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BAHCESEHIR UNIVERSITY

GRADUATE SCHOOL OF ENGINEERING

THE DEPARTMENT OF BIOENGINEERING

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MASTER'S THESIS

DALYA AL-ZEHHAWI

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Supervisor: Assist. Prof. Dr. Canan Bağcı

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This thesis was read by us, quality and content as a Master's thesis has been seen and accepted as sufficient.

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I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

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ABSTRACT

The effect of copper oxide nanoparticles on the oxidative metabolism of an *in vitro* Parkinson disease cell model

Dalya Al-Zehhawi

Bioengineering Master's Program

Thesis Supervisor: Prof. Dr. Canan Bagcı

September 2023, pages 59

Numerous acute and long-term neurodegenerative disorders are shown being affected by oxidative stress. The underlying mechanisms of the Parkinson's disease (PD), the second-most common neurodegenerative disease worldwide, is considered as oxidative stress with a significant role in the development of PD.

While copper oxide (CuO) nanoparticles (NPs) offer valuable properties for a wide range of fields, their potential toxicity and environmental impact have become subjects of significant research. CuO NPs have been studied for their toxicity, and research has shown that their small size and high surface area can enhance their reactivity and potential toxicity. Release of copper ions from CuO NPs was shown to cause oxidative stress, cell and tissue damage.

The purpose of this study is to investigate the cytotoxicity and possible oxidative stress of CuO NP on PD induced cells. For this purpose, human neuroblastoma SH-SY5Y cells were treated with 6-OHDA and different doses of CuO NP to assess toxicity.

This study suggests that CuO NPs can adversely affect the oxidative metabolism of an *in vitro* PD cell model. The increased oxidative stress may contribute to the progression of PD pathology. To clarify the underlying mechanisms and evaluate new therapeutic approaches, more study is required to lessen the negative effects of CuO NPs in the setting of PD. These findings emphasize the importance of considering the potential risks associated with the use of CuO NPs in biomedical applications and highlight the need for cautious utilization of nanoparticles in the development of novel therapeutic approaches for neurodegenerative diseases.

Keywords: Copper oxide nanoparticles, Green synthesis, Oxidative stress, Parkinson's disease (PD)

ÖZ

Bakır Oksit Nanoparçacıklarının *in vitro* Parkinson Hastalık Modelinde Oksidatif Metabolizma üzerine Etkisi

Dalya Al-Zehhawi
Biyomühendislik Yüksek Lisans Programı
Tez Danışmanı: Doç. Dr. Canan Bagci

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Çok sayıda akut ve uzun vadeli nörodejeneratif bozukluğun oksidatif stresten etkilendiği gösterilmiştir. Dünya genelinde ikinci en yaygın nörodejeneratif hastalık olan Parkinson hastalığının (PD) temel mekanizması, PD'nin gelişiminde önemli bir rol oynayan oksidatif stres olarak kabul edilmektedir.

Bakır oksit (CuO) nanoparçacıkları (NP'ler), çeşitli alanlarda değerli özellikler sunarken, potansiyel toksisiteleri ve çevresel etkileri önemli bir araştırma konusu haline gelmiştir. CuO NP'lerin küçük boyutları ve yüksek yüzey alanları, reaktivitelerini ve potansiyel toksisitelerini artırabilir. CuO NP'lerden bakır iyonlarının salınımı, oksidatif stres, hücre ve doku hasarına yol açabileceği gösterilmiştir.

Bu çalışmanın amacı, CuO NP'lerin PD oluşturulan hücreler üzerindeki sitotoksisitesini ve olası oksidatif stresini araştırmaktır. Bu amaçla, insan nöroblastoma SH-SY5Y hücreleri 6-OHDA ile tedavi edilmiş ve farklı dozlarda CuO NP'ler ile muamele edilmiştir.

Bu çalışma, CuO NP'lerin in vitro bir PD hücre modelinin oksidatif metabolizmasını olumsuz etkileyebileceğini önermektedir. Artan oksidatif stres, PD patolojisinin ilerlemesine katkıda bulunabilir. Temel mekanizmaları açıklamak ve yeni tedavi yaklaşımlarını değerlendirmek için, CuO NP'lerin PD bağlamında negatif etkilerini azaltmak için daha fazla çalışma gerekmektedir. Bu bulgular, biyomedikal uygulamalarda CuO NP'lerin kullanımıyla ilişkilendirilebilecek potansiyel riskleri düşünmenin önemini vurgulamakta ve nörodejeneratif hastalıklar için yeni tedavi yaklaşımlarının geliştirilmesinde nanoparçacıkların dikkatli kullanımının gerekliliğini vurgulamaktadır.

Anahtar Kelimeler : Bakır oksit nanoparçacıkları, yeşil sentez, oksidatif stress, Parkinson hastalığı

To my family, Mum, Dad and sisters I am so lucky to have you in my life. And to my supervisor, Dr.Canan Bagci a great thanks to you.

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To my family, and especially to my dad and my mom,

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PD	Parkinson's disease
CuO	Copper oxide
CuO NPs	Copper oxide nanoparticules
ROS	Reactive oxygen species
SNpc	Nanotechnology Promotion Center
RNS	Reactive nitrogen species
OS	Oxidative stress
NDs	Neurodegenerative diseases
XRD	X-Ray diffraction analysis
FT-IR	Fourier transform infrared spectroscopy Analysis

Chapter 1
Introduction

1.1 Overview

Nanotoxicity is caused by numerous ways, one of which is oxidative stress. Some nanometal oxides can increase the cell's production of reactive oxygen species (ROS), which causes oxidative stress. The role of oxidative stress in the development of Parkinson's disease (PD) is underlined, with various enzymes and signaling molecules involved in the disease's underlying causes. Preventive care research in Parkinson's disease is focusing on copper oxide nanoparticles (CuO NPs), which can induce protein aggregation and underlying cytotoxicity.

CuO NPs have gained attention due to their unique physicochemical properties and potential applications in various fields. However, the potential toxicity of CuO NPs has raised concerns, particularly regarding their impact on cellular processes and their association with neurodegenerative diseases.

Many neurodegenerative diseases might be caused by metal nanoparticles that lead to oxidative stress by disrupting the cellular redox system and creating intracellular reactive oxygen species. The degree to which designed nanomaterials generate ROS is determined by the chemical nature of the nanoparticles.

In the context of PD, several studies have investigated the effects of CuO NPs on oxidative metabolism using *in vitro* cell models. These models typically involve dopaminergic neuron cells or cells engineered to exhibit PD-like characteristics. It is important to note that the effects of CuO NPs on the oxidative metabolism of *in vitro* PD cell models can vary depending on various factors, including the concentration and size of the nanoparticles, the duration of exposure, and the specific characteristics of the cell model used. Therefore, the exact mechanisms and outcomes may differ between studies.

The hypothesis of this proposed study is that CuO nanoparticles might affect the oxidative stress metabolism in PD *in vitro*. The results of this study might contribute to understanding of the potentially toxic effects of CuO NPs on PD pathology. Furthermore, this knowledge could help guide the development of safety guidelines and preventive measures for individuals at risk of exposure to CuO NPs, as well as provide insights into potential therapeutic strategies for managing PD.

1.2 Objective of the study

Parkinson's disease (PD) is a chronic neurological disorder characterized by the early death of dopaminergic neurons in specific brain regions. Increased oxidative stress and reactive oxygen species are thought to be one of the likely common causes of a variety of neurodegenerative diseases and are thought to be critical in the development of PD.

This study aims to explore the relationship between CuO NPs and PD by examining the role of ROS. Previous studies suggest that exposure to CuO NPs could be a risk factor for PD. As PD is already a pandemic, environmental or occupational exposure to CuO NPs should be regulated for public health. The study has the potential to provide new insights into the role of oxidative metabolism in PD upon CuO NPs administration and toxicity in PD model cell lines. It will determine whether exposure to copper oxide nanoparticles alters the oxidative metabolism in an *in vitro* PD cell model. This information would contribute to understanding the potential impact of nanoparticles on PD pathology and provide insights into the underlying mechanisms of nanoparticle-induced cellular effects. It could also have implications for the development and safe use of copper oxide nanoparticles in various applications, including nanomedicine.

1.3. Research hypothesis

Copper oxide nanoparticles (CuO NPs) are expected to induce toxicity in an *in* vitro Parkinson's disease cell model through the generation of oxidative stress, with the severity of toxicity increasing in a dose-dependent manner.

Chapter 2
Literature Review

2.1 Parkinson's disease (PD)

Early dopaminergic neuron death in the substantia nigra, the pars compacta (SNpc), and the pervasive intracellular protein alpha-synuclein (aSyn) are characteristics of Parkinson's disease (PD), a long-term neurodegenerative disease. Dopamine deficiency in the basal ganglia is what leads to the classic Parkinsonian motor symptoms of bradykinesia, tremor, rigidity, and subsequent postural instability (Divya, Vinay Goyal, 2018). PD is the most typical form of primary parkinsonism as well as the second most typical progressive neurodegenerative disease. Around 1% of those over 50 and 2.5% of people over 70 are affected by it. (S. Esmail,2018).

Women have a 1.3% lifetime risk of having PD compared to a 2.0% risk for men. The most common kind of Parkinson's disease, commonly known as sporadic PD, often affects people over 65. Motor symptoms, including resting tremor, bradykinesia/akinesia, and overall rigidity, are connected to a dopamine deficit in the basal ganglia caused by the death of dopaminergic neurons in the substantia nigra pars compacta (SNpc). 2016 (Marketa Marvanova,2016). The pathogenic characteristics of PD include the buildup of misfolded Lewy body aggregates across the brain and the loss of neurons in the substantia nigra pars compacta (Rocca WA, 2018). The cornerstone of PD management today is pharmaceutical therapy, although these symptomatic drugs have considerable limitations in advanced disease.

2.2 (ROS) reactive oxygen species, and free radicals

Reactive oxygen species (ROS) are a group of chemical compounds that result from one or more oxygen electron reductions. ROS play a role in physiology and illness, and they can be both the cause and the result of a variety of biological events. ROS are molecules with a high reactivity due to their chemical makeup, which can be produced by the metabolism of oxygen or nitrogen. Free radicals such as superoxide radical (O_2-•),

hydroxyl radical (OH), and nitric oxide (NO) can produce ROS and reactive nitrogen species (RNS).

Other non-free radicals can also be identified, such as H_2O_2 peroxide and OOO- peroxynitrite. What is a free radical? A free radical is an atom or a group of atoms that has an unpaired electron at its outermost orbital point. This adds to the instability and reactivity of free radicals, as well as their ability to interact with molecules in a variety of ways to form both radical and non-radical species. These species are usually toxic to humans because they change the structure of molecules, resulting in damage or instability of cells and tissues. A redox reaction occurs when a free radical has lost an electron. Free radical is reduced and the stable molecule that has lost an electron is oxidized, becoming a free radical and igniting a chain reaction. Different forms of free radicals, such as ROS and RNS, are produced as a result of metabolism (Losada-Barreiro S., 2015). Acute changes in oxygen availability (hyperoxia and hypoxia, respectively) affect all organisms, resulting in the generation of ROS.

The most prevalent source of ROS is the one-electron reduction of oxygen. As a result of one electron reduction of oxygen, superoxide anion (O_2 •) is produced, which is the most common first step in all ROS-producing enzymes and a highly hazardous species. (Vicente-Gutierrez,2019).

Reactive oxygen species, such as hydroxyl and peroxyl radicals, hydrogen peroxide, and the superoxide radical anion, have long been linked to the oxidative harm to fatty acids, DNA, proteins, and other biological components. Overproduction of reactive oxygen species (ROS) has been connected to several diseases. Oxidative stress, which is produced by an imbalance between excessive ROS formation and inadequate antioxidant defenses, is connected to age-related disorders, cancer, cardiovascular, inflammatory, and neurological diseases such as Parkinson's and Alzheimer's diseases.

The long-held "free radical hypothesis of aging," developed by Denham Harman in 1956, states that the detrimental effects of ROS generated during cellular respiration at the mitochondrial level are directly involved in aging processes. However, a revision to this premise is being made right now. A rising body of data points to ROS's potential physiological function as messengers in cellular signaling, a novel theory into the diverse

and complicated chemistry of ROS that has received considerable attention in the last ten years (Katerina Krumova, 2016).

2.3 Oxidative stress

"Oxidative stress" is a broad term used in redox biology and medicine. It has drawn a lot of interest and some criticism since its debut in 1985. (Sies, H., 2018). Oxidative stress (OS) is the imbalance between the generation and destruction of ROS or RNS. (H. Fujii et al., 2011) In contrast to how Almokhtar A. Adwas et al. (2019) define oxidative stress as oxidative harm to proteins, fats, nucleic acids, and carbohydrates brought on by an imbalance between free radicals and antioxidants, antioxidants have helped protect the body from free radical damage. ROS and RNS are produced by both endogenous and exogenous sources. Inflammation mechanisms and immune cell activation, extreme exercise, ischemia, mental activity stress, malignant and infectious disorders, and aging are all examples of the endogenous creation of these species. (Ilaria Liguori *et al.* 2018).

Pollution of water, air, and land, alcohol consumption, smoking, some medicines, heavy metals, certain pharmaceuticals (tacrolimus and cyclosporine), radiation, cooking, and some solvents such as benzene are all exogenous sources of ROS. After penetrating the body, these chemicals break down into ROS (Figure1).

When oxygen interacts with specific molecules, free radicals are extremely reactive atoms or molecules with one or more unpaired electrons in their external shell. These radicals can be created in cells by losing or accepting a single electron, acting as oxidants or reductants, respectively. The terms "ROS" and "RNS" refer to oxygen and nitrogen derivatives that are reactive radicals and non-radicals, respectively.

Nowadays, oxidative stress is described as a long-term imbalance favoring the synthesis of highly reactive molecular species (oxidants) over antioxidants, resulting in redox signaling and control disruption and/or molecular damage. (Sies H., 2015).

The four levels of oxidative stress are baseline oxidative stress, low-intensity oxidative stress, intermediate oxidative stress, and strong oxidative stress. Low-intensity oxidative stress is probably detectable by the Nrf2/Keap system, which is activated by small amounts of ROS. (Lorena Meaca-Guerrero *et al.*, 2020).

Oxidative stress can be characterized based on its severity, with intensity scales ranging from physiological oxidative stress (eustress) to toxic oxidative burden that destroys macromolecules (distress) (Figure 1).

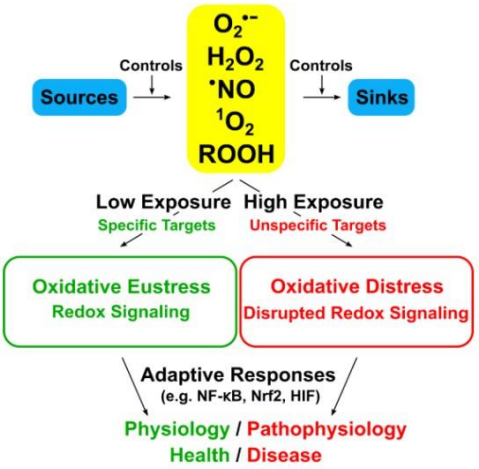

Fig 1: The relationship between oxidative stress and redox signaling. Specific (highly reactive) targets are addressed by physiological (low) oxidant exposure, whereas unspecific targets are addressed by supraphysiological (high) oxidant exposure. Adaptive responses operate as a counterbalance. Secondary reactions yield additional significant oxidants, such as ONOOH from O_2 and NO, or HOCl from H_2O_2 and Cl- Modified from Ref (Helmut Sies et.al, 2020).

The oxidative equivalents used in redox signaling target regulatory pathways, particularly those handled by transcription factors, directly or indirectly. Hydrogen peroxide has emerged as a significant redox metabolite involved in redox sensing, signaling, and control. (Sies H., 2017). In some extreme cases, the accumulation of ROS to a certain level might result in a pathological condition that can lead to a variety of diseases. (Fanjul-Moles, 2016). Superoxide radicals, hydroxyl radicals, and hydrogen peroxide are the reactive oxygen species produced by oxidative stress. The majority of oxidative DNA damage is indirect, and the adduction of radicals to the DNA might result in mutation, causing the cell to become malignant. Internal source factors for the generation of free

radicals in internal cellular metabolism include mitochondrial ETC. Furthermore, several of these reactive oxygen intermediates may act as redox-signaling secondary messengers. This may disrupt regular cellular signaling pathways due to the oxidative stress induced by copper oxide nanoparticles in the in vitro Parkinson's disease cell model, potentially leading to aberrations in cellular function.

Physiological oxidative stress (eustress) and toxic oxidative stress (distress) can be distinguished by their intensity: low exposure of cells and organisms to oxidative stress is required for redox signaling directed at specific targets, whereas elevated exposure causes redox signaling to be disrupted and/or decay, addressing unspecific targets.

The damage to essential biomolecules is caused by oxidative stress, which is defined as high ROS generation combined with a deficient defensive system (H. Sies, 2018).

The role of oxidative stress in the etiology of a wide range of diseases (Figure 2), including metabolic syndrome, atherosclerosis, cardiovascular disease, cancer, neurodegenerative disorders, diabetes, infertility, renal diseases, and gastrointestinal and hepatic diseases

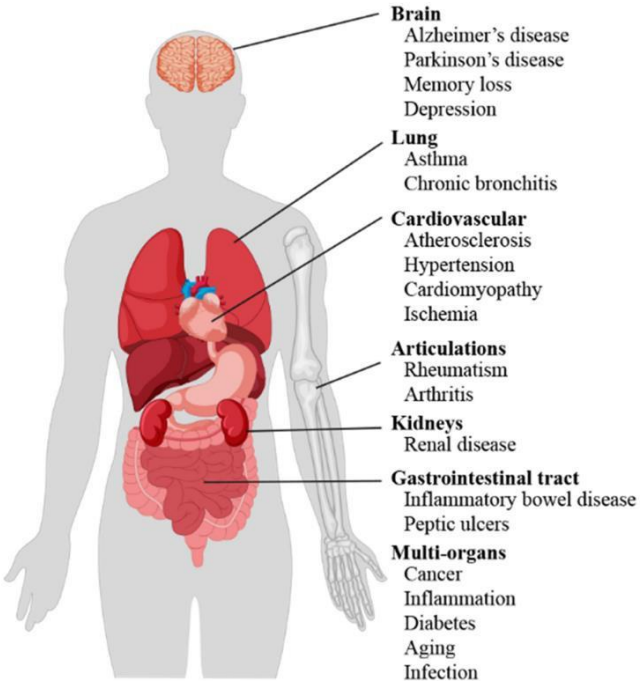

Fig 2: Human diseases caused by oxidative stress (Rosa Vona et al, 2021).

Cellular respiration at the mitochondrial level is directly engaged in aging processes, according to the long-held "free radical hypothesis of aging" proposed by Denham Harman in 1956. However, this hypothesis is currently being revised. A growing body of evidence suggests that ROS may have a useful physiological role in cellular signaling as messengers, a new paradigm in the complex and diverse chemistry of ROS that has gotten a lot of attention in recent decades (Claudio Cabello *et al.*, 2016).

2.4 Impact of Oxidative Stress on Neurodegenerative Diseases

Neurodegenerative diseases are a diverse category of conditions that are characterized by the loss of neurons over time. Although the etiology of NDs is still unknown, it has long been recognized that the oxidative stress is linked not only to normal aging but also to the development of a range of NDs, particularly Alzheimer's disease and Parkinson's disease. (Andy Wai Kan Yeung *et al.*, ,2021).

One of the most likely widespread etiologies of a variety of neurodegenerative diseases has been found to be increased oxidative stress. However, the causes of neurodegenerative illnesses are still not completely known. Cellular damage, deterioration of the DNA repair system, and mitochondrial malfunction have all been connected to accelerated aging and the emergence of neurodegenerative disorders and cumulative oxidative stress. (Geon Ha Kimetal *et al.*,2015). Most aging theories refer to the notion that chronic oxidative stress causes mitochondrial mutations, dysfunction, and oxidative damage. However, as the significance of ROS in aging and age-related disorders becomes more widely recognized, a number of debates in this topic have emerged. (Sonia Gandhi *et al.*, 2012). In the activation of pathogenic processes in brain cells, mitochondrial ROS and calcium have complementary actions. (Artyom Y. Baev *et al.*, 2022). ROS are kept in a dynamic equilibrium by antioxidant defenses and ROS-generating biological activities. Oxidative stress results when reactive oxygen species (ROS) outnumber the antioxidant defense mechanisms. This condition develops when homeostasis is disturbed, leading to an increase in ROS production, or when defense mechanisms are weaker. The brain is particularly susceptible to oxidative stress due to a trifecta of increased ROS production, weakened antioxidant defenses, and a constrained capacity for regeneration. In response to

pathogen- and damage-AMPs, such as aggregated protein and cellular debris, like phagocytes, microglia can orchestrate an "oxidative/respiratory burst". As a result, ROS forms an essential part of the tissue defense system of the microglia. (Dominic S. A. Simpson *et al.*, 2020).

2.5 Parkinson's disease and oxidative stress

Parkinson's disease (PD) is a neurological condition that progresses and is characterized by a specific loss of dopaminergic (DA) neurons in the substantia nigra, a midbrain region. The fact that the mechanism of PD is not fully known may serve to underscore the fact that PD, the second most prevalent neurodegenerative disease, is still incurable. As a result of significant research, it is now widely accepted that the etiology of Parkinson's disease is primarily influenced by genetic background, environmental factors, and aging (Minrui Weng *et al.*, 2018).

Parkinson's disease (PD) is a chronic neurodegenerative condition that is mostly identified by its outward clinical manifestations. (ROS) are believed to play a significant role in regulating the course of Parkinson's disease. Despite extensive investigation, the molecular pathways for PD start and development that depend on antioxidants are controversy (Mohsen Hemmati-Dinarvand *et al.*, 2019).

During the early stages of PD, prior to the initiation of cell death, ROS buildup is a key element of a number of harmful molecular pathways. Excessive ROS accumulation can promote or directly induce apoptosis (intrinsic and extrinsic), cytoplasmic, and autophagic cell death, among others. (Morris *et al.*, 2018).

The early stages in the etiology of Parkinson's disease continue to be oxidative damage and the subsequent mitochondrial dysfunction. It has been suggested that mitochondria, the cell's main source of adenosine triphosphate (ATP), may play a role in the onset of Parkinson's disease (PD) because of the high energy needs of dopaminergic neurons. (Benjamin G. Trist *et al.*, 2019). Due to regular electron leakage through the electron transport chain, mitochondria, which are the primary source of cellular energy ATP through the oxidative phosphorylation process, are also a significant source of ROS (Park, J.-S. et al., 2018). Most of the time, mitochondrial antioxidative systems detoxify ROS,

ensuring a balance between the production of harmful radicals and the body's ability to fight them. As oxidative stress increases due to an imbalance, mitochondrial structure's macromolecules are susceptible to oxidative damage. Accumulation of oxidative stress-damaged macromolecules in the organelle impairs the function of the mitochondria. As a result, eventually cytochrome c is released from the mitochondria and cell apoptosis is induced, as seen in the death of dopaminergic neurons in Parkinson's disease. (Heng-Chung Kung *et al.*, 2021).

In PD, oxidative stress is caused by a variety of processes and is linked to the failure of complex I of the respiratory chain as well as the existence of certain PARK genetic variations. At the same time, oxidative stress levels may play a role in the efficacy of antiparkinsonian medication and DBS therapy (Jolanta Dorszewska *et al.*, 2020).

Mutations caused by DNA oxidation may increase reactive oxygen species generation in PD patients' brains, increasing neuronal loss caused by mitochondrial electron transport chain abnormalities, antioxidant depletion, and harmful oxidized dopamine exposure. (Jolanta Dorszewska *et al.*, 2020).

2.6 Nanoparticles

Nanotechnology has created a variety of materials at the nanoscale. Particulates with a minimum diameter of 100 nm fall within the broad category of nanoparticles (NPs). Depending on the overall shape, these materials can be 0D, 1D, 2D, or 3D (Holzinger *et al.* ,2014). The importance of these materials was realized when researchers discovered that the size of a substance can influence its physiochemical qualities, such as its optical capabilities (Ibrahim Khan *et al.*,2019).

Three layers make up NPs, which are not simple molecules. These layers are the surface layer, which can be functionalized with a variety of small molecules, metal ions, surfactants, and polymers, and the interior layer, which can also be functionalized with a variety of small molecules, metal ions, surfactants, and polymers (Shin *et al.*, 2016).

Their average particle diameter, size, and charge have an impact on the physical stability and in vivo distribution of nanoparticles. Electron microscopy methods can be used to analyze polymeric nanoparticles' overall shape, which may have an impact on their toxicity. The surface charge of the nanoparticles has an impact on the physical stability and

redispersibility of the polymer dispersion as well as their in vivo performance (Sovan Lal Pal *et al.*, 2011).

2.7 Copper oxide nanoparticles (CuO NPs)

Metal nanoparticles are employed in a wide range of industries due to characteristics such a high surface area/volume ratio, variations in chemical reactivity and catalytic qualities, and selective medication delivery to target organs and tissues with ease of surface modification. Examples of applications include electrochemical processes and hydrogen production, electronic devices and communication systems, sensor design, and biomedical and medical imaging technology. They could potentially be applied to a variety of fields, including material chemistry (Castro L. *et al.*, 2014). Copper oxide nanoparticles (CuO NPs) are gaining popularity for their use in catalysis, sensing, and superconducting because of their exceptional physicochemical properties. These structures are also considered to be semiconductor photocatalytic or photovoltaic materials. Some investigations have shown that CuO NPs are antimicrobial and can be used to treat cancer (Selda Doğan Çalhan, 2020).

Actually, just trace amounts of copper (Cu), including in the essential enzymes in the human body, are needed. Enzymes like cytochrome oxidase, superoxide dismutase, and tyrosinase contain this trace element. On the other hand, C Cu-free ions have the power to harm the human body's cells, organs, and body as a whole. Therefore, Cu ions in living things should be limited (Devanthiran Letchumanan, 2021).

Copper oxides (CuOs), which are inorganic NPs, can be easily oxidized from Cu NPs. Cu and CuO NPs are widely used as anticancer, antibacterial, and antioxidant agents. This is due to NPs' ability to interact with the biological system at the cellular level for numerous responses and functions (Vaid, P.; Raizada, 2020). CuO, also known as cupric oxide, is a p-type semiconductor with a bandgap of 1.2–1.9 eV. It is a transition metal oxide that is monoclinic black and has a variety of intriguing properties, including high thermal conductivity, solar capacity, excellent stability, and antibacterial activity. Due to its beneficial characteristics, CuO has been thoroughly investigated for its wide range of potential uses, including electrochemical cells, gas sensors, magnetic storage devices, field emitters, and catalysis. (Y. Aparna, 2012). CuO is less expensive than silver, is simple to

combine with polymers, and has typically stable chemical and physical properties. CuO and other highly ionic nanoparticulate metal oxides may be particularly effective antibacterial substances because they can be manufactured with unusually large surface areas and distinctive crystal morphologies (Guogang Ren,2009).

Additionally, CuO nanostructures exhibit magnetic and superhydrophobic properties that are unique to this material. These nanostructures also hold great promise for super-hydrophobic surfaces or anode materials for lithium ion batteries, enhancing nanofluid thermal conductivity, nanoenergetic materials, and heterogeneous catalysis for complete hydrocarbon conversion to carbon dioxide (Thi Ha Tran, 2014). Some of the physical and chemical procedures utilized to create CuO NPs include microwave irradiation, the sol-gel method, chemical precipitation, solution plasma, thermal breakdown, and electrochemical reduction. The use of hazardous chemicals as reducing agents still poses a serious issue despite the simplicity and high yields of these NP synthesis methods. For the creation of CuO NPs, nonpolar solvents are sometimes used to amplify the impact (Jingjun Lyu *et al.*, 2021). As a low-cost, energy-efficient, and nontoxic alternative to chemical synthesis, biological synthesis of NPs using microorganisms such algae, fungi, bacteria, and plant leaf extracts has been proposed. Since it removes the time-consuming process of maintaining a cell culture, the use of plant extracts for NP synthesis may be preferable to alternative ecologically friendly biological approaches (Worku Wubet Andualem, 2020).

2.8 Green synthesis of nanoparticles

Two methods are employed to create copper oxide. The two fundamental categories into which these approaches can be broadly categorized are the bottom-up approach and the top-down approach. Small atomic-sized particles combine in the bottom-up approach to generate nanoscale particles, whereas in the later approach, bigger molecules are smaller molecules are broken up, and the smaller molecules result in the production of suitable nanomaterials (Figure 3).

However, in the latter process, large molecules are broken down into smaller ones, and the smaller molecules are then used to produce the necessary nanomaterials. The use of highly toxic chemicals that are dispersed in the environment or absorbed on the surface of

materials and may have negative effects in medical applications, as well as high costs, poor product efficiency, and high energy consumption, are drawbacks of copper oxide nanoparticles made by chemical or physical processes.

Despite the fact that copper oxide nanoparticles produced using green technologies are more stable, safe, cost-effective, and have a longer shelf life, copper oxide nanoparticles have been produced using a variety of biotic resources (Abdul Waris *et al.*, 2020).

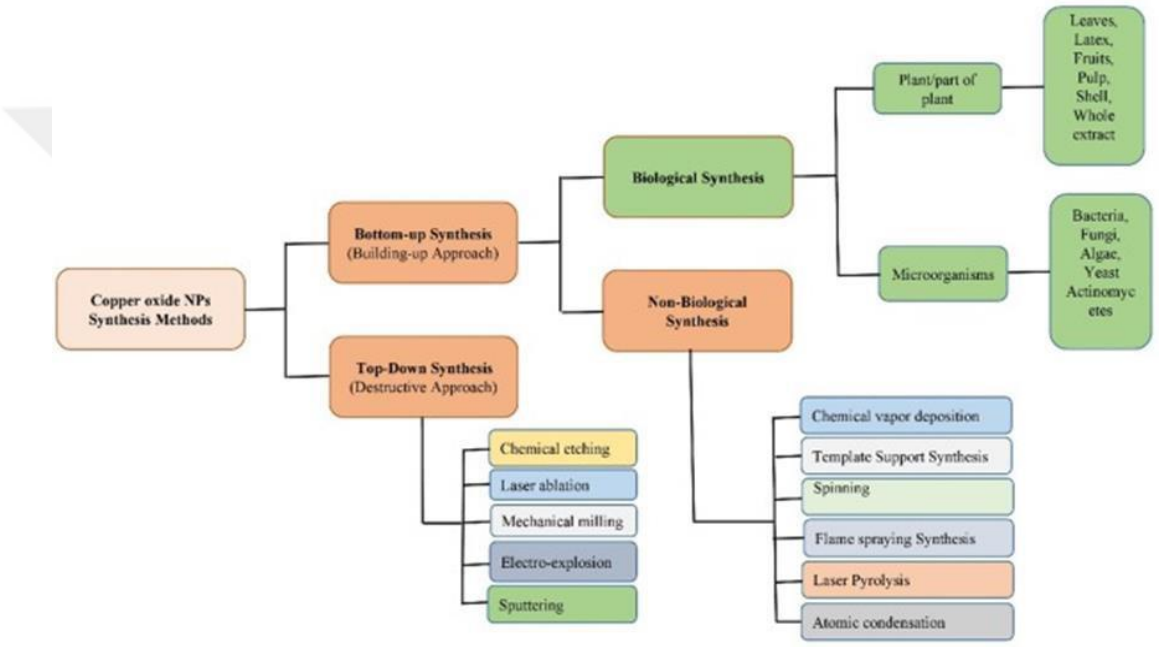

Fig 3 : **Different approaches for creating copper oxide nanoparticles**

The environmentally acknowledged "green chemistry" method has been used to the biosynthesis of nanoparticles, also known as "green synthesis," which makes use of bacteria, fungi, plants, actinomycetes, and other organisms to produce clean and ecologically acceptable nanoparticles(Aziz, W.J., 2018). A green technique for creating nanoparticles with unique features uses the mentioned organisms in the biosynthesis of nanoparticles. Nanoparticle synthesis using plant extract has advantages over other biological synthesis techniques, such as using microbes, because the rate of metal nanoparticle creation is higher, much faster, and incredibly monodispersive. (Singh, J., 2019). As a result, plant extracts are an excellent source for the creation of metal and metal oxide nanoparticles. As a result, plant extracts are an excellent source for the creation of

metal and metal oxide nanoparticles. To produce copper oxide, two techniques are used. The bottom-up approach and the top-down approach are the two basic classes into which these methodologies can be generally classified. In the bottom-up method, small atomic-sized particles come together to form nano-sized particles.

2.9 Nanoparticles and Parkinson's disease might be connected

Today, different types of nanoparticles x(NPs) are produced on a global scale and used in a wide range of goods and industries. The brain is one of the organs where NPs can accumulate after entering the body. It is crucial to protect dopaminergic striatal and substantia nigra (SN) neurons, given their heightened vulnerability, as their degeneration is closely associated with Parkinson's disease (PD). As a result, NPs may collaborate with other substances and elements to cause PD. Due to their superconductivity, Cu/CuO NPs are used in a variety of electronics, such as gas sensors, batteries, and solar cells. Despite their widespread use, little is known about the toxicity of copper/copper oxide NPs to the dopaminergic system. But many studies have shown that these NPs are dangerous. In 2005, it was demonstrated that CuO NPs could lead to oxidative stress (Radhakrishnan & Vinay Goyal, 2018) of the mitotoxic effects of these NPs.Cu-NPs' ability to harm the mitochondrial membrane's potential has been demonstrated. Since mitochondrial damage will cause apoptosis, it appears that these NPs can harm brain cells through the apoptotic pathway (Abbas Mohammadipour,2020).

Additionally, it has been discovered that these NPs can increase TGF-1, IL-1, IL-6, and TNF- alpha, which can lead to inflammation. It has also been noted that CuO NPs with a diameter of less than 50 nm may damage and induce apoptosis in respiratory epithelial cells. The impact of Cu/CuO NPs on the SN and striatum, however, has not yet been studied. Therefore, it is advised that researchers look at how these NPs affect these significant brain regions in order to ascertain whether Cu/CuO NPs and PD are connected (Baeg E, Sooklert K, *et al.*, 2018)

Parkinson's disease is linked to an increase of amyloid fibrils, which are protein clumps. It is well known that the protein synuclein's amyloid buildup plays a crucial role in the emergence of neuronal degenerative disorders in the brain (JingjunLyu *et al.*, 2021).Studies on the prevention of Parkinson's disease are concentrating on some

hazardous materials with biological uses, like copper oxide (CuO) nanoparticles that can encourage protein aggregation and underlie cytotoxicity.

2.10 Copper oxide nanoparticles' toxicity

It has been discovered that many metal oxide nanoparticles are immunotoxic, genotoxic, and cytotoxic. Despite substantial research, the mechanism of metal oxide NPs is still not entirely understood for a variety of reasons. The harmful potential of NPs is significantly influenced by the release of metal ions. Metal ions released from metal oxide nanoparticles (NPs) and the environment to which the NPs are exposed are the main contributors to toxicology (Djurišić, A.B. *et al.*, 2015).

The challenges that cause toxicity to be misunderstood include the binding of CuO NPs, their interaction with live cells, and the resulting change in surface chemistry.

To fully understand the toxicity of CuO NPs, it is vital to comprehend their characterization, surface modification, exposure paths, and toxicological mechanisms (Sania Naz *et al.*, 2019). Small NPs have more toxicity and are more likely to be internalized by cells since they are abridged in comparison to large NPs. (Sajid, M. *et al).*

CuO-NP exposure has been linked in studies to oxidative stress, DNA damage, slower growth of organisms, and cell death. CuO-NPs were discovered to cause more inflammation in mice when compared to titanium (Ti), iron (Fe), and silver (Ag) oxides. Additionally, they appeared to increase the amount of total protein, lactate dehydrogenase activity, and neutrophil and total cell migration to the lungs in BAL fluid. The negative effects of CuO on the immune system have received little research, and it is still unknown whether CuO-NPs will have any harmful effects on the human immune system (Evelyn Assadian et al., 2017). Cu-based nanoparticles (CuO NPs) release enough copper ions in the appropriate conditions to affect biological systems. When compared to bulk CuO, the toxicity of CuO NPs is 40 times higher. Despite being far more toxic than the effects of the particles themselves, Cu ions from CuO NPs have a considerable harmful effect in the growth medium. Another theory is that after penetrating, Cu ions are released into the cytoplasm and build up in lysosomes, where the pH is acidic and the solubility parameter increases, producing highly lethal Cu2+ ions. (Hui He, 2020).

Chapter 3
Materials and Methods

3.1 Required equipments & materials

Human neuroblastoma SH-SY5Y cells, Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS) as an additive, penicillin-streptomycin, trypsin, Laminar flow cabinet, fume hood, autoclave, heater, water bath, vortex, inverted light microscope, CO_2 incubator, refrigerator, centrifuge, -20°C freezer, CuO NPs synthesis**,** centrifuge, vortex, magnetic stirrer, heater, copper nitrate (Cu II Nitrate hydrate 99.999%) (CuN_2O_6), MTT [3-(4,5-dimethylthiazol-2-yl)-2,5]-diphenyltetrazolium bromide, microplate reader, 6-hydroxydopamine (6-OHDA), ascorbic acid, homogenizer.

3.2 Research Design

We report a strategy to synthesize ultrasmall CuO NPs by a green, rapid and cost-effective method. Using the neuroblastoma cell line SH-SY5Y, which Parkinson's disease, CuO NPs administered to them in varying doses to assess the toxicity by MTT assay and the oxidative stress.

3.3 Green Synthesis of CuO NPs

3.3.1 Leaf extract preparation from *Aloe vera* plant:

30 grams of *Aloe vera* plant leaves were weighed, then gently washed by distilled water. The leaves were crushed by using a mortar and pestle and then 30 ml of deionized water added to the leaves. The extract was boiled at 100–110 °C for 10 minutes. A filter paper of Whatman No.1 was used to filter the extract and it was kept at 4°C for further use (Figure 4).

3.3.2 Synthesis of CuO NPs:

0.2M copper nitrate solution was prepared by dissolving 0,94 g copper nitrate in 25 ml of distilled water. The copper nitrate solution and *Aloe vera* extract were mixed in equal volumes, then the mixture was boiled at 100^0C for about 2-3 hours. The colour change was observed as from greenish blue to the olive green with precipitate. The mixture was left in the oven for calcination at 95°C for overnight. Then the precipitate was collected in a tube and washed twice with 10 ml of distilled water by centrifugation for 10 minutes at 4.000 rpm at room temperature. Then, the supernatant was discarded, and pellet was dissolved in 10 ml distilled water. The solution was then heated at 400°C for 30 minutes in muffle furnace. The colour of the final product obtained was black indicating the formation of CuO NPs (Figure 4).

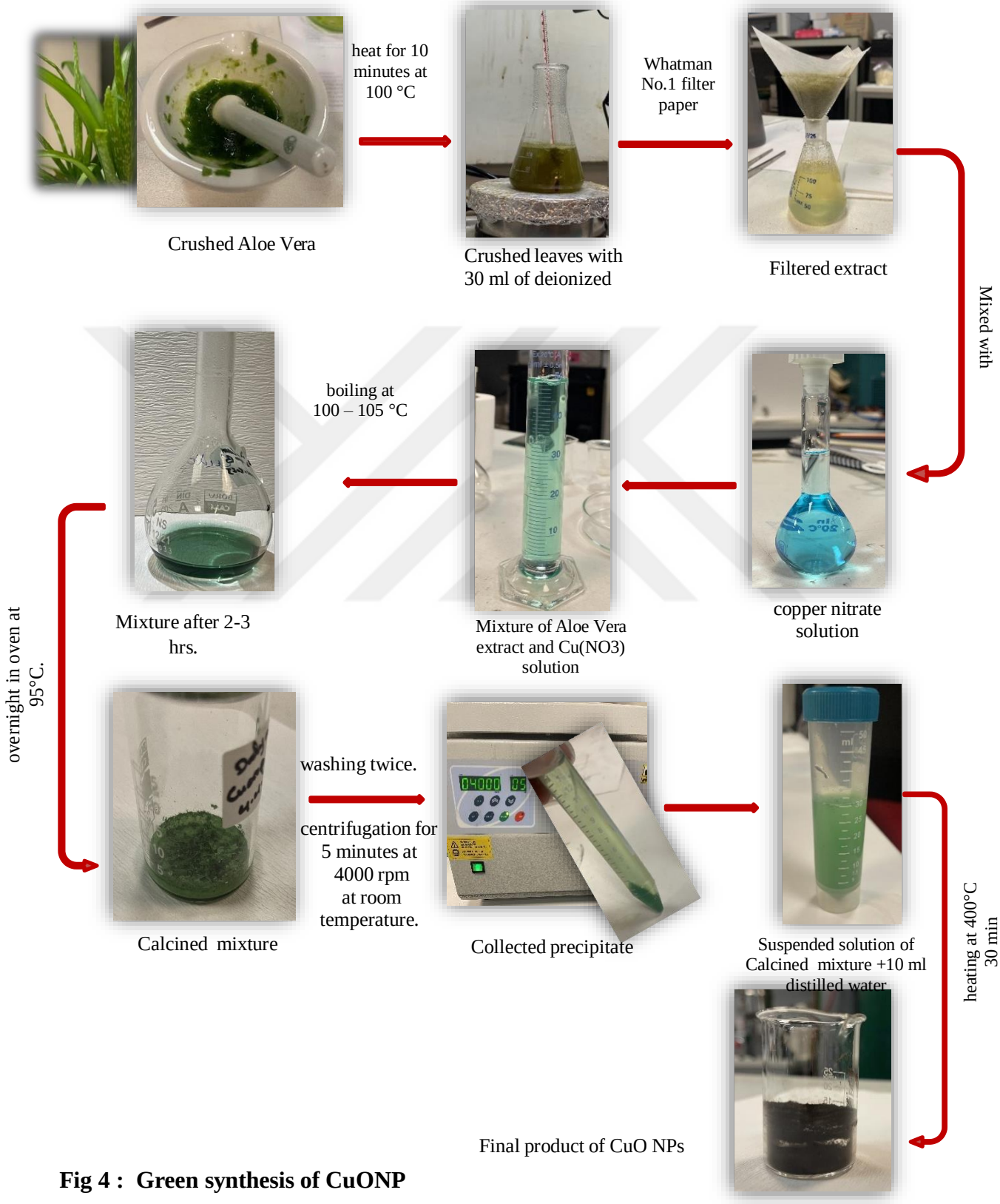

Fig 4 : Green synthesis of CuONP

3.4. Cell Subculturing (Passaging)

Human neuroblastoma cell line SH-SY5Y was used in experiments, which was a kindly gift from Assist. Prof. Dr. Mehmet Ozansoy. The SH-SY5Y cells were cultured in DMEM supplemented with FBS (10%) and penicillin antibiotics by using an incubator at 37 °C and 5% CO_2 . The medium was changed for each two days. The adherent cells were treated in 0.05% Trypsin-EDTA (Sigma-Aldrich, Germany) for 5 minutes at 37°C before being collected by centrifugation at 2.500 x g for 5 minutes.

All experiments were performed in a laminar flow hood and all equipment were thoroughly sterilized with 70% ethanol before beginning cell culture. Cell culture flasks were supposed to be at least 50% confluence when they were taken out of the incubator and examined under a microscope to make sure there is no contamination.

3.5 SH-SY5Y cell line exposure to CuO NPs

Cells were seeded into a 96-well plate at a density of 10.000 cells/well. Prior to treatment, cells were allowed to adhere to the surface of the plate for 24 hours. Corresponding CuONP concentrations (125, 250, and 500 ng /mL) were suspended in the cell culture medium and incubated for 24 hours with the cells. In each experiment, cells that had not been treated with CuO NPs were used as controls. A microscopic examination was performed to see how CuO NPs affected the cells.

3.6 SH-SY5Y cell line for mimicking PD model

SH-SY5 cells were seeded on a 96-well plate and allowed to attach and grow overnight in the incubator. The cell density was ensured to be appropriate for the subsequent experiments. Cells treated with 6-OHDA with different concentrations (10 µM, 25 µM, 50 µM,) by diluting the 10 mM stock solution with a cell culture medium.

After the 24-hour treatment period, a microscopic examination was performed to examine the SH-SY5Y cells in terms of any morphological changes, cell detachment, or other signs of cellular stress or damage whether the observed changes should confirm our PD model predictions.

3.7 Cell Viability Test

To determine the cytotoxicity, a colorimetric MTT assay was performed. After the above-mentinoned treatments, MTT solution (5 mg/ml, 50 µL/well) was used to incubate the cells in culture medium at 37°C for 3 hrs. After incubation, the violet formazan crystals were solubilized in 100 µL DMSO. The plates analyzed using a microplate reader with a wavelength of 570 nm (Figure 5).

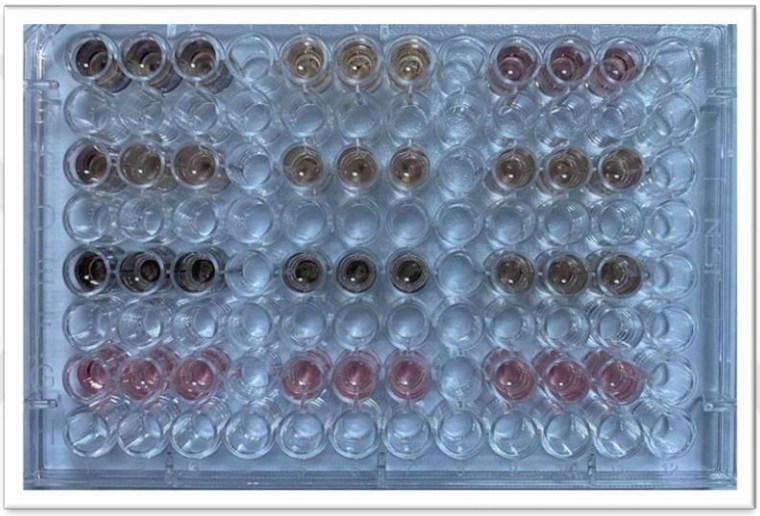

Fig 5: **MTT test**

3.8 Oxidative stress measurement

Hydrogen Peroxide Assay Kit (Abcam, AB102500) was used for the rapid, sensitive, and accurate measurement of hydrogen peroxide. The kit procedure was followed according to manufacturer's instructions. Briefly; three T25 flasks were seeded with 10^6 cells and the cells were allowed to attach for 24 hours. On the next day, CuO NP and DMSO treatment were applied and incubated for 24 hours, as the experimental group and negative control group, respectively (Figure 6). After the incubation period, cell pellet was collected by centrifugation for 15 minutes at 1000 x*g* and kept on ice for deproteinization, which is suggested in the kit protocol. A clear protein sample was obtained after homogenization and centrifugation. The samples were kept on ice to maintain their stability. Ice-cold perchloric acid (PCA) (4 M) was added to the sample to achieve a final concentration of

1 M PCA in the homogenate solution. The samples were incubated on ice for 5 minutes. This step helps the precipitation of proteins and other macromolecules. The samples were then centrifuged at 13,000 x *g* for 2 minutes at 4°C in a cold centrifuge. To neutralize the sample and remove excess PCA, ice-cold 2 M KOH was added. The amount of 2 M KOH should be 34% of the volume of the supernatant. After neutralization, it's crucial to ensure that the pH of the sample falls within the range of 6.5-8. pH paper was used to check the pH of the solution. Samples were centrifuged again at 13,000 x g for 15 minutes at 4°C. This step helps to remove any precipitated PCA and other unwanted components. The prepared samples were transferred to a 96-well plate, followed by the addition of the reaction mix to each well. OD 570 nm absorbance measurement was performed by using a microplate reader.

Although the measurements were performed, the absorbance value of samples were not in the standard range. The absorbance values were observed as lower than the lowest standard concentration (Figure 7). Thus, the oxidative stress data could not be included.

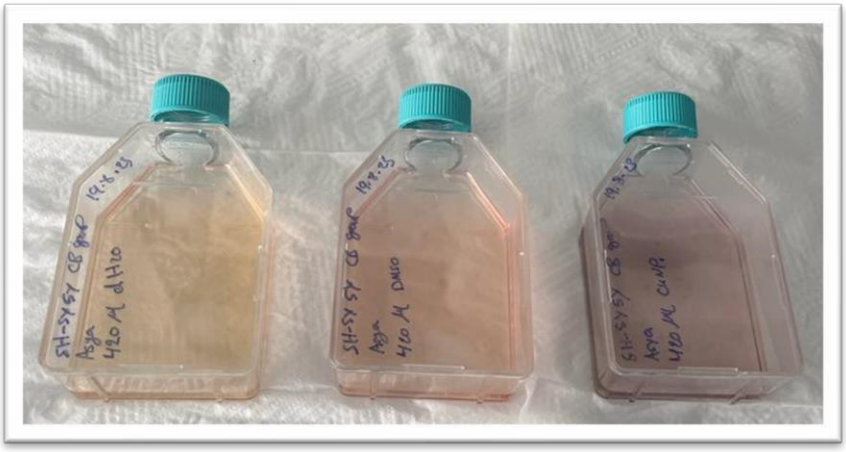

Fig 6: Positive control (dH_2O administered) , Negative control (DMSO administered), and CuONP administered sample flasks, each containing 10^6 cells seeded for oxidative stress measurement.

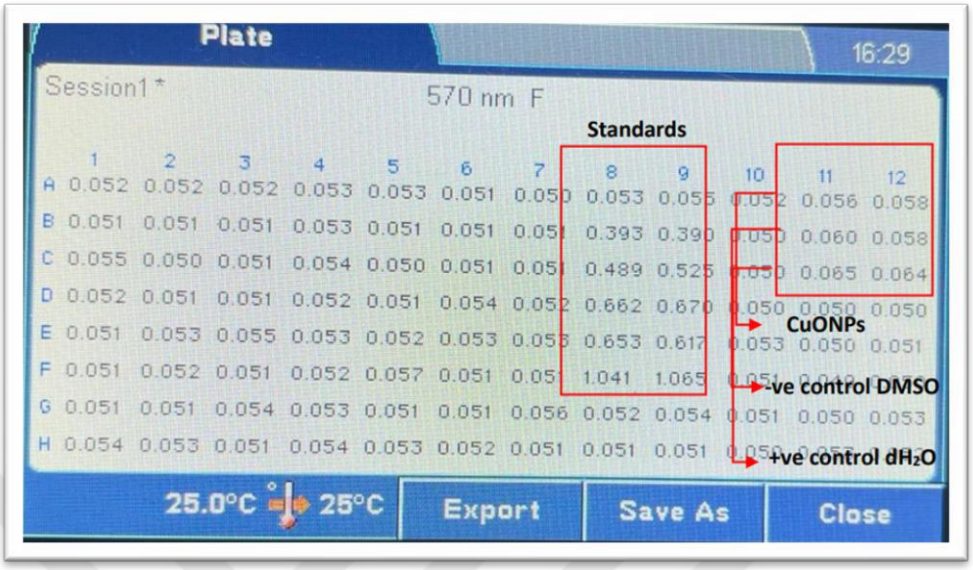

Fig 7: Oxidative stress measurement by using Ab102500 – Hydrogen Peroxide Assay Kit (Colorimetric)

3.9 Statistical Analysis

Frequency distribution for descriptive statistics, arithmetic mean and standard deviation for continuous variables were calculated by using Student's t-test. For all studies, significance value (p), which was *$p<0.05$, obtained using a paired two-tailed Student´s t-test. Data are expressed as mean ± standard error.

Chapter 4

Results

A novel, green, rapid, and cost-effective method to synthesize CuO NPs using *Aloe vera* as a reducing agent was proposed in this study. It was shown that CuO NPs led to dose-dependent decrease in cell viability when administered to SH-SY5Y human neuroblastoma cells.

4.1 : Characterization of Synthesized CuO NPs

Fourier transform infrared spectroscopy (FT-IR) analysis was used to determine the biomolecules during the synthesis of CuO NPs from an *Aloe vera* extract. Moreover, X-ray diffraction (XRD) analysis can provide valuable information about the crystallinity and phase of the synthesized CuO NPs.

4.1.1 : XRD Analysis

XRD analysis of CuO NPs revealed different peaks as shown in Figure 7. Peaks at 38.1° and 41.4 correspond with the crystalline planes [111] and [020], respectively. They show that the CuO NPs include particular crystallographic orientations. Peak at 45.5° was determined as another sign of the crystal structure, corresponding to the [020] crystalline plane. This peak's duplication suggests a high level of crystallinity. Peak at 58.2° corresponds to the crystalline plane [101]. Peak at 63.2° corresponds to [110] crystalline plane while the peak at 74.2° is showing the [020] crystalline plane (Manyasree.D *et al.*,2017).

According to the information derived from these XRD peaks, the CuO NPs synthesized via the proposed green synthesis method in this study have a monoclinic crystalline structure, and the detected peaks correspond to different crystallographic planes within that structure.

Researchers can use this XRD data to identify the specific crystallographic phase and the orientation of the crystalline domains within the CuO NPs. Additionally, this information can be used to confirm the success of the green synthesis method in producing the desired crystalline phase of CuO NPs. (Himanshu Narayan*et al.*, 2018). It’s also interesting to observe that the actual peaks for the CuO nano powder appear to have been moved by

approximately a 2θ, which may be caused by strains within the crystallites. The CuO NPs' obtained XRD spectrum is shown in Figure 8.

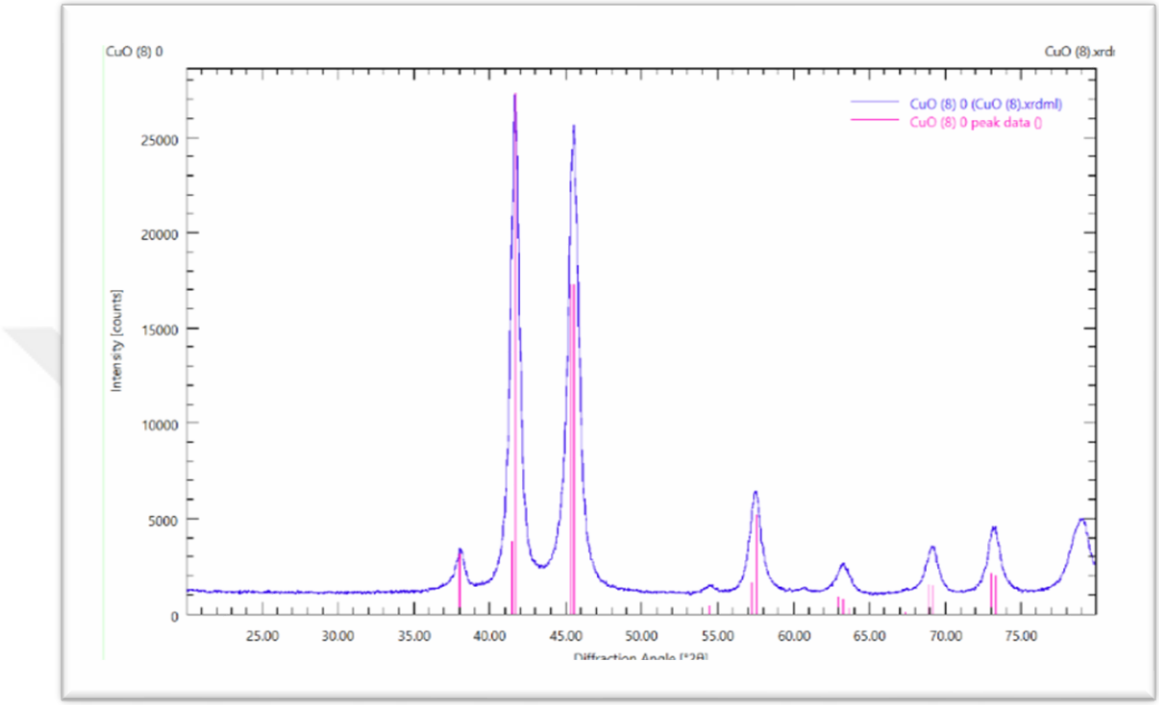

Fig 8: X-ray diffraction (XRD) patterns of CuO nanoparticles

4.1.2 :Fourier transform infrared spectroscopy (FT-IR) Analysis

The FT-IR analysis serves to the confirmation of the purity and structure of the synthesized CuO NPs by providing useful insights into the chemical composition and functional groups present. In this study, green synthesized CuO NPs represented stretching vibration of the hydroxyl (OH) groups which can be determined by the peaks between 3294 cm^{-1}, 3266 cm^{-1}, 3450 cm^{-1}. The *Aloe vera* extract, which was used to synthesize CuO NPs, likely contains these groups. The amino (-NH2) groups are represented with the peak at 1636 cm^{-1}. Alcohols, phenols, and amines are a few examples of compounds that include amino groups. The presence of these functional groups in the *Aloe vera* extract employed for the synthesis of CuO NP is shown by this peak. C-C stretching vibrations are linked to bands approximately 1600 cm^{-1}. Alkanes and hydroxyl groups' C=O stretching vibrations

are represented as the peaks around 2350 cm^{-1}. Moreover, peaks at 1000 cm^{-1} and 500 cm^{-1}, which are linked to metal-oxygen (metal-O) vibrations, give confirmation of the presence of CuO NPs. These peaks show pure CuO NPs were successfully synthesized by using *Aloe vera* extract (Figure 9).

Fig 9: FT-IR spectra of green synthesized CuO NPs by using *Aloe vera* leaf extract

4.1.3 Energy Dispersive X-Ray (EDX) Analysis

The elemental composition and purity of the biosynthesized CuO NPs were confirmed by EDX analysis . The elemental a percentage (by mass, 7.62% Cu, 54.85% O, and 37.53% C) is explored by EDX pattern, as shown Figure 9. EDX analysis confirmed the elemental composition of the biosynthesized CuO NPs, which consisted primarily of copper (Cu) and oxygen (O) in the intended proportions. Additionally, the presence of carbon (C) and oxygen (O) elements from the *Aloe vera* leaf extract used in the synthesis process was observed. Furthermore, the EDX spectra showed no other traces of elemental contaminants. These results provide important information about the purity and elemental

makeup of the CuO NPs synthesized through the proposed method in this study (Figure 10).

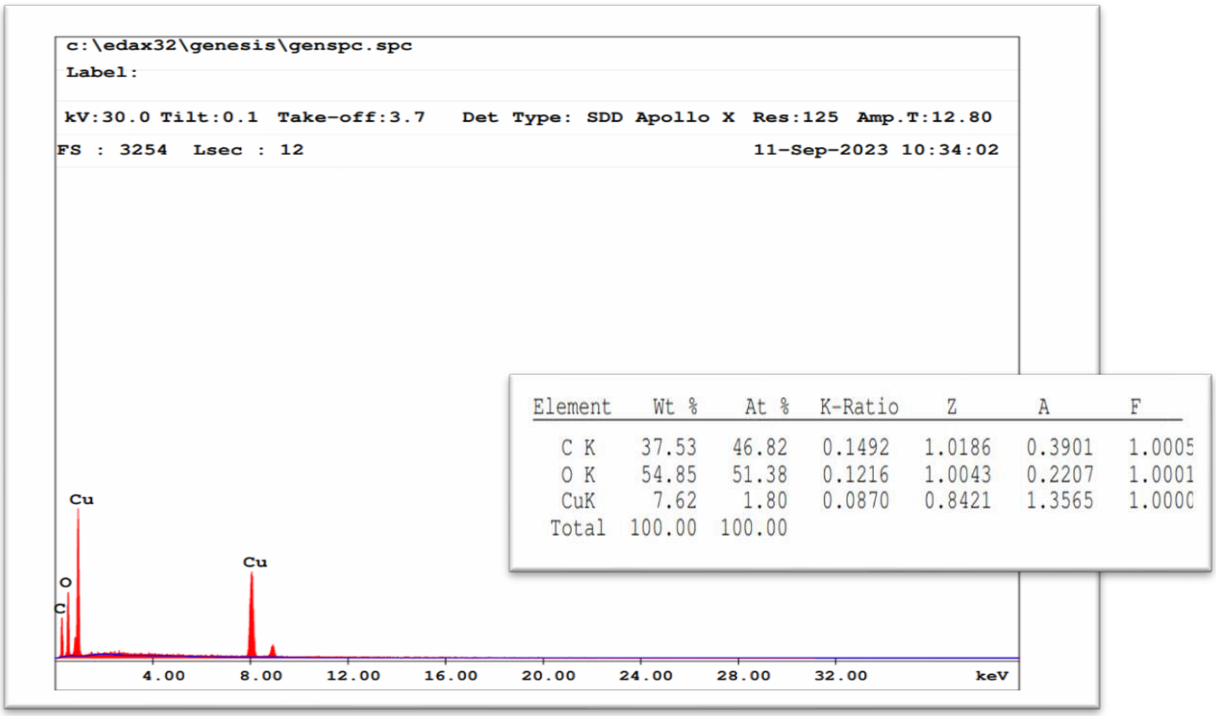

Fig 10: EDX analysis of the synthesized CuO NPs was used to confirm their elemental composition and purity.

4.1.4 Scanning Electron Microscopic (SEM) Analysis

The morphology and growth characteristics of the synthesized CuO NPs were examined using scanning electron microscopy (SEM) (Figure 10). The CuO NPs have a porous nanoscale structure and spherical shape. As demonstrated in to Figure 11, agglomeration of CuO NPs might be the result of crystallization and the oxidation of metal nanoparticles. The SEM image confirms that the CuO NPs have formed into a regular polyhedron shape. It is clearly observed that the tightly packed spherical structure formed aggregation.

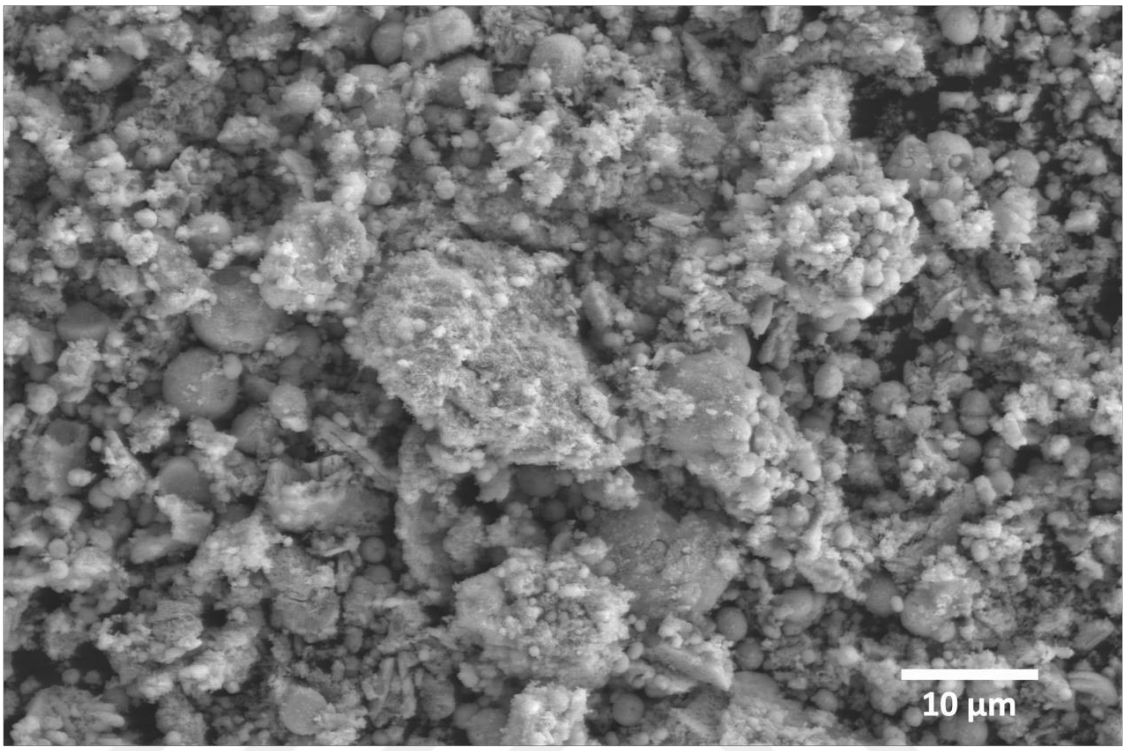

Fig 11: SEM image of CuO-NPs

4.2: SH-SY5Y cell line treatment with CuO NPs

Treatment with CuONP reduced cell proliferation and survival in a dose-dependent manner in SH-SY5Y neuroblastoma cells. Based on previous research on copper toxicity in neuroblastoma cells, the concentration range was determined as 125 ng/mL, 250 ng/mL, and 500 ng/mL (Mario Arciello *et al.*, 2005). Microscopic analysis showed that the confluency of SH-SY5Y cells reduced with increasing CuONP concentrations which might indicate that CuO NPs had an adverse impact on cell survival. Notably, after a 24-hour treatment, the highest dose of CuO NPs (500 ng/mL) almost completely inhibited the survival of SH-SY5Y cells (Figure 12, 13).

The negative impacts of CuO NPs on SH-SY5Y cells have been confirmed by the MTT assay, which measures cell viability based on mitochondrial enzyme reaction (Figure 14). Cell viability showed a decrease in a dose-dependent manner, with the clearest toxicity being shown at high CuONP doses (250 ng/mL and 500 ng/mL; $*p<0,05$, $***p<0,001$, respectively).

It was also observed that the lowest CuONP concentration (125 ng/mL) had a minimal effect on cell viability, which suggests that low CuONP concentrations may be less toxic to cells. The percentage of vital cells was calculated by comparing each sample and control with the positive control and using the following equation:

$$\% \text{ viability} = (\text{OD of test} / \text{OD positive control}) \times 100$$

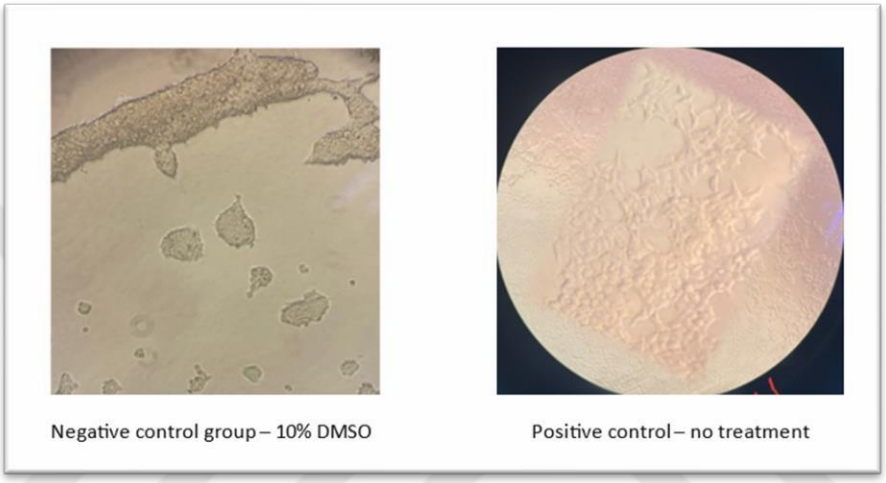

Figure 12: Microscopic examination of cell proliferation for positive and negative control of SH-SY5Y

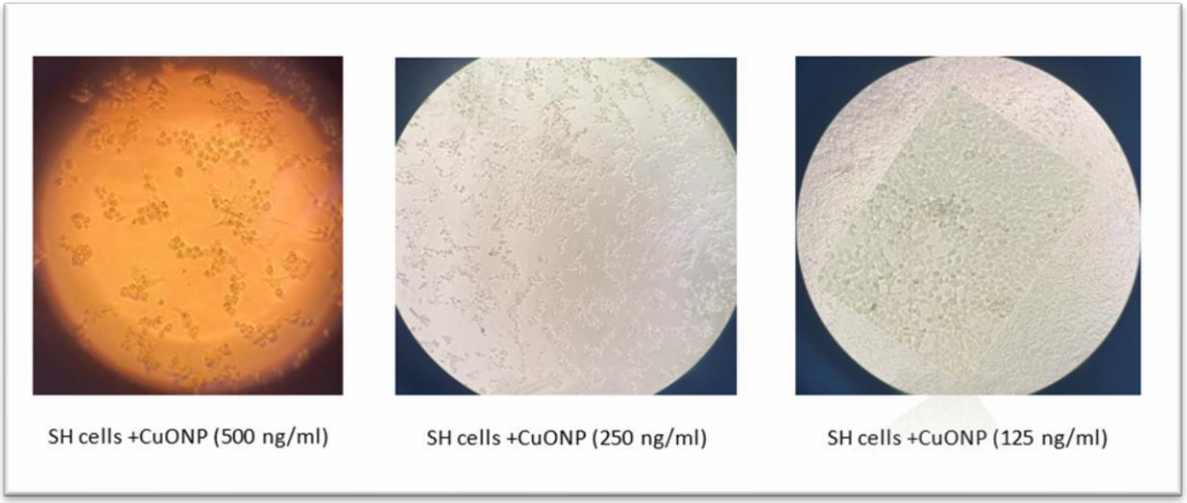

Figure 13: Microscopic examination of cell proliferation after treatment with different doses of CuO NPs for the neuronal cell line SH-SY5Y

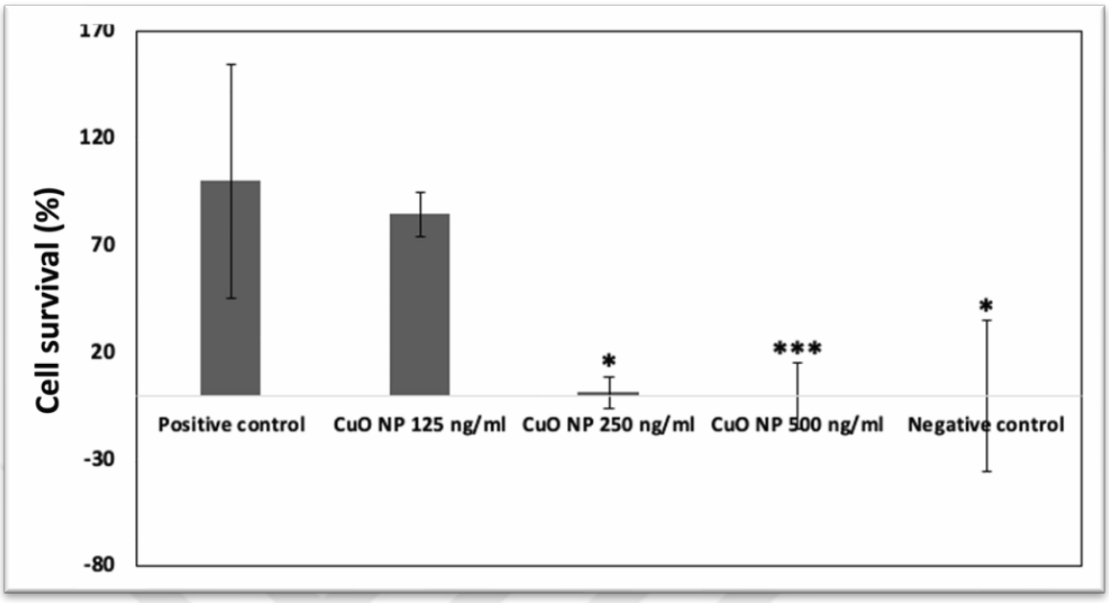

Figure 14: MTT test for cell proliferation after CuO NPs treatment of the neuronal cell line SH-SY5Y (p*<0,05, ****p*<0,001)**

4.3 PD Model and oxidative stress

We first examined to see if 6-OHDA affected the differentiation of SH-SY5Y neuroblastoma cells. For this purpose, cells were exposed to 6-OHDA for 24 hours at different concentrations from 10 to 50 µM and the cellular differentiation was assessed by microscopic examination. Our findings did not show differences in SH-SY5Y cells' differentiation when the cells exposed to 10 µM 6-OHDA while the cell viability was reduced, and the cells were dead at the 50 µM concentration. On the other hand, when 25 µM of 6-OHDA was administered to cells, cell aggregation and degradation was observed (Figure 15, 16).

To test the effect of CuO NPs on cells of PD model (6-OHDA treated cells) the nanoparticles were administered at concentrations as 25 µg/mL, 250 µg/mL, and 500 µg/mL for 24 hours. As shown in Figure 15, CuO NPs at a high concentration (500 µg/mL) induced cell death, compared to the other two concentrations. The results of MTT assay, in parallel to previously administered CuONP concentrations, confirmed that CuO NPs decreased cell survival in SH-SY5Y cells after 6-OHDA differentiation (Figure 17).

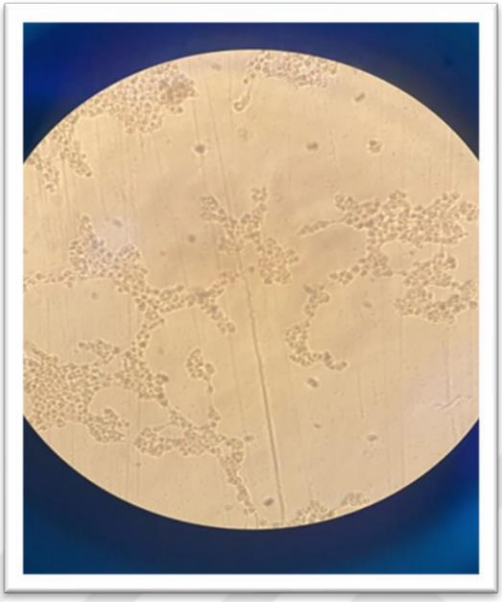

Fig 15: **SH SH-SY5Y cell line after differentiated with 25 µM of 6-OHDA**

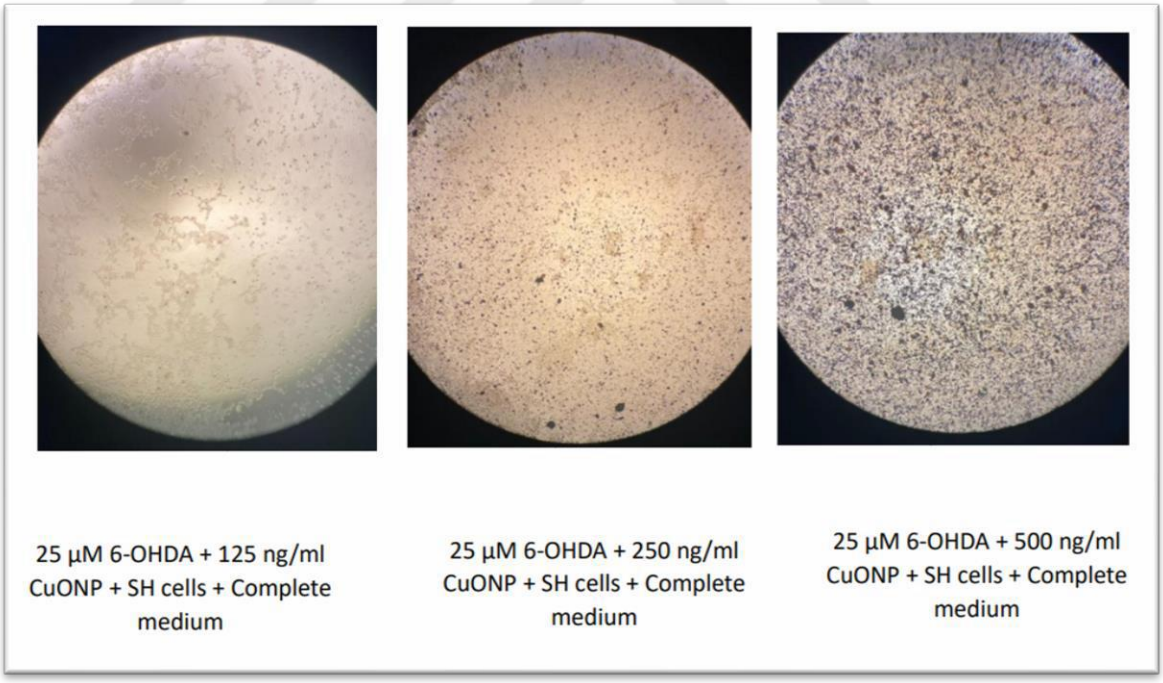

Fig 16: **Microscopic examination of cell proliferation after treatment with different doses of CuO NPs for the SH SH-SY5Y cell line after differentiated with 25 µM of 6-OHDA**

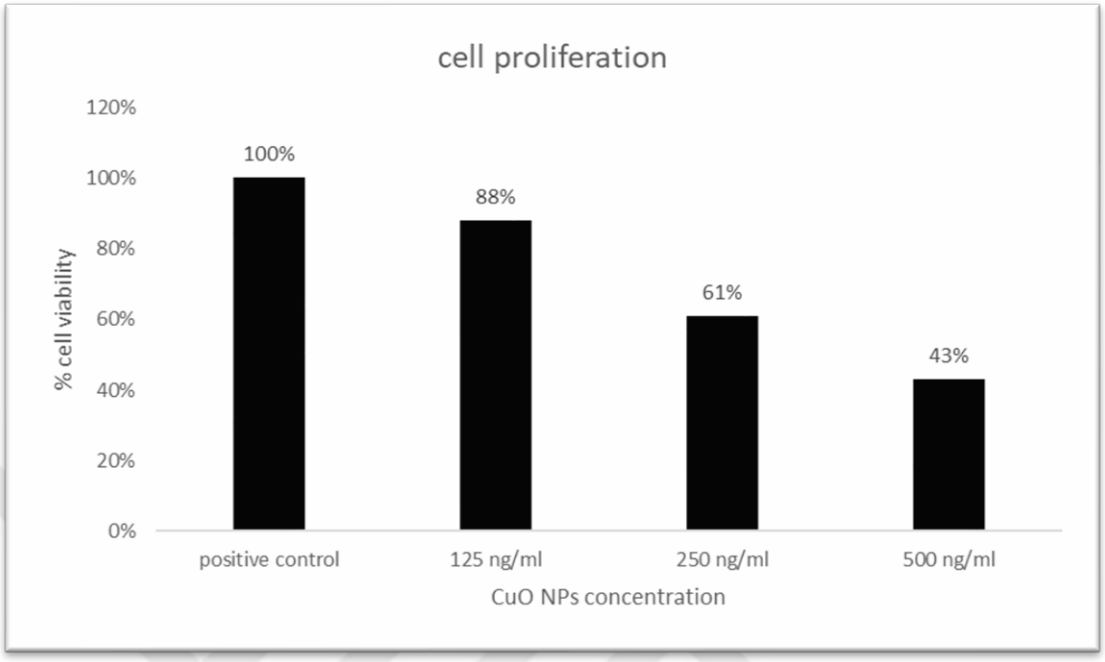

Fig 17: MTT analysis for cell survival after CuO NPs treatment of the differentiated cell line SH-SY5Y with 6-OHDA

To determine the role of oxidative stress in CuO NPs treated cells, we used a commercially available Hydrogen Peroxide Assay Kit for measuring hydrogen peroxide (H2O2) levels. However, we did not obtain any results while the standards were within the range. This may be due to several reasons which will be discussed in Chapter 5.

Chapter 5

Discussion

The CuO NPs created from *Aloe vera* leaf extract were crystallized, as demonstrated by the XRD spectrum. According to card number 801916 of the International Centre for Diffraction Data (ICDD), the peak positions demonstrated the monoclinic structure of CuO. The specific crystallographic phase can be identified, the lattice parameters can be calculated, and more information about the form and orientation of the crystalline domains within the CuO NPs can be learned with the help of this XRD data. This data can also be used to verify that the green synthesis method produced the desired crystalline phase of CuO NPs (Himanshu Naraya *et al.*,2018). It's also intriguing to note that the CuO nano powder's actual peaks seem to have moved by around a 2θ, which could be the result of crystallite strains (Vijay Kumar *et al.*, 2015).

The FT-IR analysis of CuO NPs synthesized from *Aloe vera* extract shows the presence of several functional groups and validates the CuO NPs' purity and structure. When compared to previous research, our findings are consistent with other studies, particularly the one by Suha Maher Abed *et al.* (2021) who also noted comparable peaks in the FTIR spectra of CuO NPs synthesized using copper sulphatepentahydrate ($CuSO_4$). To learn more about the chemical composition and vibrational modes of the molecules involved in the synthesis process, particular absorption peaks at various wavenumbers were used. These results support the validity of our study because they are in line with earlier work in the field (Vinod Vellora Thekkae Padil, *Miroslav Černík.*,2010).

The SEM analysis provides valuable insights into the structural characteristics of CuO nanoparticles. The large crystallite size, sharp diffraction peaks, and high peak intensity indicate a well-defined and highly crystalline structure for CuO NPs synthesized from *Aloe vera* extract. The spherical and regular polyhedron shape, along with some agglomeration tendency, describes the morphology and dispersion behaviour of the nanoparticles. These characteristics are important considerations for various applications of CuO-NPs, including catalysis, sensors, and nanoelectronics, among others (Ismat Zerin Luna *et al.*, 2015).

Our findings showed that CuO NPs cause dose-dependent toxicity in SH-SY5Y neuroblastoma cells. CuO NPs showed a negative impact on cell viability which was more prominent at higher doses. These results are in line with the concept of dose-dependent toxicity, which suggests that biological effects become more severe with increasing exposure to a toxic agent. Moreover, our findings are consistent with an earlier research on copper toxicity in neuroblastoma cells (Ying Shi *et al.*, 2020). They showed that only high-dose CuO NP had an anti-proliferative impact, with about 90% inhibition on H4 and PC12 cells during a 48-hour incubation period. (Ying Shi *et al.*, 202).

The results of our study on differentiated PD cells are also parallel with the results of a previous study (Michael J *et al.* 2016), which also observed toxicity of copper nanoparticles when added to the media used for cell culture. Our results demonstrated that CuO NPs, particularly at high concentrations, cause cell death in SH-SY5Y neuroblastoma cells after 6-OHDA administration. These findings help us figure out how these substances might be toxic when used in cell culture and PD research (Suha Maher Abed *et al.*, 2021).

However, we could not provide data on our the hypothesis stating that oxidative stress played a role in the treatment of CuO NPs due to experimental problems. Measuring reactive oxygen species (ROS) can be challenging due to the potential for artifacts and complexities associated with various detection methods. Different assays may be sensitive to various forms of ROS. ROS levels can be significantly influenced by cell culture conditions. It's important to be aware of the possibility of oxidative stress when working with cells in *in vitro* because of factors like a lack of antioxidants in the culture media and high oxygen concentrations (Michael P. Murphy *et al.*, 2022). Moreover, different cell types may respond to oxidative stress differently and have varying amounts of endogenous antioxidants (Helmut Sies, 2015). On the other hand, since ROS levels might change over time, it's important to incubate the cells with nanoparticles in different incubation time ranges. Time-course experiments are frequently helpful for detecting dynamic changes in the production of ROS or clearance (Kaushik Das, Aryadeep Roychoudhury, 2014). Experiments with proper controls are essential for distinguishing between ROS-related signals and artifacts. To create a baseline, this may require using ROS scavengers, ROS-producing compounds, or comparing treated and untreated cells. When measuring ROS,

using orthogonal approaches is an excellent way to double-check the findings and reduce method-specific artifacts. Since different assays may be sensitive to different types of ROS, using different methods can provide a more comprehensive picture of oxidative stress (Michael P. Murphy *et al.*, 2022).

Chapter 6

Conclusion and Future Work

Aloe vera extract was used to synthesize CuO NPs, and the comprehensive study provided important details about their structural and biological characteristics. X-ray diffraction (XRD) analysis demonstrated that the CuO NPs were crystalline with a monoclinic structure, further verified using ICDD database. This structural information aids in understanding the crystalline phases, lattice parameters, and crystallographic details, affirming the success of the green synthesis method. CuO NPs' chemical composition and structural integrity were confirmed by the presence of several functional groups, which were detected by FT-IR spectroscopy. This study improves our knowledge of the chemicals involved in the synthesis process and corresponds with earlier findings. The morphology of CuO NPs was shown by scanning electron microscopy (SEM), which revealed a well-defined and highly crystalline structure with a nanoparticle size of approximately 50 nm. The reported properties are important to take into consideration for potential applications in nanoelectronics, sensors, and catalysis. CuO NPs exhibited dose-dependent toxicity in SH-SY5Y neuroblastoma cells, highlighting the importance of understanding their potential adverse effects. These findings align with earlier research on copper toxicity in various cell types and provide insights into their cytotoxicity.. Reactive oxygen species (ROS) measurements can be challenging due to a variety of variables, such as assay sensitivity and cell culture conditions. Further experiments with careful consideration on experimental setup are needed to fully understand the relationship between CuO NPs and oxidative stress.

Further research is warranted to elucidate the underlying biological mechanisms responsible for the observed dose-dependent toxicity of CuO NPs. This could involve investigating cellular pathways, oxidative stress markers, and potential protective measures. CuO NPs have the potential to be employed in a variety of biomedical applications, including drug delivery, imaging, and therapeutic agents while ensuring their safety and efficacy, is an area of growing interest. Extensive studies on the toxicological aspects of CuO NPs, including their interaction with different cell types, long-term effects, and potential mitigating strategies, will contribute to a comprehensive understanding of

their impact on human health and the environment. It may be possible to optimize the optical characteristics of CuO NPs for certain applications by looking into the tunability of their optical properties based on the choice of plant extract or synthesis method.

In summary, the synthesis and characterization of CuO NPs from *Aloe vera* extract present a promising avenue for diverse applications, but further research is needed to address safety concerns, explore potential applications, and deepen our understanding of their properties and behaviour in various contexts.

References

Almokhtar A Adwas,1, Ata Sedik Ibrahim Elsayed,2, Azab Elsayed Azab,3 ,Fawzia Amhimmid Quwaydir,**and underlying neurotoxicity as a model of Parkinson's disease**,2021, ournal of Molecular Liquids 323 (2021) 115051

Andrés García-Sánchez , Alejandra Guillermina Miranda-Díaz ,and Ernesto Germán Cardona-Muñoz, **The Role of Oxidative Stress in Physiopathology and Pharmacological Treatment with Pro- and Antioxidant Properties in Chronic Diseases,**2020, Oxidative Medicine and Cellular Longevity ,Volume 2020, Article ID 2082145, 16 pages https://doi.org/10.1155/2020/2082145

Andy Wai Kan Yeung, Nikolay T. Tzvetkov, Maya G. Georgieva, Iliyan V. Ognyanov, Karolina Kordos, Artur Jóźwik, Toni Kühl, **Reactive Oxygen Species and Their Impact in Neurodegenerative Diseases: Literature Landscape Analysis**,2021, Antioxidants & RedoxSignalingVol.34,No. 5, https://doi.org/10.1089/ars.2019.7952

Araújo, I.M.S.; Silva, R.R.; Pacheco, G.; Lustri, W.R.; Tercjak, A.; Gutierrez, J.; Júnior, J.R.S.; Azevedo, F.H.C.; Figuêredo, G.S.; Vega, M.L.; Ribeiro, S.J.L.; Barud, H.S. **Hydrothermal Synthesis of Bacterial Cellulose-copper Oxide Nanocomposites and Evaluation of their Antimicrobial Activity**, Elsevier. (2017). doi:10.

Antonio, Mechanism and Applications of Metal Nanoparticles Prepared by Bio-Mediated Process, Reviews in Advanced Sciences and Engineering, Volume 3, Number 3, September 2014, pp. 199-216(18)

Ashwini Anantharaman , Liz George, Mary George, **GREEN SYNTHESIS OF COPPER OXIDE NANOPARTICLES AND ITS APPLICATIONS**,2016, Advanced Powder Technology 29(12), DOI:10.1016/j.apt.2018.09.009

Afrah Atri , Mosaab Echabaane , Amel Bouzidi , Imen Harabi ,Bernabe Mari Soucase ,Rafik Ben Chaabane, **Green synthesis of copper oxide nanoparticles using Ephedra Alata plant extract and a study of their antifungal, antibacterial activity and photocatalytic performance under sunlight**, 2023, Heliyon | VOLUME 9, ISSUE 2, E13484, FEBRUARY 2023

Benjamin G. Trist1 | Dominic J. Hare2,3 | Kay L. Double, **Oxidative stress in the aging substantia nigra and the etiology of Parkinson's disease**,2019, https://doi.org/10.1111/acel.13031

Bayram ÇİMEN, Sonya ŞENGÜL, Memduha ERGÜT, Ayla ÖZER, **Green Synthesis and Characterization of CuO Nanoparticles:Telon Blue AGLF and Methylene Blue Adsorption** ,2019, Sinop Uni J Nat Sci 4 (1): 1-20(2019) .ISSN: 2536-4383

C. Minelli, talk on 'Measuring nanoparticle properties: are we high and dry or all at sea?' at '**Nanoparticle Characterisation – Challenges for the Community' event IOP (Institute of Physics)** July 2016, book of abstracts, , LondonCastro, Laura; Blázquez, Ma Luisa; Muñoz, Jesús ángel; González, Felisa González; Ballester,

Claudio Cabello-Verrugio,1,2 Marta Ruiz-Ortega,3 Matias Mosqueira,4 and Felipe Simon2, Oxidative Stress in Disease and Aging: **Mechanisms and Therapies,2016, Hindawi Publishing Corporation Oxidative Medicine and Cellular Longevity** Volume 2016, Article ID 8786564, 2 pages http://dx.doi.org/10.1155/2016/8786564

Devarajan Alagarasan, A. Harikrishnan,M. Surendiran,Karuppusamy Indira, Amany Salah Khalifa , Basem H. Elesawy, **Synthesis and characterization of CuO nanoparticles and evaluation of their bactericidal and fungicidal activities in cotton fabrics,**2021, Applied Nanoscience , https://doi.org/10.1007/s13204-021-02054-5

Divya M Radhakrishnan, Vinay Goyal, **Parkinson's disease: A review,**2018, Neurology India, Neurological Society of India, ;66:S26-35., DOI: 10.4103/0028-3886.226451

Djurišić, A.B., Leung, Y.H., Ng, A.M., *et al.*: '**Toxicity of metal oxide nanoparticles: mechanisms, characterization, and avoiding experimental artefacts'**, 2015, 11, (1), pp. 26–44 doi:10.1155/2011/343082

Dominic S. A. Simpson 1,2ORCID andPeter L. Oliver 1, **ROS Generation in Microglia: Understanding Oxidative Stress and Inflammation in Neurodegenerative Disease**,2020, Antioxidants 2020, 9(8), 743; https://doi.org/10.3390/antiox9080743

Evelyn Assadian1,2 & Mohammad Hadi Zarei2 & Ali Ghanadzadeh Gilani1 &Mehrzad Farshin2 & Hamid Degampanah1 & Jalal Pourahmad2,**Toxicity of Copper Oxide (CuO) Nanoparticles on HumanBlood Lymphocytes** ,2017, Biol Trace Elem Res DOI 10.1007/s12011-017-1170-4

Fox, S. H., Katzenschlager, R., Lim, S. Y., Barton, B., de Bie, R. M., Seppi, K.,

Geon Ha Kim 1, Jieun E Kim 2, Sandy Jeong Rhie 3, Sujung Yoon 4, **The Role of Oxidative Stress in Neurodegenerative Diseases**,2015, http://dx.doi.org/10.5607/en.2015.24.4.325 Exp Neurobiol. 2015 Dec;24(4):325-340. pISSN 1226-2560 • eISSN 2093-8144

Helmut Sies , **Oxidative stress: a concept in redox biology and medicine**,2015, Redox Biology ,Volume 4, April 2015, Pages 180-183

Helmut Sies, **On the History of Oxidative Stress: Concept and Some Aspects of Current Development**,2018, DOI:10.1016/j.cotox.2018.01.002

Helmut Sies, **Oxidative Stress: Concept and Some Practical Aspects**,2018, Antioxidants 2020, 9(9), 852; https://doi.org/10.3390/antiox9090852

Helmut Sies,1,2 Carsten Berndt,3 and Dean P. Jones4, **Oxidative Stress**,2017, Annual Review of Biochemistry Vol. 86:715-748 (Volume publication date June 2017

Helmut Sies1,2 and Dean P. Jones, **Reactive oxygen species (ROS) as pleiotropic physiological signalling agents** 2020, Nat Rev Mol Cell Biol. 2020 Jul;21(7):363-383. doi: 10.1038/s41580-020-0230-3. Epub 2020 Mar 30.

Heng-Chung Kung 1,2 , Kai-Jung Lin 1,3 , Chia-Te Kung 4,* and Tsu-Kung Lin 1, **Oxidative Stress, Mitochondrial Dysfunction, and Neuroprotection of Polyphenols with Respect to Resveratrol in Parkinson's Disease**, Biomedicines 2021, 9, 918. https://doi.org/10.3390/biomedicines9080918

Hideki Fujii,Kentaro Nakai,Masafumi Fukagawa, **Role of Oxidative Stress and Indoxyl Sulfate in Progression of Cardiovascular Disease in Chronic Kidney Disease** ,2011, the International Symposium "Uremic Toxins and Oxidative Stress in Dialysis Patients" from the 55th Annual Meeting of the Japanese Society for Dialysis Therapy held 18–20 June 2010 in Kobe, Japan.

Himanshu Narayan1 *, Hailemicheal Alemu2, Lineo F. Maxakaza2 , Sandesh Jaybhaye3, **Copper Oxide Nanoparticles : Synthesis and Characterization,** 2018, National Conference on 'Advanced Analytical Tools for Materials Characterization' (AATMC-2018) © 2018 IJSRSET | Volume 5 | Issue 4 | Print ISSN : 2395-1990 | Online ISSN : 2394-4099

Holzinger *et al.*, M. Holzinger, A. Le Goff, S. **Cosnier ,Nanomaterials for biosensing applications: a review Front. Chem**., (2014), p. 63, 10.3389/fchem.2014.00063

https://www.sciencedirect.com/science/article/pii/S1878535217300990

Hui He1,*Zhen Zou 2,*Bin Wang 2 Ge Xu2 Chengzhi Chen 3 Xia Qin1 Chao Yu1 Jun Zhang **, DNA Damage and Cell Death via Copper Ion-Mediated P38 MAPK Activation in Vascular Endothelial Cells** ,2020, International Journal of Nanomedicine 2020:15 3291–3302.

Himanshu Narayan1 *, Hailemicheal Alemu2, Lineo F. Maxakaza2 , Sandesh Jaybhaye3,**Copper Oxide Nanoparticles : Synthesis and Characterization,**2018, National Conference on 'Advanced Analytical Tools for Materials Characterization' (AATMC-2018) ,| Volume 5 | Issue 4 | Print ISSN : 2395-1990 | Online ISSN : 2394-4099

Helmut Sies, **Oxidative stress: a concept in redox biology and medicine,**2015, Redox Biology,Volume 4, April 2015, Pages 180-183

IbrahimKhan, KhalidSaeed, IdreesKhan, **Nanoparticles: Properties, applications and toxicities,**2019, Arabian Journal of Chemistry, Volume 12, Issue 7, November 2019, Pages 908-931.

Ilaria Liguori, Gennaro Russo, Francesco Curcio, Giulia Bulli, Luisa Aran, David Della-Morte, Gaetano Gargiulo, Gianluca Testa, Francesco Cacciatore, Domenico Bonaduce, Pasquale Abete, **Oxidative stress, aging, and diseases ,** 2018, The National Center for Biotechnology Information advances science and health, 26;13:757-772. doi: 10.2147/CIA.S158513. eCollection 2018.

Jingjun Lyu a,∗, Xiaobing Long a, Tuxiu Xie b, Guijun Jiang a, Jie Jiang c, Lu Ye , Qiongzi Li , **Copper oxide nanoparticles promote α-synuclein oligomerization**

Jolanta Dorszewska1, *, Marta Kowalska1, Michał Prendecki1, Thomas Piekut1,oanna Kozłowska1, Wojciech Kozubski2,**Oxidative stress factors in Parkinson's disease** ,2020, https://doi.org/10.4103/1673-5374.300980.

Katerina Krumova and Gonzalo Cosa, **Overview of Reactive Oxygen Species,**2016, Overview of Reactive Oxygen Species , in Singlet Oxygen: Applications in Biosciences and Nanosciences, Volume 1, 2016, pp. 1-21 DOI: 10.1039/9781782622208-00001

Kovalevich J, Santerre M, Langford D. ,**Considerations for the Use of SH-SY5Y Neuroblastoma Cells in Neurobiology**, Methods Mol Biol, 2021, 2311: 9-23

Krishnan B, Mahalingam S, **Improved surface morphology of silver/coppe 537 oxide/bentonite nanocomposite using aliphatic ammonium based ionic liquid for enhanced 538 biological activities**. Journal of Molecular Liquids. 2017 Sep 1;241:1044

Kaushik Das and Aryadeep Roychoudhury, **Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants,**2014,Front. Environ. Sci., 02 December 2014 Sec. Environmental Toxicology Volume 2 - 2014 | https://doi.org/10.3389/fenvs.2014.00053

Letchumanan, D.; Sok, S.P.M.; Ibrahim, S.; Nagoor, N.H.; Arshad, N.M. **Plant-Based Biosynthesis of Copper/ Copper Oxide Nanoparticles: An Update on Their Applications in Biomedicine, Mechanisms, and Toxicity.** Biomolecules 2021, 11. doi:10.3390/BIOM11040564.

Lorena Meñaca-Guerrero1 ORCID, Amileth Suarez-Causado2 ORCID, Antonio José Díaz-Caballero3, **Reactive species of oxygen, oxidative stress and its relationship with tissular destruction in periodontitis** ,2020, Meñaca-Guerrero L, Suarez-Causado A, Díaz-Caballero AJ.

Luna, I.Z., Hilary, L.N., Chowdhury, A.M.S., Gafur, M.A., Khan, N. and Khan, R.A. **Preparation and Characterization of Copper Oxide Nanoparticles Synthesized**

via Chemical Precipitation Method. (2015) Open Access Library Journal, 2: e1409. http://dx.doi.org/10.4236/oalib.1101409.

María Luisa Fanjul-Moles1 and Germán Octavio López-Riquelme2, **Relationship between Oxidative Stress,Circadian Rhythms, and AMD**,2016, Hindawi Publishing Corporation Oxidative Medicine and Cellular Longevity Volume 2016, Article ID 7420637, 18 pages http://dx.doi.org/10.1155/2016/7420637.

Marketa Marvanova, **Introduction to Parkinson disease (PD) and its complications,**2016, MENTAL HEALTH IN GERIATRIC POPULATIONS, DOI: 10.9740/mhc.2016.09.229.

Minrui Weng 1, Xiaoji Xie 1, Chao Liu 2, Kah-Leong Lim 2, Cheng-Wu Zhang 1, Lin Li 1, **The Sources of Reactive Oxygen Species and Its Possible Role in the Pathogenesis of Parkinson's Disease**,2018, 2;2018:9163040. doi: 10.1155/2018/9163040.

Mohsen Hemmati-Dinarvand, Samira saedi,Mohammad Valilo, Ashkan Kalantary-Charvadeh, Mahmood,Alizadeh Sani, Roya Kargar, Hossein Safari, Nasser Samadi, **Oxidative stress and Parkinson's disease: conflict of oxidant-antioxidant systems**,2019,PII:S0304-3940(19)30369-6 DOI:https://doi.org/10.1016/j.neulet.2019.134296 Article Number: 134296.

Manyasree D, Kiran Mayi Peddi , Ravkumar R , **CuO NANOPARTICLES: SYNTHESIS, CHARACTERIZATION AND THEIR BACTERICIDAL EFFICACY,**2017, International Journal of Applied Pharmaceutics ISSN- 0975-7058 Vol 9, Issue 6, 2017.

Michael J. Mashock1, Tyler Zanon1, Anthony D. Kappell1, Lisa N. Petrella1, Erik C. Andersen2, Krassimira R. Hristova1, **Copper Oxide Nanoparticles Impact Several Toxicological Endpoints and Cause Neurodegeneration in Caenorhabditis elegans,**2016, PLOS ONE | DOI:10.1371/journal.pone.0167613 December 2, 2016

Michael P. Murphy, Hülya Bayir, Vsevolod Belousov, Christopher J. Chang Kelvin J. A. Davies, Michael J. Davies , Tobias P. Dick, Toren Finke, Henry J. Forman,Yvonne Janssen-Heininger, David Gems1, Valerian E. Kagan , Balaraman Kalyanaraman,Nils-Göran Larsson, Ginger L. Milne, Thomas Nyström, Henrik E.

Noha Al-Qasmi, **Facial Eco-Friendly Synthesis of Copper Oxide Nanoparticles Using Chia Seeds Extract and Evaluation of Its Electrochemical Activity**, 2021, Processes 2021, 9, 2027. https://doi.org/10.3390/pr9112027

Nilanjan Chakraborty, Jishnu Banerjee, Pallab Chakraborty, Anuron Banerjee, Sumeddha Chanda, Kasturi Ray, Krishnendu Acharya & Joy Sarkar, **Green synthesis of copper/copper oxide nanoparticles and their applications: a review** ,2022, ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/tgcl20.

Pandey, V., Mishra, G., Verma, S., Wan, M. and Yadav, R. **Synthesis and Ultrasonic Investigations of CuOPVA Nanofluid. Journal of Materials Sciences and Applications**, (2012) , 3, 664-653. ,http://dx.doi.org/10.4236/msa.2012.39097

Parkinson's disease, (2018),Mov. Disord. 33, 1248–1266. doi: 10.1002/mds.27372.

Prachi Vaid, P. Raizada, R. Saini, **Biogenic silver, gold and copper nanoparticles - A sustainable green chemistry approach for cancer therapy** ,2020, DOI:10.1016/j.scp.2020 .100247Corpus ID: 216459814Rocca WA) **The burden of Parkinson's disease: a worldwide perspective**,2018, Lancet Neurol 17: 928-929.

Poulsen, Rafael Radi,Holly Van Remmen, Paul T. Schumacker, Paul J. Thornalley , Shinya Toyokuni ,Christine C. Winterbourn, Huiyong Yin and Barry Halliwell, **Guidelines for measuring reactive oxygen species and oxidative damage in cells and in vivo,** Nature Metabolism | VOL 4 | June 2022 | 651–662 | www.nature.com/natmetab.

S. Esmail, **The Diagnosis and Management of Parkinson's Disease ,**2018, Sch J Appl Sci Res Vol: 1, Issu: 9, https://www.researchgate.net/publication/330292374

Sajid, M., Ilyas, M., Basheer,.: '**Impact of nanoparticles on human and environment: review of toxicity factors, exposures, control strategies, and future prospects', ** Environ. Sci. Pollut. Res., 2015, 22, (6), pp. 4122–4143.

Sania Naz1, Ayesha Gul1, Muhammad Zia, **Toxicity of copper oxide nanoparticles: areview study** , 2019, ISSN 1751-8741, doi: 10.1049iet-nbt.2019.0176www.ietdl.org/

Sanyog Jain, Ijeoma F. Uchegbu, Guru Betageri, and Giorgia Pastorin ; **Nanotechnology in Advanced Drug Delivery,** 2011, Hindawi Publishing Corporation ,Journal of Drug Delivery ,Volume 2011, Article ID 343082, 2 pages.

Sapana Jadoun, Rizwan Arif, Nirmala Kumari Jangid & Rajesh Kumar Meena, **Green synthesis of nanoparticles using plant extracts: a review**,2021, Environmental Chemistry Letters https://doi.org/10.1007/s10311-020-01074-x.

Selda Doğan Çalhan1* , Mustafa Gündoğan2, **Copper Oxide Nanoparticles: Synthesis, Characterization,Antimicrobial Activities and Catalytic Reduction of Methylene Blue**,2020, Doğan Çalhan S. Gündoğan M. JOTCSA. 2020; 7(2): 561-570.

Shin *et al.*, W.-K. Shin, J. Cho, A.G. Kannan, Y.-S. Lee, D.-W. Kim, **Cross-linked composite gel polymer electrolyte using mesoporous methacrylate-functionalized SiO2 nanoparticles for lithium-ion polymer batteries**., (2016), p. 26332, 10.1038/srep26332.

Sonia Gandhi1 and Andrey Y. Abramov, **Mechanism of Oxidative Stress in**

Neurodegeneration,2012, Hindawi Publishing Corporation Oxidative Medicine and Cellular Longevity Volume 2012, Article ID 428010, 11 pag doi:10.1155/2012/428010

Sonia Losada-Barreiro, Carlos Bravo-Díaz, **Free radicals and polyphenols: The redox chemistry of neurodegenerative diseases** ,2017, European Journal of Medicinal Chemistry, DOI: 10.1016/j.ejmech.2017.03.061.

Sovan Lal Pal, Utpal Jana, P. K. Manna, G. P. Mohanta, R. Manavalan**, Nanoparticle: An overview of preparation and characterization**,2011, Journal of Applied Pharmaceutical Science 01 (06); 2011: 228-234.

Stefanos Mourdikoudis , Nguyen TK Thanh, **Characterization Techniques for Nanoparticles: Comparison and Complementarity upon Studying Nanoparticle Properties,** 2018, DOI: 10.1039/c8nr02278.

Suha Maher Abed, Yumna Shakir Mahmood, Ibrahim F. Waheed , Ammar Mohammad Alwan, **Antibacterial Activity of Green Synthesized Copper Oxide Nanoparticles,**2020, Iraqi Journal of Science, 2021, Vol. 62, No. 9(Special Issue) pp: 3372-3383 DOI: 10.24996/ijs.2021.62.9(SI).8

Thi Ha Tran1 and Viet Tuyen Nguyen2, **Copper Oxide Nanomaterials Prepared by Solution Methods,Some Properties, and Potential Applications: A Brief Review**,2014, Hindawi Publishing Corporation International Scholarly Research Notices Volume 2014, Article ID 856592, 14 pages http://dx.doi.org/10.1155/2014/856592.

Vinod Vellora Thekkae Padil ,Miroslav Černík, **Green synthesis of copper oxide nanoparticles using gum karaya as a biotemplate and their antibacterial application**,2013 , International Journal of Nanomedicine 2013:8 889–898.

Vijay Kumar 1,3 & U. Shameem1 & Pratap Kollu2 & R. L. Kalyani 4 & S. V. N. Pammi, **Green Synthesis of Copper Oxide Nanoparticles Using Aloe vera Leaf Extract and Its Antibacterial Activity Against Fish Bacterial Pathogens,**2015, Springer Science+Business Media New York, DOI 10.1007/s12668-015-0171-z

Worku Wubet Andualem,1 Fedlu Kedir Sabir,2 Endale Tsegaye Mohammed,3 Hadgu Hailekiros Belay,2 and Bedasa Abdisa Gonfa, **Synthesis of Copper Oxide Nanoparticles Using Plant Leaf Extract of Catha edulis and Its Antibacterial Activity**,2020, Hindawi Journal of Nanotechnology Volume 2020, Article ID 2932434, 10 pages https://doi.org/10.1155/2020/2932434.

Xicoy H, Wieringa B, Martens G J. **The SH-SY5Y cell line in Parkinson's disease research: a systematic review**, 2017, Mol Neurodegener, 12(1): 10.

Xie H R, Hu L S, Li G Y., **SH-SY5Y human neuroblastoma cell line: in vitro cell model of dopaminergic neurons in Parkinson's disease**., 2010, Chin Med J (Engl), 123(8): 1086-1092.

Y. Aparna1, K.V. Enkateswara Rao2 and P. Srinivasa Subbarao3, **Synthesis and Characterization of CuO Nano Particles by Novel SolGel Method,**2012, 2012 2nd International Conference on Environment Science and Biotechnology IPCBEE vol.48 (2012) © (2012) IACSIT Press, Singapore DOI: 10.7763/IPCBEE. 2012. V48. 30.

Ying Shi, Alexander R. Pilozzi and Xudong Huang, **Exposure of CuO Nanoparticles Contributes toCellular Apoptosis, Redox Stress, and Alzheimer's Aβ Amyloidosis,**2020, Int. J. Environ. Res. Public Health 2020, 17, 1005; doi:10.3390/ijerph17031005 www.mdpi.com/journal/ijerph.

(www.sciencedirect.com/topics/engineering , 2021) .

Xiaoyang Mou 1, Alexander Pilozzi 2, Breeya Tailor 2, Jing Yi 2, Catherine Cahill 2, Jack Rogers 2 and Xudong Huang, **Exposure to CuO Nanoparticles Mediates NFκB Activation and Enhances Amyloid Precursor Protein Expression**,2020, Biomedicines 2020, 8, 45; doi:10.3390/biomedicines8030045