TURKISH REPUBLIC
MARMARA UNIVERSITY
INSTITUTE OF HEALTH SCIENCES

PRODUCT ION OF GRAPHENE-BASED MATERIAL LOADED WITH SELENIUM NANOPARTICLES AND INVESTIGATION OF THEIR BIOCOMPATIBILITY

ARAM ALASHTAR

MASTER'S THESIS

SUPERVISOR
ASSOC. PROF. ÖZLEM BİNGÖL ÖZAKPINAR

BIOCHEMISTRY (PHARMACY) MASTER'S DEGREE PROGRAM

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STATEMENT

Hereby I declare that this thesis study is my own work, I had no unethical behavior in any stage from planning of the thesis until writing it, I have obtained all the information in this thesis within the academic and ethical rules, I have cited all the information and comments that are not obtained with this thesis study, and these sources are also included in the list of references, I hereby declare that I have no infringement of patents and copyrights during the study and writing of this thesis

Aram ALASHTAR

ACKNOWLEDGMENT

As I conclude my research, I would like to take this opportunity to express my appreciation to those who have significantly contributed to my academic and personal development. Your consistent support and encouragement have played a crucial role in my accomplishments, and I am sincerely grateful to each of you.

First and foremost, I extend my deepest gratitude to Allah for providing unwavering guidance, blessings, and protection throughout my life. Your presence has been a source of strength, solace, and inner tranquility, and I am profoundly thankful for all that you have bestowed upon me.

I am also indebted to my beloved parents, Mustafa and Baraa, whose love and encouragement have laid the groundwork for my academic pursuits. Your sacrifices and belief in my capabilities have empowered me to navigate the challenges and uncertainties inherent in research. I am immensely grateful for your steadfast support. To my dear siblings, Ahmad, Mohammed, and Alaa, your constant encouragement and faith in my potential have been a wellspring of motivation. Your presence in my life has inspired me to excel in my academic endeavors, and I deeply appreciate the bonds of love and support that we share. Additionally, I would like to extend my appreciation to my extended family members for their unwavering love and support. Their presence in my life holds immeasurable value, and I deeply treasure the shared experiences we have had. I am sincerely grateful for the relationships we have cultivated and the affection that unites us. Their ongoing support and involvement in my life serve as a source of resilience and happiness.

I also want to convey my gratitude to the educators and mentors who have provided guidance throughout my academic journey. Their knowledge, expertise, and commitment to fostering my intellectual development have been indispensable. Their wisdom and perspectives have influenced my research and equipped me with a solid foundation for my future endeavors. I have had the privilege of learning from exceptional educators, Assoc. Prof Özlem BİNGÖL ÖZAKPINAR and Assoc. Prof Elif CALIŞKAN SALİHİ. Their mentorship, feedback, and guidance have profoundly impacted my research and significantly contributed to my academic growth.

I would like to express my sincere gratitude to the professors at Helwan University for their exceptional guidance and support during my time in Egypt. Their dedication to teaching has been truly inspiring, and their insights have significantly contributed to my education. I want to emphasize the significant impact that the professors' collective influence has had on my academic achievements. I am deeply appreciative of the role each of them has played in my educational journey, and their support and belief in me have been a constant source of strength.

I extend my heartfelt thanks once again for being an integral part of my journey, and I eagerly anticipate sharing my future successes with all of them.

CONTENTS

ABBREVIATIONS AND SYMBOLS LIST

A/H1N1	:	Influenza A virus subtype H1N1
AFM	:	Atomic Force Microscope
AuNPs	:	Gold nanoparticles
BBB	:	Blood-brain barrier
CAMHB	:	Cation Adjusted Mueller Hinton Broth
CLSI	:	Clinical and Laboratory Standards Institute
CNS	:	Central nervous system
COVID-19	:	Coronavirus disease 2019
CRC	:	Colorectal cancer
DIO	:	Iodothyronine deiodinases
ER	:	Endoplasmic reticulum
GO	:	Graphene oxide
GO-SeNP	:	Graphene oxide based selenium nanoparticles
GPxs	:	Glutathione peroxidases
H2O2	:	Hydrogen peroxide
IFNγ	:	Inflammation and interferon γ
IL	:	Interleukin
MERS-CoV	:	Middle East Respiratory Syndrome Coronavirus
MIC	:	Minimal inhibitory concentration
MSRB1	:	Methionine-R-sulfoxide reductase B1
MTT	:	3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide
NIH313	:	Embryonic mouse fibroblast
NO	:	Nitrogen oxide
NK	:	Nature killer
PBS	:	Phosphate-buffered saline
PEG	:	Polyethylene glycol
PSS	:	Physiological saline solution
RNS	:	Reactive nitrogen species
ROS	:	Reactive oxygen species
rT3	:	Reverse-triiodothyronine
SARS-CoV	:	Severe acute respiratory syndrome coronavirus

SDS	:	Sodium dodecyl sulfate
Se	:	Selenium
SeNP	:	Selenium nanoparticles
SELENOF	:	Selenoprotein F
SELENOM	:	Selenoprotein M
SELENOP	:	Selenoprotein P
SELENOK	:	Selenoprotein K
SELENOS	:	Selenoprotein S
SEPHS2	:	Selenophosphate synthetase
SPM	:	Scanning Probe Microscopes
STM	:	Scanning Tunneling Microscope
SPIONs	:	Superparamagnetic iron oxide nanoparticles
TRAIL	:	Tumor necrosis factor-related apoptosis-inducing ligand
TRH	:	Thyrotropin releasing hormone
TrxRs, TXNRD	:	Thioredoxin reductases
T4	:	Thyroxine
T3	:	Triiodothyronine
TSH	:	Thyroid-stimulating hormone
TEM	:	Transmission Electron Microscopy
WHO	:	World Health Organization

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1. ÖZET

Tezin başlığı : Selenyum nanoparçacıkları yüklü grafen esaslı malzeme üretimi ve biyouyumluluğunun incelenmesi
Öğrencinin Adı Soyadı : Aram ALASHTAR
Danışmanın Adı Soyadı : Doç. Dr. Özlem BİNGÖL ÖZAKPINAR
Programın Adı : Biyokimya (Eczacılık)

Amaç: Selenyum (Se), geniş farmakolojik yetenekleri ve fizyolojik fonksiyonları olan bir eser elementtir. Fosfolipid hidroperoksit, glutatyon peroksidaz ve tioredoksin redüktaz gibi çok sayıda antioksidan enzimin önemli bir bileşenidir. Se eksikliği bağışıklık sisteminde hasara ve onkogenez riskinin artmasına neden olacaktır. Se takviyeleri, yüksek toksisite veya düşük emilim gibi dezavantajlara sahip olduğu için yeni taşıyıcılara ihtiyaç vardır. Bu tez çalışmasının esas amacı Se yüklü grafen esaslı (GO-SeNPs) malzemelerin üretimi ve biyouyumluluğunun incelenmesidir.

Gereç ve Yöntem: Üretilen nanoparçacıkların antimikrobiyal aktivitesi oluk difüzyon yöntemi ile saptandı. Antimikrobiyal etkinlik gösteren ekstreler için minimal inhibitör konsantrasyon (MİK) tespiti yapıldı. Antiproliferatif ve sitotoksik aktivitenin belirlenmesinde MTT yöntemi kullanıldı. Malzemelerin karakterizasyonu Fourier Dönüşümü Kızılötesi Spektroskopisi (FTIR), Taramalı Elektron Mikroskobu ve Enerji Dağıtıcı X-Ray (EDX) analizi kullanılarak araştırıldı.

Bulgular: Üretilen SeNP ve GO-SeNP'leri doza bağlı olarak, özellikle 250 ve 500 µg/mL konsantrasyonlarında, kanser hücreleri üzerinde antiproliferatif etkiye sahip olduğu belirlendi. HT-29 hücrelerinde IC_{50} değeri 236,6 µg/mL olarak bulunurken; PC-3 hücrelerinde IC_{50} değeri 175,15 µg/mL olarak hesaplandı. GO'nun normal hücreler üzerindeki sitotoksik etkisinin GO-SeNP'lerde daha da azaldığı belirlendi (IC_{50}: 193,2 µg/mL). Antimikrobiyal analizler sonucunda MİK, *Klebsiella pneumoniae* hariç tüm bakteri suşlarında ve mayalarda 1000 µg/mL olarak belirlendi.

Sonuç: Se taşınımı için bu çalışmada üretilen nanopartiküllerin antiproliferatif etkilerinden dolayı kanser tedavisi için ümit vadeden ajanlar olabileceği düşünülmektedir.

Anahtar Kelimeler: Selenyum, SeNPs, antiproliferatif aktivite, antimikrobiyal aktivite, Grafen oksit

2. SUMMARY

Title of Thesis: Production of graphene-based material loaded with selenium nanoparticles and investigation of its biocompatibility
Student Name, Surname: Aram ALASHTAR
Supervisor Name : Assoc. Prof. Özlem BİNGÖL ÖZAKPINAR
Program Name : Biochemistry (Pharmacy)

Objective: Selenium (Se) is a trace element with broad pharmacological capabilities and physiological functions. It is a significant component of numerous antioxidant enzymes such as phospholipid hydroperoxide, glutathione peroxidase, and thioredoxin reductase. Se deficiency will cause damage to the immune system and increased risk of oncogenesis. New carriers are needed because Se supplements have disadvantages such as high toxicity or low absorption. The main purpose of this thesis is to examine the production and biocompatibility of Se-loaded graphene-based (GO-SeNPs) materials.

Materials and Methods: The antimicrobial activity of the produced nanoparticles was determined by the groove diffusion method. Minimal inhibitory concentration (MIC) was determined for nanoparticles showing antimicrobial activity. MTT method was used to determine antiproliferative and cytotoxic activity. Characterization of the materials was investigated using Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy and Energy Dispersive X-Ray (EDX) analysis.

Results: It was determined that the produced SeNPs and GO-SeNPs had an antiproliferative effect on cancer cells depending on the dose, especially at concentrations of 250 and 500 µg/mL. While the IC_{50} value in HT-29 cells was 236,6 µg/mL; The IC_{50} value in PC-3 cells was calculated as 175,15 µg/mL. It was determined that the cytotoxic effect of GO on normal cells was further reduced in GO-SeNPs (IC_{50}: 193,2 µg/mL). It was found that GO-SeNPs has shown its strongest antimicrobial effect at MIC of 250 µg/mL against *Klebsiella pneumoniae* and 1000µg/mL against all of the other bacteria and yeasts.

Conclusion: It is thought that the nanoparticles produced in this study for Se transport may be promising agents for cancer treatment due to their antiproliferative effects.

Keywords: Selenium, SeNPs, antiproliferative activity, antimicrobial activity, Graphene oxide

3. INTRODUCTION and OBJECTIVE

Selenium (Se) is a crucial trace element that plays a significant role in human and animal health due to its notable physical, chemical, and biological properties. It has been the subject of numerous studies and hypotheses regarding its potential therapeutic effects in treating various diseases, particularly its potential anti-cancer properties. Researchers suggest that Se may impede cancer growth (Sanmartín et al., 2012), induce G2/M cell cycle arrest (Khurana et al., 2019a), and attenuate cancer cell enzymes, potentially impacting tumor development (Kieliszek et al., 2017). Furthermore, Se may stimulate natural killer cells and inhibit angiogenesis (Lipinski, 2005).

Se deficiency can impact the immune system's function and is linked to the progression and development of infectious diseases (Lin et al., 2021). Similarly, Se deficiency has been associated with chronic degenerative diseases (Kieliszek et al., 2022), leading to intestinal cell apoptosis and significant harm to the structure and function of the intestinal mucosa (He et al., 2020). It can also harm the liver, heart, kidneys, and testes (Wang et al., 2013), as well as cause muscle disorders (Rederstorff et al., 2006) and it has been linked to angina attacks and myocardial infarction (Apryatina et al., 2022).

It has been confirmed that cancer chemopreventive and chemotherapeutic effects of Se is depend on its concentration and chemical composition. Se at high concentrations exerts a chemopreventive effect through various mechanisms, including the upregulation of glutathione S-transferase (GST) to neutralize electrophilic compounds produced during xenobiotic metabolism. Additionally, it promotes the accumulation of low molecular weight selenocompounds that can induce cytotoxic effects (Kieliszek et al., 2015). Despite the chemopreventive effectiveness of Se, which exceeds physiological requirements by 10-fold, the supranutritional doses verge on toxic levels. Therefore, excessive doses of Se may result in toxicity and development of selenosis (Vahdati & Moghadam, 2020). Additionally, the clinical application of Se is limited due to its narrow therapeutic range, poor absorption, bioavailability, and solubility (Hosnedlova et al., 2018).

In the past few decades, nanotechnology has emerged as a highly promising and rapidly advancing discipline, primarily due to its extensive utilization in the realms of applied sciences

and technology (Ferrari, 2004). Nanotechnology is regarded as a developing technological method for the environmentally sustainable production of nanoscale materials utilizing biological sources (Anu et al., 2017). Nanoparticles display unique characteristics as a result of their high surface energies and significant large surface-to-volume ratios (Prasad et al., 2013).

Recently, Se nanoparticles (SeNPs) have gained tremendous attention during the last decade's due to their excellent biological activities and low toxicity compared to other Se-containing compounds (Geoffrion et al., 2020; Toubhans et al., 2020b). Leveraging nanotechnology, SeNPs have higher biological activity, stronger antioxidant effects and lower toxicity compared with the traditional Se compounds (Chen et al., 2022a; Lin et al., 2021). SeNPs have been extensively investigated as a potential adjustment therapy in cancer, bacterial infections, and as an antioxidant agent (Varlamova et al., 2021a). Studies on many cancer cell lines have shown that SeNP induces apoptosis in cancer cells and increases the expression of pro-apoptotic genes (Toubhans et al., 2020c; Varlamova et al., 2021; Spyridopoulou et al., 2021; Liu et al., 2020).

Several fabrication methods have been reported for nanosized SeNPs synthesis with different morphologies and sizes, including chemical, physical, and biological. Studies have shown that biological methods, also known as green synthesis, successfully overcome many of the problems associated with chemical and physical methods. Green synthesis has received more attention than physical and chemical methods for several reasons: the employment of environmentally benign and non-toxic chemicals; the utilization of renewable materials; the adoption of eco-friendly solvents, particularly aqueous media, in the course of reactions; the inherent simplicity and cost-effectiveness. (Fahimirad et al., 2019; Chandra et al, 2020). Recently, common green methods have been reported to utilize yeast (Cremonini et al., 2016), aqueous plant extracts (Yang et al., 2012; Alghuthaymi et al., 2021), bacteria (Wadhwani et al., 2017; Hassan et al., 2024) and natural polymers (Boroumandet al., 2019) to fabricate SeNPs. Futhermore, it has been reported that the Se-NPs synthesized from natural compounds such as ascorbic acid are less toxic than the SeNps prepared from chemicals (Xia, 2007).

Graphene oxide (GO) is a single layer of macromolecular hexagonal rings comprising sp2 hybridized carbon atoms combined with oxygen-containing functional groups such as epoxide, carboxylic acids, and alcohols (Wang et al., 2011). This distinctive structure renders GO hydrophilic and readily dispersible in water (Yu et al., 2020), facilitating its manipulation to exhibit a range of mechanical, colloidal, and optical properties (Tarcan et al., 2020).

Additionally, the large active site of GO allows to interaction with metals through both electrostatic and coordinate approaches (Wang et al., 2014a). The presence of oxygen-containing functional groups also promotes strong interaction with cellular proteins, making GO highly compatible with cellular behavior in terms of cell growth and viability (Zhang et al., 2016; Habte & Ayele, 2019).

GO has attracted significant interest for various applications, particularly in cancer treatment (Nandi et al., 2019), drug delivery (Zarafu et al., 2018a), plasmid transformation (Wu et al., 2020), cell culture studies (Zeng et al., 2020), tissue regeneration (Șelaru et al., 2022), viral vaccines (Zhou et al., 2021), and other biomedical fields (Habte & Ayele, 2019). Numerous studies have also confirmed the antimicrobial efficacy of GO against bacteria and biofilms (Zarafu et al., 2018b). Baek et al. (2019) have demonstrated the antibacterial properties of GO, attributing its effectiveness to the increased dispersion area of metal oxides and enhanced generation of reactive oxygen species (ROS) within bacterial cells (Baek et al., 2019a). Additionally, Wang et al. (2014) have reported the antibacterial activity of synergistic components of GO and metal nanoparticles, while being less toxic to human cell lines (Wang et al., 2014b). Furthermore, the large surface area of GO makes it suitable for functionalization with nanoparticles (Jafarizad et al., 2017).

Despite the fact, that a large number of study are reported for SeNPs synthesis, no study has been found on GO-decorated SeNPs. In addition, this thesis is the first study to synthesize SeNPs using natural compounds ascorbic acid and saponin. The present study highlights (i) the synthesis of GO-SeNPs using ascorbic acid as a reductant and saponin as a surfactant (ii) characterized through UV-Visible Spectroscopy, FTIR, SEM and EDX analysis and (iii) the role of GO-SeNPs in antiproliferative and antimicrobial activity experiments.

4. BACKGROUND

4.1. Selenium

The name Selenium (Se) comes from the root *Selen* which meaning the moon in ancient Greek language, discovered in 1817 by Jones Jacob Berzelis.

Se is a nonmetal chemical with 34 atomic numbers, placed between sulphur and tellurium in the periodic tablet, the last electronic shell has 6 electrons, to be satisfied, it is free to share two electrons in its covalent bonds and easier to gain or loss electrons and involve in redox reactions.

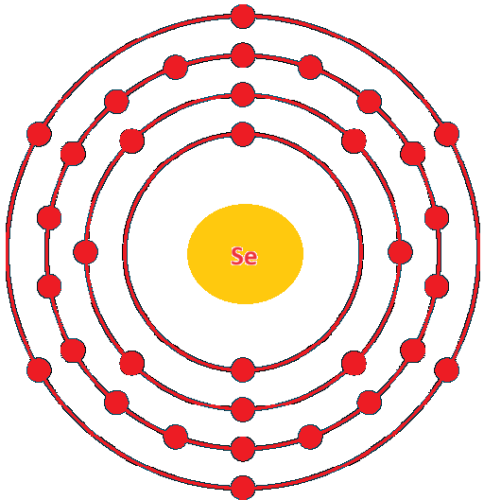

Figure 1. Selenium electronic orbitals.

Se exists in various allotropes, with the most common forms being red powder or red crystals, and a grey metallic form known as metallic selenium. It is widely distributed in the Earth's crust, occurring in pure ore or elemental state (Fan et al., 2002)

The primary sources of Se in the diet include brazil nuts, seafood, meats, cereals, grains, and dairy products. Se obtained from drinking water does not make a significant nutritional contribution.

Chemical forms of Se are selenate, (SeO_4^{-2}), selenite (SeO_3^{-2}), reduced form selenide (Se^{-2}) and elemental selenium (Se^0) (Biswas et al., 2011). The organic compounds of Se found in plants

and animals' cells primarily consist of selenomethionine and selenocysteine. These compounds are formed when Se replaces the sulfur in thionine and cysteine amino acids (Chen et al., 2022b).

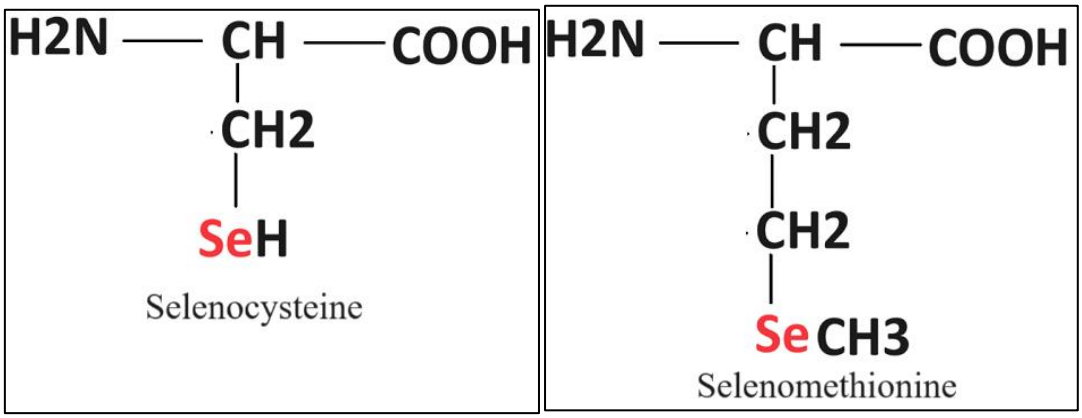

Figure 2. Selenomethionine and selenocysteine chemical forms.

4.1.1. Se in the body

Se is a crucial micronutrient, necessitating trace amounts in the human diet due to its potential toxicity at elevated levels, which can lead to various diseases and immune deficiencies when Se intake is an inadequate. Se is present in biological systems as a component of 25 selenoproteins, where it replaces the thiol group in thionine and cysteine amino acids. This type of proteins comprises numerous proteins and enzymes, including glutathione peroxidases (GPxs), thioredoxin reductases (TrxRs), and iodothyronine deiodinases (DIO) (Labunsky et al., 2014). The selenoprotein family primarily participates in various immune cell activities and redox reactions. Additionally, they play a significant role in the function and metabolism of thyroid hormones. Some members of this family, such as Selenoprotein F, H, I, and K, are not enzymes and are considered to have non-enzymatic functions. (Avery & Hoffmann, 2018).

The GPx family was first discovered in 1957, and its primary role involves safeguarding red blood cells against H_2O_2 by converting it into water, metabolizing lipid peroxidase, and breaking down organic hydroperoxides to shield cells from oxidative damage (Burk & Hill, 2010).

The chemical structure of TrxR/TXNRD members is classified within the pyridine nucleotide-disulfide oxidoreductase family. Their primary role is to maintain thioredoxin in a reduced state

(Turanov et al., 2010). Additionally, DIO enzymes are primarily involved in the activation and deactivation of thyroid hormones (Darras et al., 2012).

Selenoprotein P (SELENOP) is primarily responsible for transporting Se in the plasma (Kato et al., 1992), while selenoprotein F (SELENOF) is located in the endoplasmic reticulum (ER) organelle and is involved in the folding and secretion of proteins in the ER (Ren et al., 2018). Selenoprotein S (SELENOS) plays a role in regulating inflammatory response, oxidative stress, and ER stress (Ren et al., 2018). Selenoprotein M (SELENOM) is located in the ER and is highly expressed in the brain, suggesting potential roles as a neuroprotective agent, antioxidant, and regulator of cytosolic calcium (Guerriero et al., 2014).

Selenoproteins O, H, W, and K play a role in redox reactions and serve to prevent oxidative stress within the body, as well as in the regulation of the immune system. Meanwhile, Selenoprotein N is involved in calcium signaling. Selenoprotein I is associated with phospholipid biosynthesis. Selenoprotein R plays a crucial role in reducing methyl sulfoxide groups. Selenophosphate synthetase, which is itself a selenoprotein, plays a critical role in the synthesis of selenoproteins (Mangiapane et al., 2014).

Table 1. Selenoprotein enzymes and functions

Selenoprotein name	**Function**
Glutathione peroxidases	Hydrogen peroxide reduction
Thioredoxin reductases	Maintaining thioredoxin
Iodothyronine deiodinases	Activation and deactivation of thyroid hormones
Selenoprotein P	Transport Se in plasma
Selenoprotein F	Folding and secretion of proteins
Selenoprotein S	Regulation inflammatory response, and oxidative stress
Selenoprotein O Selenoprotein H Selenoprotein W Selenoprotein K	Oxidative stress
Selenoprotein M	Neuroprotective
Selenoprotein N	Calcium signaling
Selenoprotein R	Reduction of methyl sulphoxy groups
Selenoprotein I	Phospholipid biosynthesis
Selenophosphate synthetase	Selenoproteins synthesis

4.1.2. The antioxidant properties of Se

Oxidative stress results from an imbalance between the accumulation (ROS, which are typically generated as by-products of cellular oxygen metabolism, and the capacity of a biological system to neutralize these reactive substances (Pizzino et al., 2017).The development of a range of health conditions is associated with this imbalance. At times, the body's defense mechanisms are unable to fully counteract the damage caused by ROS. Therefore, it is crucial to incorporate natural dietary antioxidants, like polyphenols, vitamins, and trace elements, which function as scavengers of free radicals (Bjørklund et al., 2022).

The micronutrient Se possesses significant antioxidant properties, playing a role in enhancing the body's defense against oxidation, supporting the immune system, and maintaining metabolic balance. Numerous selenoproteins play a role in redox reactions and the regulation of oxidative stress. The Se-containing enzyme selenoproteins GPX1–4 and GPX6, contribute to the regulation of free radical reactions by converting hydrogen peroxide to water and organic hydroperoxides to alcohol, thereby maintaining tolerable levels of oxidative stress (Burk & Hill, 2010). The Se-dependent TrxR1-3 plays a role in reducing oxidative stress, while the GPX4 is crucial for the development of embryonic tissue and the survival of cells (Wu et al., 2013).

Selenium plays a crucial role as an antioxidant in the regulation of glucose and lipid metabolism. Furthermore, it plays a significant role in redox regulation to uphold cellular homeostasis and metabolism. Existing evidence suggests a notable association between selenium levels and positive outcomes in metabolic disorders such as hyperglycemia, hyperlipidemia, and hyperphenylalaninemia (Huang et al., 2022; Wang et al., 2017).

The benefits of Se are not limited to animal and human cells; they also play a crucial role in plant cells. Se helps to mitigate abiotic stresses, which are environmental conditions that are detrimental to plant growth and development. These stresses can lead to plant injury, destruction, and even death. Se regulates the adverse effects of abiotic stresses on plants by enhancing photosynthetic properties, promoting the synthesis of photosynthetic pigments, improving the activities of antioxidant enzymes to remove ROS, regulating cell permeability, increasing the concentrations of resistant substances such as proline and increase the soluble sugar to maintain osmotic pressure, inhibiting the uptake of harmful substances like salt and

metal ions by forming complexes or precipitates, and regulating the expression of genes related to increasing cell tolerance (Liu et al., 2023).

4.1.3. Se and immunity

The primary mechanism of Se as an immunomodulatory compound related to the activity of selenoproteins enzymes especially their roles in redox hemostasis (Avery & Hoffmann, 2018). The antioxidant properties of selenoproteins are suggested to regulate and facilitate redox-based reactions within cells, blood, and tissues by utilizing selenocysteine in their active sites. To summarize, GPxs, TXNRDs, methionine-R-sulfoxide reductase B1 (MSRB1), DIOs and Selenophosphate synthetase, have an impact on immune functions. Additionally, the non-enzymatic selenoprotein K, a transmembrane protein in the ER, is involved in ER stress, calcium flux, and plays a crucial role in the activation and proliferation of immune cells.

There is evidence suggesting that Se may have a modulating effect on the pathogenesis of inflammatory liver and gut diseases, as well as inflammation associated with cancer (Barrett et al., 2015). Additionally, Se deficiency has been clearly linked to inflammatory cytokines in various tissues, including the gastrointestinal tract and mammary glands (Avery & Hoffmann, 2018). However, the modulatory response to inflammation depends on the dosage of Se, as both high and low doses have been shown to have varying effects. For example, research in rodents has demonstrated a reduction in airway inflammation in mice with allergic asthma following Se supplementation, while suboptimal or high doses of Se have led to significantly negative responses in other mouse groups (Hoffmann et al., 2007).

Furthermore, Tsuji et al. (2015) observed an elevation in the levels of expression and translation of mRNAs carrying stress-related Selenium-based protein codes, as well as genes responsible for inflammation and interferon γ (IFNγ) activities, following the administration of sodium selenite to mice.

Se is known to have an immunostimulatory effect, which can be assessed through the functions of innate immune cells such as natural killer (NK) cell activity and T cell proliferation (Huang et al., 2012). However, there is a correlation between Se supplementation and immune response. For example, high levels of Se in the diet have been associated with increased human blood leukemia and improved vaccine activation against pathogens (Bentley et al., 2014).

Similarly, inadequate Se levels have been linked to reduced inflammatory and immune responses, as evidenced by rectal biopsies from healthy volunteers (Méplan et al., 2016). Additionally, Se has been shown to enhance the inflammatory response in patients with respiratory distress syndrome by increasing the antioxidant capacity of the lungs, thereby moderating interleukin (IL)-1β and IL-6 levels and improving respiratory function (Mahmoodpoor et al., 2019).

Additionally, a brief review was conducted to examine the impact of Se, vitamin D, and vitamin C supplementation in patients with coronavirus disease 2019 (COVID-19). The review concluded that selenium may inhibit viral mutations, enhance CD4+ T cell activity, differentiation, and proliferation, promote a Th1 phenotype, increase the cytotoxicity of CD8+ T cells and the lytic activity of natural killer cells, modulate T cell-dependent antibody production, and prevent vasoconstriction and blood coagulation, which are primarily linked to elevated mortality in COVID-19 cases. In contrast, when examining COVID-19 patients, higher levels of Se were observed in those who survived and recovered (Bae & Kim, 2020).

4.1.4. Se and thyroid hormones

Thyroid hormones, such as Triiodothyronine (T3), Tetraiodothyronine (T4), and Calcitonin, are produced and released by the thyroid gland situated in the anterior, inferior neck. Their synthesis is regulated by the hypothalamic hormone thyrotropin-releasing hormone (TRH) and the pituitary gland hormone thyroid-stimulating hormone (TSH). These hormones primarily regulate metabolic processes, bone growth, body temperature, and other physiological functions. Their effectiveness is influenced by various factors, including levels of Se and iodine.

The selenoproteins DOIs are integral membrane proteins located in the ER and plasma, responsible for the activation (DIOI1,2) and inactivation (DIO3) of T4, T3, and reverse-triiodothyronine (rT3) (Genchi et al., 2023). The conversion of T4 to T3 by removing iodine

from the outer rings is facilitated by DOI1 and 2, while DOI3 adds iodine to T3 to convert it into its active form, T4 (Figure 3).

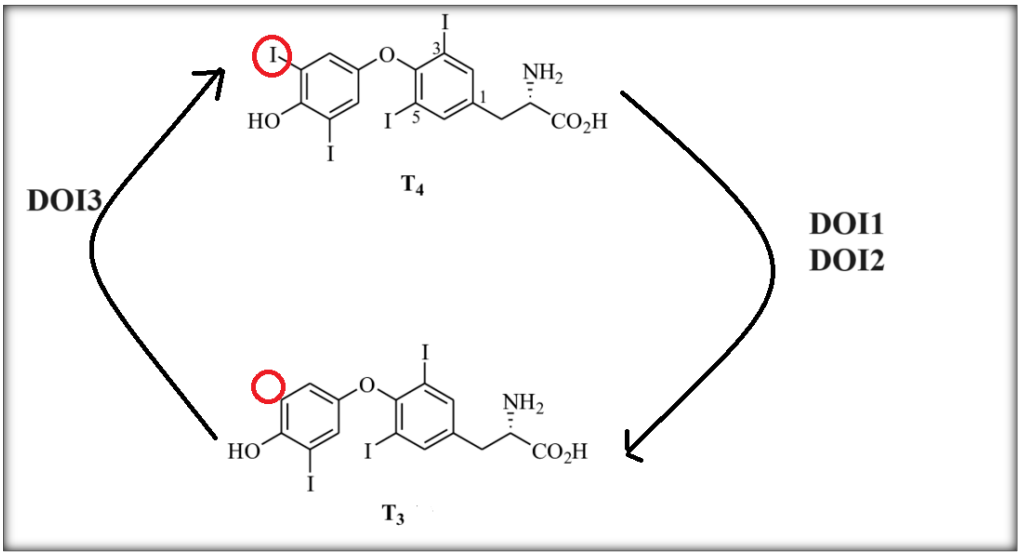

Figure 3. T4 activation and deactivation

DOIs contain selenocysteine in their active site, underscoring the significance of Se in the metabolism of thyroid hormones. This is evident from the observed iodine and Se deficiency in endemic goiter and congenital hypothyroidism in Central Africa and the Himalayas regions (Corvilain et al., 1993). Additionally, selenoproteins play a role in regulating the H2O2 produced in the angiofollicular units in the thyroid gland, thereby protecting against oxidative stress-induced damage. Such damage could potentially impact the lymphocytic infiltration of the thyroid gland, which serves as a marker in Hashimoto's disease, a condition that can affect the reproductive and menstrual cycles of approximately one-third of women (Mammen et al., 2021).

4.1.5. Se dosing, deficiency, and toxicity

Se is a crucial micronutrient with a significant importance for the health of humans, animals, and plants. It plays a vital role in biological systems as a potent antioxidant. As per the guidelines provided by The Food and Nutrition Board at the Institute of Medicine of the National Academies in the United States, the suggested daily intake of Se supplementation is 45 micrograms (µg) for adult males and females (Stoffaneller et al., 2015). The World Health Organization (WHO) suggests a daily intake of 26 µg for women and 34 µg for men, as stated

in the publication "Vitamin and Mineral Requirements in Human Nutrition" (2004). The recommended doses from WHO for different demographic groups are detailed in Table 2.

Table 1. WHO recommend Se intakes: micrograms per day (μg/day)

Group	Male	Female
0–6 months	6	6
7–12 months	10	10
1–3 years	17	17
4–6 years	22	22
7–9 years	21	21
10–18 years	32	26
19–65 years	34	26
65+ years	33	32
Pregnant women	-	28-30
Lactating women	-	35-40

Elevated consumption of Se can lead to toxicity, resulting in acute manifestations such as hair loss, nail abnormalities, gastrointestinal discomfort, halitosis, headache, muscle or joint discomfort, fatigue, and skin eruptions (MacFarquhar et al., 2010). Excessive Se intake, typically at doses of 200 μg/day or higher, has been associated with selenosis, characterized by symptoms including alopecia, dermatitis, non-melanoma skin cancer, heightened mortality, type 2 diabetes, and increased risk of prostate cancer (Rayman, 2020).

However, Se deficiency plays a significant role in human development, reproduction, and muscle strength. Inadequate Se intake has been linked to symptoms such as depressed mood,

anxiety, and confusion. In endemic regions of China, Keshan disease is associated with bone deformities and heart failure, which are correlated with Se deficiency. Insufficient Se levels may disrupt oxidative stress regulation, redox reactions, and thyroid hormone metabolism and functions. Additionally, autoimmune diseases have been linked to Se deficiency due to the high levels of cytokines in such diseases, which suppress hepatic selenoprotein synthesis and affect SELENOP secretion into the blood, leading to reduced Se metabolism and an overall suppressed Se status.

As previously noted, Se is a trace element with a narrow therapeutic range due to its potential toxicity at excessive doses and its impact on biological reactions at low doses. Additionally, Se exhibits poor absorption, bioavailability, and solubility, which limits its clinical applications. However, selenium nanoparticles (SeNPs) offer significant advantages over other Se-containing compounds in clinical protocols (Hosnedlova et al., 2018; Ikram et al., 2021; Geoffrion et al., 2020; Toubhans et al., 2020b).

4.1.6. Anti-cancer effect of Se

Cancer is characterized by a high prevalence of diagnosed cases, which is associated with detrimental impacts on individuals' well-being. Cancer occurs when the regulation of normal cell growth is disrupted, leading to malignant alterations and the infiltration of healthy tissues and organs, ultimately causing dysfunction and adverse health effects. Research in the field has primarily concentrated on interventions such as treatment, early detection, and preventive measures.

Conventional treatment options typically include chemotherapy, immunotherapy, radiation therapy, or surgical intervention. However, early diagnosis, classification, and treatment of the disease are crucial for improving the prognosis of cancer and minimizing the risk of metastasis (Ehudin et al., 2022). There has been recent emphasis on the potential of Se as an adjunct therapy in cancer treatment.

Se is recognized as a potent antioxidant due to the involvement of selenoproteins such as GPX and TXNRD in various biological pathways aimed at safeguarding cell membranes and DNA from oxidative damage, thereby contributing to the prevention or protection against cancer (Wu et al., 2021). While numerous studies have underscored the chemopreventive properties of Se,

conversely, recent research has suggested that Se supplementation may elevate the risk of cancer, particularly when combined with type 2 diabetes (Vinceti et al., 2021).

The redox reactions facilitated by selenoproteins, particularly GPX, are crucial for shielding cells from oxidative damage caused by ROS and reactive nitrogen species (RNS) such as H2O2 and nitric oxide (NO) (Schweizer et al., 2016). Furthermore, Se supplementation has been found to regulate NO-mediated apoptosis induced by toxic agents like cadmium (Liu et al., 2014).

Se has been identified as a potentially beneficial trace element within the glioma microenvironment due to its ability to maintain redox homeostasis through the regulation of selenoproteins at the molecular level. Additionally, Se has been found to enhance bioenergy metabolism, modulate the immune response, and inhibit tumor angiogenesis (Yakubov et al., 2021).

Numerous developing theories exist regarding the potential anticancer effects of Se. For instance, Se is believed to play a role in promoting cell apoptosis by sensitizing apoptotic inducers such as doxorubicin and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) (Sanmartín et al., 2012). Additionally, it has been suggested that Se induces G2/M cell cycle arrest while causing minimal harm to normal cells (Khurana et al., 2019a), potentially by protecting them from cellular stress (Watrach et al., 1984). Furthermore, Se may alter the conformation of sulfhydryl groups in proteins involved in tumor development, potentially weakening the activity of cancer cell enzymes (Kieliszek et al., 2017). Moreover, Se may directly activate natural killer (NK) cells and inhibit the formation of new blood vessels around cancer cells (Lipinski, 2005).

In order to examine the impact of Se on the treatment of ovarian cancer, researchers conducted experiments using Se^{+4}, Bis(trimethylsilyl)acetamide (BSA)-coated SeNPs, and chitosan-coated SeNPs on SKOV-3 and OVCAR-3 ovarian cancer cell lines. The findings revealed that Se was effective in inhibiting cell growth and demonstrated cytotoxic effects on the cancer cell lines. The two cell types exhibited differing responses to the tested materials, with SKOV-3 cells displaying greater sensitivity to Se^{+4} compared to OVCAR-3 cells, while OVCAR-3 cells exhibited greater sensitivity to SeNPs (Toubhans et al., 2020b).

Moreover, the toxic effects of Se may be ascribed to the generation of oxidative stress resulting from the intracellular redox cycle of Se metabolites with oxygen and cellular thiols (Turovsky,

et al., 2021b). This process can result in the production of imbalanced levels of superoxide and cellular disulfides. However, these observed effects occur at doses that are close to toxic levels (Ganther, 1999; Khurana et al., 2019b). Additionally, the potential chemopreventive advantages of Se may be associated with its antioxidant properties, inhibition of p53 protein kinase, enhancement of p53 protein expression, regulation of cell division, and protection against the toxic effects of heavy metals (Domínguez-Álvarez et al., 2022).

4.2. Nanotechnology

Nanotechnology and nanoscience have wide-ranging applications across various scientific disciplines such as computer science, chemistry, and biology. The term 'nano' derives from the Greek word for 'dwarf' and signifies a scale of one thousand millionth of a meter. The roots of nanoscience can be traced back to ancient Greece, particularly to the 5th century B.C., when early philosophers like Democritus pondered the nature of matter, considering whether it was continuous, infinitely divisible, or composed of indivisible particles, now known as atoms (Bayda et al., 2020).

Nanoparticle structures were utilized as early as the fourth century AD by the Romans, providing an early example of nanotechnology in ancient civilizations. The Lycurgus cup, housed in the British Museum, represents one of the oldest instances of dichroic glass, which exhibits varying colors under different lighting conditions. Specifically, the cup appears green in direct light and red-purple when illuminated from behind, a phenomenon attributed to the presence of nanoparticles measuring 50 to 100 nanometers in diameter. X-ray analysis has confirmed that these nanoparticles are comprised of a silver-gold (Ag-Au) alloy (Freestone et al., 2008). A similar effect is observed in late medieval church windows, which display red and yellow hues resulting from the incorporation of Au and Ag nanoparticles into the glass.

During the period spanning from the 9th to the 17th centuries, luminous "luster" ceramic glazes employed in the Islamic regions, and subsequently in Europe, were infused with nanoparticles such as silver (Ag) or copper (Cu). Italian artisans from the 13th to the 18th centuries drew inspiration from Ottoman techniques in the production of "Damascus" saber blades. They integrated cementite nanowires and carbon nanotubes into the composition of the blades to augment their robustness, longevity, and ability to retain a keen cutting edge (Reibold et al., 2006).

In 1857, Michael Faraday investigated the optical and electronic properties of colloidal suspensions of "Ruby" gold, demonstrating how gold nanoparticles produce solutions of different colors under specific lighting conditions (‘X. The Bakerian Lecture. —Experimental Relations of Gold (and Other Metals) to Light’, 1857).

In 1981, physicists Gerd Binnig and Heinrich Rohrer developed the Scanning Tunneling Microscope (STM), which is currently employed for visualizing atomic-level surfaces (Bayda et al., 2020). The STM has also been utilized for the precise manipulation of atoms and molecules, facilitating the construction of diverse structures. Notably, in 1990, Don Eigler utilized an STM to arrange 35 individual xenon atoms on a nickel surface, forming the letters of the IBM logo (IBM Zurich Research Laboratory).

Binnig and Rohrer's 1986 invention laid the groundwork for the development of Atomic Force Microscope (AFM) and Scanning Probe Microscopes (SPM), which have since become the preferred tools for researchers in the field of nanotechnology (Binnig et al., 1986). In 1985, Robert Curl, Harold Kroto, and Richard Smalley made a significant discovery regarding the existence of carbon in the highly stable form of spherical structures known as fullerenes or buckyballs. These carbon spheres, with the chemical formulas C60 or C70, are produced through the evaporation of graphite in an inert atmosphere. Subsequently, in 1991, Iijima utilized Transmission Electron Microscopy (TEM) to identify carbon nanotubes, which are hollow graphitic tubes known for their remarkable strength and flexibility (Iijima, 1991). These properties hold great promise for a wide range of applications in the field of nanotechnology (Bayda et al., 2020).

Inadvertently in 2004, a novel category of carbon nanomaterials, known as carbon dots (C-dots) with dimensions smaller than 10 nm, was identified during the refinement of single-walled carbon nanotubes by Xu (2004). The favorable attributes of this emerging material, such as low toxicity and strong biocompatibility, position it as a promising substance for utilization in bioimaging, biosensor development, and drug delivery (Xu et al., 2004).

After the significant finding of "graphene" in 2004, carbon-based materials have emerged as the fundamental constituents in a wide range of scientific and engineering fields (Bayda et al., 2020).

4.2.1. Nanotechnology benefits

The progress of nanotechnology has been facilitated by the advancement of microphysics, which offers a more comprehensive comprehension of materials at the atomic level. This has equipped us with a means to investigate the mechanisms of diseases, develop novel treatment approaches, and devise diagnostic methods. Noteworthy features of nanotechnology include its high surface area-to-volume ratio, adaptable structure, and considerable biocompatibility. The applications of nanotechnology have been broadened to encompass various domains such as industries, chemistry, medicine, food industry, biology, medicine, agriculture, and others.

Numerous researchers have extensively documented the advancements in nanotechnology for the effective treatment of colorectal cancer (CRC) in both in vitro studies using cancer cell lines and in vivo experiments involving various CRC animal models, as well as in the detection of CRC disease (Gogoi et al., 2022). Additionally, the development of nanoscaffolds, which are three-dimensional structures of polymer fibers produced at the nanoscale, has shown promise in promoting cell differentiation by incorporating different materials to create an in vitro intestinal model closely resembling the structure of in vivo intestinal tissue. Furthermore, the use of nanomaterial delivery systems has addressed the issue of off-target drug delivery to a certain extent, leading to a reduction in side effects and the required drug dosage (Fei et al., 2022).

In areas such as drug and gene delivery, nanomaterials are utilized to improve drug stability and solubility, control drug release, decrease toxicity, shield drugs from enzymatic breakdown, and ultimately achieve more effective therapeutic outcomes. Various nanocarrier systems have been employed, including polymeric nanoparticles, inorganic nanoparticles, and nanohydrogels, as well as different vectors for DNA delivery using lipids and calcium phosphate, which facilitate the gene transfer process (Hamimed et al., 2022). For instance, gold nanoparticles (AuNPs) are particularly noteworthy as highly functional drug carriers due to their strong bonding capabilities through both covalent and non-covalent conjugation. These properties ensure sustained stability during drug release from AuNPs, and AuNPs have demonstrated effectiveness in anticancer therapy when used to encapsulate the morin drug in a xenograft mouse model (Ding et al., 2020).

Metallic nanoparticles, including silver, copper oxide, gold, iron oxide, and zinc oxide (ZnO), have demonstrated antibacterial properties. Incorporating silver nanoparticles into gels has

proven effective in promoting wound healing, particularly in diabetic wounds, and reducing bacterial count. This is attributed to the slow release of silver ions, which exhibit antibacterial activity, inflammation inhibition, and modulation of the immune response (Blanco et al., 2021).

Nanotechnology shows promise in the management of neurological disorders such as Alzheimer's disease, Parkinson's disease, brain tumors, and stroke. Studies have shown the successful use of nanomaterials in treating central nervous system (CNS) disorders, with nanocarriers capable of crossing the blood-brain barrier (BBB) to deliver therapeutic agents to the CNS (Saeedi et al., 2019). Additionally, nanomedicine is being employed to address emerging infectious diseases, including severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East Respiratory Syndrome Coronavirus (MERS-CoV), influenza A virus subtype H1N1 (A/H1N1), and most recently, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Doroudian et al., 2021).

The applications of nanotechnology and nanoparticles are extensive and diverse, encompassing a wide range of uses that cannot be easily enumerated.

4.3. SeNPs

Nanoparticles play a role in mitigating toxicity, minimizing side effects, improving bioavailability, and optimizing drug release and targeting. Among the various types of inorganic nanoparticles, nano minerals such as silver, gold, cerium, iron, selenium, titanium, and zinc demonstrate greater efficacy and favorability compared to conventional forms.

SeNPs exhibit significant potential as nanomaterials for various biological purposes, such as antioxidant, anticancer agents, and drug delivery vehicles. Numerous studies have highlighted their efficacy in treating diseases, including their anticancer, antioxidant, antimicrobial, anti-biofilm, and neuroprotective properties (Bisht et al., 2022).

Se possesses a limited therapeutic range and exhibits suboptimal absorption, bioavailability, and solubility. SeNPs offer advantages over elemental Se. Administration of SeNPs has been shown to reduce Se-related toxicity and mortality in rodent models (Zhang et al., 2001). Additionally, the use of SeNPs appears to notably alleviate liver injuries caused by high Se doses, as evidenced by alterations in hepatotoxicity biomarkers (Wang et al., 2007).

Lots of study talking about the anticancer effects of SeNPs as they effect the growth of cancer cell in cell culture trails, the presence of the SeNPs inside the cancer cells causes ROS

production and cytotoxicity, for example SeNPs were found to alleviate N-nitrosodiethylamine induced hepatocarcinoma by reducing DNA damage (Khurana et al., 2019c; Zhang et al., 2023).

In a recent *in vivo* investigation involving five colon cancer cell lines (HCT-116, HT-29, Caco-2, SW620, and CT26), it was observed that lentinan-functionalized-SeNPs demonstrated significant cytotoxic effects, with the highest sensitivity observed in HCT-116 cells. Additionally, LNT-SeNPs were found to inhibit the proliferation of HCT-116 cells by influencing the mitochondria-mediated apoptotic pathway and inducing cell cycle arrest in the G0/G1 phase (Gao et al., 2022).

The utilization of the probiotic strain *Lactobacillus casei* ATCC 393 for the synthesis of SeNPs has demonstrated efficacy in inhibiting the proliferation of colon cancer cells both *in vitro* and *in vivo*. The pro-apoptotic properties of SeNPs and their ability to induce immunogenic cell death in colon cancer cells have been validated. Furthermore, the impact of SeNPs on the growth of Caco-2 cells and their potential to stimulate caspase activation show promise for the potential use of SeNPs as an adjunct therapy for cancer treatment (Spyridopoulou et al., 2021).

In recent studies, the antimicrobial attributes of SeNPs have been validated (Miglani et al., 2021), along with its cooperative impact when utilized in conjunction with conventional antibiotics such as Cipro (Nikam et al., 2022) and linezolid (Han et al., 2021). Moreover, it has been identified as a promising candidate in antiviral applications (Liu et al., 2022).

Furthermore, researchers have explored the use of SeNPs as a drug delivery system, demonstrating notable antibacterial and anticancer properties. By using niosome-loaded SeNPs, the study involved the investigation of the effects of this novel system on breast cell lines, revealing an upregulation of genes associated with the apoptotic process. Additionally, the system exhibited significant antibacterial and anti-biofilm effects against *Staphylococcus aureus, Enterococcus fecalis*, *Escherichia coli,* and *Pseudomonas aeruginosa* (Haddadian et al., 2022).

However, the main constraint of SeNPs is their limited uptake by cells. Extensive efforts have been made to overcome this challenge by attaching targeting ligands to the external surface of the nanoparticles. Surface capping agents play a critical role in controlling the size and stability of SeNPs, enhancing their targeting of cancer cells, improving cellular uptake, and increasing their availability and effectiveness. The inclusion of amphoteric ligands, such as polyethylene

glycol (PEG) and graphene, has been shown to significantly assist in the production of nanoparticles (Zheng et al., 2012).

4.3.1. SeNPs synthesis

SeNPs can be synthesized using a variety of techniques, such as physical, chemical, and biological methods.

In chemical synthesis, the reduction of key Se sources, such as Na_2SeO_3, is a crucial step. It is important to highlight that SeNPs have a tendency to aggregate in aqueous environments, thus necessitating modification with suitable stabilizing agents. These agents may encompass polysaccharides, quercetin, gallic and ascorbic acids, polyvinyl alcohol, and analogous compounds.

Common physical methods for material removal include laser ablation and ultrasound. Laser ablation involves the use of laser pulses to eliminate material from a surface by disrupting polymer chains through localized heating. In a liquid environment, colloidal selenium can be generated through the ejection of small particles from the solid surface when a Rayleigh wave of specific amplitude travels from the laser-irradiated point across the Se surface.

The production of nanoparticles through the utilization of plant extracts and microorganisms has become a promising alternative to conventional chemical and physical methods for generating SeNPs. A significant benefit of utilizing plant-based SeNP biosynthesis is its environmentally sustainable and cost-efficient nature, as it utilizes natural stabilizers and reductants.

Aloe vera leaf extracts have demonstrated efficacy in the synthesis of SeNPs by acting as potent reductants and stabilizers owing to their abundant content of sterols, polysaccharides, vitamins, phenolic compounds, lignin, flavonoids, and proteins. Furthermore, the utilization of plant extracts from diverse sources such as *Vitis vinifera*, *Allium sativum*, *Dillenia indica*, fresh citrus and lemon fruits, *Roselle* plants, *Cinnamomum zeylanicum* bark, *Prunus amygdalus* leaves, and others has been successfully documented in scientific literature for the biosynthesis of SeNPs (Varlamova et al., 2021c).

4.4. GO

Graphene is a single layer of macromolecular hexagonal rings composed of sp2 hybridized carbon atoms, which imparts exceptional stability and strength (Geim &amp et al., 2007). Its minimal thickness renders it the thinnest known material, making it a potential candidate for utilization in flexible electronics and transparent conductors. Furthermore, its high electron mobility and limited scattering of charge carriers contribute to its outstanding electrical conductivity, while it also demonstrates remarkable thermal conductivity (Castro et al., 2009; Geim & Novoselov, 2007).

GO, or graphene oxide, is the oxidized form of graphene resulting from the combination of carbon atoms with oxygen, forming functionalized groups such as epoxide, carboxylic acids, and alcohols (Wang et al., 2011). This distinctive structure confers hydrophilicity to GO, enabling its easy dispersion in water (Yu et al., 2020). Additionally, this structure facilitates the manipulation of GO's mechanical, colloidal, and optical properties (Tarcan et al., 2020), and provides a large active site for metals through both electrostatic and coordinate approaches (Wang et al., 2014a).

In this particular context, GO has attracted considerable attention for various applications, particularly in the realm of cancer treatment (Nandi et al., 2019), drug delivery (Zarafu et al., 2018a), plasmid transformation (Wu et al., 2020), cell cultures (Zeng et al., 2020), tissue regeneration (Șelaru et al., 2022), viral vaccines (Zhou et al., 2021), and other biomedical fields (Habte et al., 2019). Additionally, the extensive surface area of GO renders it a suitable candidate for nanoparticle decoration (Jafarizad et al., 2017).

GO has garnered considerable attention in the biosensing field, with numerous studies focusing on its integration onto sensor surfaces, either independently or in conjunction with other nanomaterials, resulting in extensively documented composite structures. For example, Arfin and Rangari (2018) developed a glassy carbon electrode modified with ZnO-functionalized GO (GO–ZnO/GCE) for the electrochemical detection of phenol. Similarly, He et al. (2018) demonstrated the effectiveness of a TiO2-based GO composite-modified glassy carbon electrode for tartrazine detection. Furthermore, GO and ionic liquid modified electrodes have shown promise in the detection of genes such as breast cancer 1 (BRCA1) gene (Işın et al., 2022).

Numerous studies have confirmed the antimicrobial effectiveness of GO against bacteria and biofilms (Zarafu et al., 2018b). Baek et al. (2019) have demonstrated the antibacterial properties of GO, attributing its efficacy to the increased dispersion area of metal oxides and enhanced generation of ROS within bacterial cells (Baek et al., 2019a). Additionally, Wang et al. (2014) have reported the antibacterial activity of synergistic components of GO and metal nanoparticles, which are also less toxic to human cell lines (Wang et al., 2014b). Graphene-based materials have been effectively utilized for the dispersion, stabilization, and controlled delivery of various nanomaterials and pharmaceutical agents, including antibiotics. Moreover, their outstanding biocompatibility has facilitated a wide range of antimicrobial applications, such as antibacterial packaging, wound dressings, and disinfection (Ji et al., 2016).

Clinical trials have presented substantial evidence supporting the antibacterial properties of GO, particularly when combined with nanohybrids such as zinc oxide (ZnO). For example, GO-based ZnO and GO-based TiO2 have shown effectiveness against multidrug-resistant *Escherichia coli* (Baek et al., 2019b). Additionally, Riyal et al. (2022) have verified the antibacterial effectiveness of GO combined with SeNPs against *Staphylococcus aureus*, *Proteus vulgaris*, and *Bacillus subtilis* bacteria (Riyal et al., 2022).

Furthermore, clinical trials have approved the effective applications of GO nanocomposites in cancer hyperthermia therapy and antiviral treatment. GO sheets decorated with superparamagnetic iron oxide nanoparticles (SPIONs) functionalized with polyethylene glycol (PEG) or chitosan (CS) exhibited superparamagnetic behavior. The resulting nanocomposites demonstrated low toxicity to L929 cells. Additionally, GO/SPION with PEG eradicated cancer cells when exposed to a magnetic field, while GO/SPION with CS effectively neutralized the SARS-CoV-2 virus, suggesting its potential as a material against COVID-19 (Kohzadi et al., 2022).

Moreover, the presence of oxygen-containing functional groups in GO ensures strong interaction with cellular proteins, making it highly compatible with cellular behavior in terms of cell growth and viability (Zhang et al., 2016; Habte et al., 2019). The biocompatibility of graphene and its derivatives is intricate and depends on various factors, including the type, size, and shape of the graphene nanomaterial, as well as the experimental conditions. Therefore, it is crucial to investigate these properties using both *in vitro* cell cultures and *in vivo* animal models. Some studies have shown that GO did not penetrate A549 cells (human alveolar basal epithelial

cells) and did not exhibit apparent cytotoxicity. However, GO induced dose-dependent oxidative stress in cells, resulting in a slight decrease in cell viability at higher concentrations. These effects were found to be dependent on both the dosage and the size of GO particles. Similarly, GO displayed dose-dependent toxicity towards human fibroblast cells and mice (Liao et al., 2018). Their findings revealed that GO doses below 20 μg/mL showed no discernible toxicity in human fibroblast cells. Conversely, doses exceeding 50 μg/mL exhibited pronounced cytotoxicity, leading to reduced cell adhesion and cell apoptosis. In *in vivo* experiments with mice, administration of low doses (0,1 mg) and moderate doses (0,25 mg) of GO showed no evident toxicity, while a high dose (0,4 mg) resulted in chronic toxicity, leading to mouse mortality and the formation of lung granulomas (Yang et al., 2011).

4.5. GO–SeNP

The GO–SeNP composite exhibited enhanced electrical properties attributed to the electrical conductivity. Consequently, a hybrid system comprising Se nanorods and GO composite exhibits potential as a cathode material for durable batteries and other energy storage devices (Ahmad et al., 2020). Additionally, the development of a GO-SeNPs composite serves as an effective acetone gas detector (Shar et al., 2020).

The GO-SeNP nanocomposite was developed and evaluated for its effectiveness against *Staphylococcus aureus*, *Proteus vulgaris*, and *Bacillus subtilis* bacterial strains. The findings indicated that the synthesized nanocomposites demonstrated notable antibacterial properties, showing promise as an effective antibacterial agent (Riyal et al., 2022).

5. MATERIALS and METHODS

5.1. Materials

Ascorbic acid (Sigma-Aldrich, USA)

Graphite

Phosphoric acid (Sigma-Aldrich, USA)

Potassium permanganate (Sigma-Aldrich, USA)

Saponin (Sigma-Aldrich, USA)

Sodium selenite (Na_2SeO_3)

Sulfuric acid (Sigma-Aldrich, USA)

5.2. Synthesis of GO

Graphene oxide (GO) was produced using modified Hummer's method (Wang et. al., 2017). Initially, phosphoric acid (20 mL) was gradually added to sulfuric acid (100 mL) under magnetic stirring at room temperature. Subsequently, graphite (2 g) was incorporated into the solution. Following this, potassium permanganate (8 g) was slowly added to the mixture, resulting in a noticeable change in dispersion color to dark green. After a three-day period, hydrogen peroxide (3 drops) was added to stop the reaction. After thirty minutes of stirring, the resulting product displayed a brief bright yellow color. The resulting mixture was filtered, washed with 5% hydrochloric acid (200 mL), and then rinsed with distilled water through a filter membrane until reaching a pH value of 7. Finally, the synthesized GO was dried in an oven at 85°C.

5.3. Production of GO- SeNPs

GO (20 mg) was dispersed in distilled water using ultrasonic treatment to achieve a uniformly dark dispersion. In a separate container, sodium selenite (2 g), ascorbic acid (1 g), and saponin (25 mg) were dissolved in distilled water. The sodium selenite was then added to the GO dispersion, followed by the gradual addition of ascorbic acid (1 mL) and saponin in a drop-wise manner, repeated twenty times until the solution exhibited a dark brown/orange hue. After 24

hours of thorough mixing, the solution underwent centrifugation at 11,000 rpm for 20 minutes, and the supernatant was discarded. The resulting GO-SeNP was subsequently washed with ethanol and deionized water using centrifugation, and the residual material was dried in an oven at 85–90 degrees Celsius. The same procedural steps were followed to generate a control sample (SeNP) without the incorporation of GO dispersion.

5.4. Characterization of the Nanomaterials

The absorption spectra of the nanomaterials were recorded using a UV-Visible Spectrophotometer. The structure of the materials was characterized using Fourier Transform Infrared Spectroscopy (FTIR). Additionally, the morphology and elemental composition of the materials were investigated using Scanning Electron Microscope (SEM) with Energy Dispersive X-Ray (EDX) analysis.

5.5. Determination of the biocompatibility of SeNPs and GO- SeNPs

5.5.1. Cell culture studies

In this study, human colorectal adenocarcinoma (HT-29), human prostate cancer (PC-3), human glioblastoma multiforme (U251 MG), and mouse embryonic fibroblast (NIH3T3) cell lines were utilized. HT-29, NIH3T3, and U251-MG cells were maintained in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin, while PC3 cells were cultured in RPMI 1640 medium. The cells were maintained at 37°C with 5% CO_2. Upon reaching 80-90% confluence, the cells were detached from the flask surface using Trypsin-EDTA and transferred into 25 cm^2 flasks.

5.6. Determination of the antiproliferative activity

The MTT method was used to determine the antiproliferative activity of the prepared nanomaterials. MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) is a quantitative method used to measure cell proliferation, cell viability, and cytotoxicity. This method relies on the reduction of tetrazolium salts catalyzed by mitochondrial enzyme systems. Tetrazolium salts such as MTT, XTT, and WST-1 are typically colorless compounds that, when acted upon as substrates, yield colored products due to the mitochondrial activity of viable cells. MTT, a specific tetrazolium salt, forms insoluble blue-purple formazan salts upon binding to the succinate dehydrogenase (SDH) enzyme in the mitochondria of living cells. In contrast, deceased or cells with impaired mitochondrial activity do not generate the purple-blue formazan

product. Formazan salts are readily soluble in organic solvents and can be quantified for optical density spectrophotometrically. The cytotoxicity of the test substance is ascertained by comparing the spectrophotometrically obtained value with the value representing 100% viability.

Preparation of MTT solution

The Vybrant MTT Cell Proliferation Assay Kit, commercially obtainable from Thermo Fischer in the United States, was employed for the experiment. 5 mg of MTT dye was dissolved in 1 mL of sterile phosphate-buffered saline (PBS) by thorough vigorous vortexing. The light-sensitive MTT solution was then stored in a dark environment at a temperature of +4°C until it was utilized.

Preparation of cell cultures in 96-well microplates

The cells were counted utilizing a hemocytometer, and $1x10^4$ cells was seeded into each well. Subsequently, the cells were cultured in the wells for a duration of 24 hours, with 200 µL of culture medium added to each well.

Adding the samples

At the end of 24 hours, prepared samples were added to the wells at concentrations of 20-50-100-250 and 500 µg/mL. The samples used in the study and their contents are as follows: GO, SeNPs and GO-SeNPs.

MTT Assay

After incubating the samples for 24 hours, the culture medium was completely removed. Subsequently, the wells were rinsed with 200 µL of PBS. Following this, 100 µL of fresh culture medium and 10 µL of MTT solution were added, and the plate was left to incubate for 4 hours. Upon completion of the incubation period, 100 µL of sodium dodecyl sulfate (SDS) was introduced to dissolve the formazan salts produced by MTT, and the plate was further incubated for 12 hours. The absorbances at a wavelength of 570 nm were then measured using a microplate reader. The average absorbance values from the control wells lacking any substance were recorded and added together, constituting the reference value of 100%.

% Cell Viability = (Sample Absorbance Value / Control Absorbance Value) ×100

5.7. Determination of the antimicrobial activity of GO-SeNPs

The microdilution method was employed to investigate the antimicrobial activity of GO, SeNPs and GO-SeNPs (CLSI 2020, CLSI 2012, Berkow, 2020).

In our study, 6 reference bacterial strains (*Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 4352, *Escherichia coli* ATCC 25923, *Salmonella typhimurium* ATCC 14028, *Staphylococcus aureus* ATCC 25922 and *S90028, Staphylococcus epidermidis* ATCC 12228 and 4 standard yeast strains (*Candida albicans* ATCC 90028, Candida *tropicalis* KUEN 1021, *Candida parapsilosis* ATCC 90018 and *Candida glabrata* ATCC 90030) were used in order to determine the antimicrobial activity of GO, SeNPs and GO-SeNPs.

Bacteria were inoculated on tryptic soy agar while yeasts were inoculated on sabouraud dextrose agar, incubated at 37 °C for 24 hours. Microorganism suspensions were prepared from colonies in 0.85% NaCl physiological saline solution (PSS).

5.7.1. Detection of minimal inhibitory concentration (MIC) of bacteria

The minimal inhibitory concentration (MIC) for bacteria was performed in accordance with the standards of the Clinical and Laboratory Standards Institute (CLSI). Cation-adjusted Mueller Hinton Broth (CAMHB) served as the medium for the experiment. A bacterial suspension was prepared from colonies in a 24-hour bacterial culture, following the McFarland 0.5 turbidity standard, and the final inoculum concentration was adjusted to $5x10^5$ CFU/mL. Sterile U-based microdilution plates were filled with 100 µL of CAMHB. Subsequently, 100 µL of nanomaterials were added to the first wells, and serial dilutions were performed. Then 5µL of the bacterial suspension was added to the wells and the plates were incubated at 37 °C for 24 hours (CLSI 2020).

5.7.2. Detection of minimal inhibitory concentration MIC of yeasts

Yeast suspensions were prepared from 18-24-hour agar cultures using PSS and adjusted to a concentration of 2×10^3 CFU/mL based on the McFarland 1 standard. (CLSI 2012, Berkow,2020). RPMI 1640 for yeasts was added to all wells of a sterile 96-well microplate, followed by the addition of 100µL of GO, SeNPs, and GO-SeNP to the first wells, with subsequent 12 serial dilutions. Bacteria and yeast suspensions were then added to all wells, and the microplates were incubated at 35°C for 18-24 hours for bacteria and 24-48 hours for yeasts, in accordance with CLSI (2012) and Berkow (2020).

Following the incubation period, the MIC value, representing the lowest concentration at which growth was inhibited, was determined for both bacteria and yeasts. Likewise, CAMHB and RPMI were used as negative control. Meropenem and Fluconazole were used as positive control (CLSI 2020, CLSI 2012, Berkow, 2020).

6. RESULTS

6.1. Characterization of Nanoparticles

According to the absorbance spectra, the main spectrum of GO has a strong absorption peak at 200-250 nm, and weak absorption at 305 nm. The characteristic absorption spectrum of SeNP is depicted in a sharp peak at 250–280 nm, coupled with the pronounced dark brown/orange coloration observed in the aqueous colloidal dispersion, which provides clear evidence of the successful formation of our product. Also, it was an indication for reduction of sodium selenite into elemental selenium. UV-visible absorption spectra of GO, SeNPs and GO-SeNPs are presented in Figure 4.

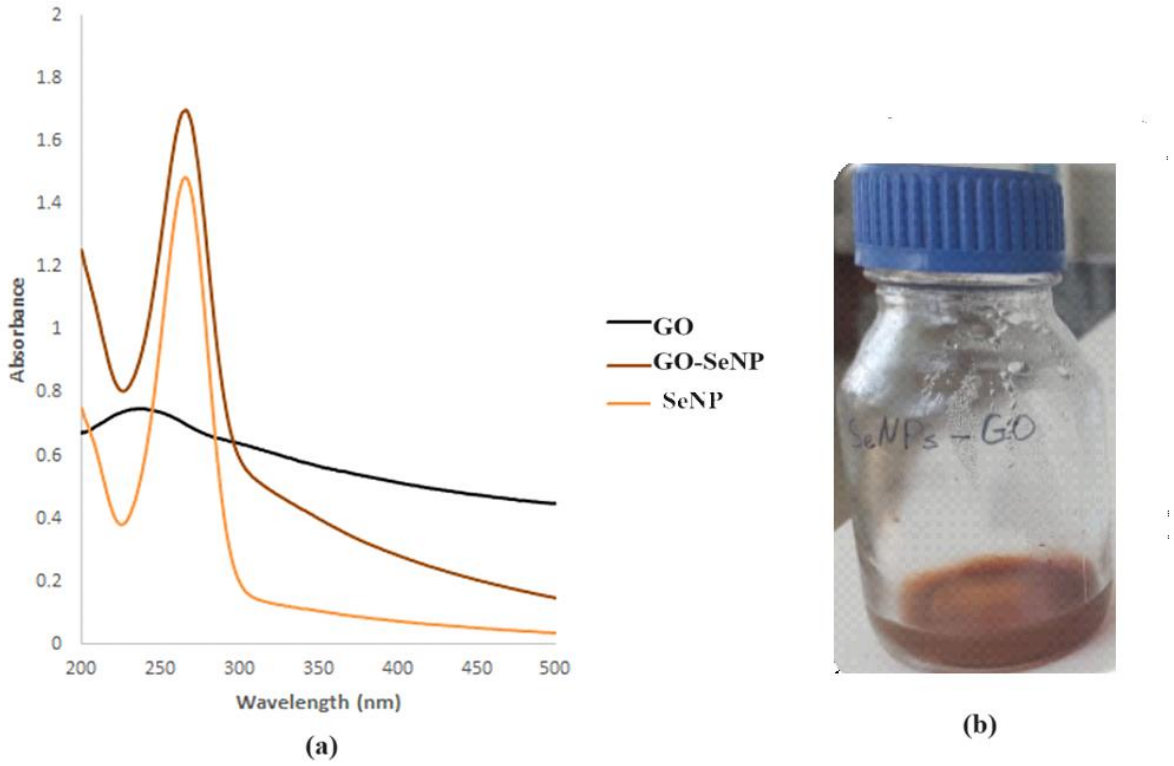

Figure 4. (a) UV-Visible absorption spectra of GO, GO-SeNP and SeNP; **(b)** Photo of GO-SeNP dispersion

The FTIR analysis revealed prominent peaks for GO-SeNPs at 2900 and 2800 cm^{-1}, which are commonly attributed to C-H stretching vibrations, and at 1400, 1200, and 1000 cm^{-1}, indicative of the presence of the C-O functional group. Figure 5 illustrates the FTIR spectra of the generated samples (GO, SeNPs, and GO-SeNPs).

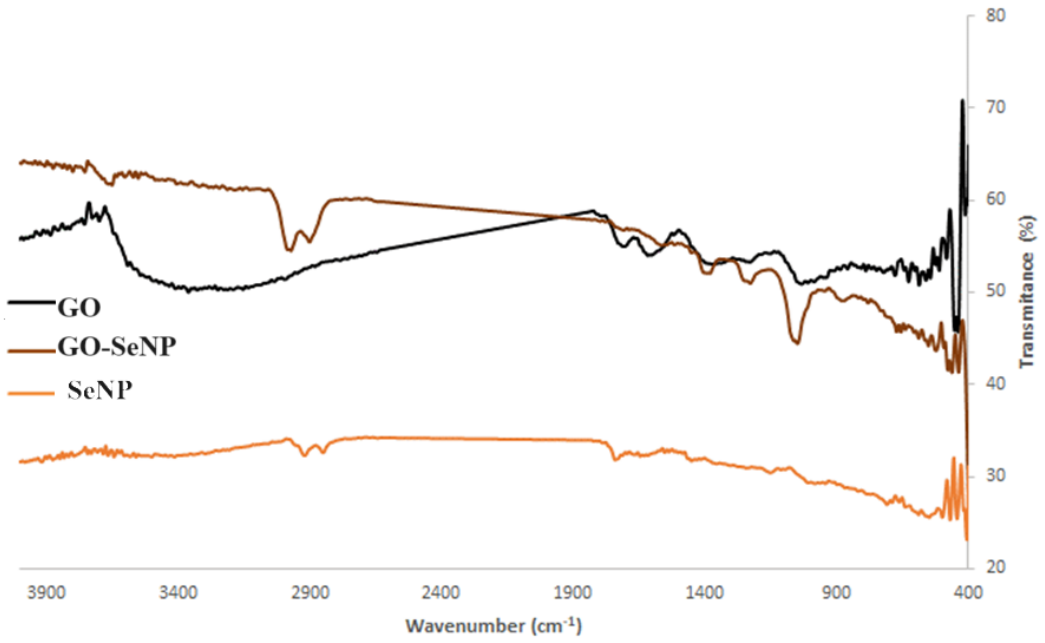

Figure 5. FTIR spectra of GO, SeNPs and GO-SeNPs.

SEM-EDX analyses were conducted to examine the morphological characteristics and chemical composition of the nanomaterials. The EDX findings were presented in Figure 6, while SEM images of GO and GO-SeNPs were provided in Figure 7.

The SEM image illustrates the surface structure of GO, revealing its flake-like configuration. SEM-EDX analysis confirms the elemental composition of GO, predominantly comprising carbon and oxygen. The notable peaks are observed at approximately 2 keV and 18 keV. The EDX analysis reveals the presence of carbon and oxygen in the GO sample, with carbon accounting for 31,51% and oxygen constituting 68,49% of the sample.

The SEM-EDX analysis of the SeNPs indicates that the nanoparticles are predominantly composed of Se, with a minor presence of carbon (C) and oxygen (O). The weight percentages of these elements are as follows: Se: 100%. The existence of Se in the nanoparticles suggests successful synthesis.

The SEM image illustrates the spherical morphology of SeNPs, with a size range of approximately 50-100 nm and a uniform dispersion, indicating minimal aggregation.

Additionally, the EDX spectrum reveals elemental peaks corresponding to the sample composition, with weight percentages of 19,71% C, 26,24% O, and 54,05% Se. Furthermore, the SEM image depicts a GO layer on the SeNPs.

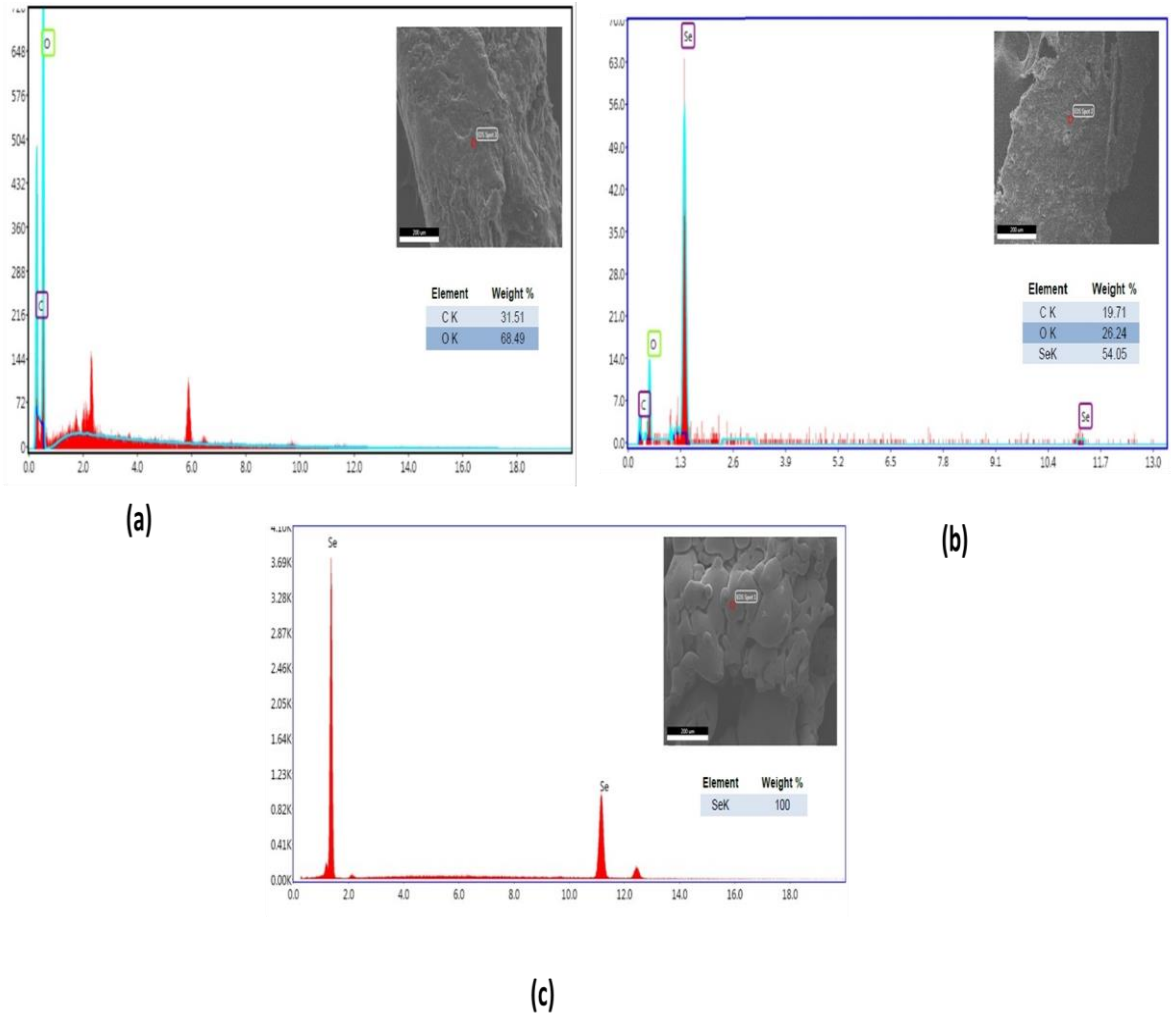

Figure 6.SEM-EDX analysis results of GO (a), GO-SeNPs (b) and SeNPs (c).

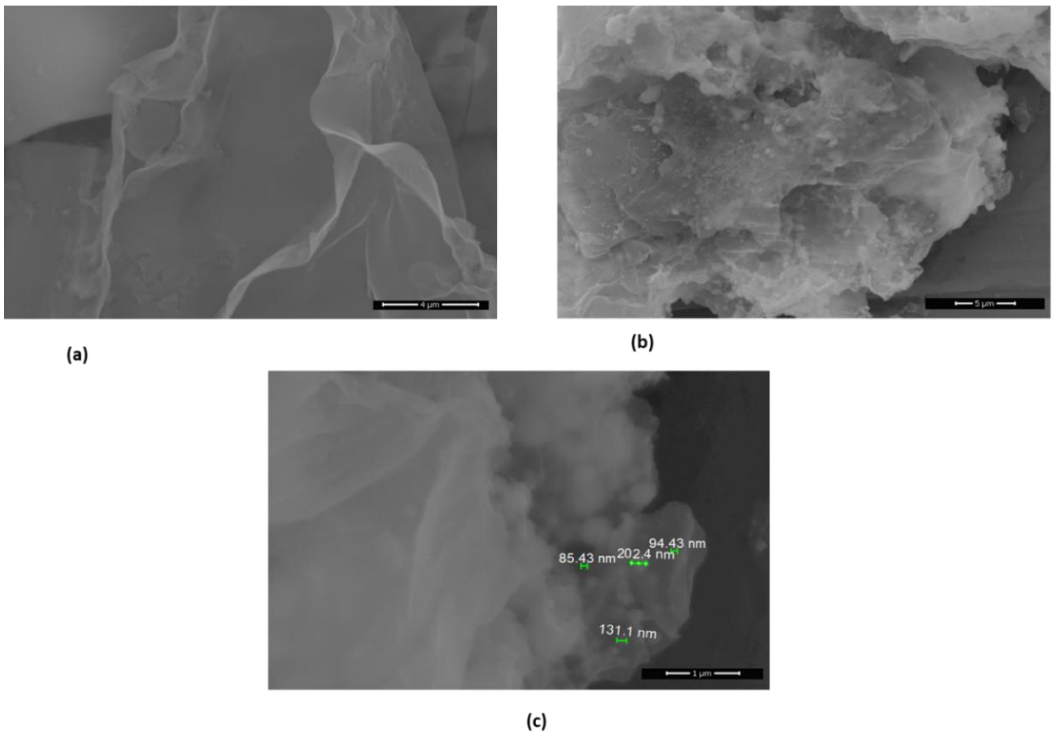

Figure 7. The SEM images of GO (a) and GO-SeNPs (b, c).

6.2. Cell viability

The antiproliferative effects of nanoparticles on cancer cell lines (HT-29, PC3, and U251-MG) and non-tumorigenic mouse embryonic fibroblast cell line (NIH3T3) have been detected using the MTT assay.

MTT results revealed that the synthesized SeNPs exhibited a dose-dependent inhibition of cell proliferation across all examined cancer cell lines (Fig 8). Notably, the inhibitory effect was more prominent at a dosage of 500 µg/mL, particularly evident in the PC-3 and HT-29 cell lines (43,29%±3,16 and 27,30%±6,34, respectively). In this study, it was established that SeNPs induced dose-dependent cytotoxicity in mouse fibroblast cells, with the most pronounced inhibition observed at a dosage of 500 µg/mL. Conversely, SeNPs exhibited no cytotoxic effects up to 250 micrograms; however, it is noteworthy that they elicited a significant 34,35% ±5,13 inhibition on PC-3 cells at a dose of 250 µg/mL.

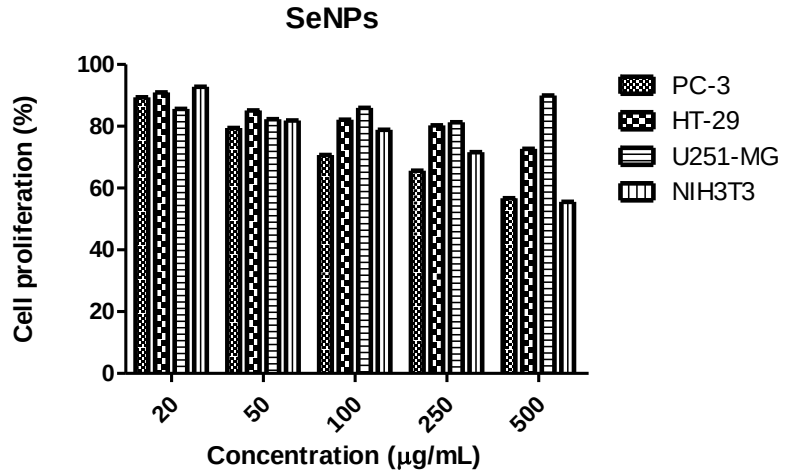

Figure 8. The antiproliferative effects of SeNPs on cancer cell lines and mouse embriyonic fibroblast cells (NIH3T3).

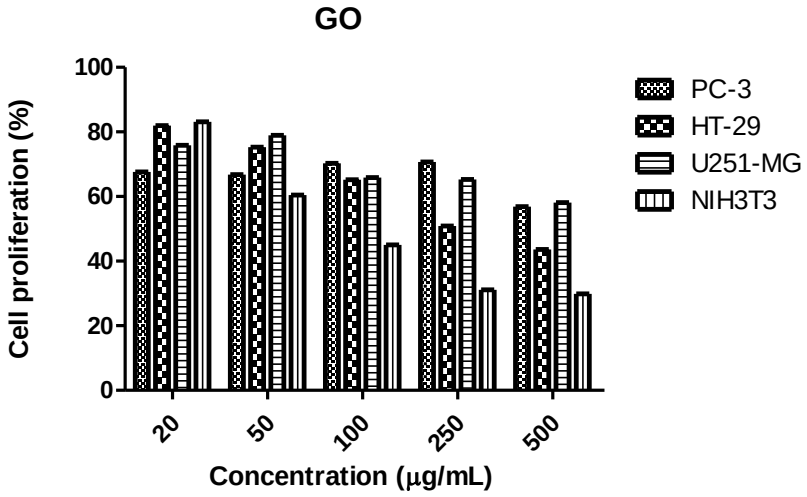

Figure 9. The antiproliferative effects of GO on cancer cell lines and mouse embriyonic fibroblast cells (NIH3T3).

GO, like SeNPs, exhibited a dose-dependent inhibition of cell proliferation in cancerous cell lines (Fig 9). Its most pronounced inhibitory effects were observed in PC-3 and U251-MG cells when administered at a dosage of 500 µg/mL (43,15% ±2,5 and 41.84% ±2,05, repspectively) Notably, the IC_{50} value of GO in HT-29 cells was determined to be 317,7 µg/mL. In contrast to its impact on cancer cells, GO showed a cytotoxic effect on normal cells at lower concentrations. IC_{50} value was measured as 94,52 µg/mL.

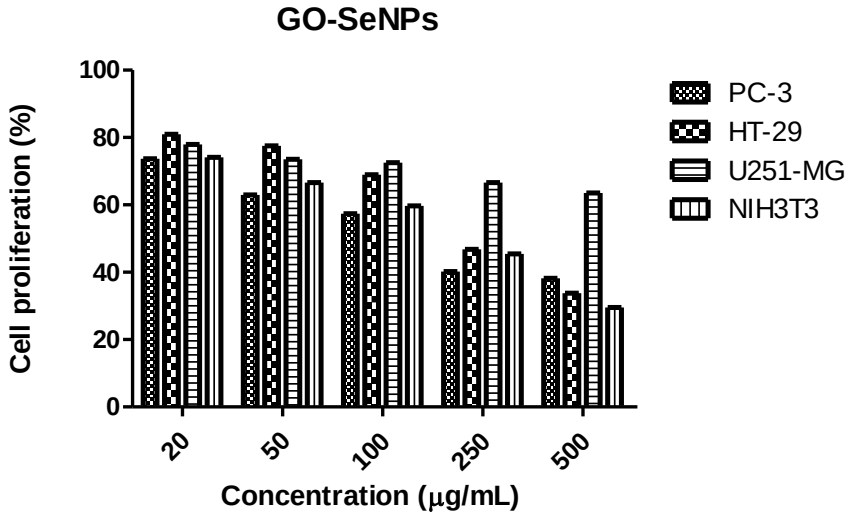

Figure 10. The antiproliferative effects of GO-SeNPs on cancer cell lines and mouse embriyonic fibroblast cells (NIH3T3).

As seen in Fig 10, it was determined that G0-SeNPs inhibited the proliferation of cancer cells more than SeNPs in a dose-dependent manner. While the IC50 value on HT-29 cells was found to be 236,6 µg/mL; The IC50 value on PC-3 cells was calculated as 175,15 µg/mL. It was also determined that the cytotoxic effect of GO on normal cells was further reduced in GO-SeNPs (IC50: 193.2 µg/mL).

6.3. MIC Results

After the incubation period of our bacterial strains (*Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC4352, *Escherichia coli* ATCC25922, *Salmonella typhi* ATCC15313, *Staphyloccus aureus* ATCC25923, *Staphylococcus epidermidis* ATCC11228), and yeast strains (*Candida albicans* ATCC 90028, *Candida tropicalis* KUEN 1021, *Candida parapsilosis* ATCC 90018 and *Candida glabrata* ATCC 90030), the MIC values has been shown in (Table 3).

MIC was 1000 µg/mL in all bacterial strain except in *Klebsiella pneumoniae* it was 250 µg/mL, the same as in yeast strains as it was 1000 µg/mL.

While SeNPs display almost the same effects at 1000 µg/mL for the bacterial strains excluding *Klebsiella pneumoniae* and *Salmonella typhi* it was 500 µg/mL, at the same time MIC of SeNPs in fungal strain *Candida albicans* and *Candida parapsilosis* has not been detected, unlike in *Candida glabrata* and *Candida tropicalis* it was 1000 µg/mL.

Our produced GO-SeNPs nano system shows MIC about 1000 µg/mL in the bacterial strains, other than *Klebsiella pneumoniae* it was 250 µg/mL, the same time results in yeast strains, were differentiated, in *Candida albicans*, MIC has been detected in our used concentrations and it was 250 µg/mL in *Candida parapsilosis*, while it was 1000 µg/mL in *Candida glabrata* and *Candida tropicalis*.

Table 2. Detection of minimal inhibitory concentration (MIC) Values (µg/mL). GO: Graphene oxide Se-NPs: Selenium nanoparticles, GO-Se NPs: Graphene based selenium nanoparticles, ND: Not Done

MIC (µg/mL)	**GO**	**Se-NPs**	**GO - Se NPs**	**Meropenem**	**Fluconazole**
***Pseudomonas aeruginosa* ATCC 27853**	1000	1000	1000	0,25	ND
***Klebsiella pneumoniae* ATCC 4352**	250	500	250	4,25	ND
***Escherichia coli* ATCC 25922**	1000	1000	1000	2,0	ND
***Salmonella typhi* ATCC 15313**	1000	500	1000	4,25	ND
***Staphyloccus aureus* ATCC 25923**	1000	1000	1000	0,06	ND
***Staphylococcus epidermidis* ATCC 11228**	1000	1000	1000	0,25	ND
***Candida albicans* ATC 90028**	1000	>1000	>1000	ND	3,125
***Candida glabrata* ATCC 90030**	1000	1000	1000	ND	6,25
***Candida parapsilosis* ATCC 90018**	1000	>1000	250	ND	1,56
***Candida tropicalis* KUEN 1021**	1000	1000	1000	ND	1,56

7. DISCUSSIONS and CONCLUSION

Selenium (Se) is a trace element with broad pharmacological capabilities and physiological functions. It is a significant component of numerous antioxidant enzymes such as phospholipid hydroperoxide, glutathione peroxidase, and thioredoxin reductase (Labunsky et al., 2014). It is also a micronutrient that has important roles in human health, improving cardiovascular health or immunomodulatory functions, protecting of the liver restricting the progression of cancer, preventing neurodegeneration and regulating thyroid hormone metabolism (Liu et al., 2012; Barret et al., 2015; Genchi et al., 2023).

The deficiency of Se will cause damage to the immune system and an increased risk of oncogenesis. Se supplements traditionally used in cancer treatment have disadvantages such as high toxicity or low absorption. Therefore, the development of new systems that enhance the bioavailability of Se-containing compounds and allow for controlled release in the organism has become an important requirement in this field.

Therefore, this study aims to describe a solution-phase approach to the synthesis of selenium nanoparticles on graphene oxide surface by reducing sodium selenite salt with ascorbic acid in the presence of saponin as stabilizing agent for the first time.

Originally, sodium selenite solution was colorless. The color change of the reaction mixture from colorless to reddish confirms the successful synthesis of SeNPs. It was also confirmed by the gradual increase in optical phenomena within the characteristic peak between 200 and 400 nm depending on the reaction time (Cui et al., 2018; Sivakumar and Jeganathan, 2018). A red shift occurs in the absorption spectra of SeNPs due to the increase in particle size (Zong-Hong and Wang CR, 2005). In our study, the color of sodium selenite turned into dark brown/orange with the addition of GO and exhibited absorption at 250 and–280 nm. The appearance of brownish orange colour indicates the reduction of sodium selenite and formation of SeNPs similar to the results reported earlier. Anu et al. (2017) produced selenium nanoparticles (SeNPs) by a green synthesis method using *Allium sativum*. Their research revealed the visible maximum of UV at 260 nm. Similarly, Boroumand et al. (2019) produced SeNPs resulting from the chemical reduction of sodium selenite with ascorbic acid in the presence of polyvinyl

alcohol (PVA) or Chitosan as stabilizing agents. UV–visible absorption spectra exhibited peaks at 264 nm for PVA-SeNPs and 310 nm for Chitosan-SeNPs. Likewise, Gunti et al., (2019) have prepared SeNPs from *Emblica officinalis* fruit extract by green synthesis approach and observed UV-visible absorption maximum at 270 nm.

The FTIR spectra shows the functional groups, bonds, and interactions within the synthesized materials. In the present study, FTIR spectra of GO, SeNPs and GO-SeNPs have been examined. There were some similarities in the FTIR spectra of the SeNPs and GO-SeNPs. The strong absorption peaks of C-H stretching vibrations were observed in the spectra for SeNPs and GO-SeNPs at 2900 and 2800 cm^{-1}. Additionaly, in the FTIR spectrum of the GO-SeNPs, the characteristic absorption peaks at 1400, 1200, and 1000 cm^{-1} were due to the C–O functional groups (Çalışkan Salihi, et. al., 2016). Result indicates that GO was successfully combined with the SeNPs. Taken together, these results confirmed that we had synthesized selenium nanoparticles decorated on GO.

SEM analysis indicated that SeNPs and GO-SeNPs were spherical in shapes with nearly no aggregation and the size of GO-SeNPs ranged from 50 to 100 nm. The findings were consistent with findings from previous research. Alhawiti (2022), Ramamurthy et al (2013), and Boroumand et al (2019) have green synthesized SeNPs using different natural substrat such as sitric acid, plant extract and polymer and observed optimum size of SeNPs as 67-83, 50-150, 48-66, respectively. The diversity in the size of bio-synthesized nanoparticles is influenced by the chemical constituents of the biomaterial utilized (Akhtar et al., 2013). As a result, the size variation of SeNPs in our study differed from previous findings.

The elemental composition and purity of nanoparticles were also achieved through the utilization of EDX spectra. SeNPs displayed the highest Se content at 95.74% among nanoparticles. According to our findings, our study revealed the highest selenium content in nanoparticles, reaching 95%. Anu et al. (2020) found that the Se content in the SeNPs derived from the leaf extract of *Cassia auriculata* was determined to be 82%. Furthermore, EDS analysis indicated that when GO was employed, the Se content in SeNPs was 55%. The other peaks being observed in EDS spectrum were carbon and oxygen. There were no obvious peaks for other elements or impurities. Recent studies on the synthesis of SeNPs different sources support the data obtained in this study (Joshi et al., 2019; Alhawiti, 2022). These results confirm the decoration of Se-NPs on the surface of GO.

Numerous studies have verified the anticancer properties of SeNPs produced through environmentally friendly green synthesis methods (Ramamurthy et al., 2013; Wadhvani et al., 2017; Cui et al., 2018; Gunti et al., 2019). In this study, the growth inhibition of GO, SeNPs and GO-SeNPs in PC-3, HT-29, U251-MG cells was evaluated by the MTT assay. According to our results, GO and other nanoparticles have inhibited the cell viability in dose-dependent way. However, GO has inhibited cell viability at much lower concentration compared to SeNPs. Furthermore, SeNPs without GO functionalization demonstrated much lower cytotoxic effect on all of the cancer cells. MTT results obtained from this study showed that GO surface decoration enhanced the antiproliferative activity of SeNPs in PC-3 and HT-29 cancer cells. Similarly, Yang et al (2012) found that SeNPs decorated with Spirulina polysaccharide showed stronger antiproliferative effects than normal SeNPs. Taken together, the results suggest that the strategy to use GO as a surface decorator could be an effective way to enhance the cellular uptake and anticancer effects of nanomaterials. GO-SeNPs may be a potential candidate for further evaluation as a chemopreventive and chemotherapeutic agent against human cancers.

According to our findings, GO-SeNPs have cytotoxic effect on healthy cell line NIH3T3, which is scientifically increased dose dependent manner, however, it demonstrates a cell protection property against GO toxicity.

SeNPs were not biocompatible by testing over healthy cell line and exhibit dose dependent toxicity, a slight increase of antiproliferative characters of our nanoparticles by increasing the concentration over colon cancer cell line HT-29, while PC-3 cells were more sensitive, but a negative impact of anticancer effect in U251-MG cell.

The ability of SeNPs and GO-SeNPs to inhibit bacterial growth was tested by reference strains with different concentrations of nanoparticles by using the agar well diffusion assay and microdilution methods. Based on our findings, the efficacy of GO-SeNPs in terms of antimicrobial activity is weak, as we need high concentration (1000 µg/mL) comparing with the used antibacterial control agent meropenem. However, it is noteworthy that the bacterial strain *Klebsiella pneumoniae* was more sensitive (MIC of GO-SeNP was 500 µg/mL). This observation contrasts with the recent study of Riyal et al (2022) when they use *Staphylococcus aureus* to approve the positive antibacterial effects of GO-SeNP, this disagreement is maybe duo to using different methods to detect antibacterial effect, our used experiment is more sensitive method, while Riyal et al (2022) used agar well diffusion assay.. Parallel to our study,

Cremonini and colleagues demonstrated in their work that chemically synthesized SeNPs showed activity against *Pseudomonas aeruginosa* ATCC 27853 strain at a concentration greater than 512 µg/mL MIC. Similarly, the antifungal property of GO-SeNP is poor or significantly not detectable compared with our antifungal control fluconazole, but *Candida parapsilosis* is more sensitive against GO-SeNP than the other used yeast strains.

Consequently, in the present study, GO-decorated SeNPs were successfully synthesized using ascorbic acid and saponin. The synthesized GO-SeNPs exhibited amorphous nature, spherical shape and nano-size. The GO-SeNPs have exhibited antiproliferative activity compared to GO and SeNPs. Also, GO-SeNPs shown weakly antimicrobial activity. In general, the potential of GO-SeNPs makes them a promising biomedical agent, exhibiting both antimicrobial and anticancer properties. Nevertheless, additional comprehensive research is necessary to completely understand the exact mechanisms responsible for their antimicrobial and anticancer effects.

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9. SCIENTIFIC ACTIVITIES

Poster:

Investigation of the antiproliferative and antimicrobial properties of green synthesized selenium nanoparticles decorated on graphene oxide.

5th International Conference on Nanomaterials, Nanofabrication and Nanocharacterization (NANOMACH), April 18-24, 2024, Muğla Turkey.