowing et al., 2008) and is widely researched as enzyme immobilization (Barik et al., 2008). As a result, understanding the cellular toxicity of SiO_2 NPs is critical for rationally designing this material for medicinal purposes. Although SiO_2 NPs are often regarded as biocompatible materials for biomedical and biotechnological applications, they can aggregate and remain in the kidney, heart, spleen, liver and brain following ingestion, inhalation, or skin application (Zhou et al., 2019). It is thought that it may also have insecticidal effects by blocking the

digestive system of the insect and causing malformation in its external morphology (Thabet et al., 2021). Inhalation of crystalline SiO_2 promotes silicosis and lung cancer by inducing inflammation and oxidative stress (Donaldson and Borm, 1998; Peretz et al., 2006). Also, in the tritrophic food chain, plants, harmful insect species and their predators or parasitoids are directly or indirectly affected by each other. The basis of this food chain varies depending on the plants' uptake of silicon from the soil and the different mechanisms of its uptake into the cells (Cooke and Leishman, 2016).

The presence of organic and inorganic pollutants in the environment causes deterioration, a severe issue that endangers the global ecosystem (Zulfiqar et al., 2019; Zeeshan et al., 2021). Environmental pollutants can be defined as substances that harm the environment as a result of human activities and natural consequences. These pollutants include heavy metals and pesticides. Pesticides are chemicals used to control harmful organisms in agriculture and can cause serious damage to nature. SiO_2 NPs are effective against heavy metals, pesticides, detergents, etc. By adsorbing these environmental pollutants, it can cause more toxic effects.

Cadmium has become a pollutant in the environment because of industrial expansion and modern advances in technology (Satarug, 2019). Cadmium is a very toxic heavy-metal pollutant that is known to cause cancer in humans (Henkel et al., 2004) and is easily absorbed by organisms, and its toxicity has been investigated in both prokaryotes and eukaryotes (Zalups et al. 2003; Heng et al. 2014), so that it is one of the most dangerous heavy metals, with considerable bioaccumulation (Singh et al., 2020). It is present on the soil surface in quantities ranging from 0.1 to 1.0 ppm and has been shown to be toxic in a variety of animal and human tissues. It is widely utilized in a range of sectors, including batteries, paint, and jewelry, but the low allowed limit (0.01 mg/L) endangers both human health and the environment (Nawrot et al., 2010; Eichler et al., 2014). Although cadmium

is a non-essential material, it is widely understood that it accumulates in biological systems and produces toxicity, a major environmental problem (Kalman et al., 2010; Liao et al., 2011). Several different forms of cadmium exposure have been reported throughout the last century, and cadmium is prevalent in the environment due to a variety of human activities (Rahimzadeh et al., 2017). Toxic metals harm humans, plants, and animals because they persist in the environment (Afzal et al., 2019). As a result, it is easily transported and stored throughout the body (Hajeb et al. 2014).The mechanisms responsible for the toxic effects of cadmium vary depending on the cell type and can cause different damages in living things (Fornazier et al., 2002). Damages such as inhibiting enzymatic activity, damaging the cell membrane and DNA, preventing protein synthesis, denaturing proteins and preventing cell division occur. Cadmium-related toxicity can cause increased oxidative stress (El-Sharaky et al., 2007; Shaikh et al., 1999; Nunia and Goyal, 2007; Bolkent et al., 2007). Many investigations have found that lipid peroxidation mediated by oxidative damage is a significant mediator of cadmium toxicity (Warren et al., 1999).

Cadmium sulfate is a pesticide containing heavy metals and is used for plant protection in agriculture. The release of cadmium sulfate into the environment can cause soil and water pollution. Cadmium absorbed by plants accumulates in the soil, enters the food chain and can have toxic effects on all living things. Pesticides are widely used to increase efficiency in agricultural production. However, the release of these chemicals into the environment can cause negative effects on water resources, soil and air quality. The release of pesticides into the environment can reduce biodiversity and disrupt the balance of ecosystems. This may lead to adverse effects and even death of organisms in the food chain. Concerns have been expressed concerning the combined toxicity of airborne NPs and other pollutants to human health. Cadmium has been shown in animal experiments to increase the production of reactive oxygen species such as H_2O_2, superoxide anion

radicals, and hydroxyl radicals. It is a cadmium element that impairs the antioxidant defense mechanism in tissues and raises lipid peroxidation levels (Stajn et al., 1997; El-Sharaky et al., 2007).

Pollutant-nanoparticle interactions, as well as synergistic toxicity, are often overlooked. The goal of this research is to give a review of the combined toxicity of NPs and copollutants in order to emphasize the lack of evidence in the current literature and suggest that this issue deserves immediate attention. However, NPs released indiscriminately into the environment may interact with or absorb other pollutants on their surfaces, allowing them to enter the body (Abbas et al., 2020). This may cause a significant alteration in the toxicological profile. As a result, while these interactions are difficult to characterize, they need serious consideration.

Abamectin is a natural substance derived from the soil bacterium *Streptomyces avermitilis* (Burg et al., 1979) that is effective in cattle and sheep at extremely low doses (200 mg/kg) and is a widely used insecticide and anthelmintic. Moreover, it is a type of pesticide or insecticide and is used to control insect pests on agricultural crops. It is generally used by farmers all over the world due to its effectiveness and broad-spectrum effect. It is especially effective against pests such as thrips, whiteflies, aphids and spider mites.Abamectin affects the nervous system of insects, causing fatal consequences. It specifically targets glutamate-gated chloride channels and gamma-aminobutyric acid (GABA) gated chloride channels.

1.1. Oxidative Stress and Antioxidant Defense System

Many compounds are known to produce free radicals, which cause oxidative damage. Oxidative stress (OS) is a largely mechanism for immunotoxicity. NPs cause oxidative stress by increasing reactive oxygen species and membrane lipid peroxidation, while

decreasing intracellular glutathione (GSH) levels. OS damages biological components, causing cell death by apoptosis. Nanomaterial features such as size, shape, and deformability can influence immune cells and, as a result, oxidative stress responses (Shvedova et al. 2003). Oxidative stress is generated by an imbalance between molecules eliminated by the antioxidant defense system and free radicals, which are continually created in the body. Biomarkers are utilized in toxicology studies to evaluate xenobiotic exposure and its influence on the environment or organism. External substances (heavy metals, pesticides, etc.), radiation, infectious microorganisms (viruses, bacteria, parasites, etc.), and environmental stress (seasonal fluctuations, water pollution, etc.) all cause an increase in free oxygen radicals in target species. Factors can also contribute to oxidative stress. When an organism is exposed to an environmental pollutant, it is initially impacted at the biochemical and biomolecular levels (enzyme, DNA), with the effect manifesting at higher organizational levels. A biomarker is often characterized as a detectable cellular, bodily fluid, tissue, biochemical or physiological alteration (van der Oost et al., 2003). Environmental pollutants, such as NPs, change the organism's oxidative stress levels and impair immune cell function. Foreign chemical that enter the body are neutralized by immune system cells, which are seen as a sign of the organism's health and a defense against xenobiotics. Oxidative stress is described as "disruption of oxidative balance" caused by an increase in reactive oxygen species such as $\cdot OH$, H_20_2, and O^{-2} as a result of a shortage of antioxidants.Reactive oxygen species damage the double bond-containing groups of intracellular protein and lipid structures, as well as the double bonds of bases in DNA, which causes oxidative stress. Then, they initiate chain oxidation reactions by breaking off a hydrogen atom. Cell death or damage occurs as a result of oxidation reactions and damage to macromolecules such as intracellular DNA, lipids and proteins (Berlett and Stadtman, 1997, Özcan et al., 2015).

ROS are known as forms of oxygen that are generally composed of free radicals and are more chemically active compared to normal oxygen. Free radicals are high-energy, unstable molecules with one or more unpaired electrons in their outermost atomic orbitals. Certain kinds of ROS can harm the organism (Diplock, 1998). Therefore, free radicals; Damage occurs to many biological molecules in the cell such as coenzymes, lipids, DNA, and proteins (Diplock, 1998). Antioxidants are effective by removing oxygen and transition metal ions, suppressing or scavenging ROS, and breaking the chain reaction that has begun (Halliwell, 1997).

The first line of defense antioxidants against OS in cells, which primarily include superoxide dismutase, catalase, glutathione S-transferase, and glutathione peroxidase, are particularly effective against the superoxide anion radical, which is continuously produced by various processes in normal body metabolism. It is important in the entire defense strategy of antioxidants against ($*O_2$) (Ighodaro and Akinloye, 2018). In the antioxidant defense system, SOD, CAT and (GPx) are found in all mammalian cells that metabolize oxygen (Weydert and Cullen, 2010).

1.1.1. Superoxide dismutase (SOD)

Superoxide dismutase is the cell's first detoxifying enzyme and strongest antioxidant. It is an crucial endogenous antioxidant enzyme that works as the body's first line of defense against reactive oxygen species. It catalyzes the conversion of two molecules of superoxide anion into hydrogen peroxide and molecular oxygen therefore decreasing the potentially harmful superoxide anion. Superoxide dismutase is a metaloenzyme, which means it requires a metal cofactor to operate correctly. There are several enzyme types depending on the kind of metal ion needed as a cofactor for Superoxide dismutase. (Fridovich, 1995; Dringen, 2005; Ighodaro and Akinloye, 2018).

$O2^{\cdot-} + O2^{\cdot-} + 2H+ \rightarrow H_2O_2 + O_2$

SOD generally binds metal ions such as zinc, copper, iron, and manganese. Superoxide dismutase are classed as three types, Fe-SOD, which is found in prokaryotes and the chloroplasts of several plants, Mn-SOD, which is mainly found in prokaryotes and eukaryotic mitochondria and SOD, which is prevalent in eukaryotes and more widely distributed.

In addition to the cytoplasm, Cu/Zn SOD is also found in peroxisomes and chloroplasts. Gill et all., 2010; Karuppanapandian et all., 2011; Ighodaro and Akinloye, 2018.

1.1.2. Catalase (CAT)

Catalase is a hemoprotein with four heme groups in its structure. Catalase is present in small counts in the cytoplasm and in large counts in peroxisomes. Catalase breaks down H_20_2 into water and oxygen molecules. It prevents the formation of hydroxyl free radical by eliminating the H_20_2 formed within the cell (Dawn BM., 1996).

Catalases are made up of four protein subunits, each containing a heme group (Fe^{3+}-protoporphyrin) connected to the active portion. Any subunit also has a molecule of reduced NADPH, which protects the enzyme from its substrate hydrogen peroxide (Cross et al., 1987; Dimascio et al., 1989; Nakai et al., 1997). Storage, freeze-drying, and exposure to acid and alkali cause the subunits to separate and lose enzyme activity. Catalase is found in all organs in mammals, however it is most concentrated in the liver and erythrocytes. It is mostly found in peroxisomes, with trace levels in mitochondria, chloroplasts, and endoplasmic reticulum (Hess et al., 2000). Azide or cyanide reduce CAT activity, although their action is nonspecific and inhibits a wide range of enzymes. The most widely used inhibitor is aminotriazole (Cross et al., 1987); (Dimascio et al., 1989).

CAT protects biological systems from hydrogen peroxide, which causes damage, by converting it to O_2 and H_2O via a dismutation reaction process identical to SOD (Cross et al., 1987; Goldman et al., 1989; Lowry et al., 1951).

$CAT\text{-}Fe^{+3} + H_2O_2 \rightarrow$ Compound I

Compound I $+H_2O_2 \rightarrow CAT\text{-}Fe^{+3} + 2H_2O + O_2$

In the presence of H_2O_2, the CAT enzyme performs peroxidase-like activities. Thus, chemical I converts alcohols like methanol and ethanol into their aldehydes, formaldehyde and acetaldehyde (Dimascio et al., 1989).

$AH_2 + H_2O_2 \rightarrow A + 2H_2O$

The most commonly used method for detecting CAT activity is spectrophotometric observation of hydrogen peroxide degradation at 240 nm. The variations in absorbance per unit time is a measure of CAT activity. It is important to use low amounts of substrate during analysis to prevent inactivation of the enzyme. Other methods are determinations made by oxygen electrode or polarography based on the measurement of the amount of O_2 formed as a result of the reaction (Aust et al., 1993; Melchiorri et al., 1996; Şener et al., 2006; Anandakumar et al., 2007).

1.1.3. Glutathione Peroxidase

GPx is the most powerful antioxidant against oxidative stress in vertebrates and is a cytosolic enzyme that catalyzes the reduction of H_20_2 and lipid peroxides, consists of four subunits (tetramers) and contains 4 selenium atoms (Manduzio et al., 2005). The GSH-Px enzyme was first found in animal tissue by Mills in 1957. While it is mostly not found in higher bacteria and plants, it has been reported to be seen in some fungi and algae (Cross et al., 1987); (Kalaiselvi et al., 2005). The decrease in GPx activity causes an increase in

hydrogen peroxide level and cell damage (Schaeffer and Stainer, 1978, Ursini et al., 1982).

This enzyme exists in two distinct forms. One of them is selenium dependent GSH-Px (Se-GSH-Px), which has selenium covalently linked in the form of selenocysteine at its active site. Se-GSH-Px is effective against both organic hydroperoxides and hydrogen peroxide. It aids in the reduction of different hydroperoxides, notably hydrogen peroxide, by oxidising GSH. The GSH-Rd enzyme converts oxidized glutathione (GSSG) back into Glutathione Peroxidase. The selenium-dependent GPx enzyme is generally inactive in living Lepidoptera species.

H_2O_2 (ROOH) + 2 GSH → GSSG + 2 H_2O (ROH)

GSSG + NADPH + H+ GSH-Rd 2 GSH + NADP+

A various type of enzyme consists of proteins that do not require selenium for catalysis processes and have little or no activity towards hydrogen peroxide. These group of enzymes is called glutathione Peroxidase and is expressed as protein that enable glutathione Peroxidase to combine with electrophilic compounds (Cross et al., 1987).

1.2. Playing a Role in Detoxification in Insects

1.2.1. Cytochrome P450 Monooxygenase (P450) Enzyme

P450 enzyme is one of the important metabolic systems in insects that provides anabolism and catabolism of substances such as hormones, acids, steroids or pesticides (Feyereisen, 1999). The P450 enzyme is present in many living things such as insects, plants, birds, bacteria and mammals. The main oxidase event is carried out within the P450 system. P450 enzyme is found in mitochondria and endoplasmic reticulum in eukaryotes. The first total P450 in insects was detected in 1967 (Scott, 1999). To date, P450 monooxygenases

have been identified in more than 100 insect species. The P450 enzyme in insects has functional properties such as growth, development, nutrition, resistance and tolerance to pesticides (Van Pottelberge et al., 2008). Therefore, since P450 enzyme inhibitors indirectly affect hormone synthesis, they cause changes in the morphology, development and lifespan of holometabolous insects (Soderlund, 1990).

In insects, P450 has been detected in most tissues, especially in the intestine and malpygian tubes. P450 enzyme level is high in the adult stages throughout the life cycle of insects. P450 level increases in the egg and larval stages and decreases in the pupa stage (Feyereisen, 1999). In insects, as in vertebrates, the P450 family plays a role in the biological transfer of harmful substances taken into the body. When some insecticides are taken into the insect body, detoxification occurs as as a result of selection and biotransformation within the population (Krieger, 2001).

1.2.2. Glutathione S-transferase (GST) Enzyme

GST enzymes are present in aerobic organisms such as insects, fish, birds, vertebrates, and invertebrates (Konanz and Nauen, 2004). GSTs are a type of enzyme that effectively detoxifies foreign substances introduced into the body. The role of GSTs in pesticide metabolism was initially established in the detoxification of organic phosphorus compounds. GSTs were next tested in insects against organic chlorinated and cyclodiene pesticides. GSTs contribute significantly to insect resistance to organic phosphorus pesticides (Susurluk, 2008). GST enzyme is isolated from insects of the order Diptera, Lepidoptera, Coleoptera, and Hymenoptera. In insects, the GST enzyme defends cellular membranes from oxidative damage as well as pesticide detoxification (Yu, 2008).

GSTs are a group of functional enzymes that conjugate glutathione (GSH). This enzyme catalyzes the combination of glutathione and electrophilic substrates. GSTs carry out a

variety of functions, including the replacement of certain groups in glutathione and the insertion and removal of additional groups. In cockroaches, houseflies, and numerous agricultural pests, enhanced GST enzyme activity has been linked to pesticide resistance. GST activity in mosquitos increased, particularly in trials using DDT, a chlorinated hydrocarbon insecticide (Vontas et al., 2000). The fact that GST is dissolved and stable in tissues allows its characterization and purification in many insect species. Most information about the biochemistry of GST in insects is obtained from studies on house flies. Two different GST enzymes, GST1 and GST2, have been purified in houseflies. Similar GST purification studies have been performed in various insect species in the orders Lepidoptera and Diptera.

1.2.3. Acetylcholinesterases Enzyme

Acetylcholinesterase (AChE) is a non-specialized enzyme that hydrolyzes acetylcholine, which has a lipotrophic effect, in tissues, either free or combined with phospholipids. Acetylcholine (ACh) plays a role in carrying nerve impulses from nerve endings to the affected organ or from the nerve ending to a second nerve cell. It is also an ester of great biological importance, providing bioelectric current through nerve and muscle cells. Organic phosphorus, carbamate, chlorinated or some other chemicals administered to the organism prevent acetylcholine from combining with its receptors (Alon et al., 2008). They examine the transporters at the nerve and muscle junction in different ways. By suppressing AChE, they delay the response of the muscles to the nerves by ensuring that ACh remains at the junction of the nerves and muscles for a long time. The prevalent use of these substances leads to the development of various resistant individuals in insects, causing imbalanced muscle and nerve functions in the organism. Organic phosphorus and carbamates work as analogues of acetylcholine and attach to the active site of acetylcholine (Anazawa et al., 2003).

Although acetylcholine is hydrolyzed, the insecticides remain stable as an enzyme-substrate complex. These insecticides cause desensitization of acetylcholine receptors by blocking the transmission of nerve impulses through acetylcholinesterase inhibition (Hollingworth and Dong, 2008). Increased acetylcholine concentration in synapses and more stimulated central nervous system cause death in insects (Çakır and Yamaner, 2005).

1.3. Phenoloxidase

Phenoloxidase (PO) is thought to serve a critical immunological role in insects against a wide range of parasites and diseases. Phenoloxidase is a copper containing enzyme that catalyzes the oxidation of monophenols to o-diphenols and thereafter to o-quinones. These are critical steps in the synthesis of melanin, which is located in the cuticle and required for the encapsulation of foreign substances. Quinones are then transformed in a process that results in the creation of the brown-black melanin pigment. Quinones and melanin play a role in insect pathogen defense mechanisms such as nodule formation and encapsulation. The most impactful humoral immune response in insects is melanization (Lee and Anstee, 1995). The enzyme phenoloxidase catalyzes the creation of the dark pigment melanin, which is then converted into an active form by the serine proteases. Hemocytes in insects are the only source of phenoloxidase (Ashida and Brey, 1998). When cells are lysed, inactive phenoloxidase (PO), which is produced by hemocytes, accumulates in the callus or surrounding the encapsulated intruder. Melanization forms a coating around the alien object that entirely separates it from its environment and prevents it from contacting the outside world. Many of the metabolic mechanisms that promote melanin production are shared by insects and both mammals (Nappi and Christensen, 2005). Melanin also helps with wound healing and cuticle sclerotization.

1.4. Hemolymph and Hemocytes in insects

While multicellular animals have both innate and acquired immunity, insects have only innate immunity (Er, 2011). Different hemocytes produced by insects have various functions. These hemocytes are stated under subheadings below. Hemocytes are mostly found in hemolymph, which is analogous to mammalian blood, but they are also found subcutaneously throughout the digestive system and fat body. Hemocyte concentration changes throughout life and is regulated by microorganism-induced stress (Arteaga Blanco et al., 2017). During an infection, active hemocytes go to the injured area. In general, insects have a wide range of hemocytes with different histological, functional characteristics and morphological (Gupta, 1985). *G. mellonella* has at least six distinct cell types: prohemocytes, granulocytes plasmatocytes, coagulocytes, spherulocyte, and oenocytoids (Boman and Hultmark, 1987).

1.4.1. Prohemocyte

It contains a nucleus that covers the entire cell and a small cytoplasmic region (Strambeanu et al., 2014). Prohemocytes are stem cell-like cells involved in the immune system of *G. mellonella*. These cells, which are generally small and round, have large nuclei and little cytoplasm. Prohemocytes are the precursors of other types of hemocytes and can differentiate into different types of hemocytes when necessary. They are round or oval cells, 4-10 μm wide and 4-14 μm long (Gupta, 1985; Chapman, 1998; Brehelin and Zachary, 1986; Giulianini et al., 2003). The cytoplasm is completely filled with the nucleus and contains ribosomes and mitochondria, but other organelles such as the golgi apparatus and the endoplasmic reticulum are generally scarce (Rowley and Ratcliffle, 1981; Levin, 2007). Since their main function is to divide and they contain centrioles and microtubules, they are considered active hemocytes and are considered the precursors of other hemocyte types (Gupta, 1985; Rowley and Ratcliffe, 1981; Cociancich et al., 1994;

Ribeiro and Brehelin, 2006). Their rates vary between 1-7%, depending on the total count of hemocytes in circulation (Levin, 2007).

1.4.2. Plasmocyte

They are generally small cells, spherical or oval in shape, and have long and thin cytoplasmic extensions. The prohemocyte type with thin and long cytoplasmic extensions is called pseudopodia. They are found individually or in groups in the hemolymph. It is the most abundant cell form in hemolymph. Plasmocytes adhere to foreign surfaces, perform phagocytosis and form capsules (Er, 2011; Brey et al., 1993; Strand et al., 2006; Russell et al., 1996). It is among the most common hemocyte types in *G. mellonella* and has an significant role in immune responses. The shape of these cell round or round, have prominent nuclei, and contain a small count of granules in their cytoplasm. Plasmatocytes are spherical or oval and polymorphic, varying in size from 3 μm to 40 μm. (Rowley and Ratcliffe, 1981; Brehelin and Zachary, 1986; Chapman, 1998; Silva et al., 2002). Although it varies depending on insect species, their ratio to to overall count of hemocytes in circulation varies intermediate approximately 30-60% (Cociancich et al., 1994). When plasmatocytes are examined by transmission electron microscopy, few pseudopods, pinocytotic vesicles, vacuoles, and polyribosomes can be observed, and they also have an underdeveloped golgi apparatus. While plasmatocytes do not contain granules in many Lepidoptera species, they may contain granules in *G. mellonella* (Ribeiro and Brehelin, 2006; Ribeiro et al., 1996; Schmit and Ratcliffe, 1977).

1.4.3. Granulocytes

They are oval or spherical cells ranging in size from 4 to 45 μm (Levin, 2007). They are numerous in hemolymph preparations and constitute between 30-65% of the total hemocyte population (Öztürk et al., 2018; Miranpuri et al., 1991). It has a small nucleus in its cytoplasm (Browne et al., 2013). These hemocytes have a full developed

endoplasmic reticulum and golgi apparatus, numarous ribosomes, and few organelles such as mitochondria and microtubules (Neuwirth, 1973). Granulocytes are hemocytes that contain distinct granules in their cytoplasm. These cells play an crucial role in inflammatory responses and defense mechanisms against pathogens.

1.4.4. Spherulocyte

They are cells of different shapes, 5-25 μm in size, oval or round (Chapman, 1998; Levin, 2007). Spherule cells (spherulocytes) are non-motile cells with a size of 12-18 μm and a round or oval shape. They are known for their global structure (Hüdaverdi, 2011). The functions of spherulocytes in the immune system are not yet fully known (Ribeiro et al., 2006; Kaya, 2015). Spherulocytes are hemocytes that have large spherical granules. These cells play important roles in the immune response and homeostatic processes.

1.4.5. Oenocytoids

Oenocytoids are large hemocytes with a round or oval shape, up to 54 μm in diameter (Rowley and Ratcliffe, 1981; Levin, 2007). Oenocytoid cells constitute approximately 1-2% of the total count of hemocytes (Rowley and Ratcliffe, 1981). Although they are the largest of the hemocytes found in Lepidoptera, they are readily lysed cells, except for *G. mellonella* (Er, 2011; Chapman, 1998; Gupta, 1985). Golgi apparatus, mitochondria, and vesicles were underdeveloped in oenocytoids with a small nucleus (Wu et al., 2016). These cells with homogeneous cytoplasm contain numerous free ribosomes (Brehelin and Zachary, 1986). They are found in very small counts in hemolymph (Brehelin and Zachary, 1986).

1.5. Apoptosis And Necrosis,

Apoptosis is a type of genetically controlled cell death that eliminates waste and damaged cells from the tissue environment while preserving tissue creation. Normally, silicon

dioxide nanoparticles and heavy metals such as cadmium are heavy metals with specific physiological roles regulating development at the cell, organ and organism level. However, as urbanization and industrialization progressed, heavy metals evolved into exceedingly dangerous environmental contaminants. These environmental toxins can harm the organism's physiological and genetic development, disrupt normal metabolism, and cause cancer.

Chemical pollutants, nanoparticles and heavy metals can cause apoptosis as well as necrosis (Palupi et al., 2016). Apoptosis causes changes at the cellular and nuclear levels, including DNA breaks and the disintegration of the cell membrane due to cytoplasm shrinkage. Early apoptosis is characterized by increased cytoplasmic free Ca^{2+} concentration and caspase-3 activation (Ermak and Davies, 2001; Li et al., 2014; Porter and Janicke, 1999).

While apoptotic cells turn into apoptotic bodies, the apoptotic cell is phagocytosed by surrounding cells. Necrosis, the other cell death, is a pathological form of cell death and that causes in inflammation with the development of an unregulated process (Coşkun and Özgür, 2011). Although the causes of necrosis are hypoxia, they are mostly various heavy metals and insecticides. Inflammation is observed in necrosis as a consequens of cell and organelle disintegration and the spread of cytoplasm and nuclear content into the intercellular space. In inflammation in vertebrates, macrophages and neutrophils migrate to the area and as a result of this event, the necrotic tissue undergoes phagocytosis (immunological phagocytosis). There are some differences between apoptosis and necrosis (Altunkaynak and Özbek, 2008).

Heavy metal pollution can have detrimental effects in animals not only on reproduction, growth, survival and metabolism, reproduction, moreover on the nature immune system. Therefore, many invertebrates, including insects, are considered suitable model organisms

to investigate the toxicity of heavy metals and determine environmental pollution levels (Wu and Yi, 2015) and are also used as useful bioindicators of environmental contamination (Borowska and Pyza, 2011). . In our study, by comparing the effects of SiO_2 nanoparticles and these pollutants on *G. mellonella*, it will be evaluated whether nanoparticles trigger similar biological stress mechanisms as environmental pollutants. This review will help us understand how insect antioxidant defense systems respond to such stressors and will provide important information in the field of environmental toxicology.

Figure 1.2. *G. mellonella* larvae

The greater wax moth *G. mellonella* (Lepidoptera: *Pyralidae*) (Fig 1.2.) is a model organism utilized in several research on the cytotoxicity of nanomaterials and Nanoparticles. *G. mellonella* is a moth species that acts as a model organism in scientific study. It is regarded as a popular and useful model in such and toxicological investigations. *G. mellonella* is a holometabolous insect with 4th life instars: larva, egg, adult, and pupa (Smith 1965; Fasasi and Malaka 2006; Ellis et all., 2013; Swamy, 2008; Desai et al., 2019; Kwadha et al., 2017; Hosamani et al., 2017). The whole cycle time for egg, larva, pupa, and adult is roughly 8 weeks at 29-39°C and high humidity (Ellis et al.,

2013). During spawning, females deposit 50-150 clustered eggs, and hatching rates vary with temperature (Ellis et al. 2013). In reality, warm temperatures promote greater development than cold temperatures, and eggs cannot withstand temperature extremes. They have a spheroidal shape and are white to light pink in color. Transformation into larvae depends on temperature and takes between 3 and 30 days (Charrière and Imdorf, 1997). Larvae are 1-23 mm long, cream-white in color with a reddish head, darkening as they grow. They go through around 8-10 molting cycles. The larva is made up of a sequence of segments split into three anatomical parts: the head, thorax, and abdomen. The thorax contains six legs, whereas the abdomen houses prolegs. (Wojda et al. 2020). Wax moth larvae hatch and grow to be about 1-3 mm length and 0.12-0.15 mm in diameter. Before pupation, late instar larvae measure 25-30 mm in length and 5-7 mm in diameter. *G. mellonella* larvae have an immune system that is similar to mammals' innate immune response, and in recent years, they have been employed as model organisms to study the virulence mechanisms of human pathogens (Rossoni et al., 2019; Lapointe et al., 2020; Harding et al., 2012; Djainal et al., 2020). *G. mellonella* larvae have a wide surface area and are easy to isolate hemolymph, making them ideal for immunological research (Pereira et al. 2018). They may be cultivated in great quantities in the laboratory without the need for specialized equipment (Wojda 2017). The larvae's size allows for precision pathogen injection and sampling of organs including as hemolymph, hemocytes, fat body, trachea, and midgut for further examination. *G. mellonella* larvae provide 20-40 µl of hemolymph, which can be collected from a group of caterpillars and used to purify bioactive compounds (Mak, Zdybicka-Barabas and Cytryn´ in 2010). Furthermore, ethical considerations that have emerged recently are minimal or non-existent for invertebrate models, and insect may be mass generated in a relatively short period of time. Furthermore, *G. mellonella* can live at temperatures ranging from 25 to 37 °C, making it useful for infection investigations and allowing experiments to simulate mammalian systems (Sugeçti and Büyükgüzel, 2018; Rossoni et

al., 2019). Additionally, the utilization of *G. mellonella* allows for research in a variety of fields, including vector management, biological control, and environmental toxicology. The immune system of *G. mellonella* larvae is physically and functionally similar to the mammalian innate immune response, making it an appropriate model for such studies (Pereira et al., 2018). As a result, nanotoxicology research using this insect will aid in determining the potential consequences humans and on the ecosystem (Zorlu et al. 2018). The use of this organism has been thoroughly evaluated in a count of research, and the findings are generally transferable to people. However, *G. mellonella* does not fully represent human biology, therefore the findings' direct relevance to humans should be carefully considered.

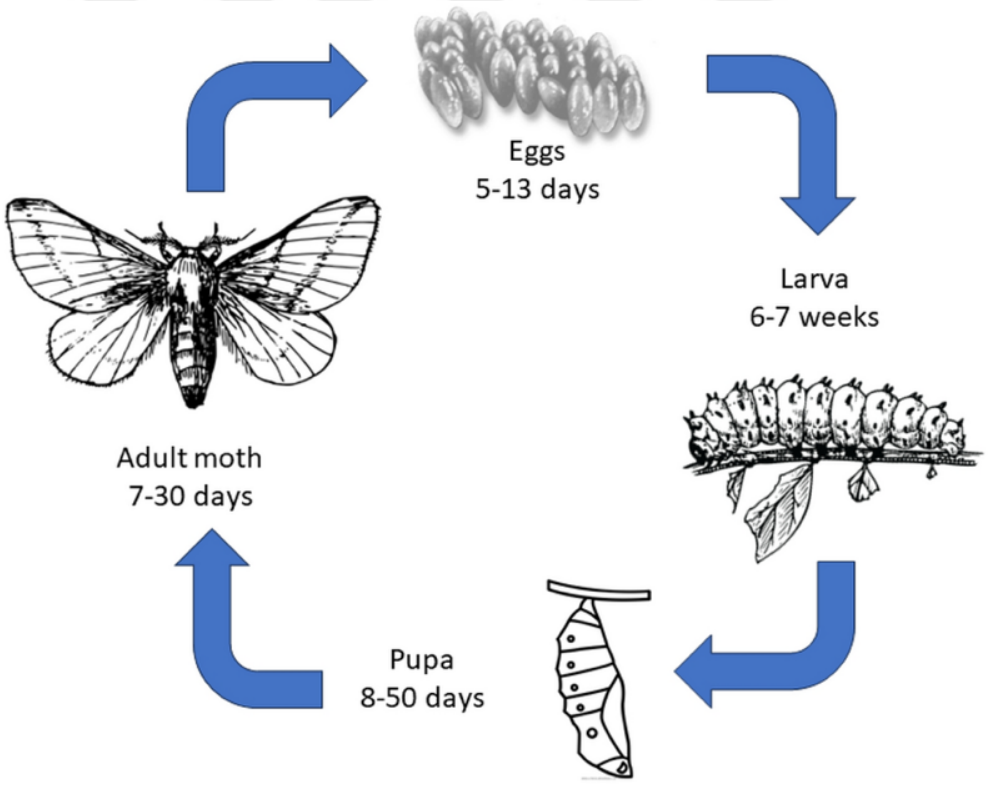

Figure 1.3. Schematic diagram of lifecycle of *G. mellonella*. (Boschi et al., 2023)

2. THEORETICAL FOUNDATION AND LITERATURE REVİEW

Li et al. (2005), investigated the effects of cadmium on antioxidant enzymes in *Oxya chinensi*s. They found that GPx (Glutathione peroxidase) enzyme activity decreased with increasing Cd levels, whereas SOD enzyme activity increased.Due to the study, they indicated that SOD showed a moderate detoxification effect and GPx showed a low detoxification effect.

Ahamed et al. (2010), fed Ag NPs at certain doses to third stage larvae of *Drosophila* and reported that oxidative stress and apoptosis increased as a result.

El-Shenawy (2010), investigated their cytotoxicity in isolated male rat hepatocytes. The study showed that incubation of hepatocytes with 10 or 100 μM of each insecticide for 2 h significantly reduced cell viability. The activities of antioxidant enzymes such as SOD, GSH-Px and GST were decreased by phenythrothion incubation more than endosulfan and abamectin. The same treatment decreased the antioxidant glutathione level and increased the lipid peroxidation level. This study revealed that phenythrothion showed dose-dependent pathological symptoms and high dose caused significant damage in oxidative and antioxidant parameters in isolated hepatocytes.

Borowska and Pyza (2011), exposed *Musca domestica* (Diptera: *Muscidae*) larvae to low and high semi-sublethal doses of each metal (Cu 5 and 1000 mg/kg, Zn 100 and 2000 mg/kg, Cd 3 and 50 mg/kg, Pb 20 and 10,000 mg/kg). They reported that the count of prohemocytes, i.e. stem cells, increased, whereas amount of granulocytes responsible for phagocytosis decreased.

Teleb (2011), in a study on *Schistocerca gregaria* (Orthoptera: Acrididae), applied different doses (50, 100 and 500 ppm) of an insecticide called Nomolt and examined its

effects on total haemocyte count and various haemocyte types.Therefore, he found an increase in the count of prohemocytes, precocitoids, plasmatocytes and granulocytes in the haemolymph, but a decrease among count of spherulocytes. It was determined that spherulocytes were the most response cells to Nomolt, while procoagulocytes were the least affected cells and reported that the total count of haemocytes decreased.

Emre et al.(2013), examined the impact of different cadmium concentrations (1.25, 2.50, 5.00, 10.00, 20.00 and 40.00 mg/100g food) on protein, glycogen, lipid, catalase and superoxide dismutase in a study with *G. mellonella*. They reported that SOD enzyme activity increased at high cadmium concentrations and CAT enzyme activity decreased at all concentrations.

Dubovskiy et al. (2013), in a study on *G. mellonella* and *Leptinotarsa decemlineata* larvae, found that sublethal and half-lethal doses (LC10 and LC50) of primiphos methyl, an organophosphorus insecticide, increased phenoloxidase activity and increased the count of encapsulation and haemocytes.

Ahamed (2013), studied the oxidative stress, cytotoxicity and apoptosis induction of silicon dioxide NPs in human skin epithelial and human lung epithelial cells. It was demonstrated that the messenger RNA levels of apoptotic genes (caspase-3 and caspase-9) rose in a dose-dependent manner. Furthermore, both cell types exposed to silicon dioxide NPs showed considerably increased caspase-3 and caspase-9 enzyme activity. This study found that silicon dioxide NPs promoted cytotoxicity and death in A431 and A549 cells, probably via ROS generation and oxidative stress.

Wu and Yi (2015), reported that the application of low concentrations of Cr and Pb heavy metals to *Galleria mellonella* larvae increased the total count of haemocytes, phagocytic activity and encapsulation, along with CAT, SOD, and peroxidase enzyme activities in the haemocytes.

Li et al. (2015), studied the combined impacts of ZnO NPs and Cu NPs in a human hepatoma cell line and discovered that ZnO NPs greatly boosted the cytotoxic impact of Cu NPs. However, co-incubation with a medium containing just zinc ions had no effect on the cytotoxicity caused by Cu NPs, demonstrating that the increase in toxicity was due to the particle shape of ZnO NPs. As a result, research into binary nanoparticle combinations appears to be extremely important for gaining a better knowledge of pollutant combined toxicity.

Benavides et al. (2016), in their study *on Carassius auratus* exposed to ZnO and Al_2O_3 NPs for 7, 14 and 21 days, found that SOD and CAT activities in gill and liver showed a crucial increase between 7-14 days and a crucial decrease between 14-21 days at all NP doses.

Abd El-Aziz and Fahmy (2015), investigated the effects of abamectin and haematoporphyrin on the oxidative stress indicator malondialdehyde content and antioxidant enzyme activity (catalase, glutathione-Stransferase) in environmentally friendly *Spodoptera littoralis* 4th stage larvae. Abamectin treatment substantially reduced GSH levels on days 3 and 4, whereas haematoporphyrin treatment boosted them on days 1 and 2. The activity of the antioxidant enzymes CAT and GST did not follow a consistent pattern in relation to abamectin and haematoporphyrin. These findings suggested that exposure to abamectin and haematoporphyrin reduced enzymatic antioxidant defence capability in stage 4 *S. littoralis* larval tissues.

Suganya et al. (2016), investigated the antioxidant parameters of Spodoptera litura larvae after 24-48 h in food media containing different concentrations of Cd and Pb metals. The larvae were fed with Cd 0.3, 0.44 and 0.50 mg or Pb 0.48, 4.8 and 9.6 mg concentrations for 48 hours and they found that there were changes in antioxidant enzyme activities. When the data obtained were compared with the control group, they observed that enzyme activities like SOD, CAT and GST increased with increasing doses.

Subala et al. (2017), examined the time-dependent effects of melatonin on abamectin-induced oxidative stress in the *Spodoptera litura* model. The larvae were divided into five groups: (1) control, (2) Melatonin group (4.3×10-5 M/100 ml of food), (3) Abamectin group 1.5 ml/L, (4) Melatonin applied group (PM) (4.3×10-5 M/100 ml of food) 1.5 ml/L before Abamectin application, and (5) Melatonin applied group (TM) after Abamectin application. As a result, the ABA group demonstrated substantial alterations in ROS and carbonyl concentration in the midgut. GST, SOD, POX, CAT, and APOX levels were found to be elevated in PM's midgut. As a result, they observed that indolamine may have an important role in lowering abamectin toxicity in S. litura in a time-dependent way.

Zeng et al. (2018), in a model of human bronchial cells, he demonstrated that CeO2 nanoparticles were able to diminish arsenite presence by adsorption, resulting in reduced toxicity.

Sendi et al. (2018), added different concentrations of cadmium and copper to the food of *Helicoverpa armigera* (Lepidoptera: Noctuidae) larvae and examined their effects on haemocyte counts. As a result of the experiment, they observed that the total haemocyte count decreased at 12.5 mg/kg Cd concentration, while there was a significant increase at 50 mg/kg Cd and Cu concentrations. In the differential haemocyte count analysis, they reported that prohemocytes decreased at 12.5 mg/kg Cd and Cu concentrations, while granulocytes increased at Cd concentration.

Martin-Folgar and Martinez-Guitarte (2019), investigated the damage of heavy metals like cadmium and copper on *Chironomus riparius* species belonging to the Diptera order. Cd and Cu doses were applied to insects both separately and in mixture. In the experiment, the effects on apoptosis mechanism (caspase) and immune system (PO, Defensin) were examined. As a result of the experiment, no difference was observed in antiapoptotic genes involved in apoptosis, while the mixture of Cd and Cu with the highest dose was found to cause significant differences on effector caspases. It was emphasised that this

situation causes changes in the immune system of the insect by affecting the apoptosis mechanism and may jeopardise the possibility of survival

Wang et al., 2019, subjected human lung cells to either Si NPs, methylmercury, or a mixture of the two. They discovered that the intracellular silicon and mercury concentration in the combination group was much greater than in the single exposure groups.

Kara et al. (2021), injected various concentrations of Al_2O_3 NPs (10, 50 and 100 µg/mL) into *G. mellonella* last stage larvae with the help of a hamilton injector and examined the total haemocyte count by exposing them for 2, 4 and 8 hours. According to the results obtained, they reported that total haemocyte count decreased at all concentrations compared to the control.

Meşe et al., 2020 injected ZnO NP at 30 mg/l and 30 µg/mL dosages onto *G. mellonella* last instar larvae via the prolegs. According to the findings, AChE activity in the fat body of *G. mellonella* larvae exposed to ZnO NP increased and decreased statistically significantly in both application groups as compared to the control.

Tunçsoy et al. (2021), examined the effects of CuO NP on some biochemical parameters of fourth stage *G. mellonella* larvae. According to the data obtained, they found that SOD activity in the midgut and adipose tissue decreased, CAT activity increased, but there was no crucial change in GPx activity.

Eskin (2022), investigated the effects of (SiO_2 NP) nanoparticles on total haemocyte count (THC) and haemocyte viability in *G. mellonella* larvae. Applying 60 and 100 µg / 10 µl SiO2 NPs dramatically reduced THC levels in larvae within 48 hours compared to the control group. After 48 hours, the haemocyte viability findings showed that the 100 µg/10 -180 µg/10 µl SiO_2 NPs groups had a greater percentage of dead cells than the control group (2.92%).

Teng et al. (2020), It was discovered that co-exposure to cadmium chloride affected the surface charge of ZnO NPs (due to Cd2+ adsorption on the nanoparticle surface), increasing both maternal-fetal pollution transmission and embryotoxicity

3. METHODOLOGY

3.1. Characterisation of Nanoparticles

The characterisation of metal nanoparticles was carried out by electron microscopy (SEM) and X-ray diffraction (XRD) analyses at Çukurova University Central Research Laboratory.

3.2. Galleria mellonella

G. mellonella (L.) is a pest of the order Lepidoptera, family Pyralidae. The larvae of this species cause significant damage to beekeeping habitats, beehives, honeycombs and stored wax, resulting in reduced yields. A variety of chemicals are used to control this species, and the residual problems are harmful to both the environment and human health. More recently, this species has been used as a model organism, particularly to study the impact of microbial diseases on human health. The fact that the rat, which is widely used as an experimental model organism in medical research, is difficult and expensive to breed and has allowed the use of invertebrates as model organisms. Species such as *G. mellonella* are excellent model organisms because they are easy and inexpensive to culture. In addition, the fact that *G. mellonella* larvae are commercially viable and can be produced quickly and easily at room temperature sets this species apart from other invertebrate organisms. Finally, *G. mellonella* has a natural immune system comparable to that of mammals, making it an appropriate model organism.

G. mellonella has 4 life stages: egg, larva, pupa and adult. The larval stage consists of seven instars, with an average duration of 24 days. The experiment used *G. mellonella* larvae at the end of their developmental period. The pupa stage has an average length of 16.10 mm and a width of 3.50 mm. The final stage larvae construct white cocoons to

protect the pupa inside the hive. Lacking a digestive tract as adults, they stop eating and die after producing eggs.

Figure 3.1. *G. mellonella* larvae.

3.3.Preparation of *G. mellonella* Stock Culture and Obtaining Experimental Larvae

The study used larvae from the *G. mellonella* stock culture at the Animal Physiology Laboratory, Department of Biology, Çukurova University. To maintain the stock culture, Bronskill's, 1961) nutrient combination (bran, honey, clean water, glycerol and honeycomb) was placed in 5-litre glass jars and mature *G. mellonella* insects were released into the jars. The jar cap openings were lined with tulle and the lids were pierced to facilitate air circulation. The prepared jars were incubated in the culture chamber under ambient conditions of 28 ± 2˚C temperature and 70 ± 5% relative humidity, with a daily photoperiod of 12:12 (dark:light). Adult insects in the jar were allowed to breed and lay eggs on the meal. Larvae emerging from these eggs after hatching were used in the tests.

3.4. Determination of LD_{50} Value of SiO_2 NPs

The last instar *G. mellonella* larvae treated with SiO_2 NPs were removed from the feeding medium and separated into control and treatment groups. The larvae were injected with

various concentrations of 50, 100, 250, 400, and 500 µg/ml using a Hamilton micro-injector. The concentrations used were determined according to the previous studies. Following treatment to the last instar larvae, they were put in pteri dishes. Following the application of SiO_2 NPs, control and treatment groups of 10 larvae each were established and the experiments were repeated three times at different times. The total count of dead larvae in the applied groups was recorded 120 h after nanoparticle administration. The LD_{50} values of silica nanoparticles for *G. mellonella* last instar larvae were then determined using the probit analysis method in SPSS 21. statistical data software.

3.5. Preparation of solutions to be used in the experiment

Last instar larvae of *G. mellonella* were removed from the growth media and weighed in groups of ten using a precision balance. Care was taken to ensure that the control and treatment groups, consisting of selected individuals, were uniform. The experiment was divided into five groups: control and application groups;

1) control
2) Silicon dioxide nanoparticles (SiO_2 NPs) (LD_{50}: 396 µg/ml)
3) cadmium sulphate ($CdSO_4$) (environmental concentration: 10 µg/L)
4) Abamectin (Agrimec®EC, Syngenta®; analytical standard) (ambient concentration: 10 µg/L)
5) SiO_2 NPs+ $CdSO_4$
6) SiO_2 NPs+Abamectin

The sheets holding the eggs deposited by adult *G. mellonella* females were placed in jars containing regular diet, and the larvae were allowed to eat in this nutritious environment until they reached the last instar stage. The larvae that had reached the last instar were removed from the diet medium. The Hamilton injector was used for the microinjection

procedure. For the application procedure, SiO_2 NPs (LD_{50}: 396 µg/ml), cadmium sulphate, abamectin and mixture groups (SiO_2 NPs + $CdSO_4$; SiO2 NPs + abamectin) were administered to the larval proleg. For the control group, distilled water was applied with the same procedure.

3.6. Dissection of the Tissues

After application, the larvae were placed on ice for 2-3 minutes to slow their movements and cleaned with 95% ethyl alcohol. The larvae were dissected by pinning their backs to the polystyrene and cutting them with alcohol disinfected micro scissors along the central axis in front of the prolegs. The fat body was first separated with forceps, followed by removal of the midgut (Fig. 3.2.). The extracted fat body and midgut were placed in Eppendorf tubes containing homogenisation buffer (20 mM; pH 7.6).

Figure 3.2. Dissection of *Galleria melonella* larvae

Homogenization Buffer;

Table 3.1. The substances used in the homogenization buffer

Tris(hdyroxymethyl)aminomethane	2,4228 gr
EDTA	0,2923 gr
Sucrose	171,2 gr
KCl	11,2 gr
Dithiotreitol (DTT)	0,1543 gr

3.7. Homogenization of Tissues

Fat body samples were homogenized in an ultrasonic homogenizer set to +4 °C. The supernatant was then transferred to another tube and centrifuged at 12.000 g for 45 minutes at +4 °C. It was centrifuged again at 10,249 rpm.

Midgut samples were first homogenized with ultra turrax before being centrifuged using the same techniques as fat tissue. Supernatants from both tissues were sorted into Eppendorf tubes for Bradford determination (100 µl).

Figure 3.3. Ultrasonic homogenizer

Filtration was carried out at the end of each of these steps. Low molecular weight proteins were removed during the filtering process using Sephadex® G-25 gel columns (Fig.3.4.) (Gonzalez-Reya et al, 2014). The columns were cleaned 5 times with homogenization buffer, then 2500 µl of material was added and filtered entirely. After filtering, 3500 µl of buffer was added and the filtered samples were split into Eppendorf tubes for enzyme activity. The samples were then maintained in a deep freezer at -80 °C until enzyme activities were determined.

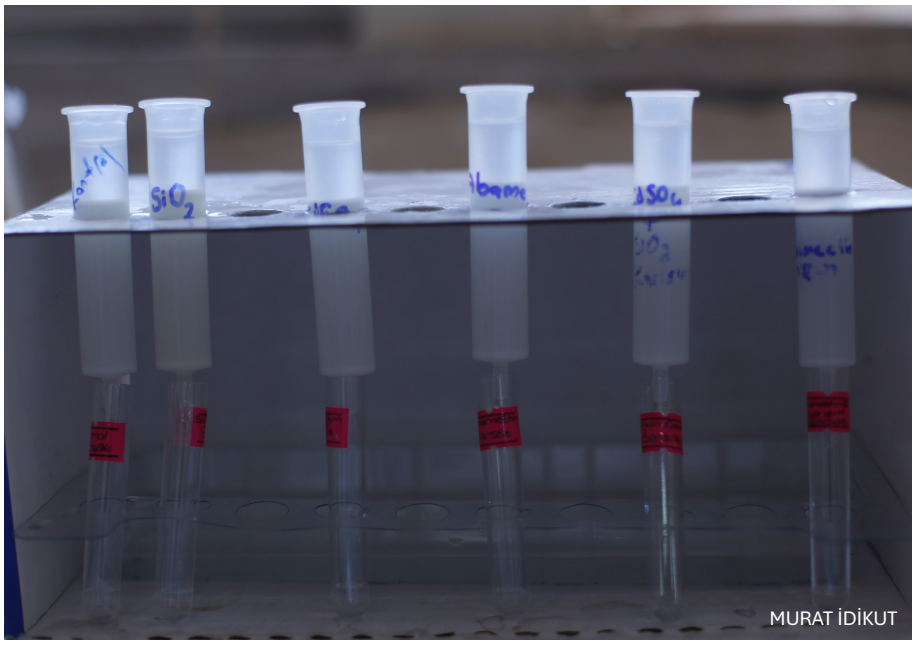

Figure 3.4. Filtration of homogenates using Sephadex® G-25 gel columns.

3.8. Determination of Antioxidant Enzyme Activities of Tissues

3.8.1. Determination of Superoxide dismutase (SOD) Enzyme Activity

The method of McCord and Fridovich (1969) was used for superoxide dismutase (SOD) enzyme activity. The determination of superoxide dismutase (SOD) activity is based on the measurement of the reduction of cytochrome c by xanthinoxidase/hypoxanthine system. 1 unit of SOD is expressed as the amount of enzyme accumulated by inhibiting 50% of the cytochrome c reduction. SOD activity is obtained by defining the total protein concentration as U mg^{-1}.

Solutions;

Phosphate buffer;

850 ml Sol B +100 ml Sol A : pH 7.8

Phosphate buffer;

Solution A: KH_2PO_4 6.8 gr

EDTA 0.0292 gr 1L ultrapure water

Solution B; K_2HPO_4 8.7 gr EDTA : 0.0292 gr

1L ultrapure water

850 ml Sol B+ 100 ml Sol : pH 7.

Hypoxanthine;

Hypoxanthine: 20.4 gr

Ultrapure water 100 ml. Heated at 50 °C until completely dissolved.

It is heated at 50º C until completely dissolved.

Sitochrome c oxidases;

Cytochrome c: 37.5 gr

Phosphate buffer: 20 ml

Ksantin oksidaz (56 Um/ml);

Xanthine: 50 µl (38.5 U/ml)

Phosphate buffer: 2 ml

Xanthine oxidase test;

The xanthine oxidase test was performed before the samples were read. Measurements were repeated three times for one minute at 550 nm absorbance.

Table 3.2. The amount of solutions used for the xanthine oxidase test

phosphate buffer	2700 µl
Hypoxanthine	100 µl
Sitochrome c oxidases	100 µl
Xanthine oxidase	100 µl
Total	3000 µl

Measurement of samples;

Samples were kinetically measured using a spectrophotometer,recording absorbance at 550 nm over a period of 1 minute.

Table 3.3. The amount of solutions used for the (SOD) Enzyme Activity

Solution	**Volume (µl)**	**Curve(µl)**
phosphate buffer	2650	2800
Hypoxanthine	100	100
Cytochrome c oxidase	100	100
sample	50	-
Xanthine oxidase	100	-
Total	3000	3000

3.8.2. Determination of Catalase (CAT) Enzyme Activity

Greenwald (1985) method was used for catalase (CAT) enzyme activity. Catalase activity is based on the measurement of the decreasing absorbance released by the breakdown of H_2O_2 at 240 nm absorbance for one minute. Changes in absorbance per unit time using a

constant count (ε240= 40 $M^{-1}cm^{-1}$) were taken as the measurement of catalase activity. Enzyme activity is given in units of nmol min^{-1} mg^{-1} protein.

Solutions;

Phosphate buffer;

250 ml Sol B +50 ml Sol A: pH 7,8 Complete with 1liter ultrapure water. Adjust pH 7,5 with HCl.

Solution A: KH_2PO_4 10.9gr

1L ultrapure water

Solution B; K_2HPO_4 13.92gr

1L ultrapure water

250 ml Sol B + 50 ml Sol A: pH 7.8

It is completed with 1L ultrapure water. The pH is adjusted to 7.5 with HCl.

H_2O_2:

In a separate flask, add 1.7 ml of 30% H_2O_2 and add the prepared phosphate buffer to 100 ml. This solution is placed in a separate bottle.

Measurement of samples:

In a spectrophotometer, the samples were measured kinetically at 240 nm absorbance every 10 seconds for 1 minute.

Table 3.4. The amount of solutions used for the CAT Enzyme Activity

Solution	**Volume (µl)**	**Curve (µl)**
phosphate buffer	1900	3000
H_2O_2	1000	-
sample	100	-
Total	3000	3000

3.8.3. Determination of Glutathione peroxidase (GPx) Enzyme Activity

The glutathione peroxidase enzyme activity was determined using Lawrence and Burk's (1976) techniques. Total glutathione peroxidase is evaluated in the presence of reduced glutathione, glutathione reductase and cumene hydroperoxide as substrates after NADPH oxidation at 340 nm. The GPx activity is measured in nmol min^{-1} mg^{-1} of total protein content.

Solutions;

Phosphate buffer;

250 ml Sol B + 50 ml Sol A: pH 7,8

Solution A: KH_2PO_4 13.6 gr

1L ultrapure water

Solution B; K_2HPO_4 17.4 gr

1L ultrapure water

250 ml Sol B + 50 ml Sol A: pH 7.8

GSH (reduced glutathione);

GSH : 0,123 grr

phosphate buffer : 10ml

Cumene hydroperoxide;

Add up to 100 ml of ultrapure water.

Cumene (%80) : 0,563 ml

Ethanol : 20 ml

GR (glutathione reductase);

GR: 38,4 µl (38,5 U/ml)

phosphate buffer : 1,78 ml

NADPH;

NADPH : 4 mg

phosphate buffer : 2 ml

Cumene hydroperoxide test:

Table 3.5. The amount of solutions used for the cumene hydroperoxide test

Solution	**Volume (µl)**
phosphate buffer (100mM)	450
GR (20 U/ml)	50
GSH (40 mM)	50
NADPH (2,4 mM)	50
Tris buffer*	300
Cumene hydroperoxide	100
Total	1000

*Tris HCl buffer used for homogenisation of the sample

Measurement of samples:

Sample was kinetically measured using in a spectrophotometer, recording absorbance at 340 nm over a period of 1 minute.

Table 3.6. The amount of solutions used for the GPx Enzyme Activity

Solution	Volume (µl)	Curve (µl)
Phosphate buffer (100mM)	450	1000
GR (20 U/ml)	50	-
GSH (40 mM)	50	-
NADPH (2,4 mM)	50	-
Sample	300	-
Cumene hydroperoxide	100	-
Total	1000	1000

3.9. Determination of Detoxification Enzyme Activities

3.9.1. Determination of Acetylcholinesterase (AChE) Enzyme Activity

AChE (AChE: EC: 3.1.1.7) is an enzyme that catalyses the cleavage of acetylthiocholine to acetate with thiocholine. AChE activity was determined by measuring the intensity of the yellow colour given by DTNB resulting from the reaction between thiocholine and DTNB in a spectrophotometer at a wavelength of 412 nm (Ellman et al., 1961).

Solutions

Tris HCl :100 mM pH: 8.0

Tris (hydroxymethyl)-aminomethane ($NH_2C(CH_2OH)_3$) :12,11gr

Ultrapure water 1000 mL

The pH was adjusted with HCl solution, the solution is always stored in the cold (4 ºC).

Acetylcholine solution (ATC) (10mM);

Acetylcholine ($C_7H_{16}NOS.I$) : 5,784 mg

Ultrapure water : 2 mL

DTNB (1Mm);

DTNB ($C_{14}H_8N_2O_8S_2$) 5.5'-ditiyo-bis (2 nitrobenzoat): 1,5852 mg

Tris HCl 100 Mm, pH: 8.0 : 4 mL

%10 Triton;

Triton X100 ($C_{34}H_{62}O_{11}$) : 10 µl

Ultrapure water : 10 mL

Measurement of samples

After shrinking the plastic test tubes, the samples were placed and weighed again and the weights of the tissues were calculated. Tris HCl solution was added at a ratio of 1:5 [tissue (g): Tris HCl (mL)]. 10% Triton 10 times the volume of Tris HCl solution was added to the mixture in µl. Samples were homogenised for 2 minutes. The total homogenate was centrifuged at 12000 g for 30 min at +4 ºC. Part of the supernatant obtained at the end of centrifugation was used for the determination of total protein and the remaining part was stored in a deep freezer at -80 ºC for the determination of AChE activity.

Measurement of AChE activity of samples

Samples were kinetically measured using a spectrophotometer, recoring absorbance at 405 nm absorbance over a period of 1 min.

Table 3.7. The amount of solutions used for the AChE Enzyme Activity

Solution	**Volume (µl)**	**Curve (µl)**
Tris HCl	2250	2250
DTNB (1 mM)	300	300
Sample	150	150 µl Tris HCl is added.
Mixture stand at room temperature for 5 minutes.		
ATC (10 mM)	300	300

3.9.2. Determination of Cytochrome P450 (Cyt P450) Enzyme Activity

4-nitroanisole (PNOD) was utilized as a substrate to determine cytochrome P450 monooxygenase enzyme activity (Rose et al., 1995).

Solutions

Sodium phosphate buffer 0.1 M, pH: 7.8

Sol A (0,2 M): 31,21 gr NaH_2PO_4. H_2O

NaH_2PO_4. H_2O is weighed and dissolved in some 1% acetone.

The volume is completed to 1 litre.

Sol B (0,2 M): 53,59 gr Na_2HPO_4. $7H_2O$

Na_2HPO_4. $7H_2O$ is weighed and dissolved in some 1% acetone.

The volume is completed to 1 litre.

Buffer: 91,5 mL Sol B + 8,5 mL Sol A pH: 7,8

The volume was completed to 200 mL after pH control.

Then over this mixture;

EDTA : 0,0744 gr

Phenylthiourea : 0,0304 gr

DTT : 0.0308 gr was added and homogenisation buffer was prepared.

4-nitroanisol (2 mM)

4-nitroanisol: 61 mg

1 mL was dissolved in 100% Ethanol.

625 mikroliter of the mixture was added to 125 milliliter of warm sodium phosphate buffer.

NADPH (9.6 mM)

NADPH: 12 mg

After weighing, 1500 µL sodium phosphate buffer is added.

Measurement of samples

Kinetic measurement was performed at 405 nm at 15 second intervals for 15 minutes at 30°C.

Table 3.8. The amount of solutions used for the Cyt P450 Enzyme Activity

Solutions	**Volume (µl)**	**Curve (µl)**
Sodium phosphate bumper	-	900
4-nitroanisol	1000	1000
Sample	900	-
Mixture was incubated at 30°C for 30 minute.		
NADPH	100	100
Total	2000	2000

3.9.3. Determination of Glutathione-S-transferase (GST) Enzyme Activity

Total GST activity catalysing the conjugation of CDNB (1-chloro-2,4-dinitrobenzene) with reduced glutathione was measured. The change in absorbance at 340 nm was recorded and the enzyme activity was calculated as mmol conjugated CDNB/min/mg protein using a molar extinction coefficient (ε) of 9.6 mM^{-1} cm^{-1} (Habig et al, 1974).

Solutions

KH_2PO_4/K_2HPO_4 tampon (0,2 M; pH: 7.9)

KH_2PO_4 68.05 gr (0.2 M)

After weighing, it was completed to 500 mL.

K_2HPO_4 34.84 gr (0.2 M)

After weighing, it was completed to 1000 mL.

Phosphate buffer: 980 mL of 0.2 M K2HPO4 solution was taken and 0.2 M KH2PO4 solution was added until pH 7.9 (approximately 20 mL).

CDNB (0.2 mM, room temperature)

For a total solution of 25 mL;

0.04135 gr CDNB was dissolved in 10 milliliter ethanol and vortexed.

15 milliliter of ultrapure water was added and stored at room temperature.

GSH

0.01255 gr GSH was dissolved in 5 mL ultrapure water.

Measurement of samples

Table 3.9. The amount of solutions used for the GST Enzyme Activity

Solution	Volume (µl)
Phosphate buffer	1850
GSH	50
CDNB	50
Sample	50
Total	2000

3.10. Calculation of molar absorbance coefficient (ε) and Enzyme activity

The optical density (mO.D.) obtained from the kinetic reading was expressed as (nmol 4-nitroanisole min-1 mg-1 protein) (Rose et al. 1991; 1995). The extinction coefficient (ελ) of 4-nitroanisole was used as the product in this conversion. To calculate the extinction coefficient of 4-nitroanisole, different concentrations were prepared. These were read at 405 nm in the same spectrophotometer. A regression equation was obtained from the absorbance values obtained in the experiments and the slope was used as the extinction coefficient of 4-nitroanisole (Temizkan and Arda, 2008; Hephızlı, 2011). This value was calculated using the enzyme unit (U) in the sample (Temizkan and Arda, 2008).

3.11. Determination of Total Protein Amount

The Bradford method is derived from the formation of a blue coloured complex by binding amino acid residues such as tryptophan, arginine, histidine, tyrosine and phenylalanine in proteins to Coomassie brillant blue G-250 dye. The absorbance of the coloured solution is measured at 595 nm (Bradford, 1976).

Stock protein solution was prepared from bovine serum albumin at 1 mg/mL. Solutions were prepared by dilution the stock protein solution at 0, 10, 20, 30 and 50 µg/L concentrations. The absorbance values of the standards were determined by the method used to measure the sample protein concentrations and used to construct the standard graph. After the solutions were prepared, they were mixed with vortex for 5 seconds.

Standard Protein solutions;

Table 3.10. The amount of solutions used for the standart protein

Concentration (µg/L)	0		20	30	50
BSA (ml)	0	0,05	0,1	0,15	0,25
Ultrapure water (ml)	5	4,95	4,9	4,85	4,75

Bradford Reagent;

It was prepared by adding 40 ml of ultrapure water to 10 ml of Bradford reagent.

Preparation of samples

The supernatants obtained after homogenisation of the tissues were first diluted 1/100 and then again 1/5.

1st dilution: 1/100; 990 µl ultrapure water
10 µl sample

2nd dilution: 1/5; 200 µl ultrapure water
50 µl sample diluted 1/100

3.12. Reading standards and samples

250 µl of the prepared standard solutions were added to another tube and 750 µl of Bradford reagent was added. The absorbance of the samples at the 595 nm was

measured using a spectrophotometer. The same amount of ultrapure water was used as a curve.

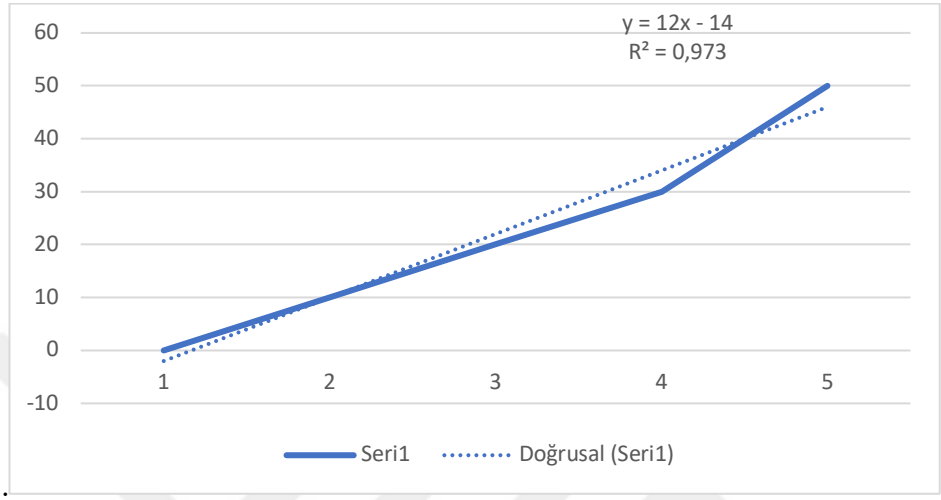

Figure 3.5. Linear relationship between protein concentration and absorbance.

3.12. Determination of Hemocyte Types of *G. mellonella* Larvae

3.12.1. Total Hemocyte Count

After 120 hours, *G. mellonella* larvae were placed at -20°C for 15-20 seconds to stop their movements and extract haemolymph. The larvae were disinfected with 95% ethanol before being excised from the top of the first prolegs using fine-tipped microscissors. 10 µl of haemolymph was then transferred to Eppendorf tubes containing N-phenylthiourea (Sigma Aldrich) (PTU). 4 µl of haemolymph was transferred to an ice-filled Eppendorf tube containing 36 µl of anticoagulant (0.186 M NaCl, 0.017 M Na_2EDTA, 0.098 M NaOH and 0.041 M citric acid, pH 4.5). 1:10 µL of the 10% diluted cell solution was added to the Neubauer haemocytometer. The haemocytes were counted using a Leica

DM750 microscope and the count of haemocytes per ml of haemolymph was determined using the Jones method (1962).

Neubauer hemocytometer (Fig 3.6.) is a counting slide with two distinct counting sections divided by a depression. Each counting area contains four 1 mm^2 portions (big squares) at its corners. These are cut into sixteen medium-sized squares (1/4 x 1/4 = 1/16 mm2). The huge square in the center is split into 25 smaller squares. Medium-sized squares are encircled by double lines. Each central square is split into sixteen smaller squares. Thus, it was reported that the center region consisted of 25 major squares and 400 little squares. A little square has an area of 1/400 mm^2 (1/20 x 1/20). The margins outside of the counting area are 0.1 mm higher than the counting area surface.When the coverslip of the hemocytometer is placed on the counting area, a gap of 0.1 mm remains between the coverslip and the counting area surface. Thus, the volume of each square between the slide and coverslip was calculated.

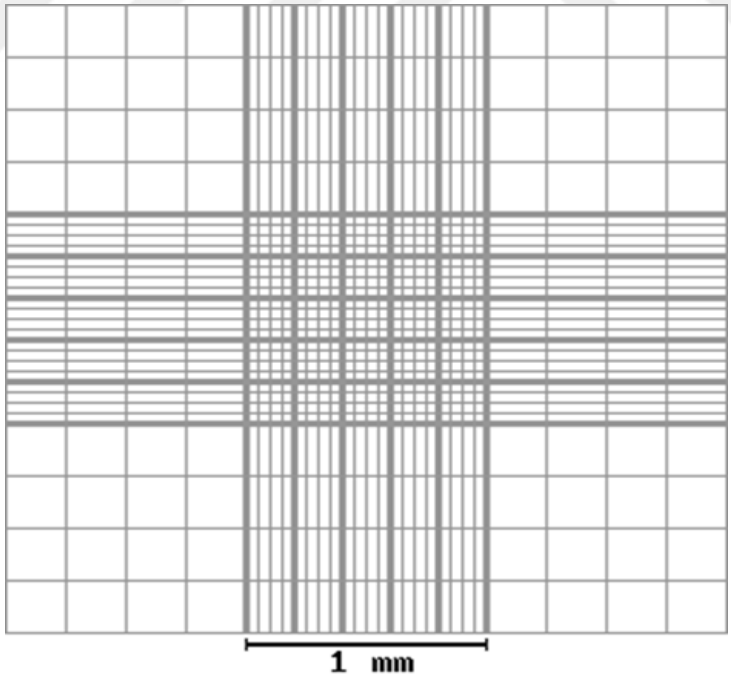

Figure 3.6. Neubauer Hemocytometer.

The computation was conducted using the following formula. The volume of the biggest square ($1mm^2$) is 1 X 1. In the experimental groups, the overall count of hemocytes was calculated by counting the complete 1 mm^2 square on the hemocytometer slide. The total count of hemocytes per milliliter was calculated using the formula shown below: Count of cells per milliliter: count of cells counted in the big square x dilution coefficient multiplied by 10^4.

3.12.2. Differential Hemocyte Count

After 120h of the application, the larvae were transported to -20°C to extract hemolymph. The motions were slowed down by waiting 15-20 seconds. After disinfecting the larvae with 95% ethanol, they were cut from the top of the first proleg with fine-tipped microscissors and 5 µl hemolymph was collected using a micropipette. The recovered hemolymph was distributed on slides that had previously been cleaned with alcohol and allowed to dry at room temperature.

The slides were immersed in a 3:1 solution of methanol and acetic acid for 5 minutes before being allowed to dry. Hemocytes were stained with a 10% Giemsa staining solution (MERCK Giemsa's Azure Eosine and Methylene Blue Solution). After fixing, the dry slides were immersed in Giemsa dye and allowed to stain for 5 minutes. After the staining process was complete, the slides were rinsed with clean water. After drying, it was put through xylene. To create a permanent preparation, it was coated with entellan, and the hemocyte types were identified and counted using a Leica DM750 microscope.

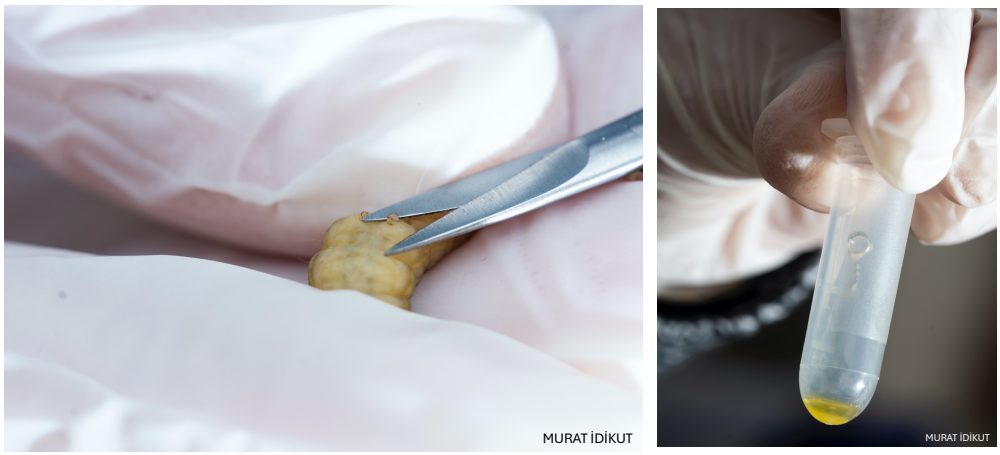

Figure 3.7. Hemocyte collection process from *G. mellonella*.

3.13. Phenoloxidase (PO) Enzyme Activity

To determine phenoloxidase activity, 8 microlitres of haemolymph and 400 microlitres of ice-cold phosphate buffered saline (PBS, pH 7.4) are added to a plastic Eppendorf tube. In another Eppendorf tube, 100 microlitres of buffered haemolymph was added, followed by 100 microlitres of 10 mM L-dopa substrate. This mixture was incubated for 20 minutes at 25°C and the kinetic absorbance was measured at 490 nm with a UV spectrophotometer at 5-minute intervals from 0 to 30 minutes. For PO, one unit is the amount of enzyme that causes an increase in absorbance of 0.001 per minute.

3.14. Apoptotic Index

To extract haemolymph, larvae were held at -20°C for 15-20 seconds to inhibit movement. After disinfection with 95% ethanol, larvae with restricted movement were excised from the top of the first proleg with a fine-tipped micromacass and 5 µl of haemolymph was collected with a micropipette. 5 µl AO, 5 µl EB and 5 µl haemolymph were combined in the Ependorf tube and mixed thoroughly. 5 µl of the mixture was applied to slides cleaned

with 70% ethyl alcohol. Slides were allowed to dry for 1-2 minutes before examination under a fluorescence microscope equipped with a blue filter.

3.15. Data collection and analysis

Statistical data from the control group and the groups receiving metal nanoparticles were compared using the Student Newman Keul's (SNK) test in the SPSS 21 programme. A p-value of <0.05 is considered statistically significant.

4. ANALYSIS AND DISCUSSION

4.1. ANALYSIS

4.1.1. Characterization of SiO_2 NPs

4.1.1.1. SEM analysis

The characterization of the shape, surface and morphology of SiO_2 nanoparticles is carried out by scanning electron microscope (SEM) analysis at Çukurova University Central Research Laboratory (ÇÜMERLAB). The results are shown in Figure 4.1. The SEM analysis results show that SiO_2 Nanoparticles are spherical in structure.

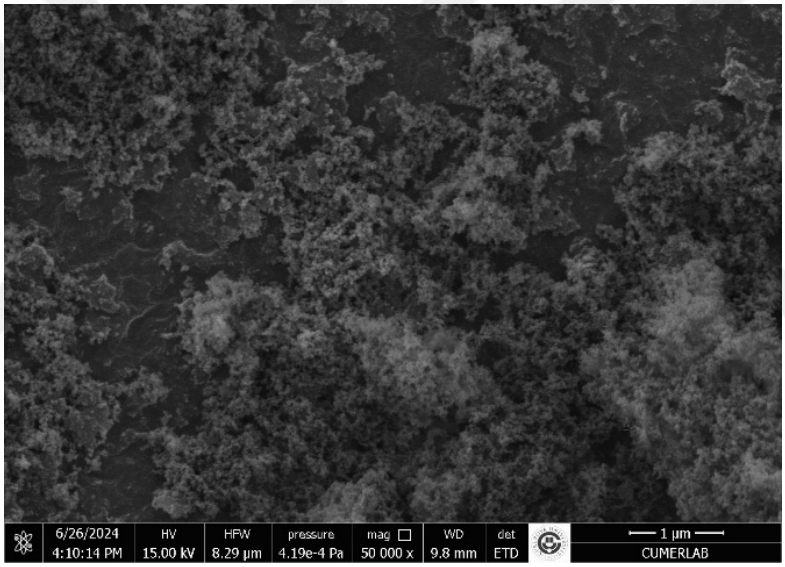

Figure 4.1. SiO_2 NPs SEM analysis.

4.1.1.2. XRD analysis

The characterization of the crystal and molecular structure of SiO_2 nanoparticles is carried out by X-Ray diffraction analysis (XRD) analysis at Çukurova University Central Research Laboratory (ÇÜMERLAB). The results are shown in Figure 4.2.

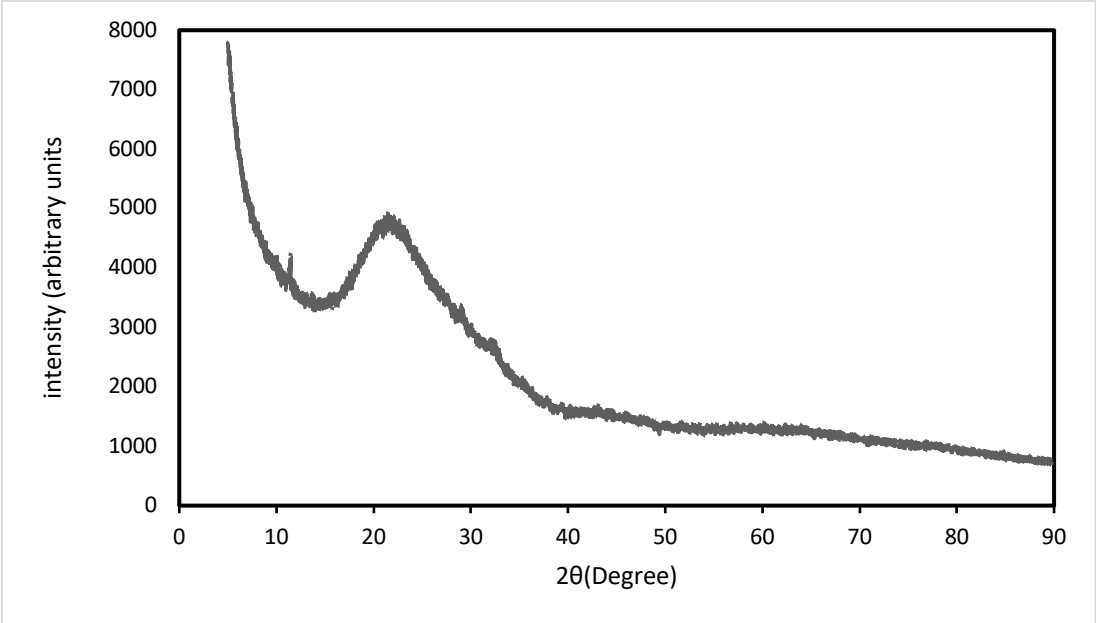

Figure 4.2. SiO_2 NPs XRD analysis.

4.1.2. Determination of Enzyme Activities in *G. mellonella* Larvae

4.1.2.1. CAT Enzyme Activity

The effects of SiO_2 and $CdSO_4$ singly and in mixtures on the CAT enzyme activity in the fat body and midgut of *G. mellonella* are presented in Table 4.1 and shown in Figure 4.3 and Figure 4.4. There was an increase in CAT activity in the midgut in the mixture applied group according to the control and this increase was found to be statistically significant (37.85-fold) ($p<0.05$). As for fat body, exposure to $CdSO_4$ and mixture resulted in a 1.94-fold and 1.55-fold decreases, respectively, while an increase occurred in the SiO_2 NPs applied group (1.22-fold) ($p<0.05$).

Table 4.1. CAT activity of SiO_2 NPs and $CdSO_4$ singly and their mixtures of *G. mellonella* larvae (Mean ±Standard Error;) *

	CAT ACTIVITY (µmol dk^{-1} mg^{-1} protein) ($\overline{X}$ ± $s\overline{x}$)*	
Concentration(µg/mL)	**Midgut**	**Fat body**
Control	2,67 ± 0,00 a	433,09 ± 11,33 a
SiO_2	1,78 ± 0,00 a	526,51 ± 2,88 b
$CdSO_4$	4,90 ± 0,25 a	223,61 ± 1,14 c
$SiO_2 + CdSO_4$	101,06 ± 5,93 b	278,90 ± 5,41 d

*SNK indicates that there is a statistically notable variation (P<0.05) between the data given with different letters.

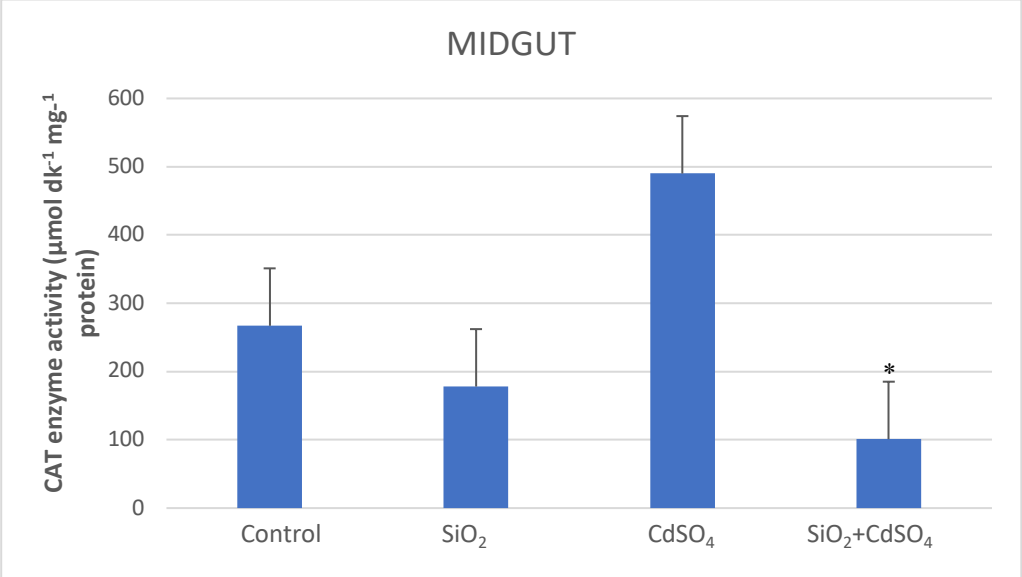

Figure 4.3. CAT enzyme activity in the midgut of *G. mellonella* larvae using SiO_2 NPs and $CdSO_4$ singly and their mixtures

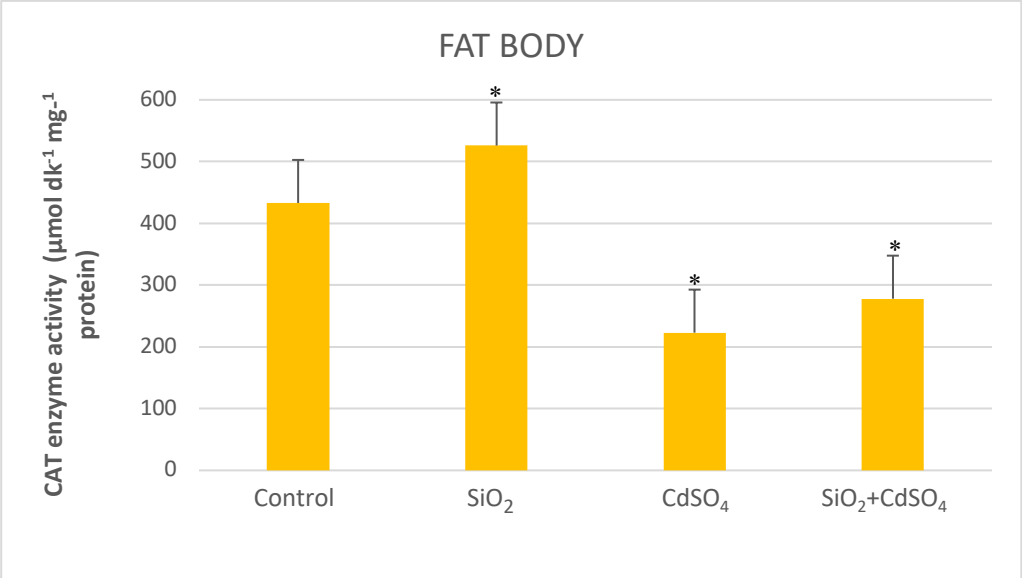

Figure 4.4. CAT enzyme activity in the fat Body of *G. mellonella* larvae using SiO_2, $CdSO_4$ singly and their mixtures

The effects SiO_2 NPs and Abamectin singly and in mixtures on CAT activity in the fat body and midgut of *G. mellonella* are presented in Table 4.2 and shown in Figure 4.5 and Figure 4.6. there was an increase in CAT actşvşty in the midgut in the mixture applied group according to the control and this increase was found to be statistically significant (33.53-fold) ($p<0.05$). The decreases in CAT activity in fat body was observed in the Abamectin and mixture applied groups, this decrases resulted in a 2,15-fold and 1.18-fold, respectively ($p<0.05$).

Table 4.2. CAT activity of SiO_2 NPs and Abamectin singly and mixtures of *G. mellonella* larvae (Mean ±Standard Error;) *

	CAT ACTIVITY (µmol dk^{-1} mg-^{1} protein) ($\overline{X} \pm s\overline{x}$)*	
Concentration(µg/mL)	**Midgut**	**Fat body**
Control	2,67 ± 0,00 a	433,09 ± 11,33 a
SiO_2	1,78 ± 0,00 a	526,51 ± 2,88 b
Abamectin	2,22 ± 0,25 a	201,76 ± 5,90 c
SiO_2 + Abamectin	89,52 ± 2,28 b	368,49 ± 11,20 d

*SNK indicates that there is a statistically notable variation ($P<0.05$) between the data given with different letters.

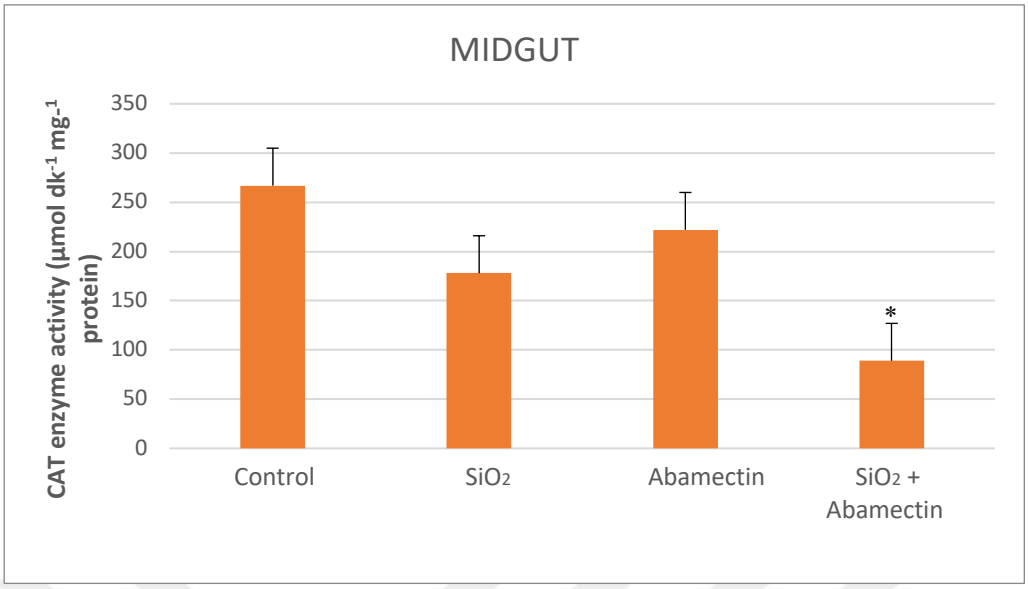

Figure 4.5. CAT enzyme activity in the midgut of *G. mellonella* larvae using SiO_2 NPs and Abamectin singly and their mixtures.

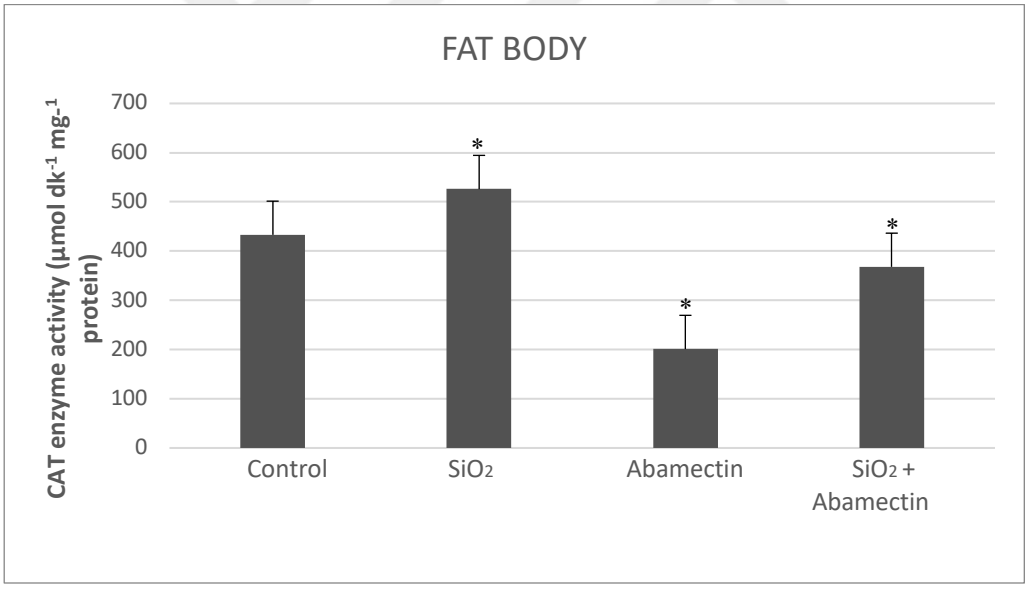

Figure 4.6. CAT enzyme activity in the fat body of *G. mellonella* larvae using SiO_2 NPs and Abamectin singly and their mixtures.

4.1.2.2. SOD Enzyme Activity

The effects of SiO_2 NPs and $CdSO_4$ singly and in mixtures on the SOD enzyme activity in the midgut and fat body of *G. mellonella* are presented in Table 4.3 and shown in Figure 4.7 and Figure 4.8. There was a significant decrease in SOD activity in the midgut in the SiO_2 NPs

applied group compared to the control (1.25-fold; $p<0.05$), while increases was observed in the $CdSO_4$ and mixture applied groups. This increases resulted in 1.5-fold and 1.7-fold, respectively ($p<0.05$). As for fat body, a decrease in SOD activity was observed in the SiO_2 NPs applied group compared to the control, but increases was detected in the $CdSO_4$ and mixture applied groups (1.44-fold and 1.24-fold, respectively) ($p<0.05$).

Table 4.3. SOD activity of SiO_2 NPs and $CdSO_4$ singly and their mixtures of *G. mellonella* larvae (Mean ±Standard Error;) *

	SOD ACTIVITY (U mg^{-1} protein) ($\overline{X} \pm s\overline{x}$)*	
Concentration (µg/mL)	**Midgut**	**Fat Body**
Control	71,28 ± 0,01 a	71,15 ± 0,01 a
SiO_2	56,98 ± 0,03 b	14,22 ± 0,00 b
$CdSO_4$	106,99 ± 4,13 c	99,67 ± 0,03 c
$SiO_2 + CdSO_4$	121,14 ± 4,13 d	85,43 ± 0,02 d

*SNK indicates that there is a statistically notable variation ($P<0.05$) between the data given with different letters.

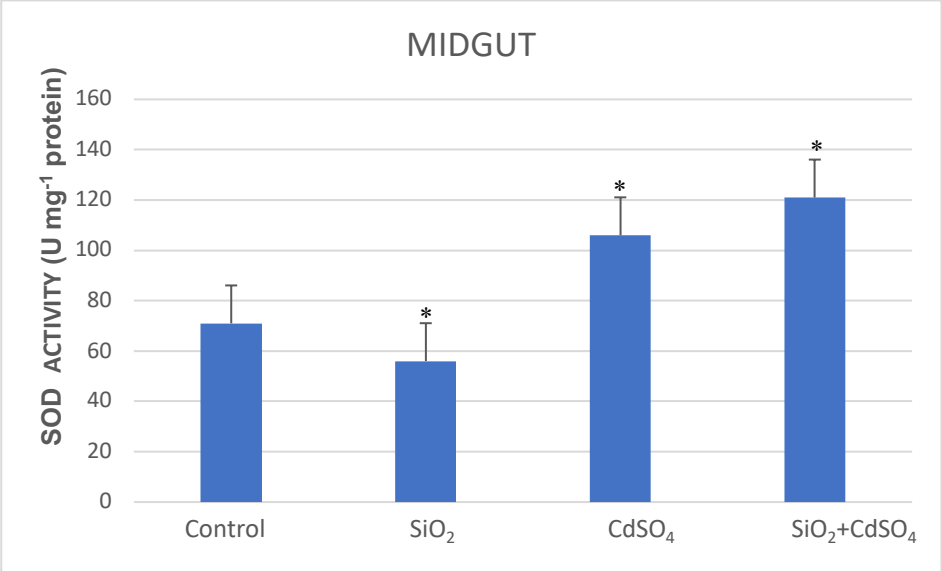

Figure 4.7. SOD enzyme activity in the midgut of *G. mellonella* larvae using SiO_2 NPs and $CdSO_4$ singly and their mixtures

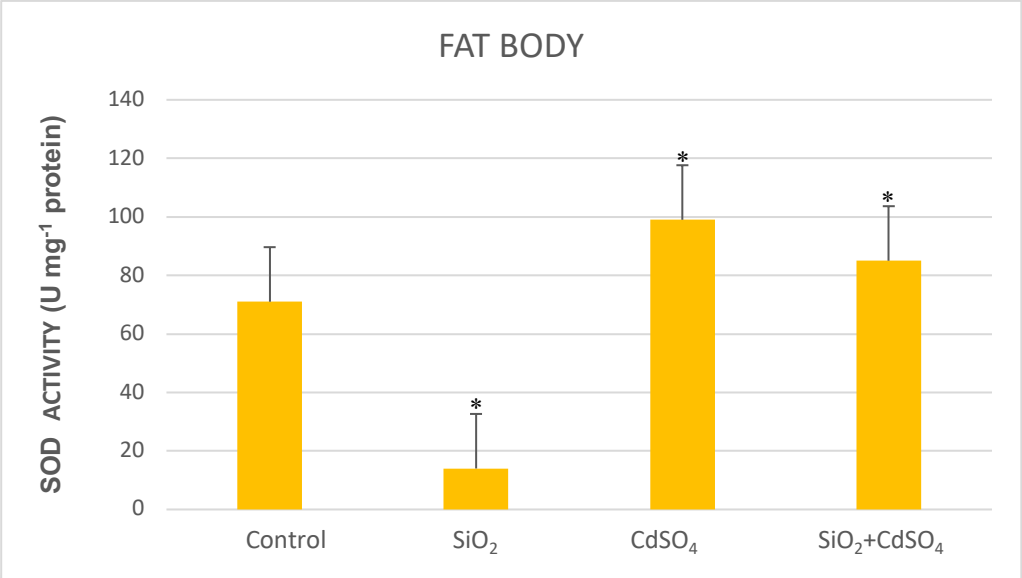

Figure 4.8. SOD enzyme activity in the fat body of *G. mellonella* larvae using SiO_2 NPs and, $CdSO_4$ singly and their mixtures

The effects of SiO_2 NPs and abamectin singly and in mixtures on the SOD enzyme activity in the midgut and fat body of *G. mellonella* are presented in Table 4.4 and shown in Figure 4.9 and Figure 4.10. There was a significant increases in SOD activity in the midgut in the abamectin applied group compared to the control (1.8-fold), while a decrease was observed in the mixture group (2.5-fold) ($p<0.05$). As for fat body, decreases in SOD activity were detected in the abamectin and mixture applied groups compared to the control (1.66-fold and 1.53-fold, respectively) ($p<0.05$).

Table 4.4. SOD activity of SiO_2 NPs and Abamectin singly and their mixtures of *G. mellonella* larvae (Mean ±Standard Error;) *

	SOD ACTIVITY (U mg^{-1} protein) ($\overline{X} \pm s\overline{x}$)*	
Concentration(µg/mL)	**Midgut**	**Fat Body**
Control	71,28 ± 0,01 a	71,15 ± 0,01 a
SiO_2	56,98 ± 0,03 b	14,22 ± 0,00 b
Abamectin	128,40 ± 0,02 c	42,75 ± 0,00 c
SiO_2 + Abamectin	28,50 ± 0,01 d	46,47 ± 2,07 d

*SNK indicates that there is a statistically notable variation ($P<0.05$) between the data given with different letters.

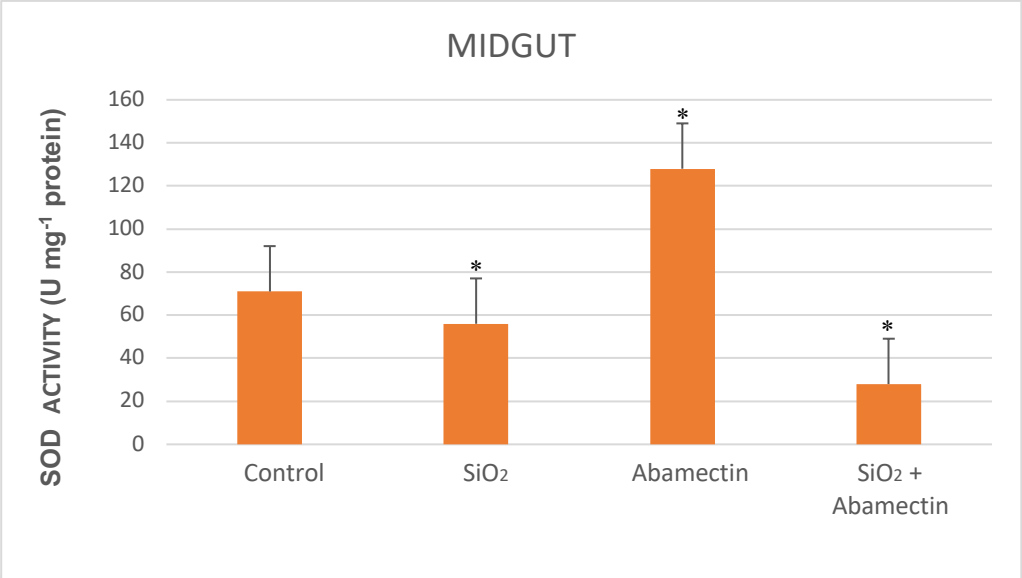

Figure 4.9. SOD enzyme activity in the midgut of *G. mellonella* larvae using SiO_2 NPs and Abamectin singly and their mixtures

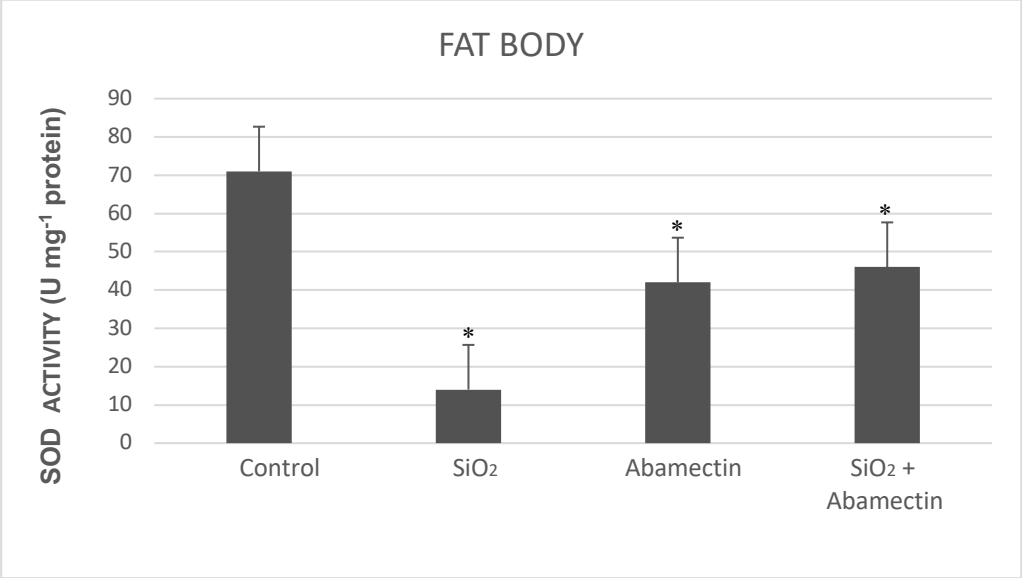

Figure 4.10. SOD enzyme activity in the fat body of *G. mellonella* larvae using SiO_2 NPs and Abamectin singly and their mixtures

4.1.2.3. GPx Enzyme Activity

The effects of SiO_2 NPs and $CdSO_4$ singly and in mixtures on GPx enzyme activity in the midgut and fat body of *G. mellonella* are presented in Table 4.5 and shown in Figure 4.11 and Figure 4.12. There was a decrease in GPx activity in the midgut in the SiO_2 NPs applied groups compared to the control (2.13-fold), but an increase was observed in the mixture applied group

(2.9-fold) ($p<0.05$). As for fat body, a significant decrease in GPx activity was only detected in the $CdSO_4$ applied group compared to the control (1.76-fold) ($p<0.05$).

Table 4.5. GPx activity of SiO_2 NPs and $CdSO_4$ singly and mixtures of *G. mellonella* larvae (Mean ±Standard Error;) *

	GPx ACTIVITY (nmol dk^{-1} mg^{-1} protein) ($\overline{X} \pm s\bar{x}$)*	
Concentration(µg/mL)	**Midgut**	**Fat body**
Control	7,33 ± 0,76 a	35,28 ± 1,46 a
SiO_2	15,63 ± 0,36 b	36,87 ± 2,01 a
$CdSO_4$	6,92 ± 0,27 a	20,07 ± 0,73 b
$SiO_2 + CdSO_4$	21,23 ± 0,82 c	34,33 ± 0,54 a

*SNK indicates that there is a statistically notable variation ($P<0.05$) between the data given with different letters.

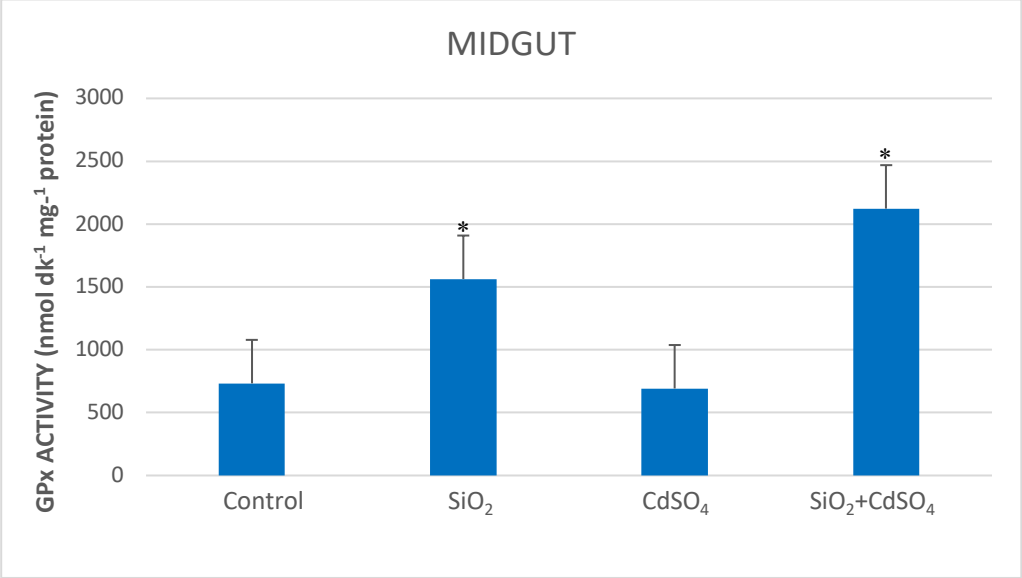

Figure 4.11. GPx activity in the midgut of *G. mellonella* larvae using SiO_2 NPs and $CdSO_4$ singly and their mixtures.

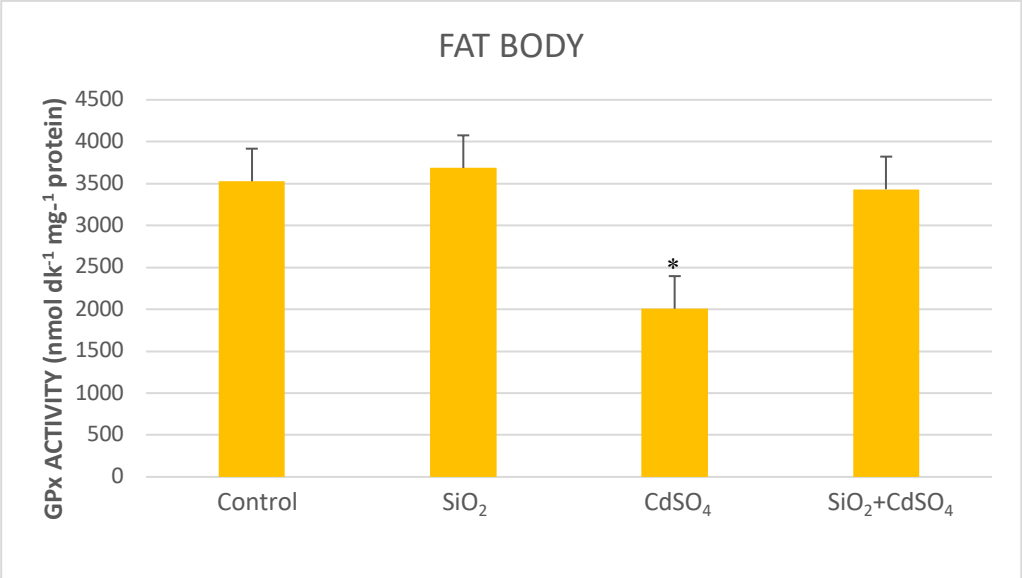

Figure 4.12. GPx activity in the fat body of *G. mellonella* larvae using SiO_2 NPs and $CdSO_4$ singly and their mixtures.

The effects of SiO_2 NPs and abamectin singly and in mixtures on the GPx activity in the midgut and fat body of *G. mellonella* are presented in Table 4.6 and shown in Figure 4.13 and Figure 4.14. There was a significant increase in GPx activity in the midgut in the mixture applied group compared to the control (4.68-fold). As for fat body, significant decreases in GPx activity were detected in the Abamectin and mixture applied groups compared to the control (1.40-fold and 1.39-fold, respectively) ($p<0.05$).

Table 4.6. GPx activity of SiO_2 NPs and Abamectin singly and mixtures of *G. mellonella* larvae (Mean ±Standard Error;) *

	GPx ACTIVITY (nmol dk^{-1} mg-^{1} protein) ($\overline{X} \pm s\overline{x}$)*	
Concentration(µg/mL)	**Midgut**	**Fat Body**
Control	7,33 ± 0,76 a	35,28 ± 1,46 a
SiO_2	15,63 ± 0,36 b	36,87 ± 2,01 a
Abamectin	6,76 ± 0,36 a	25,14 ± 0,73 b
SiO_2 + Abamectin	34,33 ± 0,54 c	25,46 ± 1,64 b

*SNK indicates that there is a statistically notable variation ($P<0.05$) between the data given with different letters.

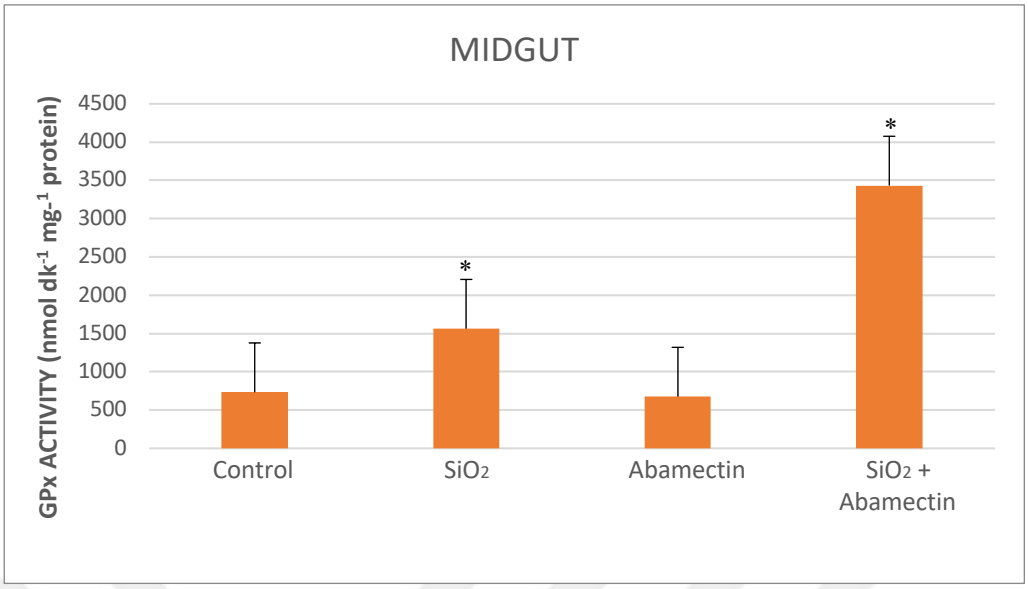

Figure 4.13. GPx activity in the midgut of *G. mellonella* larvae using SiO_2 NPs and Abamectin singly and their mixtures.

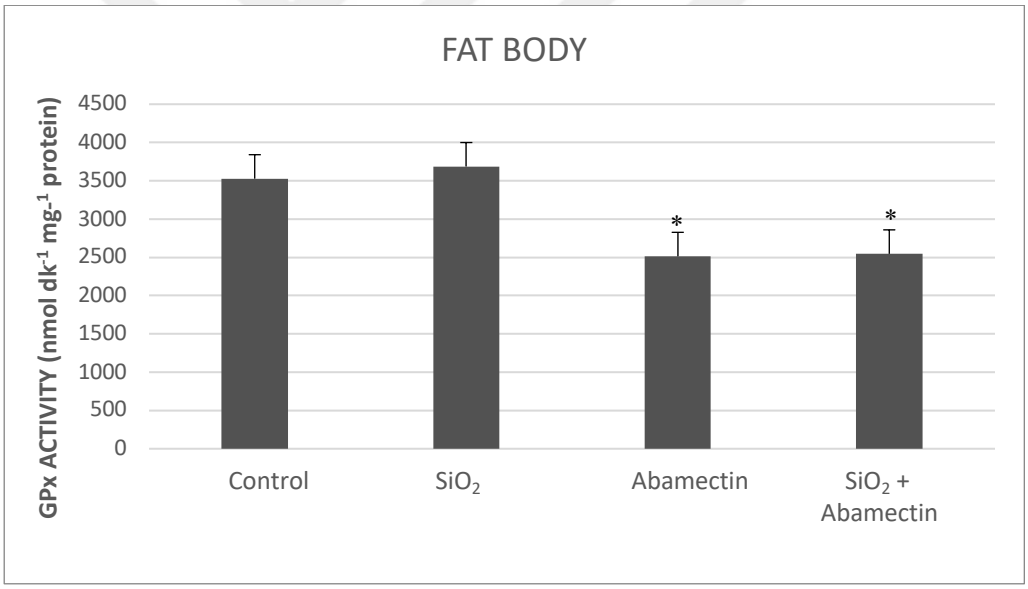

Figure 4.14. GPx activity in the fat body of *G. mellonella* larvae using SiO_2 NPs and Abamectin singly and their mixtures

4.1.3. Determination of Detoxification Enzyme Activities

4.1.3.1. AChe Enzyme Activity

The effects of SiO_2 NPs and $CdSO_4$ singly and in mixtures on AChE enzyme activity in the midgut and fat body of *G. mellonella* are presented in Table 4.7 and shown in Figure 4.15 and Figure 4.16. Increases were observed in all applied groups according to the control (4.51-fold, 1.92-fold and 4.51-fold, respectively) ($p<0.05$). As for fat body, an increase was only detected in the mixture applied group and this increase resulted in 3.50-fold ($p<0.05$).

Table 4.7. AChE activity of *G. mellonella* larvae with SiO_2 NPs and $CdSO_4$ singly and their mixtures (Mean ±Standard Error;) *

	AChE ACTIVITY (U/dk/mg protein)	
Concentration(µg/mL)	**Midgut ($\overline{X} \pm s\overline{x}$)***	**Fat body($\overline{X} \pm s\overline{x}$)***
Control	5,65 ± 0,00 a	5,71 ± 0,00 a
SiO_2	25,46 ± 1,63 b	8,57 ± 1,65 a
$CdSO_4$	10,86 ± 0,37 c	5,71 ± 0,00 a
$SiO_2 + CdSO_4$	25,46 ± 1,63 b	20,00 ± 1,65 b

*SNK indicates that there is a statistically notable variation ($P<0.05$) between the data given with different letters

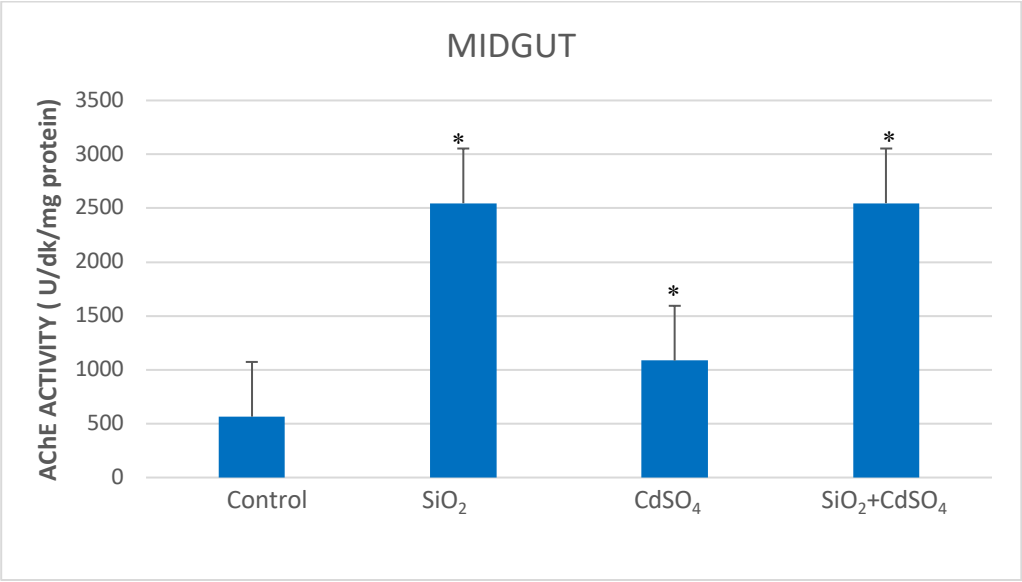

Figure 4.15. AChE activity in the midgut of *G. mellonella* larvae using SiO_2 NPs and $CdSO_4$ singly and their mixtures.

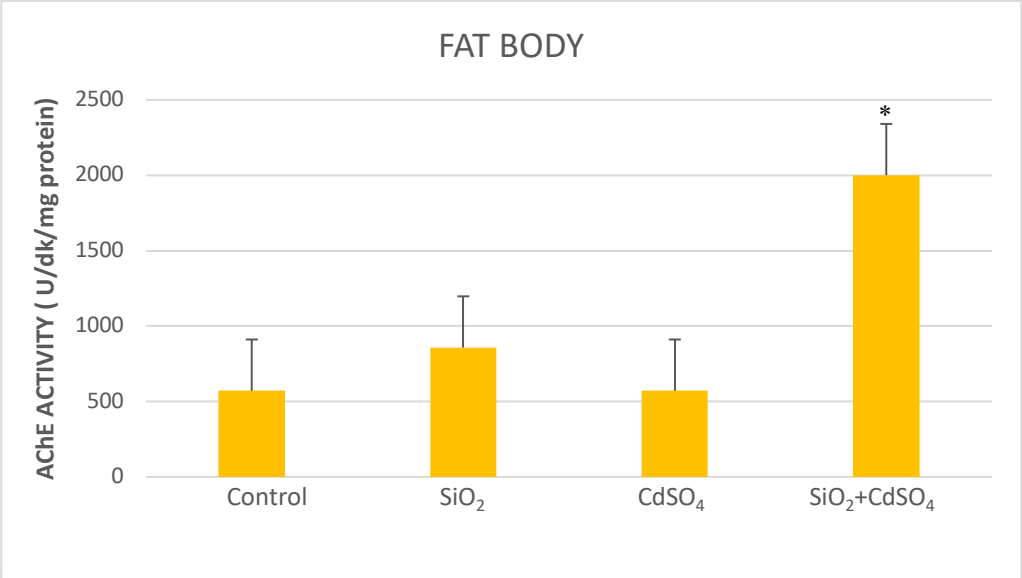

Figure 4.16. AChE activity in the fat body of *G. mellonella* larvae using SiO_2 NPs and $CdSO_4$ singly and their mixtures

The effects of SiO_2 NPs and Abamectin singly and in mixtures on AChE enzyme activity in the midgut and fat body of *G. mellonella* are presented in Table 4.8 and shown in Figure 4.17. and Figure 4.18. Increases in AChE activity in fat body were detected in the abamectin and abamectin with SiO_2 NPs applied groups compared to the control (both 2.50-fold) ($p<0.05$).

Table 4.8. AChE activity of SiO_2 NPs and Abamectin singly and in mixtures of *G. mellonella* larvae (Mean ±Standard Error;) *

	AChE ACTIVITY (U/dk/mg protein) ($\overline{X} \pm s\bar{x}$)*	
Concentration(µg/mL)	**Midgut**	**Fat body**
Control	5,65 ± 0,00 a	5,71 ± 0,00 a
SiO_2	25,46 ± 1,63 b	8,57 ± 1,65 ab
Abamectin	5,66 ± 0,00 a	14,27 ± 1,65 b
SiO_2 + Abamectin	5,65 ± 0,00 a	14,28 ± 1,64 b

*SNK indicates that there is a statistically notable variation ($P<0.05$) between the data given with different letters.

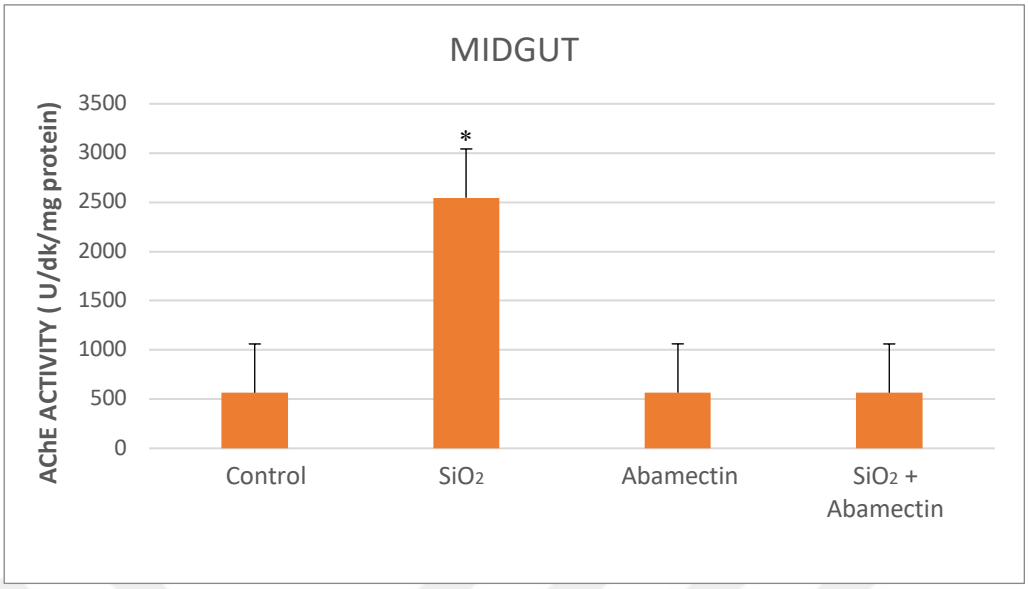

Figure 4.17. AChE activity in the midgut of *G. mellonella* larvae using SiO_2 NPs and Abamectin singly and their mixtures

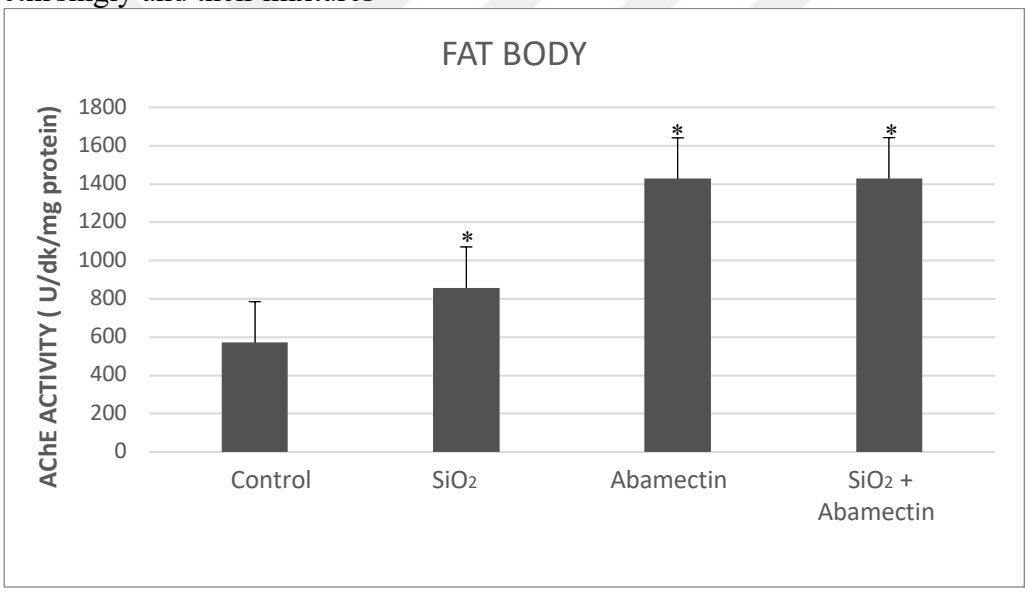

Figure 4.18. AChE activity in the fat body of *G. mellonella* larvae using SiO_2 NPs and Abamectin singly and their mixtures

4.1.3.2. CYT P450 Enzyme Activity

The effects of SiO_2 NPs and $CdSO_4$ singly and in mixtures on the Cyt P450 enzyme activity in the midgut and fat body of *G. mellonella* are presented in Table 4.9 and shown in Figures 4.19 and 4.20. There were decreases in Cyt P450 enzyme activity in the midgut in all applied groups compared to the control (1.32-fold, 1.41-fold nd 1.37-fold, respectively; $p<0.05$), while

increases were detected in fat body in all applied groups compared to the control (1.06-fold, 1.14-fold and 1.11-fold, respectively; $p<0.05$).

Table 4.9. CYT P450 activity of *G. mellonella* larvae with SiO_2 NPs and $CdSO_4$ singly and their mixtures (Mean ±Standard Error;) *

	CYT P450 ACTIVITY (pmol/dk/mg protein) ($\overline{X} \pm s\overline{x}$)*	
Concentration (µg/mL)	**Midgut**	**Fat body**
Control	230,59 ± 0,73 a	149,69 ± 0,13 a
SiO_2	174,22 ± 4,96 b	159,04 ± 1,59 b
$CdSO_4$	163,70 ± 2,53 b	170,20 ± 1,08 c
$SiO_2 + CdSO_4$	168,52 ± 0,68 b	165,70 ± 1,42 d

*SNK indicates that there is a statistically notable variation ($P<0.05$) between the data given with different letters.

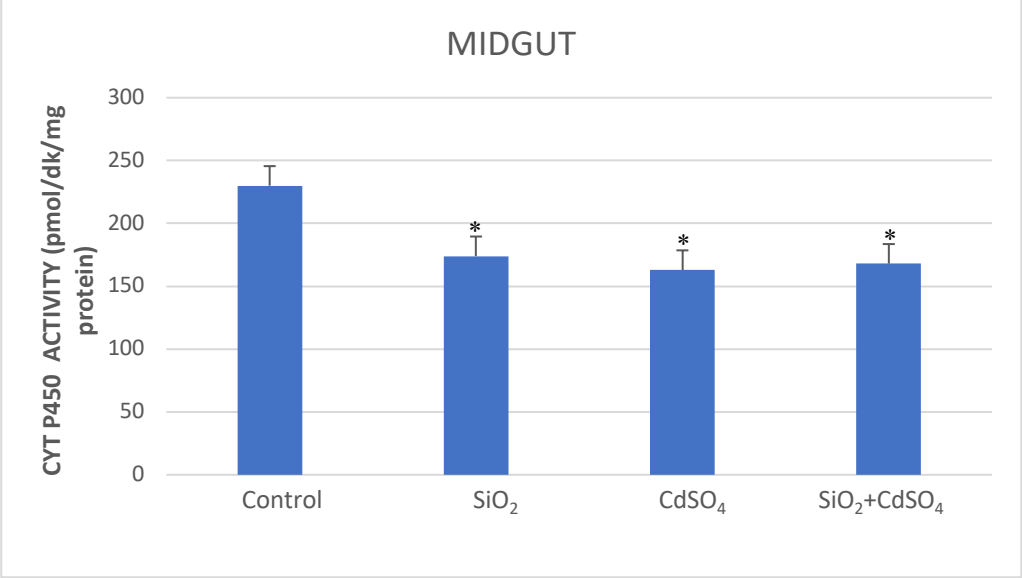

Figure 4.19. Cyt P450 activity in the midgut of *G. mellonella* larvae using SiO_2 NPs and $CdSO_4$ singly and their mixtures

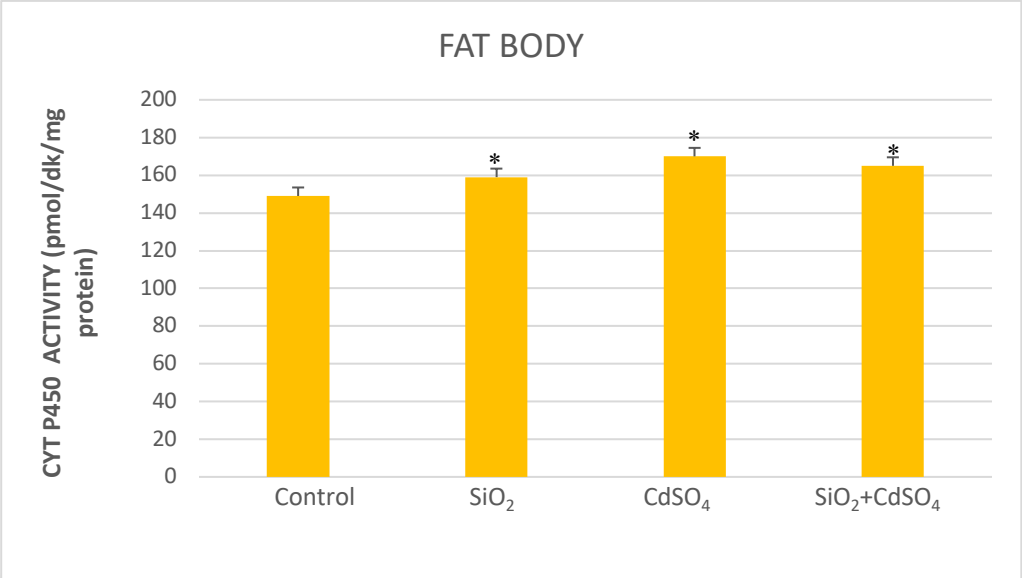

Figure 4.20. Cyt P450 activity in the fat body of *G. mellonella* larvae using SiO2 NPs and $CdSO_4$ singly and their mixtures

The effects of SiO_2 NPs and Abamectin singly and in mixtures on the Cyt P450 enzyme activity in the midgut and fat body of *G. mellonella* are presented in Table 4.10 and shown in Figures 4.21 and 4.22. There were decreases in Cyt P450 enzyme activity in the midgut in all applied groups compared to the control (1.32-fold, 1.04-fold and 1.44-fold, respectively; $p<0.05$). On the other hand, increases in fat body were detected in all applied groups compared to the control (1.06-fold, 1.21-fold and 1.13-fold, respectively; $p<0.05$).

Table 4.10. CYT P450 activity of SiO_2 NPs and Abamectin singly and their mixtures of *G. mellonella* larvae (Mean ±Standard Error;)*

	CYT P450 ACTIVITY (pmol/dk/mg protein) ($\overline{X} \pm s\overline{x}$)*	
Concentration (µg/mL)	**Midgut**	**Fat Body**
Control	230,59 ± 0,73 a	149,69 ± 0,13 a
SiO_2	174,22 ± 4,96 b	159,04 ± 1,59 b
Abamectin	225,15 ± 6,40 c	181,39 ± 0,00 c
SiO_2 + Abamectin	160,07 ± 1,25 d	169,83 ± 0,06 d

*SNK indicates that there is a statistically notable variation (P<0.05) between the data given with different letters.

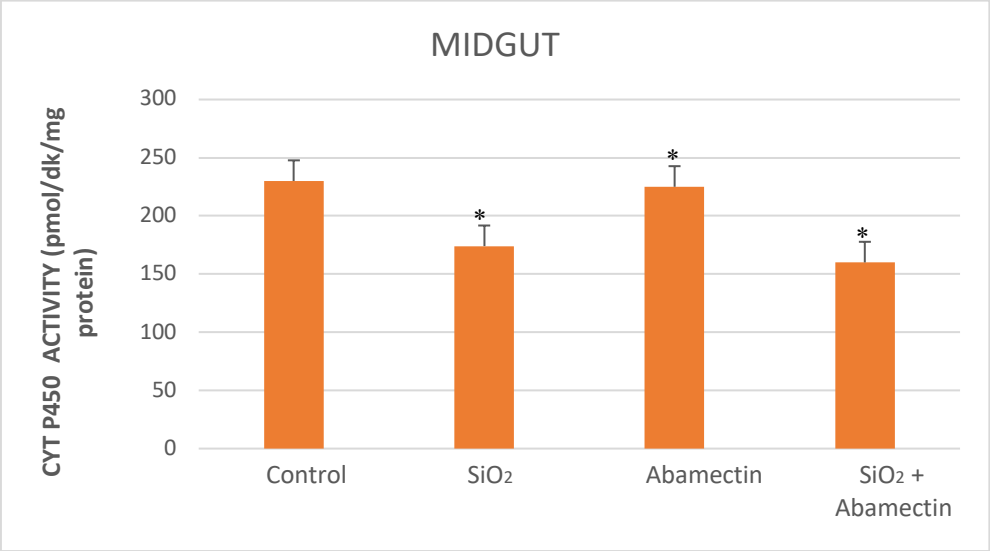

Figure 4.21. Cyt P450 activity in the midgut of *G. mellonella* larvae using SiO_2 NPs and Abamectin singly and their mixtures

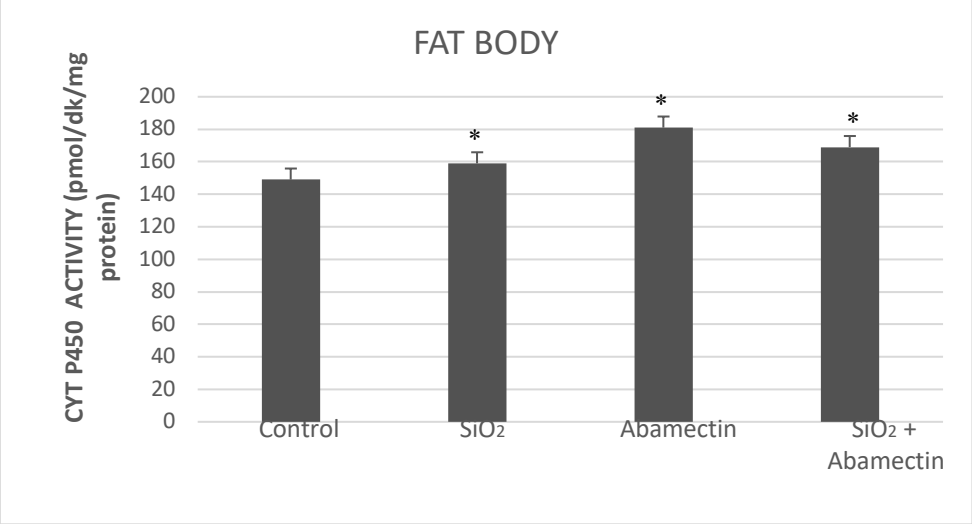

Figure 4.22. Cyt P450 activity in the fat body of *G. mellonella* larvae using SiO_2 NPs and Abamectin singly and their mixtures

4.1.3.3. GST Enzyme Activity

The effects of SiO_2 NPs and $CdSO_4$ singly and in mixture on the GST enzyme activity in the midgut and fat body of *G. mellonella* are presented in Table 4.11 and shown in Figures 4.23 and 4.24. Decreases in GST activity in the midgut were detected in all applied groups compared to the control (1.6-fold, 1.6-fold and 2-fold, respectively; $p<0.05$). Also, decreases in fat body were observed in all applied groups compared to the control (1.57-fold, 1.27-fold and 3.36-fold, respectively; $p<0.05$).

Table 4.11. GST activity of SiO_2 NPs and $CdSO_4$ singly and their mixtures of *G. mellonella* larvae (Mean ±Standard Error;) *

	GST ACTIVITY (mmol dk^{-1} mg^{-1} protein) ($\overline{X} \pm s\overline{x}$)*	
Concentration(µg/mL)	**Midgut**	**Fat body**
Control	0,08 ± 0,00 a	0,47 ± 0,01 a
SiO_2	0,05 ± 0,00 b	0,30 ± 0,00 b
$CdSO_4$	0,05 ± 0,00 b	0,37 ± 0,02 c
$SiO_2 + CdSO_4$	0,04 ± 0,00 b	0,14 ± 0,00 d

*SNK indicates that there is a statistically notable variation ($P<0.05$) between the data given with different letters.

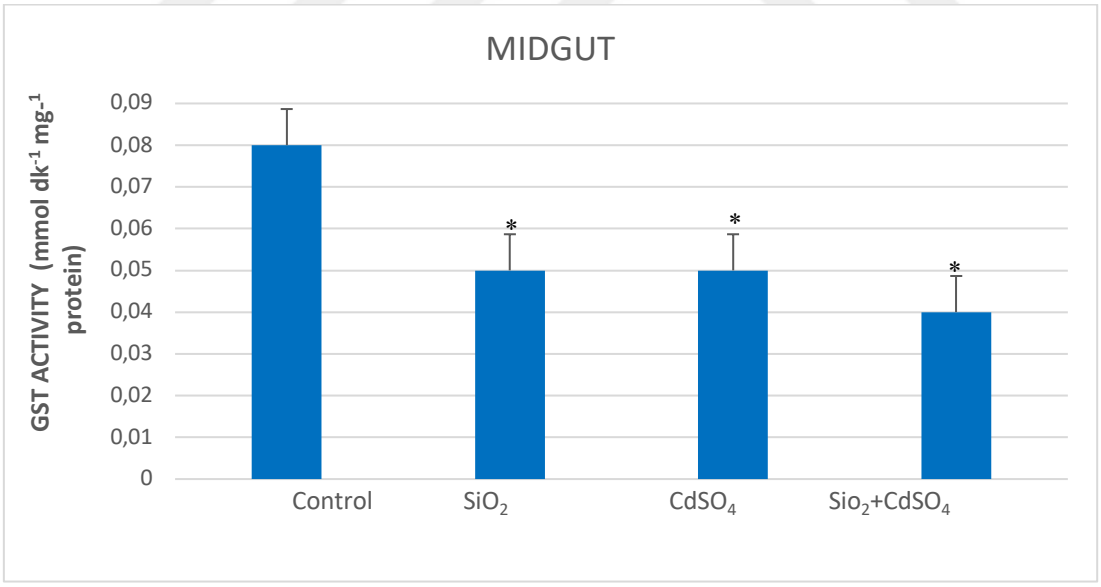

Figure 4.23. GST activity in the midgut of *G. mellonella* larvae using SiO_2 NPs and $CdSO_4$ singly and their mixtures

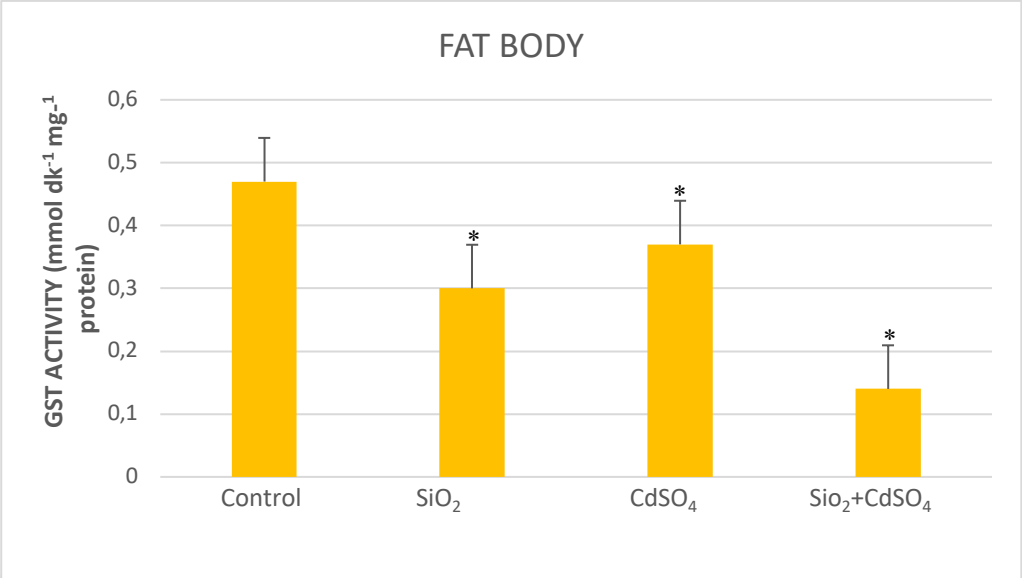

Figure 4.24. GST activity in the fat body of *G. mellonella* larvae using SiO_2 NPs and $CdSO_4$ singly and their mixtures

The effects of SiO_2 and Abamectin singly and in mixtures on the GST enzyme activity in the midgut and fat body of *G. mellonella* are presented in Table 4.12 and shown in Figures 4.25 and 4.26. Increases in GST activity in the midgut were detected in the abamectin and abamectin with SiO_2 NPs applied groups compared to the control (3.88-fold and 7.75-fold, respectively; $p<0.05$). A decrease in GST activity in fat body was observed in abamectin applied groups compared to the control and this decrease resulted in 1.27-fold ($p<0.05$).

Table 4.12. GST activity of SiO_2 NPs and Abamectin singly and their mixtures of *G. mellonella* larvae (Mean ±Standard Error;) *

	GST ACTIVITY (mmol dk^{-1} mg^{-1} protein) ($\overline{X} \pm s\bar{x}$)*	
Concentration(µg/mL)	**Midgut**	**Fat body**
Control	0,08 ± 0,00 a	0,47 ± 0,01 a
SiO_2	0,05 ± 0,00 b	0,30 ± 0,00 b
Abamectin	0,31 ± 0,00 c	0,37 ± 0,01 c
SiO_2 + Abamectin	0,62 ± 0,01 d	0,44 ± 0,01 a

*SNK indicates that there is a statistically notable variation ($P<0.05$) between the data given with different letters.

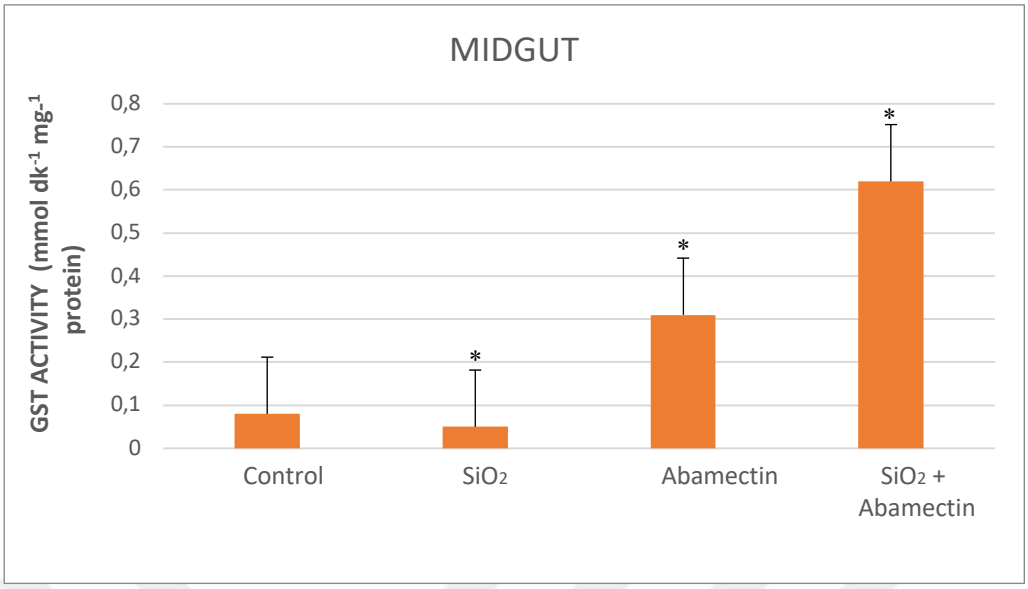

Figure 4.25. GST activity in the midgut of *G. mellonella* larvae using SiO_2 NPs and Abamectin singly and their mixtures

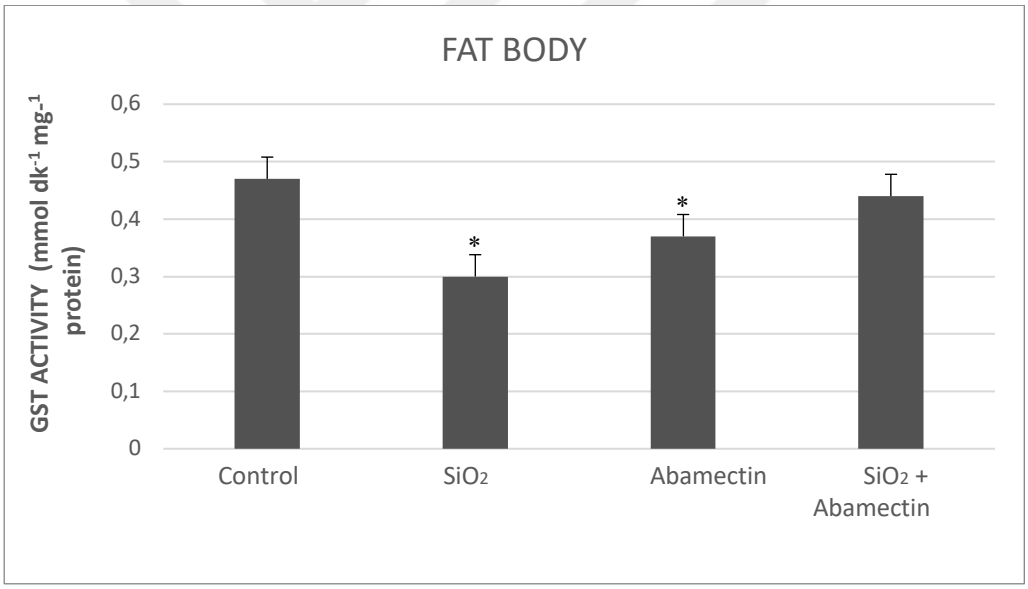

Figure 4.26. GST activity in the fat body of *G. mellonella* larvae using SiO_2 NPs and Abamectin singly and their mixtures

4.1.4. Total Hemocyte Count

Effects of SiO_2 and $CdSO_4$ singly and in mixtures on total hemocyte counts of *G. mellonella* last instar larvae are shown in Table 4.13 and Figure 4.27. There was a significant decrease in SiO_2 and $CdSO_4$ singly applied groups compared to the control. However, an increase was detected in the mixture applied group compared to the control ($p<0.05$).

Table 4.13. Total hemocyte count in hemolymph of *G. mellonella* larvae using SiO_2 and $CdSO_4$ singly and their mixtures (Mean ±Standard Error;) *

Concentration(µg/mL)	Total Hemocyte Count ($\overline{X} \pm s\overline{x}$) *
Control	1175,00 ± 14,43 a
SiO_2	725,00 ± 14,43 b
$CdSO_4$	900,00 ± 28,86 c
$SiO_2 + CdSO_4$	1725,00 ± 14,43 d

*SNK indicates that there is a statistically notable variation ($P<0.05$) between the data given with different letters.

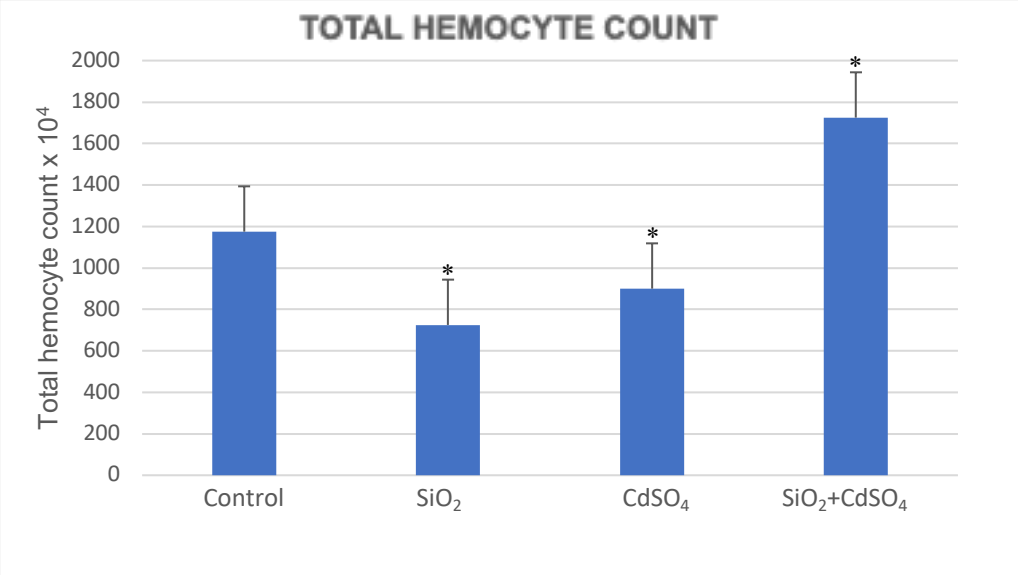

Figure 4.27. Total count of hemocytes in the hemolymph of *G. mellonella* larvae

Effects of SiO_2 and Abamectin singly and in mixtures on total hemocyte counts of *G. mellonella* are shown Table 4.14 and Figure in 4.28. Significant decreases occurred in all applied groups compared to the control ($p<0.05$).

Table 4.14. Differences in the total count of hemocytes of SiO_2 NPs and Abamectin singly and their mixtures in the hemolymph of *G. mellonella* larvae (Mean ±Standard Error;) *

Concentration(µg/mL)	Total Hemocyte Count ($\overline{X} \pm s\overline{x}$) *
Control	1175,00 ± 14,43 a
SiO_2	725,00 ± 14,43 b
Abamectin	1075,00 ± 14,43 c
SiO_2 + Abamectin	975,00 ± 14,43 d

*SNK indicates that there is a statistically notable variation ($P<0.05$) between the data given with different letters.

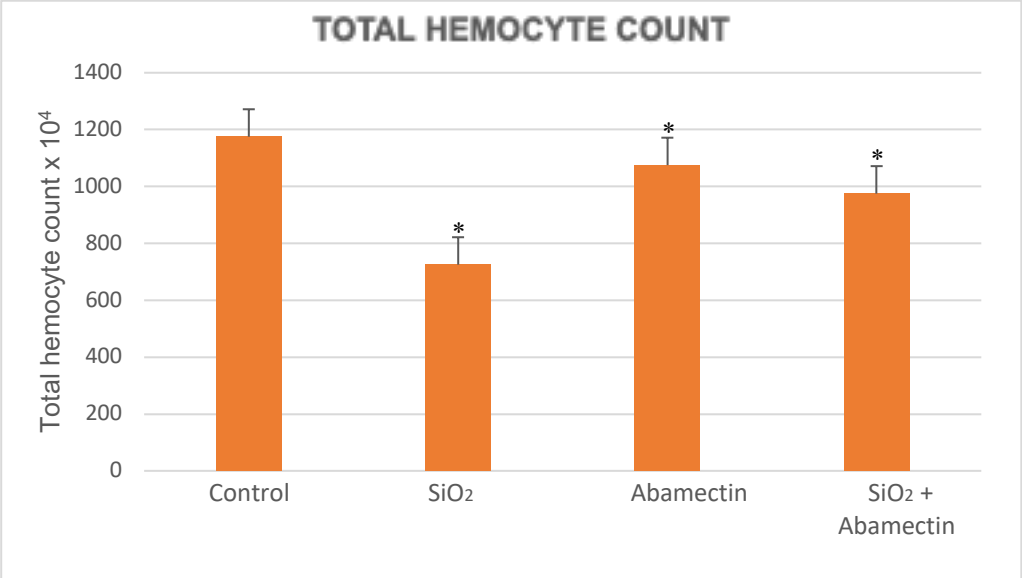

Figure 4.28. Total count of hemocytes in the hemolymph of *G. mellonella* larvae

4.1.5. Determination of Hemocyte Types of *G. mellonella* Larvae

Different kinds of hemocytes in *G. mellonella* last instar larvae were detected by examining preparations stained with Giemsa stain. It was observed that the detected hemocytes had light-dark blue cytoplasms and light-dark pink nuclei. Granulocyte, plasmatocyte, prohemocyte, oenocytoid and spherulocyte hemocyte types were determined by examining the larvae's hemocytes under a Leica DM750 light microscope.

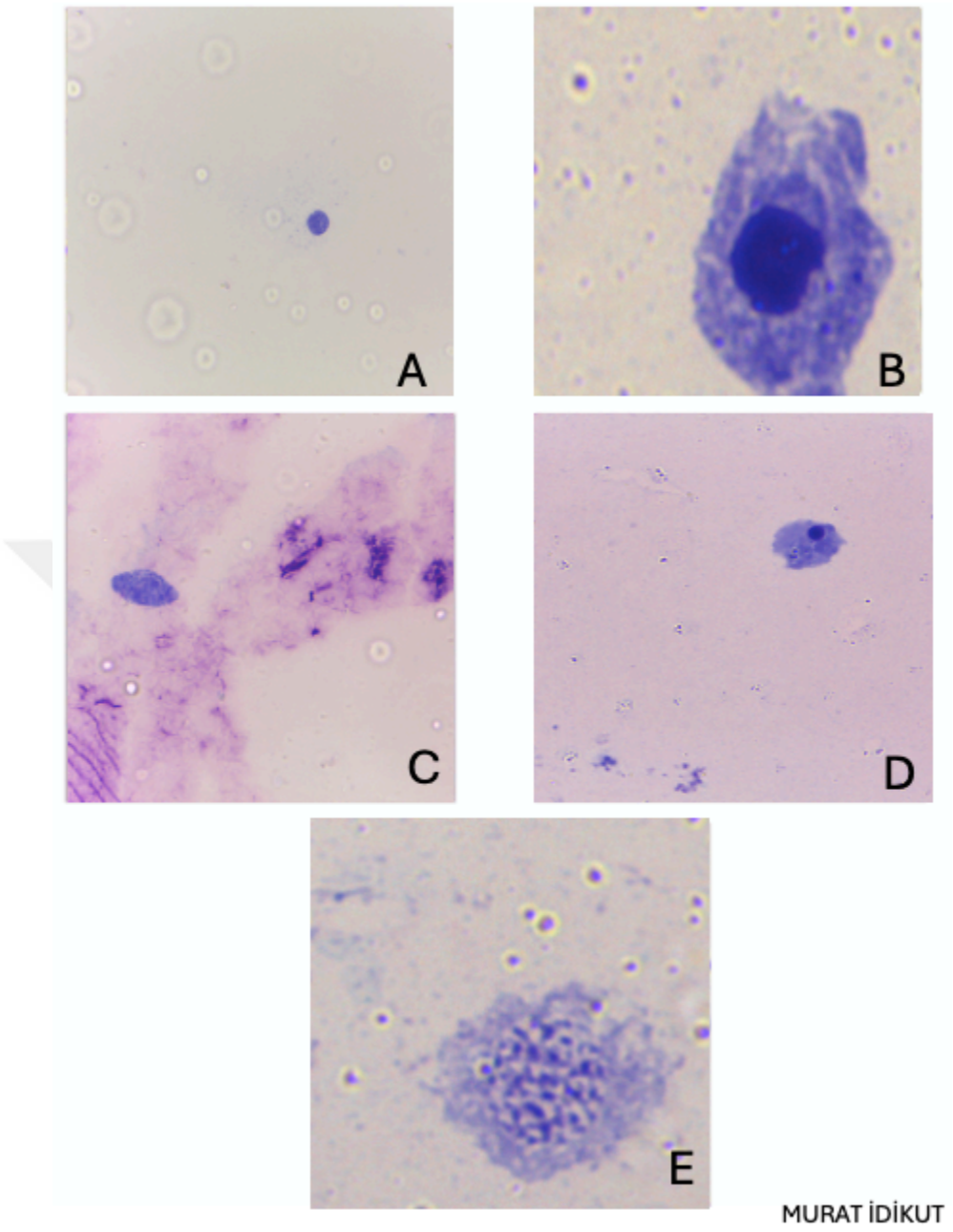

Figure 4.29. A: Prohemocyte cells, B: Granulocyte cells , C: Plasmatocyte cells, D:Eunocytoid cells, E:Spherulocyte cells of *G. mellonella* (Size Bar: 10 µm;X100)

4.1.6. Differential Hemocyte Count

The effects of SiO_2 NPs and $CdSO_4$ singly and their mixtures on the differential hemocyte count in the hemolymph of *G. mellonella* last instar larvae are shown in Table 4.15 and Figure 4.30. In plasmatocyte count, increases were observed in SiO_2 NPs and $CdSO_4$ singly applied groups, while a significant decrease was detected in mixture applied group ($p<0.05$). As for prohemocyte count, decreases were observed in SiO_2 NPs and $CdSO_4$ singly applied groups, while a significant increase was detected in mixture applied group ($p<0.05$). A decrease in granulocyte counts were observed in all applied groups compared to the control ($p<0.05$). When the spherulocyte counts were compared to the control, there was an increase in the application groups of $CdSO_4$ and mixture, but a decrease was observed in the SiO_2 group. When the oenocytoid counts were examined, an increase was detected in the $CdSO_4$ applied group compared to the control ($p<0.05$).

Table 4.15. Differences in the count of differential hemocytes of SiO_2 NPs and $CdSO_4$ singly and their mixtures in the hemolymph of *G. mellonella* larvae (Mean ±Standard Error;)($\overline{X} \pm s\overline{x}$) *

Concentration (µg/mL)	Plasmatocytes	Prohemocytes	Granulocyte	spherulocyte	Oenocytoids
Control	576,66 ± 7,51 a	279,00 ± 7,21 a	149,66 ± 4,91 a	21,00 ± 0,57 a	0,00 ± 0,00 a
SiO_2	728,66 ± 2,02 b	234,00 ± 8,08 b	70,00 ± 1,15 b	18,66 ± 0,33 b	0,00 ± 0,00 a
$CdSO_4$	652,00 ± 7,50 c	141,00 ± 2,30 c	139,66 ±1,45 c	23,66 ± 0,88 c	23,00 ± 0,57 b
$SiO_2 + CdSO_4$	567,00 ± 16,16 a	303,66 ± 2,60 d	77,66 ± 1,45 b	72,66 ± 0,33 d	0,00 ± 0,00 a

*SNK indicates that there is a statistically notable variation ($P<0.05$) between the data given with different letters.

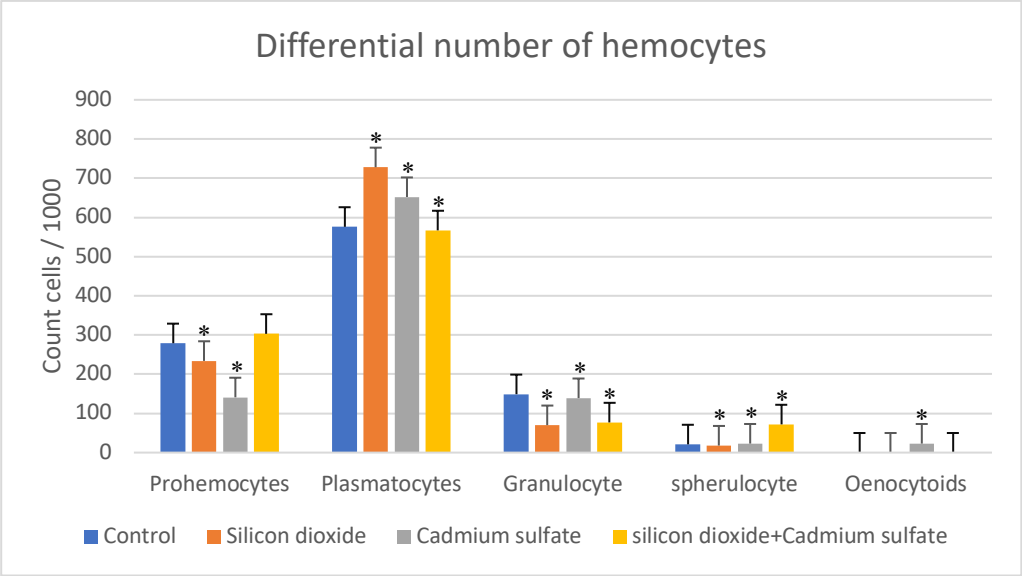

Figure 4.30. Differential hemocyte counts of *G. mellonella* larvae exposed to SiO_2 NPs and $CdSO_4$ singly and in mixtures

The effects of SiO_2 NPs and abamectin singly and their mixtures on the differential hemocyte counts in the hemolymph of *G. mellonella* last instar larvae are shown in Table 4.16 and Figure 4.31. In plasmatocyte count, it was detected that increases were observed in all applied groups compared to the control, otherwise in prohemocyte count decreases were detected in all applied groups ($p<0.05$). Also, decreases were observed in granulocye count in all applied groups ($p<0.05$). On the other hand, in spherulocyte count there are increases in abamectin and mixture groups, while spehurolcyte count decreased in SiO_2 NPs applied groups ($p<0.05$). In oenocytoid count, increases were only detected in abamectin and mixture groups ($p<0.05$).

Table 4.16. Differences in the count of differential hemocytes of SiO_2 NPs and Abamectin singly and their mixtures in the hemolymph of *G. mellonella* larvae (Mean ±Standard Error;) ($\overline{X} \pm s\overline{x}$) *

Concentration (µg/mL)	Plasmatocytes	Prohemocytes	Granulocyte	spherulocyte	Oenocytoids
Control	576,66 ± 7,51 a	279,00 ± 7,21 a	149,66 ± 4,91 a	21,00 ± 0,57 a	0,00 ± 0,00 a
SiO_2	728,66 ± 2,02 b	234,00 ± 8,08 b	70,00 ± 1,15 b	18,66 ± 0,33 b	0,00 ± 0,00 a
Abamectin	663,00 ± 3,46 c	161,66 ± 4,05 c	140,00 ±1,15 c	49,00 ± 0,57 c	12,00 ± 0,57 b
SiO_2+Abamectin	649,66 ± 10,10 c	258,33 ± 10,98 ab	137,66 ± 2,60 c	73,00 ± 0,57 d	23,66 ± 0,88 c

*SNK indicates that there is a statistically notable variation ($P<0.05$) between the data given with different letters.

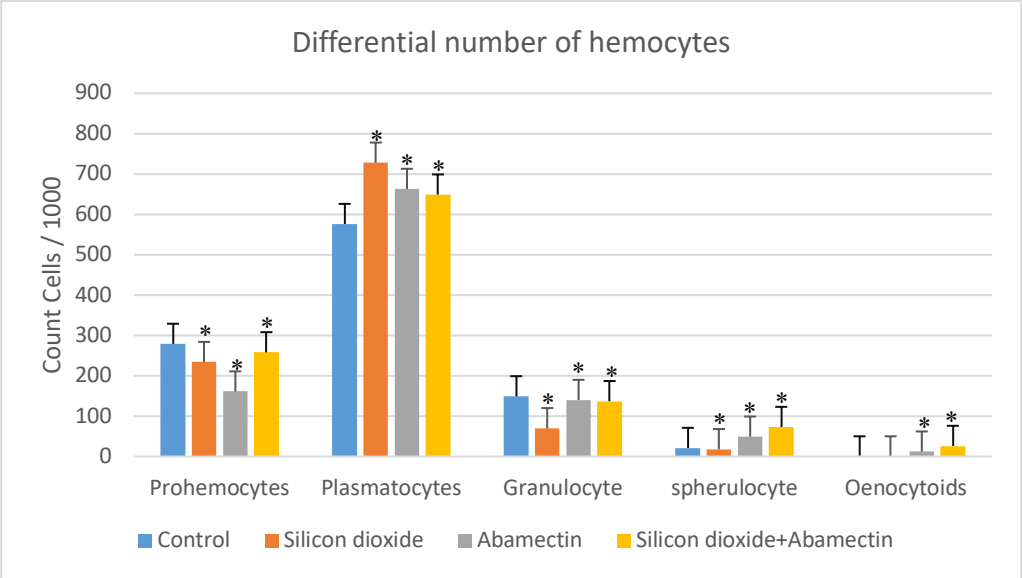

Figure 4.31. Differential hemocyte counts of *G. mellonella* larvae exposed to SiO_2 NPs and abamectin singly and in mixtures

4.1.7. Determination of Phenoloxidase Enzyme Activity in *G. mellonella* Larvae

The effects of SiO_2 NPs and $CdSO_4$ singly and in mixture on the phenoloxidase enzyme activity in the hemolymph of *G. mellonella* last instar larvae are presented in Table 4.17 and Figure 4.32. In the study, increases occurred in applied groups compared to the control and these increases resulted in 2.82-fold, 2.18-fold and 2.73-fold, respectively ($p<0.05$).

Table 4.17. Phenoloxidase Activity of SiO_2 NPs and $CdSO_4$ singly and their mixtures in the hemolymph of *G. mellonella* larvae (Mean ±Standard Error;)* ($\overline{X} \pm s\overline{x}$) *

Concentration (µg/mL)	Phenoloxidase Activity
Control	51,55 ± 2,70 a
SiO_2	145,22 ± 2,71 b
$CdSO_4$	112,49 ± 0,02 c
$SiO_2 + CdSO_4$	140,49 ± 0,00 b

*SNK indicates that there is a statistically notable variation ($P<0.05$) between the data given with different letters.

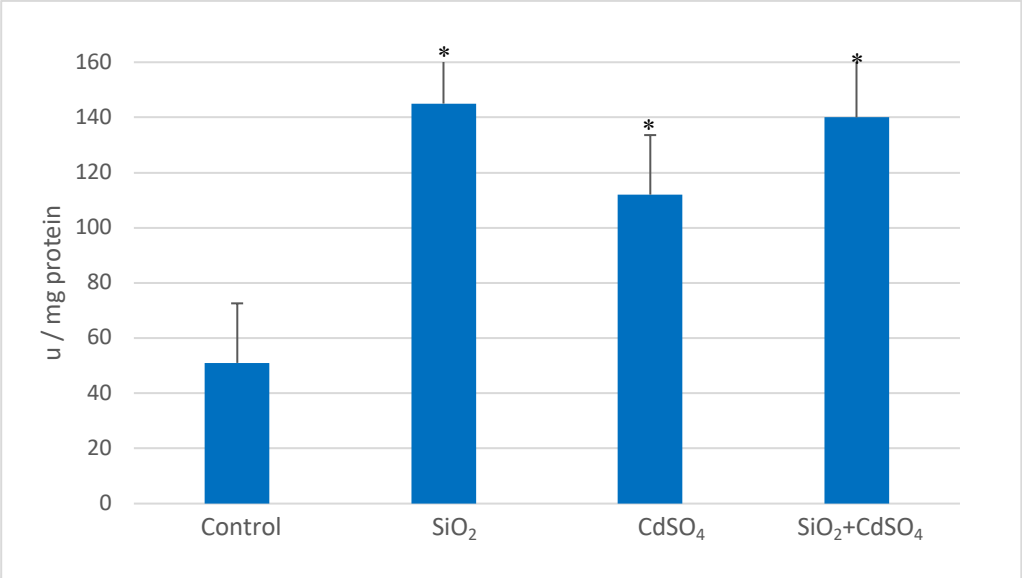

Figure 4.32. Phenoloxidase Activity in the hemolymph of *G. mellonella* larvae

The effects of SiO_2 NPs and Abamectin singly and in mixture on the phenoloxidase enzyme activity in the hemolymph of *G. mellonella* are presented in Table 4.18 and Figure 4.33. In the study, an increase occurred in the SiO_2, Abamectin, SiO_2 + Abamectin mixture application groups compared to the control group and was found to be statistically significant.

Table 4.18. Phenoloxidase Activity of SiO_2 NPs and Abamectin singly and their mixtures in the hemolymph of *G. mellonella* larvae (Mean ±Standard Error;) *$(\overline{X} \pm s\overline{x})$ *

Concentration(µg/mL)	**Phenoloxidase Activity**
Control	51,55 ± 2,70 a
SiO_2	145,22 ± 2,71 bc
Abamectin	134,18 ± 8,28 b
SiO_2 + Abamectin	161,46 ± 4,07 c

*SNK indicates that there is a statistically notable variation (P<0.05) between the data given with different letters.

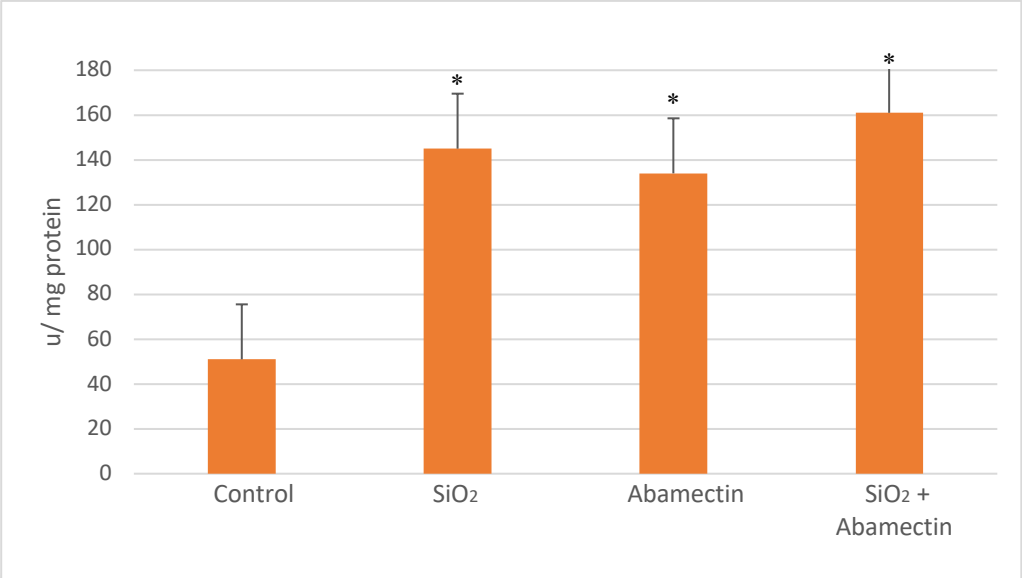

Figure 4.33. Phenoloxidase Activity in the Hemolymph of *G. mellonella* larvae

4.1.8. Determination of Apoptotic Index Amount in Larvae

In the hemolypmh of *G. mellonella* larvae, apoptotic index was determined according to the cells under microscope described below;
These cells are:

1. Live cells. The nucleus is green, while the cytoplasm might be orange or red.
2. Early apoptosis: The cell membrane remains intact, but chromatin condenses and fragments.
3. Late apoptosis is sometimes known as secondary necrosis or apoptotic necrosis. Ethidium Bromide penetrates cells with compromised membrane integrity and turns the nucleus orange.
4. Necrosis: the nucleus is orange.

The effects of SiO_2 NPs and $CdSO_4$ sinlgy and in mixtures on the apoptotic index of *G. mellonella* are presented in Table 4.19 and Figure 4.34. There were decreases in the count of live cells and early apoptosis in all applied groups (SiO_2, $CdSO_4$, SiO_2 + $CdSO_4$) compared to the control ($p<0.05$). Otherwise, significant increases in the count of late apoptosis was observed in all application groups compared to the control ($p<0.05$).

Table 4.19. Apoptotic Index of SiO_2 NPs and $CdSO_4$ singly and their mixtures in the hemolymph of *G. mellonella* larvae (Mean ±Standard Error;) * ($\overline{X} \pm s\overline{x}$)*

Concentration(µg/mL)	Live cells	Early Apoptosis	Late Apoptosis
Control	29,00 ± 0,57 a	265,00 ± 6,92 a	41,66 ± 4,33 a
SiO_2	2,33 ± 0,33 b	91,66 ± 1,45 b	213,66 ± 1,45 b
$CdSO_4$	0,00 ± 0,00 c	26,66 ± 0,88 c	272,66 ± 7,21 c
$SiO_2 + CdSO_4$	0,00 ± 0,00 c	12,66 ± 0,88 d	330,00 ± 6,24 d

*SNK indicates that there is a statistically notable variation (P<0.05) between the data given with different letters.

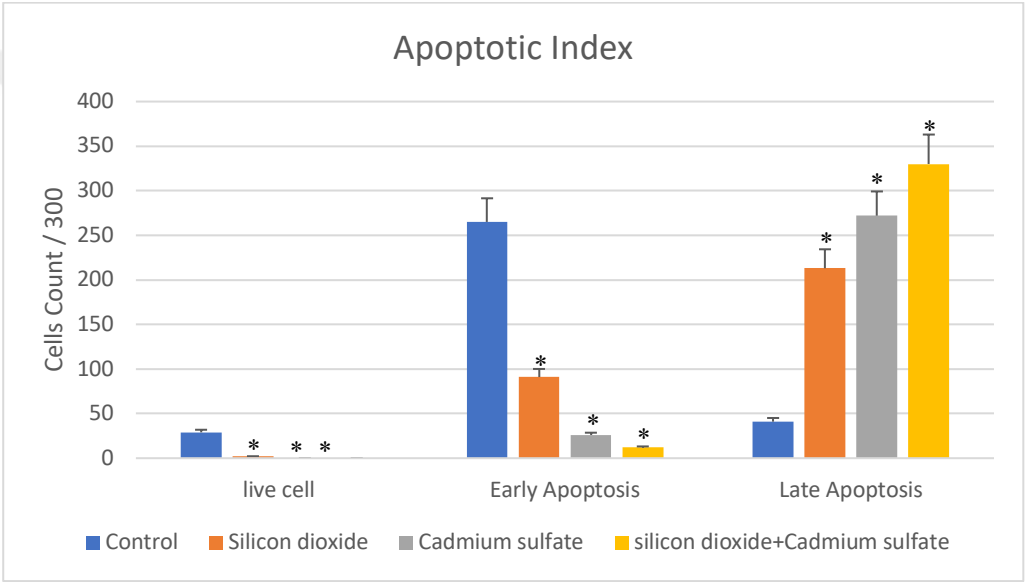

Figure 4.34. Apoptotic Index in the hemolymph of *G. mellonella* larvae

The effects of SiO_2 NPs and Abamectin singly and in mixtures on the apoptotic index in the hemolymph of *G. mellonella* last instar larvae are presented in Table 4.20. and Figure 4.35. There were decreases in the count of live cells and early apoptosis in all application groups (SiO_2, Abamectin and SiO_2 + Abamectin) compared to the control ($p<0.05$). On the other hand, significant increases in the count of late apoptosis were observed in all application groups compared to the control ($p<0.05$).

Table 4.20. Apoptotic Index Amount of SiO_2 NPs and Abamectin singly and their mixtures in the hemolymph of *G. mellonella* larvae (Mean ±Standard Error;)* ($\overline{X} \pm s\overline{x}$)*

Concentration(µg/mL)	Live cells	Early Apoptosis	Late Apoptosis
Control	29,00 ± 0,57 a	265,00 ± 6,92 a	41,66 ± 4,33 a
SiO_2	2,33 ± 0,33 b	91,66 ± 1,45 b	213,66 ± 1,45 b
Abamectin	0,00 ± 0,00 c	21,66 ± 1,45 c	292,00 ± 5,77 c
SiO_2 + Abamectin	0,00 ± 0,00 c	6,66 ± 0,33 d	372,00 ± 0,57 d

*SNK indicates that there is a statistically notable variation (P<0.05) between the data given with different letters.

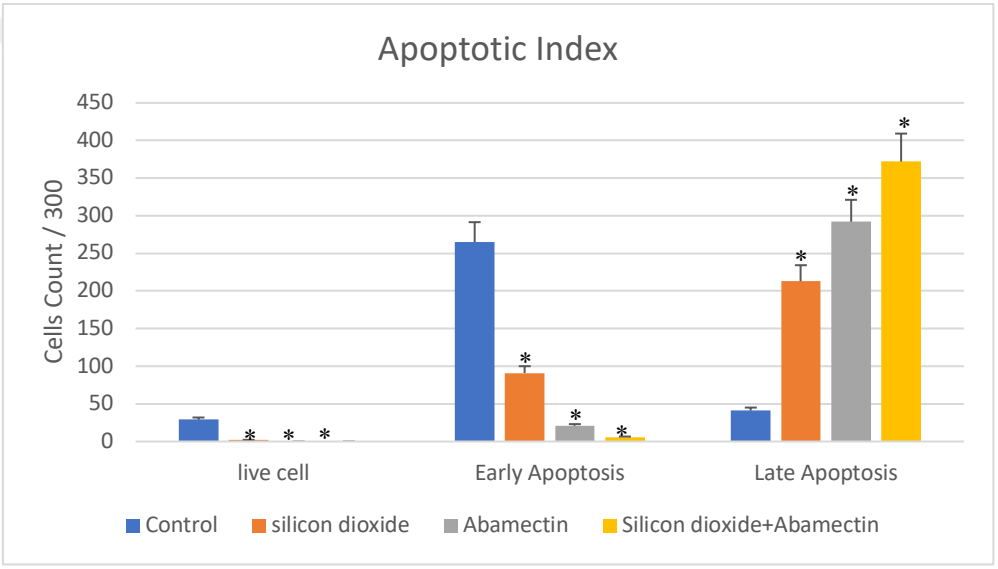

Figure 4.35. Apoptotic Index in the hemolymph of *G. mellonella* larvae

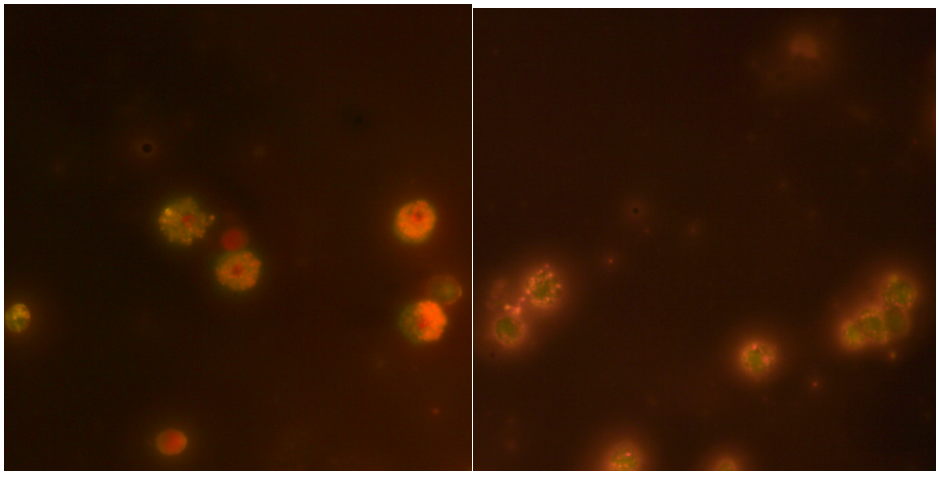

Figure 4.36. Apoptosis in the hemolymph of *G. mellonella* larvae

4.2. DISCUSSION

In recent years, the unintentional use of nanoparticles and heavy metals in a variety of settings, including industry and agriculture, has led to problems such as disruption of ecological balances and harm to non-target organisms. Furthermore, these nanoparticles, heavy metals, pesticides, and their interactions have the potential to produce significant issues in organismal systems. The impacts of SiO_2 NPs, $CdSO_4$, and abamectin on the antioxidant enzymes CAT, SOD and GPx, together with the detoxification enzymes Cyt P450, GST, and AChE activities in the midgut, and fat body were investigated. Moreover, the study looked at the changes in differential and total hemocyte counts, apoptotic index in the hemolymph by analyzing the immunological effects of silicon dioxide nanoparticles, $CdSO_4$, and abamectin singly and mixture applications on *G. mellonella* last instar larvae, both singly and in combination.

In insects, external factors like nanoparticles, chemicals such as heavy metals, pesticides, some microorganisms and radiation taken into the body outside of normal metabolic activities cause an increase in free radicals in the body and lead to oxidative stress. This stress causes damage to cellular components and affects cell processes. Free radicals damage macromolecules in cells and activate their defensive mechanisms. SOD, CAT, and GPx are antioxidants that act as the first defense in neutralizing any molecule that has the potential to become a free radical or that might cause the generation of more radicals (Ighodaro and Akinloye, 2018). The SOD enzyme catalyzes the conversion of the $O_2^{\cdot-}$ radical into H_2O_2 and O_2 (McCord and Fridovich, 1969). The resulting toxic hydrogen peroxide (H_2O_2) is transformed into water and oxygen by the action of the catalase enzyme, or into $OH^{\cdot}$ (hydroxyl) radical by the Fenton and Haber Weiss reaction in the presence of metal ions (Gomes, 2012). Catalase is more effective at higher concentrations of H_2O_2. In cases of lower H_2O_2 concentration, GPx is more effective (Duthie et al. 1989). In the study, it was detected that CAT activity increased in both mixture groups in midgut of the larvae. In fat body, CAT activity decreaed apart from SiO_2 NPs applied group. As for the SOD activity in the midgut, it was determined that there was a decrease in the groups in which SiO_2 NPs was applied singly and in mixture with abamectin, but an increase was observed in the groups in which $CdSO_4$ and abamectin were applied singly and SiO_2 NPs+ $CdSO_4$ mixture group. In fat body, it was also investigated that SiO_2 NPs application decreased in SOD activity and increased in the groups in which $CdSO_4$ was applied singly and in mixture

with SiO_2 NPs. When abamectin was applied singly and in mixture with SiO_2 NPs, it was determined that a decrease occurred in SOD activity in fat body. In GPx activity, it was determined that there was an increase in both groups in the midgut and a decrease in fat body. In the previous studies, Tuncsoy et al. (2021), found that TiO_2 and CuO NPs enhanced the total protein quantity and antioxidant enzyme activities in *G. mellonella*, indicating an increase in oxidative stress. In an another similar study, Emre (2013) detected that when *G. mellonella* was exposed to an environmental pollutant and developed oxidative stress. These induced cellular stress and damage by raising intracellular ROS levels. They showed that heavy metals like cadmium sulfate impair cellular redox equilibrium, resulting in increased oxidative stress.

The P450 enzymes are mainly involved in the first stage of the metabolism of xenobiotics, whereas the GST enzymes are involved in the second step and are responsible for the modification and conjugation of polar compounds. GST plays a role in protecting cellular integrity, preventing oxidative stress reactions and DNA damage by catalysing endogenous and exogenous xenobiotics (Mao et al. 2019). In the present study, AChE enzyme activies were increased exposed to SiO_2 NPs and $CdSO_4$ singly and in mixture in the midgut and fat body of the larvae. Also, in the fat body, AChE enzyme activities increased in the SiO_2 NPs and Abamectin singly and in mixture applied groups. As for GST activities, in all experimental groups GST activities were decreased apart from SiO_2 NPs and abamectin singly and in mixture applied groups. While Cyt P450 enzyme activities in the midgut were decreased in all applied groups, in the fat body it was increased. As a result, it was observed that SiO_2 NPs increased the toxic effects of both abamectin and Cd and led to alterations in detoxification enzymes, AChE, GST and Cyt P450.

Nanoparticles and heavy metals can accumulate in insects tissues, resulting in oxidative stress and cell membrane damage. It is known that environmental pollutants such as nanoparticlesi pesticides and heavy metal can be found together in the environment and might be more hazardous if they were singly. Mese et al. (2022), investigated the effects of Cu and Zn combinations on *G. mellonella*. Significant decreases in CAT activity were found in the Zn and mixture treated groups. The decreases were linked to the synergistic effects of metal combinations and elevated oxidative stress. Silica nanoparticles and other metal oxide nanoparticles can be incorporated into pesticide formulations, increasing their effectiveness in

combating insects. These nanoparticles can kill insects by directly inflicting physical damage or by causing oxidative damage. Moreover, it was also detected that silica nanoparticles can induce synergistic effects when applied together with heavy metals. Guo et al. (2013) determined that Cd accumulation in mouse liver increased as a result of Cd mixture with low concentrations of SiO_2 NPs and increased the hepatotoxic effect of cadmium. Moreover, Lu et al. (2015) applied SiO_2 NPs and lead in a mixture and as a result, it was determined that while cellular oxidative stress and DNA damage did not occur in lung adrenocarcinoma (A549) cells at the non-toxic concentration of silica nanoparticles singly, when applied in a mixture with lead, oxidative stress and DNA damage in cells increased compared to the application of lead singly. As a result, they reported that the mixture application produced a synergistic effect. In an another study regarding synergistic effects of SiO_2 NPs , Yang et al. (2018) reported that the effects of SiO_2 NPs and methyl mercury singly and in a mixture on human cardiac muscle cells (AC16) caused high toxic effects on cell viability and cell membrane damage. In addition, while ROS caused changes in MDA formation, it caused a decrease in SOD and GSH-Px activities. Moreover, they detected that it caused an increase in cellular apoptosis in heart muscle cells.
Invertebrates have been used as an important model organism in toxicity studies, especially in recent years, due to their ability to be intermediate consumers in food chains. Mostly, the effects of environmental pollutants are measured by their effect on oxidative damage or mortality, but hemocytes are a more convenient tool. Hemocytes have a very important role in the immune system of invertebrates, and insect hemocytes have similar properties to blood cells in vertebrates, making them an important material for immunological studies (Impellitteri et al., 2022). In this study, THC decreased when exposed to SiO_2 NPs, $CdSO_4$ and Abamektin in singly. Nonetheless, it was determined that THC increased in the groups where SiO_2 NPs and $CdSO_4$ were applied together. By causing oxidative stress in the cells, both SiO_2 NPs, $CdSO_4$ and abamectin may have caused damage or death to the haemocytes. It was known that oxidative stress can cause damage to cell membranes, DNA and proteins and can lead to cell death. On the other hand, in the SiO_2+$CdSO_4$ mixture group may have caused an increase in the count of haemocytes by increasing the activity of the immune system. This can be interpreted as the body's defence mechanism against toxic substances.

As for DHC, it was determined that the count of plasmatocytes increased in the groups in which SiO_2, $CdSO_4$ and abamectin were applied singly and in the groups in which abamectin was

applied in mixture with SiO_2 NPs. Plasmatocytes are blood cells capable of phagocyte (Beckage, 2008). It is thought that the increase in the count of plasmatocytes in these treated groups occurred in order to destroy foreign substances in the blood circulation. Moreover, it is thought that the reason for this increase is that plasmatocytes accumulate metals on the hemocoel wall with their ability to adhere, and this may be related to the blood cell's resistant effect against metals (Sendi et al., 2018). It was aslo determined that the count of prohemocytes decreased in SiO_2 NPs and $CdSO_4$ applications singly, while the count of prohemocytes increased when applied as a mixture. This increase suggests that SiO_2 NPs increases the toxic effect of Cd and the count of prohemocytes, which are haematopoietic cells, increases for defence purposes. On the other hand, a decrease was observed in the groups in which abamectin was applied singly and in mixture with SiO_2 NP. In previous studies, it was reported that insecticides decreased the count of prohemocytes (Ghasemi et al 2014). It is thought that prohemocytes may have differentiated into plasmatocytes in order to phagocytise foreign substances entering the body and as a result, the percentage of prohemocytes in the blood circulation may have decreased (Mir et al., 2020; Tuncsoy et al., 2021). In the study, decreasing prohemocyte and increasing plasmatocyte counts in the treatment groups support this. Granulocytes decreased in all applied groups. It is thought that this blood cell decreased because granulocytes are responsible for phagocytosis and can digest foreign substances taken in by hydrolytic enzymes. Besides, spherulocytes increased in all treatment groups. Although many comments have been made about the regulation of melanisation phagocytosis coagulation cell adhesion of spherulocytes, it has not been clarified yet (Stączek et al., 2020). As for eunodytoids, it was determined that an increase occurred in the groups where $CdSO_4$, abamectin and abamectin were applied as a mixture with SiO_2 NPs. Oenocytoids contain the enzyme phenoloxidase, which is responsible for melanisation in the immune system. Studies have reported that phenoloxidase is synthesised in oenocytoids and released into plasma when they are lysed (Vogelweith et al., 2016). It is suggested that the increase in oenocytoids in the groups treated with $CdSO_4$ and abamectin singly or in combination is due to the toxic effects of these substances. This is supported by the fact that the same application groups in the study showed an increase in phenoloxidase enzyme activity. In a study conducted by Çoğal et al. (2021), it was determined that there was a visible decrease in the total hemocyte counts of *G. mellonella* last instar larvae applied with Al_2O_3 NPs compared to the control group. Considering the important role of hemocytes in insect immunity, these results indicate that Al_2O_3 NPs have

suppressive effects on the immune system of *G. mellonella*. In addition, it was determined that there was a decrease in the count of granulocytes, prohemocytes, spherulocytes and oenocytoids, which are hemocyte types, while there was an increase in the count of plasmatocytes. It is thought that the reason for the increase in plasmatocytes is that these cells accumulate metals by adhering them to the hemocoel wall and tend to resist metals (Sendi et al., 2018). Eskin (2023) conducted a study with SiO_2 NPs and reported that it has a considerable influence on the insect's total hemocyte count and vitality. SiO_2 NPs were found to be 50% deadly at a dosage of 411.93 µg/10 µl. greater doses (100 and 180 µg/10 µl) resulted in considerably greater cell death rates than the control group. Adult growth time and longevity were considerably decreased in larvae fed low concentrations of SiO_2 NPs.

Phenoloxidase (PO) is a typical metal enzyme, which requires metal ions as prosthetic groups to enable the full exertion of its activity and is essential for insect immunological system (Lv et al., 2021). It is known that physical injury or tissue damage can also stimulate phenoloxidase activity. In the event of an injury, the insect's immune system responds swiftly and activates defensive systems surrounding the affected region. This is linked to the insect's increased immunological response to speed up wound healing or cope with stress. Temperature fluctuations, environmental pollutants, and other stressors can all enhance phenoloxidase activity. Xu et al. (2021) showed that ZnO NPs can trigger a protective immune response in *G. mellonella*. This response involves an increase in phenoloxidase activity. In an another research conducted by Wu and Yi (2015), it was analysed that environmental pollutants such as chromium (Cr) and lead (Pb) have high toxicity on immune and antioxidant system of *G. mellonella*. Antioxidant enzymes (CAT,SOD, Peroxidase), THC and phenoloxidase activities were increased with increasing concentrations of dietary Cr and Pb. Based on our results, it can ben concluded that in mixture applied groups due to high levels of oenocytoids, phenoloxidase activities were increased. Thus, hemocytes may have disintegrated in the presence of SiO_2 NPs, abamectin and $CdSO_4$ and their mixtures, leading to the release of phenoloxidase enzyme into hemolymph.

As for apoptotix index, there was a decrease in the count of living cells and early apoptosis in SiO_2 and $CdSO_4$ singly and in mixture applied groups compared to the control, and an increase in the count of late apoptosis was observed in the same groups. There was also a decrease in

the count of live cells and early apoptosis in SiO_2 and Abamectin singly and in mixture applied groups compared to the control, and an increase in the count of late apoptosis was observed in all application groups compared to the control. The reason for the gradual decrease in living cells is their transformation into early and late apoptosis due to the toxic effects of nanoparticles, heavy metals and pesticides. The reason for the gradual decrease in early apoptosis can be considered as its transformation into late apoptosis. In a research conducted by Eskin et al. (2022) with CuO NPs, it was determined that there was an increase in apoptotic counts in *G. mellonella* and no necrotic death occurred.

According to studies done with several insect species including *G. mellonella* larvae, blood cells in insects exposed to environmental contaminants such as nanoparticles, heavy metals, and pesticides change the morphological, histochemical, biochemical, and immunological defense systems. Knowing the observed toxic effect mechanism of silicon dioxide nanoparticle (SiO_2 NP), $CdSO_4$, Abamectin, and their mixtures on insects is thought to allow the development of new chemical methods in the fight against harmful insects that have less adverse impact on non-target organisms and the environment.

Due to the advancement of nanotechnologies, NPs are increasingly being employed and dispersed into the environment, either unintentionally or purposely. Consequenly, humans may be exposed to increasingly large counts of these particles. Furthermore, NPs can enter the body through oral exposure as well as inhalation, and co-ingestion of NPs with other contaminants such as pesticides can also have a negative effect on human health, particularly on the gastrointestinal tract (Cao et al., 2021, 2019; Dussert et al., 2020; Shi et al., 2010).

Although they are constantly exposed to many pollution sources, the interactions between contaminants and the consequent cumulative toxicity have not received adequate attention in the literature. However, NPs discharged into the environment may interaction with other contaminants or absorb them on their surfaces, allowing them to enter the body (Khan et al., 2021). Even if the toxicity of individual compounds is well understood, it has been proposed that when such substances are combined, unanticipated detrimental impact may arise (Zheng et al., 2012; Silins and Högberg, 2011), implying a collective toxicity. A co-exposed contaminant

may impact the cell membrane (in terms of fluidity, hydrophobicity, physical integrity or permeability,), increasing NP internalization and toxicity (Deng et al., 2017; Liu et al., 2018). According to Deng et al. (2017), NPs can be co-exposed to a wide range of copmounds, including organic contaminants, metal/metalloid ions, inorganic ligands, and other NPs. The ability of NPs to adsorb a co-pollutant can have a significant impact on the toxicity of the NP or pollutant, particularly by promoting pollutant entrance into the body via the absorption of NPs-pollutant complexes. Once inside, complex contaminants can be released into the body, increasing their concentration and consequently bioavailability and toxicity. According to Lu et al. (2015), harmful substances can be adsorbed on the surface of nanoparticles, allowing them to benefit from the carrier effect and reach the alveoli, causing more damage. Nonetheless, other pollutants can lessen NP toxicity by scavenging the ROS they create. Contaminants, on the other hand, have the potential to exacerbate the negative effects of nanoparticles by producing more ROS. (Deng et al., 2017). In conclusion, we determined that SiO_2 NPs may be absorved the $CdSO_4$ and abamectin and increased the toxicity of these environmental pollutants.

5. CONCLUSION

The physiological, biochemical and immunological effects of silica nanoparticles (SiO2), cadmium sulphate (CdSO4) and abamectin, singly and in combination, on *Galleria mellonella* last instar larvae were determined in this study by detecting variations in differential and total haemocyte counts, apoptosis index in larval haemolymph. Changes in antioxidant and detoxifying enzyme activities in the midgut and fat body were also detected.

Significant decreases and increases in the activities of Cyt P450, an important detoxifying enzyme, GST and AChE, enzymes responsible for neurotransmission, were detected in response to changes in the amount of silica nanoparticles, CdSO4 and abamectin, as well as CAT, SOD and GPx, indicators of oxidative stress.

The use of sublethal doses in research is considered an ecologically acceptable method of pest control. The determination of LD_{50} values helps to prevent the overuse of nanoparticles, leading to better results. Using nanoparticles with low LD_{50} values in combination has several advantages, including minimising air pollution, slowing down the development of resistance and reducing costs. Although there were variations between tissues in the research, changes in antioxidant enzymes and detoxification enzymes were generally found as a result of the mixture.

6. RECOMMENDATIONS

Because of its mammalian-like immune system, G. mellonella has become a popular model organism in recent years. It improves statistical power and reduces research costs by allowing easy in vitro culture and the use of large groups of larvae. The study of the effects of NPs, heavy metals and pesticides used in agriculture on the antioxidant defence system, an essential parameter in insect physiology, will contribute to many future genotoxic, physiological and toxicological researches.

These studies show that the use of nanoparticles in combination with pesticides has significant potential to develop more efficient and sustainable pest management strategies in many areas. In addition, the use of nanoparticles with other heavy metals is also important for combined toxicity studies. However, more research is needed on the broader ecological effects and safety of these combined interactions.

As a result, this work will inform future research on the combined toxicity of nanoparticles used in a variety of fields, including industry, medicine and agriculture, with other environmental pollutants. This study of the combined toxicity of nanoparticles, heavy metals and pesticides used in agriculture has good implications for other living organisms, the environment and human health. It will shed light on research that could have an important impact.

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