University of Kerbala
College of Science
Department of Chemistry

Biosynthesis of Zinc Oxide Nanoparticles *Ziziphus-spina christi* Leaves Extract: Characterization and Their Effects in Male Rats Exposed to Adenine

A Thesis
Submitted to the Council of the College of Science-University of Kerbala
as a Partial Fulfillment of the Requirement for the Master Degree in
Chemistry Science

By
Hayder Hussein Abbas
B.Sc. Chemistry/College of Science /University of Kufa (2009)

Supervised by

Prof. Dr. Narjis Hadi Al-Saadi	**Prof. Dr. Ayyed Hameed Hasan**
Ph. D. Biochemistry	**Ph. D. Animal Physiology**

2023 A.D   **1445 A.**

Certification of Supervisor

We certify that this thesis "**Biosynthesis of Zinc Oxide Nanoparticles** *Ziziphus-spina christi* **Leaves Extract: Characterization and Their Effects in Male Rats Exposed to Adenine**" was prepared under our supervision at the Department of Chemistry, College of Science, University of Kerbela, as partial fulfillment of the requirements for the degree of Master of Chemistry.

Signature:

Name: **Dr. Narjis Hadi Al-Saadi**

Title: Professor

Address: University of Kerbala /College of Science / Department of Chemistry

Date: 15 / 1 0 / 2023

Signature

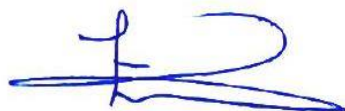Name: **Dr. Ayyed Hameed Hasan**

Title: Professor.

Address: University of Kerbala / College of Dentistry

Date: 17 / 1 0 / 2023

Report of the Head of Chemistry Department

According to the recommendations presented by the Supervisor and the Postgraduate Studies Director, I forward this thesis "**Biosynthesis of Zinc Oxide Nanoparticles *Ziziphus-spina christi* Leaves Extract: Characterization and Their Effects in Male Rats Exposed to Adenine"** for examination.

Signature:

Name: **Prof. Dr. Luma Majeed Ahmed**

Head of Chemistry Department

Address: University of Kerbala /College of Science/ Department of Chemistry.

Date: 25/ 10/ 2023

Examination Committee Certification

We, the examining committee, certify that we have read this thesis "**Biosynthesis of Zinc Oxide Nanoparticles *Ziziphus-spina christi* Leaves Extract: Characterization and Their Effects in Male Rats Exposed to Adenine**" and examined the student (Hayder Hussein Abbas) in its contents and that in our opinion; it is adequate as a thesis for the degree of Master of Science in Chemistry.

Signature:

Name: **Rana Majeed Hameed**

Title: Assist. Prof. Dr.

Address: University of   Kerbala, College of Medicine

Date:17/10 / 2023

(Chairman)

Signature:

Name: **Dr. Amer Hasan Abdullah**

Title: Assist. Prof. Dr.

Address: University of Mustansiriyah, College of Sciences, Department of Chemistry

Date:25/10/ 2023

(Member)

Signature:

Name: **Dr. Afaf Khairi Esmail**

Title: Assist. Prof. Dr.

Address: University of Kerbala, College of Sciences, Department of Biology

Date:15/10/ 2023

(Member)

Signature:

Name: **Dr. Narjis Hadi Al-Saadi**

Title: Professor

Address: University of Kerbala, College of Sciences, Department of Chemistry

Date:15 /10 / 2023

(Member & Supervisor)

Signature:

Name: **Dr. Ayyed Hameed Hasan**

Title: Professor

Address: University of Kerbala, College of Dentistry

Date:17/10/ 2023

(Member & Supervisor)

Approved by the Council of the College of Science

Signature:

Name: **Dr. Jasem Hanoon Hashim Al-Awadi**

Title: Professor

Address: Dean of College of Science, University of Kerbala.

Date:26 /10 / 2023

Dedication

To the messenger of mercy, the Prophet Muhammad "Allah blessing and peace be upon him and his pure family" ...

To my homeland, Iraq, and its noble people...

To my loving mother, who was of help and support for me, and her blessed supplication had the greatest effect in facilitating the search ship to dock on this image...

To those with whom I have shared all my life, and from whom I derive my pride and determination, my dear mother, dear brother (Karrar) and sisters (Fatimah and Zainab)...

To the spring that never stops giving, who always weaves my beautiful happiness with strings from her great heart ......

my dear wife (Enas)

To my beloved sons who were patient with me through hardship and fatigue... (Adam, Aula and Akram) ....

To family, friends, and everyone who benefits from this work....

Hayder

Acknowledgments

Summary

Zinc oxide nanoparticles (ZnO-NPs) have been widely studied in recent decades due to t eir nano-medicine applications based on t e p ysiological and bioc emical agents often added as a food supplement in animal diets. T is study aimed to synt esis and c aracterization of (ZnO-NPs) by t e biological met od using t e plant extract and t en investigated its effects in adenine-exposed male rats. Biosynt esized ZnO-NPs were synt esized using t e crude aqueous extract of *Ziziphus-spina christi* leaves and zinc nitrate exa ydrate $(Zn(NO_3)_2.6H_2O)$. T e reaction medium was maintained wit a temperature of 60 °C and a pH of 9. T e c ange of color from yellow to brown indicates t e production of ZnO-NPs. T e produced ZnO-NPs were c aracterized using several tec niques. Ultraviolet-visible (UV-Vis) spectrum s owed t e maximum peak at 362 nm, t is was strong evidence for t e forming of ZnO-NPs. Fourier Transform-Infra Red (FT-IR) spectrum revealed t e appearance of absorption peaks between 400 and 500 cm^{-1} returned to Zn-O bonding. X-ray diffraction (XRD) analysis revealed t at t e exagonal Wurtzite structure for nanoparticles wit an average crystallite size was 38.177 nm. Transmission electron microscopy (TEM) s owed t at nanoparticles ad a sp erical s ape and fell in t e range of 35 - 45 nm in diameter, also t e images of scanning electron microscope (SEM) s owed t e semisp erical s ape and t ese particles are in a ig ly agglomerated form wit an average particle size was ranged at diameter: D_1 (39.74 nm), D_2 (42.25 nm), and D_3 (48.19 nm). Finally, atomic force microscopy (AFM) displays a uniform surface wit cone-like grains covering t e surface of ZnO-NPs, and t e surface roug ness in t e range of 42 to 45 nm. T irty-six male rats were used in t is study and divided randomly into six equal groups (six rats eac). Group-I

received orally dimet yl sulfoxide (5% v/v/day) alone for 30 days and served as t e control group, group-II administrated adenine orally (100 mg/Kg. Bw/day) for 30 days, group III received *Ziziphus-spina christi* leaves extract (10 mg/Kg. Bw/day) for 30 days, group-IV was administrated ZnO-NPs (10 mg/Kg. Bw/day) for 30 days, group-V was co-administrated adenine (100 mg/Kg. Bw/day) plus *Ziziphus-spina christi* leaves extract (10 mg/Kg. Bw/day) for 30 days and t e last group-VI received t e adenine combined wit ZnO-NPs in t e same previous doses for 30 days. At t e end of t e experiment, t e kidney function tests (uric acid, urea, and creatinine) and liver function tests (alanine aminotransferase ALT, aspartate aminotransferase AST and alkaline p osp atase ALP) were determined. Antioxidant status (superoxide dismutase SOD, catalase CAT, total antioxidant capacity TAC, and reduced glutat ione GSH) and oxidative stress (Malondialde yde MDA and nitric oxide NO) were performed. Furt ermore, istopat ological alterations of kidney and liver tissues were investigated. *In vivo* of t is study, t e synt esized ZnO-NPs and *Ziziphus-spina christi l*eaves extract at a dose (10 mg/Kg. Bw) were ex ibited t e potential effects against adenine-exposed male rats, t ese confirmed by ameliorative growt rats and t e significant decreases in kidney and liver function parameters, uric acid, urea, creatinine, ALT, AST, and ALP in comparison to adenine exposed rats ($P \leq 0.05$). Significant elevation was also demonstrated in antioxidants levels SOD, CAT, TAC, and GSH associated wit depletion in oxidative stress MDA and NO in bot treatment groups fift and sixt w en compared to adenine group rats. Moreover, t ere were improvements in t e marked istological c anges of t e kidney and liver by comparing wit t e adenine group. In conclusion, *Ziziphus-spina christi* leaves extract and ZnO-NPs ave ameliorative effects against adenine-exposed male rats, implying t at t ey may be used safely against kidney and liver

damage, and no significant effects were observed in normal renal and epatocyte tissues, indicating t at t ey may be powerful antioxidant, anti-inflammatory, and antitoxic agents for biomedical applications.

List of Contents

List of Tables

List of Figures

IX

List of Symbols and Abbreviations

Abbreviations	Meaning
ADA	Adenosine deaminase
AFM	Atomic Force Microscopy
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AMP	Adenosine monophosphate
ANOVA	Analysis of Variance ANOVA
APRT	Adenine phosphoribosyl transferase
AST	Aspartate amino transferase
ATP	Adenosine triphosphate
Å	Angstrom
Bw	Body weight
BUN	Blood urea nitrogen
Caspase 3	Protein coding gene
CAT	Catalase
$CHCl_3$	Chloroform
CH_3COOH	Acetic Acid
cm^{-1}	Wavenumber
$CuSO_4.5H_2O$	Cupric sulphate pentahydrate
CP	Cisplatin
CO_2	Carbon dioxide
C_5H_5N	The chemical formula of adenine
D	The average crystalline size in Debye–Scherrer's equation
DHA	2-8-Dihydroxyadenosine
DHBS	3,5-dichloro-hydroxybenzene-1-sulfonic acid
DMN	Dimethyl nitrosamine
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid

eq.	Equation
ESRD	End Stage Renal Disease
FAD	Flavin adenine dinucleotide
FDA	Food and Drug Administration
$FeCl_3$	Ferric c loride
$FeSO_4.7H_2O$	Ferrous sulfate epta ydrate
Fe^{2+}, Fe^{3+}	Ferrous and ferric ions respectively
TPTZ	(2,4,6-tris(2-pyridyl)-1,3,5-triazine)
FT-IR	Fourier transform infrared spectroscopy
FWHM	Full widt at alf maximum
GMP	Guanine monop osp ate
GPx	Glutat ione peroxidase
GR	Glutat ione reductase
Griess I	Sulfanilamide
Griess II	Naphthyl ethylenediamine
gm	Gram
g/dL	Grams per deciliter
GTP	Guanine trip osp ate
GSH	Glutat ione reductase
H&E	Hematoxylin and Eosin stain
G2 (HepG2)	Hepatoma cell line
H_2SO_4	Sulfuric acid
H_2O_2	Hydrogen peroxide
HCl	Hydroc loric acid
HGPRT	Hypoxant ine-guanine p osp oribosyl transferase
HNO_3	Nitric acid
IMP	Inosine monop osp ate
IL-6	Interleukin-6
k	Is t e s ape factor or Sc erer`s constant (0.98)

KD	Kidney disease
Kg	Kilogram
KI	Potassium Iodide
KOH	Potassium ydroxide
$(K_3Fe\ (CN)_6)$	Potassium ferrocyanide
Kv	Kilovolt
mA	Milliampere
MDA	Malonalde yde
μmol/L	Micromole per litter
μmol/mL	Micromole per milliliter
mL	Milliliter
μL	Microliter
mM	Millimolar
MO-NPs	Metal oxide nanoparticles
NAD	Nicotinamide adenine dinucleotide
NaOH	Sodium ydroxide
Na_2CO_3	An ydrous sodium carbonate
$\overset{+}{N}H_4$	Ammonium ion
NPs	Nanoparticles
$NaNO_2$	Sodium nitrite
NO	Nitric oxide
$\overset{.}{O}_2$	Superoxide anion
p	Probability
pH	"Potential of ydrogen" (or "Power of ydrogen")
PBS	P osp ate-buffer saline
PNP	Purine nucleoside p osp orylase
RF	Renal failure
RNA	Ribonucleic acid
RNS	Reactive nitrogen species

ROS	Reactive oxygen species
SD	Standard deviation
SEM	Scanning electron microscopy
SOD	Superoxide dismutase
STZ	Streptozotocin
SPR	Surface plasmon resonance
TAA	T ioacetamide
TAC	Total antioxidant capacity
TBA	2-T iobarbituric acid
TCA	Tric loroacetic acid
TNB	2-nitro-5-t iobenzoic acid
TNF-α	Tumor Necrosis Factor-alp a proteins
U/ml	Units per milliliter
UV	Ultraviolet
UV-vis.	UV-visible spectroscopy
WST-1	Water-soluble tetrazolium salt
XDH	Xant ine de ydrogenase
XMP	Xant osine monop osp ate
XRD	X-ray diffraction
ZnO-NPs	Zinc oxide nanoparticles
Zn (NO$_3$)$_2$.6H$_2$O	Zinc nitrate exa ydrate
Z.-spina christi	*Ziziphus-spina christi*
(0D) NPs	Zero-Dimensional nanoparticles
(1D) NPs	One-Dimensional nanoparticles
(2D) NPs	Two-dimensional nanoparticles
(3D) NPs	T ree-dimensional nanoparticles
λ	Wavelengt
Θ	Is t e Bragg's diffraction angle
β	Is t e XRD peak full widt at alf maximum

Chapter One

Introduction

and

Literature Review

1-1 Introduction

In t e few past years, nanoparticles ave been different applications because t ey are reliable to provide a broad range of unusual uses and improved tec nologies for multiple applications suc as industry, environment, medicine, and communications [1]. One of t e reasons be ind t e intense interest is t at nanoparticle permits t e preparation of substances w ere at least one dimension of t e structure normally ranged from1to100 nanometers, w ic is 1.000 times smaller t an a micron. Nanoparticles are comparable to naturally occurring proteins and molecules in t e uman cell, and t e great interest is due to t eir small size and large surface area to volume ratio [2], [3]. T e biological activity of nanoparticles increased as t e total surface area of t e particles increased, w ic gives rise to some of t e superior properties to t eir bulk p ase suc as antimicrobial, catalytic, electronic, magnetic, and optical properties [4], [5].

Medicinal plants are a great role to useful for medicine purposes to reduce diseases and improve uman ealt . T e medicinal advantages of t ese plants involved in some c emical compounds t at produce a specific p ysiological action t at can be used in t e synt esis and production of drugs, due to t e acceptability, compatibility, adaptability, and t e little or no side effects of t ese natural drugs on t e uman body [6]. Biologically synt esized nanoparticles from plant extracts are usually prepared from t e biological part of plants. Plant parts, for instance, leaf, root, stem, and seed, are widely used for synt esizing metal-NPs. Moreover, plant extracts contain bioactive compounds (p ytoc emicals) suc as flavonoids, followed by saponins, carbo ydrates, alkaloids, steroids, coumarins, quinines, fats and p enolic compounds, etc. [7]. P ytoc emical constituents are playing a

significant role in reducing t e metallic ions and later stabilizing t em [8].

Metal oxide nanoparticles (MO-NPs) ave been widely studied in recent years because t ey are ig valuable materials in various fields among t em drug and ealt -related industries [9]. Some of MO-NPs were utilized in t e t erapy and clinical practice as well as promoting wound ealing and antioxidant, nanoparticles of oxides suc as titanium oxide (TiO_2), silver oxide (AgO), zinc oxide (ZnO), magnesium oxide (MgO), cerium oxide (CeO_2), iron oxide (Fe_2O_3), cadmium oxide (CdO), nickel oxide (NiO) and zirconium oxide (ZrO) [10]. T ese MO-NPs are t e most promising candidates for biomedicine, wit a considerable amount of researc data available in recent literature regarding t eir biological *in vitro* and *in vivo* activity. Moreover, due to t eir reduced size, MO-NPs can interact on t e more in-dept level wit various cellular structures compared to t eir bulk counterparts, and, more importantly, t ey do not cause toxicity due to t eir ig ly improved biocompatibility [11]. Among metal oxide nanoparticles, ZnO-NPs w ic are a nontoxic, biocompatible biomaterial, wit unique abilities t at may vary depending on t eir size, s ape, orientation, morp ology and aspect ratio.

Biological synt esis of ZnO-NPs using plant extracts or microorganisms suc as bacteria and fungi as attracted t e great consideration from many researc ers because of t e advantages it as over t e c emical and p ysical met ods, Plant extracts ave been more advantageous over t e microbes and fungi because it does not require ig isolation cost, cultivation, and maintenance, additionally can be obtained an agreeable yield in a s ort time [12].

T e improvement effects of ZnO-NPs on oxidative stress were observed in rats, birds and fis w en t e toxic materials led to a

significant increase in malonalde yde (MDA) and decrease in superoxide dismutase (SOD) and catalase (CAT) activities, w ile concentrations of MDA was significantly reduced and activities of SOD and CAT significantly increased after treatment wit ZnO-NPs [13-15]. Zinc concentration was increased from t e dissociation of ZnO-NPs, w ere t e zinc is t e core constituent of antioxidant enzymes (as superoxide dismutase) and it is a protector for sul f ydryl groups and impair lipid peroxidation by displacing transition metals (as copper and iron) from catalytic sites [16]. Finally, ZnO-NPs are protected cell membranes against oxidative damage, decrease free radicals and lipid peroxidation levels, as well as increase t e antioxidant enzyme levels, and t en protect against kidney diseases [17].

1-2. Literature review

1-2-1 *Ziziphus-spina christi* Plant

Traditional plants use as a main source of popular medicines by many people in the world. So, in many developing countries, a large proportion of the population depends on traditional plants, and concern much research on medicinal plants in order to meet health care needs. Although modern medicine may exist side by side with such traditional practices, herbal medicines have often maintained their popularity for historical and cultural reasons [18]. The high concentrations of phytochemical contents in some plants draw attention to evaluate their possible health enhancing effects [19]. Due to artificial drugs are not always inexpensive or safe, many previous studies were performed to investigate new natural molecules that have vital activity, which is considered to be safe and without side effects [20], [21]. An example of such plants is *Ziziphus -spina christi* that belongs to the Rhamnaceous family (Figure 1-1). It is commonly in Arabic the tree called Sider and the fruit (Nabag) indicating the specific importance of this plant to local people [22]. *Ziziphus -spina christi* is consisting of about a hundred varieties of deciduous or evergreen trees and shrubs distributed throughout the tropical and subtropical regions of the world [23], from which twelve species are cultivated [24]. *Z.-spina christi* is a spiny shrub and sometimes a tall tree, reaching a height of 20 m that strongly resists heat and drought [25]. However, little research has been conducted on the rich nutritional content of its fruits and leaves, as well as on its well-known antimicrobial effects [26]. Moreover, *Z.-spina christi* is still growing along with several plants, and it is one of the important fruits that grows in the dry parts of tropical Asia and Africa [27].

Figure 1-1: *Ziziphus-spina christi* **tree.**

1-2-2 Constitutes of *Ziziphus -spina christi*

The c emical nature of plants is based upon t e p ytoc emical compounds. Plants wit a ig concentration of c emical constituents can be t erapeutically active or inactive. Active components may be found in eac part of t e plant (leaf, flower, stem, bark, root, fruit, and seed) [28]. P ytoc emicals are classified into primary and secondary metabolites. Primary metabolites suc amino acids, proteins, carbo ydrates and c lorop yll, w ile secondary metabolites are flavonoids, alkaloids, saponins, tannins, steroids, coumarins, quinines, fats and p enolic compounds (Figure 1-2) [29]. *Z.-spina christi* is ric in many biologically important compounds t at were investigated by t e p ytoc emical analysis w ic indicated t e presence of flavonoids, alkaloids, tannins, coumarins, carbo ydrates, and fats [30]. In addition, leaves also are ric in calcium, iron, and magnesium [31].

5

Flavonoides

Alkaloides

Phenolic Compounds

Coumarines

Tannins

Terpenoids
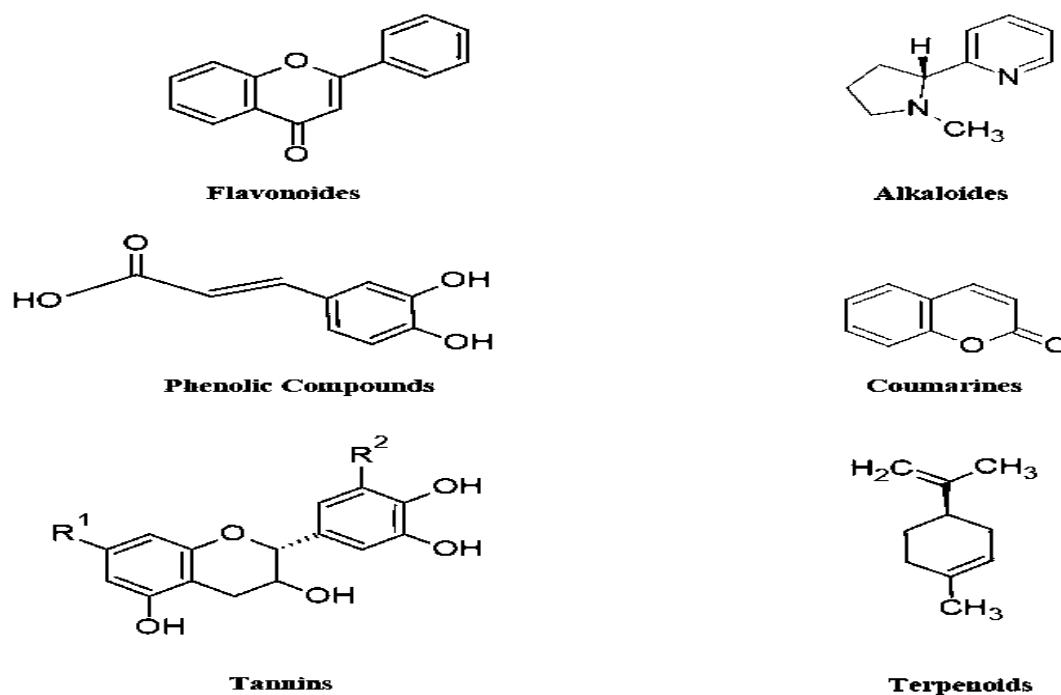
Figure 1-2 The basic chemical structure of the essential phytochemicals of *Z. spina-christi* leaves extract [32]**.**

1-2-3 Biological Applications of *Ziziphus -spina christi*

The majority of rural people have no knowledge of modern medicine and depend mostly on folk therapies, often produced by plants [33]. *Z. spina-christi* is known for its medicinal properties as a hypoglycemic, hypotensive, anti-inflammatory, antimicrobial, antioxidant, anti-tumor, kidney and liver protective agent, and as an immune system stimulant [34]. The contents of *Z. spina-christi* fruits are good diets and rich in diverse vitamins that are used in folk medicine to treat blisters, bruises, chest pains, dandruff, fractures, headaches, and mouth problems [35]. It has been described as anti-laxative, astringent, diuretic and tonic[36] while the seeds are used to relieve pain and cut off vomiting and abdominal pain associated with pregnancy [37]. The roots are used to recover and prevent skin diseases [38]. Some pharmacological analysis studies indicated that the aqueous extract of *Z. spina-christi* root bark has antinociceptive activity in mice and rats, as well as reduces the tension and anxiety in mice [39], [40].

Z. spina-christi extract has also been reported to possess a protective effect against aflatoxicosis and anti-conceptive properties in rats and have a calming effect on the central nervous system [41], [42]. Moreover, the extract of *Z. spina-christi* stem bark has antidiarrheal effects in rats [43]. Finally, fruits have cleaning properties such as purifying the stomach, removing impurities from blood as well as being restorative for the health system [44].

1-2-4 Effect of *Ziziphus spina- christi* on Kidney Diseases

Ziziphus-spina christi is one of the most widely used medicinal herbs in the world. Unlike pharmaceutical medicines, which have been created for a specific condition regardless of their side effects, the entire plant of *Z.-spina christi* not only has no side effects, but also the presence of some minerals and vitamins in the diet is very important, and the deficiency in their normal levels may be cause many diseases, among them kidney diseases [45]. *Z.-spina christi* leaves were regarded a high-value resource, and numerous studies have shown that the phytochemicals content is exceptional, including flavonoids and phenolic compounds which are crucial compounds for the improvement of renal failure due to their antioxidant and anti-inflammatory agents [46 - 48]. It has been demonstrated that significant decrease in the level of urea, creatinine, uric acid, and also free radicals. Because of the improved preservation of bio-active components of *Z.-spina christi* leaves, it can be considered as a more appropriate ingredient for food enrichment [49]. Moreover, an extract of *Z.-spina christi* could be used not only as a safe potential natural functional food ingredient or as a therapeutic drug in the treatment of kidney diseases but also it is effective in reducing both lipid peroxidation products and reactive oxidative stress accompanying renal failure [50], [51].

1-3 Nanoparticles

Nanotec nology deals wit t e design, production and c aracterization of nano-meter sized objects w ic is extended to broad area in medical, c emical and engineering applications due to its unique properties [52]. Nanoparticles (NPs) are basically defined as small objects ave t e size approximately of 1-100 nm to create and use structures t at ave novel properties [53]. T ey can also be designed to improve t e p armacological and t erapeutic effects of t e drugs, as well as ave a very ig surface area and t ey permit many functional groups to be ad ered to t em w ic in turn, can bind to tumor cells. Additionally, t ey ave proven to be an excellent replacement for radiation and c emot erapy as t ey can easily be used to remove t e tumor [54]. On ot er and, biomolecules derived NPs are getting famous because of t e growing demand of biocompatible and biodegradable NPs. Moreover, biological NPs are easily available and non-immunogenic. Apart from t eir own unique functions, biomolecules can conjugate wit ot er inorganic NPs to generate special biomolecule-NPs ybrids. Biomolecules suc as proteins, nucleic acids, lipids and polysacc arides-based NPs ave been used in various applications [55], [56].

Recent studies ave developed a number of nanoparticles suc as metals oxides, semiconductors and polymeric particles utilized in molecular imaging and particulate delivery ve icles [57], [58]. Liposomes, silica nanoparticles, micelles and c itosans play an important role in drug delivery wit minimized side effects. T ey ave also been utilized as anticancer agents. So, basically, nanotec nology deals wit construction of artificial cells, enzymes and genes or repair in t e synt esis of protein [59]. Furt ermore, t ese particles possess ig ly favorable optical and c emical properties for biomedical

imaging and t erapeutic applications, a ig density of t erapeutic agents can often be encapsulated, dispersed or dissolved in t ese nanoparticles, w ic in turn depends on t e preparation process to yield different properties and release c aracteristics of t e entrapped agent [60]. Finally, nanoparticles are good candidates to be s own as adjuvant for vaccines and advantageous features of nanoparticles include increased interaction of drug molecules wit epit elial cells can be ac ieved leading to maximal absorption of t e drug molecule [61].

1-3-1 Classification of Nanoparticles

In t e field of NPs synt esis and fabrication tec niques in t e past two decades ave ripened t e classification of nanosized particles. Any particle is classified as nanoparticle w en at least one of t e dimensions of particles are in t e order of nanometers. Generally, NPs are broadly classified into four different categories based on t eir dimensionality, regardless of t eir location or c emical properties [62].

- Zero-Dimensional (0D) NPs: All t ree dimensions of t e NPs are in t e nanoscale; i.e., up to 100 nm. quantum dots (carbon, grap ene, inorganic) and ot er sp erical NPs (noble metals, fullerenes, polymers, metal organic framework are some popular examples of 0D NPs class [63]. Due to t eir c emical inertness, biocompatibility, optical stability, cell permeability, and wavelengt -dependent p otoluminescence, t ey are interesting for biomedical and optical electronic applications [63].

- One-Dimensional (1D) NPs: (One dimension between 1 and 100 nm). In t is class, nanotubes, nanorods, nanowires, and nanofibers are made of polymer, carbon, metals, and metal oxides [64]. Due to t eir important surface-to-volume ratio and small pores, t ey are used for filtration and catalysis and as scaffolds and super-absorbents for wound dressing and tissue engineering [65].

- Two-dimensional (2D) NPs: (Two dimensions > 100 nm) include metal oxides, platelet-like forms, grap ene (grap ene oxide and re-reduced grap ene oxide), silicates, black p osp orus, exagonal boron nitride, boron nanos eets, etc. [66]. T eir p ysicoc emical, biological, and optical properties explain t eir uniform s ape, surface c arge, and ig surface-to-volume ratio [67].

- T ree-dimensional (3D) NPs: (No dimension in t e nanoscale range) include nanopores powders, nanowire bundles, nanotube bundles, nanolayers, and nanostructured electrodes. Muc researc as been done on t e development, fabrication, and evaluation of 3D NPs for storage devices (super capacitors and batteries) for wastewater treatment and electroc emical conversion [68]. T ese complex NPs are important components of biomedical devices, solar cells, and microelectromec anical systems [69].

1-3-2 Synthesis Approaches of Nanoparticles

T ere are many developments in science and engineering t at ave progressed very fast toward t e synt esis of nanoparticles to ac ieve unique properties t at are not t e same as t e properties of bulk materials. T e synt esized particle reveals interesting properties at t e dimension below 100 nm wit stable form, mostly from several effects, temperature, pH, pressure, time, cost of preparation and pore size [70]. T ese effects are t e quantization of electronic states apparent leading to very sensitive size-dependent effects suc as optical and magnetic properties and t e ig surface-to-volume ratio modifies t e t ermal, mec anical, and c emical properties of nanoparticles[71].

T ere are t ree general approac es for t e synt esis of nanoparticles in t e function of t e starting raw materials: top-down, bottom-up, and ybrid approac es [72].

- Top-Down Approac : In t is approac , nanoparticles are produced by breaking down t e bulk material into nanomaterials (Figure 1-3), as it bases on converting large pieces of metal by grinding and crus ing into nanoparticles, and t en addition of specific material led to stability of nanoparticles [73].
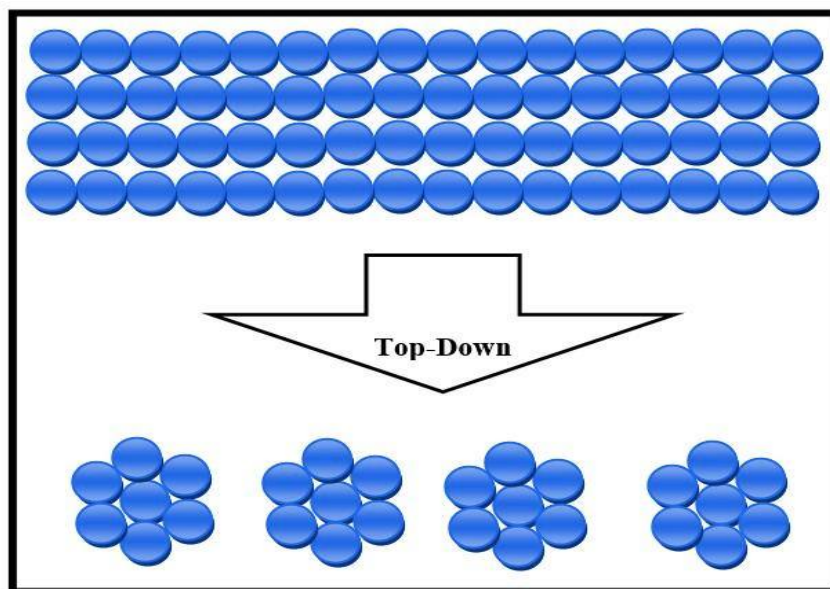
Figure 1-3: Top-down synthesis method of nanoparticles [74]**.**

- Bottom-Up Approac : In t is approac , a very small size material is converted into a larger size material (Figure 1-4), as it depends on t e atom-to-atom or molecule-to-molecule assembly process [75].
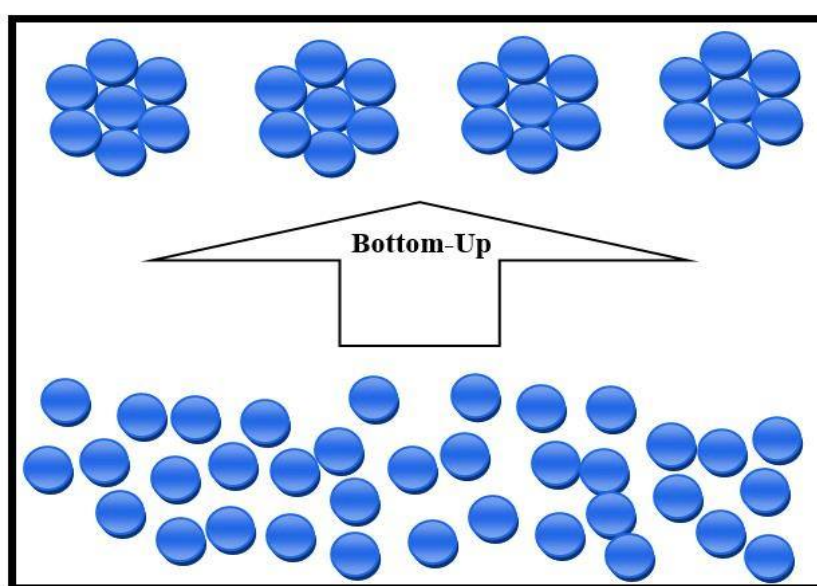
Figure 1-4: Bottom-up synthesis method of nanoparticles [74]**.**

- Hybrid Approac es: In t e ybrid approac , top-down and bottom-up fabrication tec niques are combined to develop nanostructured platforms. T ese ierarc ically organized structures are generally difficult or impossible to fabricate using only top-down or bottom-up tec niques [76].

1-3-3 Synthesis Methods of Nanoparticles

Nanoparticles are synt esized by variety met ods, w ic include p ysical, c emical and biological met ods., and t ese met ods can be broadly classified into bottom-up and top-down approac es (Figure 1-5). Depending upon t e requirements, t e synt esis met ods of nanoparticles are selected. Every met od as some advantages as well as disadvantages; t e production met od is selected based upon t e availability of t e facilities. P ysical met ods are suitable for small-scale production w ereas c emical met ods are selected w ere t e cost of production is a concern. Biological synt esis met ods ave different significance compared to p ysical and c emical met ods as t ey are non-toxic, gives rapid synt esis, low in waste production and also give large-scale production [77].

1-3-3-1 Physical Synthesis Methods:

P ysical met ods include arc plasma, t ermal evaporation, p ysical   vapor deposition, ultrasonic irradiation, and laser ablation. T ese processes are c emically pure and tec nically simple, w ic makes t em ideal for carrying out industrial processes at ig production rates [78]. For example, arc plasma w ic is based on electrical arc disc arge synt esis, is one of t e most commonly used p ysical met ods for converting bulk materials into nanomaterial via condensation and evaporation [79]. On t e ot er and, metal oxide (e.g., ZnO) nanoparticles can be synt esized using t e t ermal evaporation met od [80].

1-3-3-2 Chemical Synthesis Methods:

Hydrot ermal sol-gel, c emical vapor deposition, solvot ermal processes, polymerization, and ot er c emical precipitation tec niques are examples of c emical tec niques for t e synt esis of NPs [81]. Wet c emical synt esis, w ic is based on t e p ysical states of t e solid and liquid p ases, is t e most commonly used met od for producing NPs [82]. During industrial-scale wet c emical synt esis, capping agents/stabilizers are used extensively in spite of t eir toxicity to control particle size and to prevent t e agglomeration. T e parameters of t e ydrot ermal and solvot ermal tec niques used for t e synt esis affect t e structure, morp ology, composition، and assembly of t e resulting ZnO-NPs. Using t e ydrot ermal tec nique to t e synt esis of ZnO-NPs [83], and described t e solvot ermal tec nique to synt esis gallium-indium-ZnO-NPs for electrolyte-gated transistors [84].

1-3-3-3 Biological Synthesis Methods:

Biological met ods are promising alternatives to p ysical and c emical synt esis met ods because t ey are eco-friendly [85]. Biological synt esis met ods include microorganism-assisted, biotemplate-assisted, and plant extract-assisted biogenesis are green synt esis approac es for nanoparticle synt esis [86]. Many plant biomolecules, including enzymes, vitamins, polysacc arides, organic acids, amino acids, and proteins, are used for nanoparticle synt esis in a medium enric ed wit metal ions. T e first step involves t e preparation of leaf tinctures and t e use of biomolecules from plant extracts and various microorganisms, including fungi, bacteria, and lactobacilli. Many active compounds are present in plant extracts, for instance, alkaloids, p enols, terpenoids, quinines, amides, flavonoids, proteins, and alco ols [87]. Some of t em contribute to t e green

synt esis of metallic nanoparticles by reducing metal cations to nanoparticles (i.e., reducing agents, suc as flavonoids and p enols) and by concomitantly performing stabilization functions to avoid nanoparticle aggregation. Several previous studies based on biological met ods to t e synt esis metal oxide nanoparticles among t em, ZnO-NPs [88], [89].
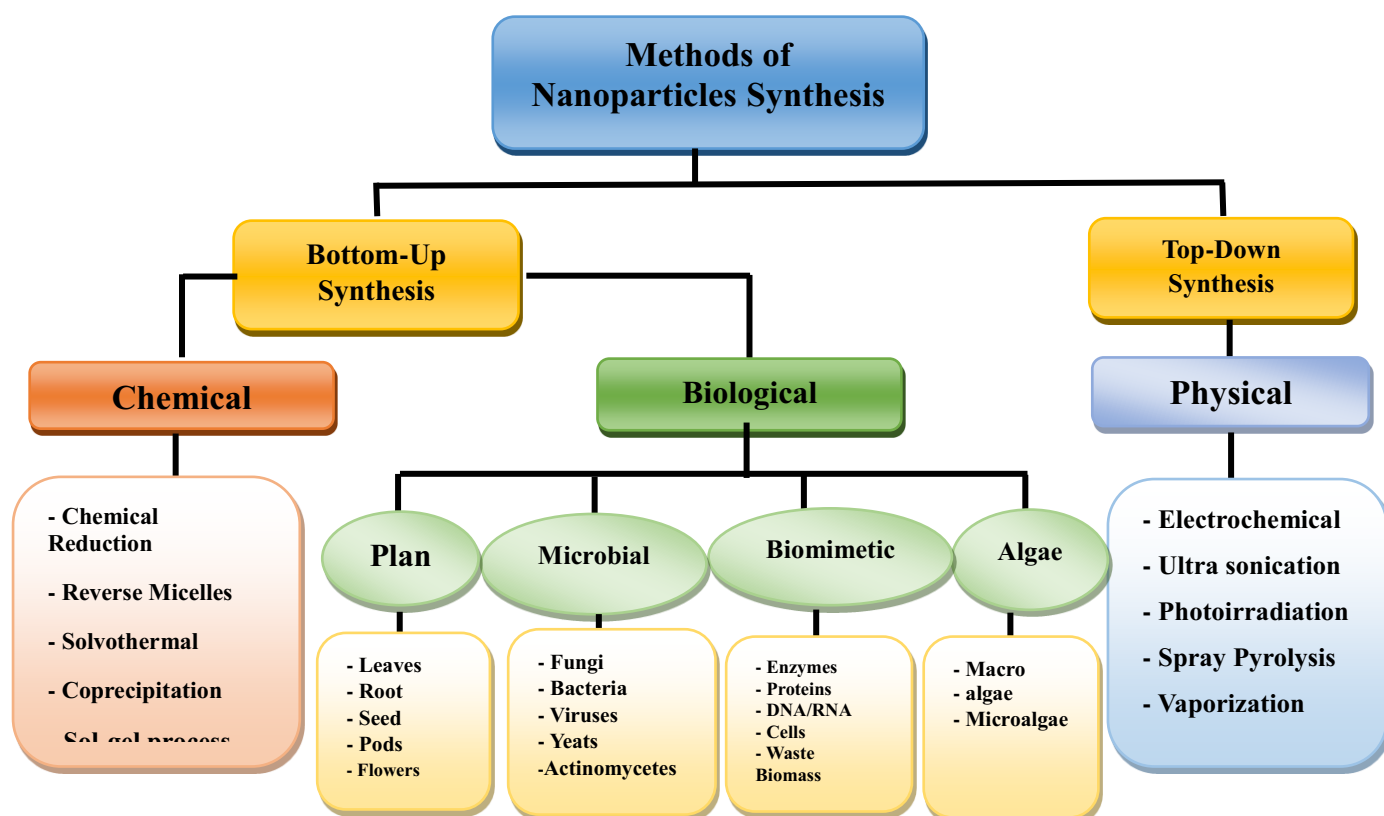
Figure 1-5: Scheme the synthesis methods of nanoparticles [90]**.**

1-3-4 Synthesis of Nanoparticles from Plants

Green synt esis of NPs using t e natural plant extracts was important to explored t e role of biological components, essential p ytoc emicals (e.g., flavonoids, alkaloids, terpenoids, amides, and alde ydes) as reducing agents and solvent systems [91]. Extraction is a solid–liquid separation process for isolating specific plant components in w ic t e plant components (i.e., t e solid object) are dissolved and entrapped in t e solvent (i.e., t e liquid). T e plant extract concentration, pH, temperature, and t e time of incubation are known

to affect t e size, s ape, and yield of t e NPs. Compared wit t e ot er previously described met ods, t e use of plant extracts for NPs synt esis is particularly interesting because it is less expensive, more environmentally friendly, can be scaled up for industrial applications, and can be performed wit out ig pressure, energy, temperature, or toxic c emicals [92]. Indeed, for NPs synt esis, plant extracts are simply mixed wit a metal salt solution at ambient temperature for a few minutes. Any plant part can be used to synt esize NPs, suc as leaves, stems, stalks, and flowers (Figure 1-6). Some of t e examples of synt esis of ZnO-NPs using plant extract include t e use of *Lepidium sativum* seed [93], *Cassia auriculata* leaf [94], *Amomum longiligulare* Fruits [95], *Phoenix dactylifera* root [96], etc. Leaf extracts are environmentally friendly, non- azardous, and non-toxic reducing agents. Researc s ould now focus on w et er plant biomass or extracts can be used to fabricate noble metal (silver, gold, platinum, and palladium) NPs wit a specific s ape and size. Indeed, due to t e great plant diversity, it is not known w et er t ey could be useful for t e production of suc NPs [97].
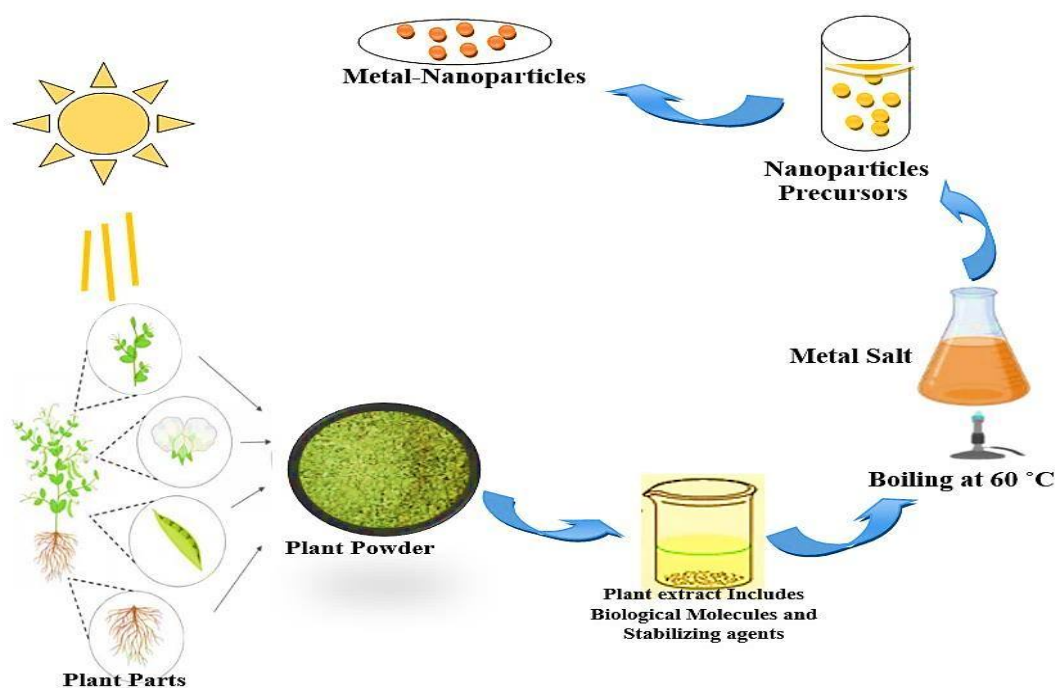
Figure 1-6: Biosynthesis of metal-nanoparticles from plant extracts [98]**.**

1-3-5 Characterization of Nanoparticles

The characterization is necessary to identify the properties of the synthesized NPs. The experimental methods that are used to quantify the dimensions, crystal orientation, elemental composition, and other physical and chemical properties of synthesized NPs are termed as characterization techniques [99]. Various *in vitro* and *in vivo* techniques are used to characterize a new system and predict its clinical efficacy. These techniques enable efficient comparison across NPs and facilitate a product optimization process [100]. Nowadays, many more types of NPs are synthesized than only a decade ago, and in higher amounts than before, requiring the development of more precise and credible protocols for their characterization. However, such characterization is sometimes incomplete. This is because of the inherent difficulties of NPs to be properly analyzed, compared to the bulk particles (e.g., too small size and low quantity in some cases following laboratory-scale production) [101]. In fact, quite often a wider characterization of NPs is necessary, requiring a comprehensive approach, by combining techniques in a complementary way. Different techniques may be used to identified NPs, in order to know if in some cases the use of only one or two of them is enough to provide reliable information when studying a specific parameter (e.g., particle size). Moreover, these techniques are sometimes exclusive for the study of a particular property, while in other cases they are combined [102]. So, to discuss all these techniques in a comparative way, considering factors such as their availability, cost, selectivity, precision, non-destructive nature, simplicity and affinity to certain compositions or materials. The techniques are always studied in depth according to their availability. There are techniques provide further information on the structure, elemental composition, optical properties, functional groups and other common and more specific physical properties of the nanoparticle samples (Figure 1-7). Examples of these techniques include Uv-vis.

spectroscopy, Fourier Transform Infrared (FT-IR) and x-ray Diffraction (XRD). Ot er tec niques w ic provide information on t e size, morp ology and crystal structure of t e NPs are microscopy-based tec niques for example, scanning electron microscopy (SEM), transmission electron microscopy (TEM) and atomic force microscopy (AFM) [103].
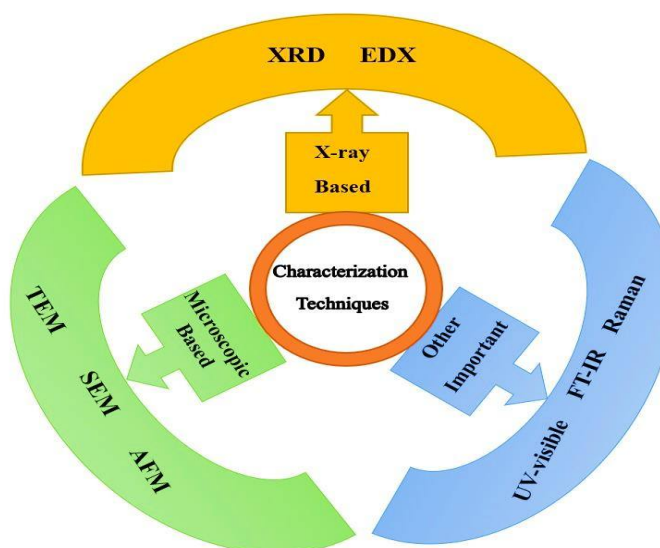
Figure 1-7: Techniques used in nanoparticles characterization [104].

1-4 Zinc Oxide Nanoparticle (ZnO-NPs)

Zinc oxide is an inorganic compound wit t e formula ZnO as a w ite powder, and is a metal oxide semiconductor. ZnO-NPs, as one of t e most important metal oxide nanoparticles ave been extensively studied and popularly employed in various fields due to t eir peculiar p ysical and c emical properties [105]. ZnO-NPs are nontoxic, insoluble in water wit density of 5.606 g.cm^{-1} and appear as a w ite powder wit refractive index of 2.0041. It also possesses a ig boiling and melting points w ic are 2360 ^{o}C and 1975 ^{o}C respectively [106]. ZnO-NPs as t ree main crystal structures under ambient condition (pressure and temperature), exagonal wurtzite, cubic zinc blende and cubic rock salt (Figure 1-8), T e exagonal wurtzite structure is t e most t ermodynamically stable and ence most common among t e

t ree structures [107]. T e exagonal wurtzite structure, w ere oxygen atoms (blue sp eres) are tetra edral coordinated to zinc atom (yellow sp eres). T e wurtzite structure as exagonal unit cell as s own in figure 1-8a.

(a) Hexagonal-Wurtzite   (b) Cubic Zinc Blende   (c) Cubic Rock Salt
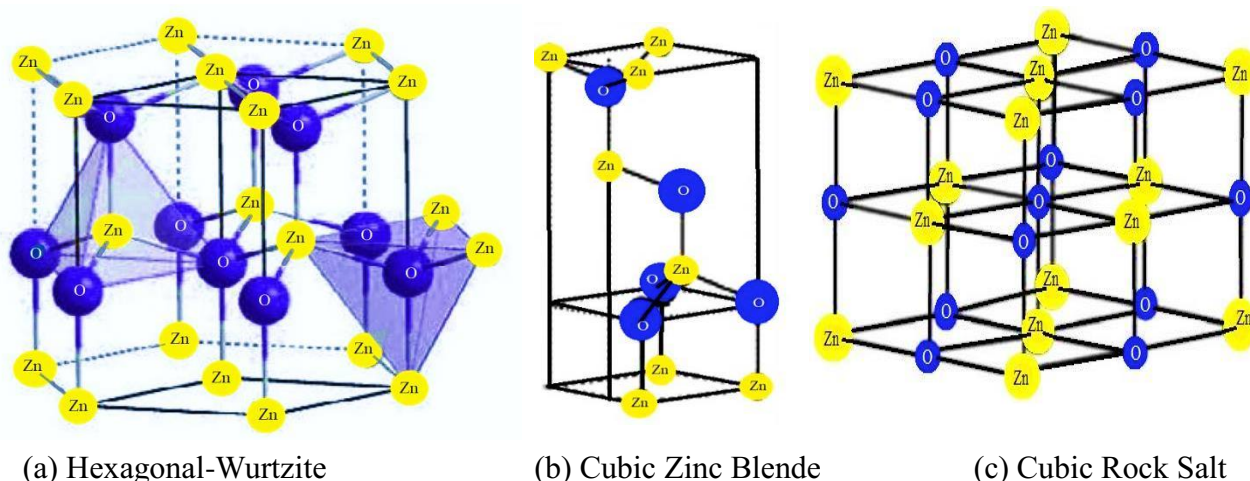
Figure 1-8: Main crystal structures of ZnO-NPs [108]**.**

In Hexagonal-Wurtzite, two interpenetrating exagonal-close-pack (cp) sublattices are alternatively stacks along t e c-axis. One sublattice consists of four Zn atoms and t e ot er sublattice consists of four oxygen atoms in one unit cell; every atom of one kind is surrounded by four atoms of t e ot er kind and forms a tetra edron structure [109].

1-4-1 Biosynthesis of Zinc Oxide Nanoparticles

P ysical and c emical met ods for ZnO-NPs preparations ave been widely developed. ZnO-NPs can be synt esized using aqueous or alco olic plant extracts, microorganisms (bacteria, fungi, aquatic algae, and yeasts) and ot er biosynt esis met ods [110]. A broad variety of plant extract are used for t e biosynt esis of ZnO-NPs [111-113]. ZnO-NPs prepared by biosynt esis met ods ex ibited strong potential for biomedical applications suc as its excellent anticancer and antibacterial activity. In general, for t e ZnO-NPs synt esis, t e desired quantity of zinc oxide, zinc nitrate, zinc acetate, or zinc sulfate is used

and dissolved in distilled water. To t e zinc solution, a defined volume of plant extract is added and mixed well in a magnetic stirrer. Plants consist of p ytoconstituents like flavonoids, amino acids, terpenoids, alkaloids, polyp enols, and polysacc arides w ic can act as reducing agents as well as capping or stabilizing agents. Also, t ese p ytoconstituents reduce metal ions or metal oxides into zero valence metal nanoparticles. Hence, no capping or stabilizing agents are needed in t is green approac to nanoparticle synt esis [114].

1-4-2 Optimization of ZnO-NPs Synthesis

Different factors like temperature, pH, time, and bot concentrations of metal ion and plant extract ave important roles in obtaining desired size, s ape, and stability, and en ancing t e product yield of t e nanoparticle [115]. Temperature plays a very crucial role in all reactions. it as recently been found t at temperature also plays a role in determining t e size, s ape, and yield of nanoparticles synt esized using plant extracts. Bot t e reaction and particle

owever, t e average particle size decreases, and t e particle conversion rate steadily rises as t e temperature rises [116]. T e best results for ZnO-NPs synt esis are obtained by varying t e pH of t e reaction medium wit in t e desired range [117]. T e synt esis of nanoparticles is greatly dependent on t e concentrations of substrate (metal salt) and biomass (plant extract) [118]. As example, w en plant extract and zinc salt are combined, t e color c anges from yellow to brown, indicating t e first appearance of ZnO-NPs. Zinc salt as a significant impact on t e size and structure of ZnO-NPs. As t e concentration of salt increases, t e surface plasmon bands s ift to a ig er frequency, indicating aggregation [119]. T e incubation time of t e reaction determines t e efficiency of ZnO-NPs [120]. Formation of

ZnO-NPs is confirmed wit UV spectroscopy based on its surface plasmon resonance (SPR) effect. Different morp ologies of ZnO-NPs are nanoflower, nanoflake, nanorods, nanowires, and nanobelt [121].

1-4-3 Biological Applications of Zinc Oxide Nanoparticles

T e biological activity of ZnO-NPs depends on many factors, surface c emistry, size distribution, particle morp ology, and particle reactivity in solution. T erefore, t e development of ZnO-NPs wit controlled structures t at are uniform in size, morp ology, and functionality is essential for various biomedical applications [122]. Moreover, ZnO-NPs are soluble in biological fluids and subsequently release zinc ions. On oral administration first pass effect, gastrointestinal barrier, kidney, liver, and gut wall function decreases t e bioavailability of ZnO-NPs; w ereas on intravenous administration s ows 100% bioavailability. Different factors like pH, particle size, concentration, and t e presence or absence of organic compounds influence t e solubility of ZnO-NPs [123]. Dissolution, absorption, and distribution of ZnO-NPs depend upon t e exposure dose amount and do not depend upon t e particle size of t e nanoparticle. On oral administration, ZnO-NPs are distributed in t e kidney, liver, and spleen w ereas on intraperitoneal administration, zinc oxide nanoparticles are distributed in t e lungs, kidney, spleen, eart, and liver. Kidneys and t e liver are t e common target organ in bot administrations [124]. Distribution of ZnO-NPs in t e organs depends upon t e type of experimental animals, route of exposure, and p ysicoc emical properties of t e nanoparticles [125].

1-4-4 Antioxidant Activity of Zinc Oxide Nanoparticles

Zinc oxide nanoparticles ex ibit antioxidant properties because of t e electron density transfer at oxygen and t e property rely on t e structural configuration of t e oxygen atoms [126]. T e naturally

obtained substance s ows t e ig protective activity of natural antioxidants from ig er plants against several diseases w ic originated from oxidative processes [127]. In t e biological system, over production of ig ly reactive radical species or t eir precursor. leads to oxidative stress w ic as been observed in various disease suc as cancer، cardiovascular disease, renal diseases, diabetes and art ritis. In few studies antioxidant and free radical scavenging activities of ZnO-NPs in biological system as been described. Many studies ave s own t at ZnO-NPs plays a significant role against various toxic effects, including cytotoxicity، genotoxicity, inflammation, and oxidative stress, also known to ave anti-inflammatory properties by blocking pro-inflammatory cytokines, proved t at ZnO-NPs synt esized from t e plant extract ave anti-oxidant and anti-inflammatory properties and t ey are used in several disease prevention and t erapies [128], [129].

1-5 Kidney and Liver Toxicity

The kidney filters all harmful substances and metabolites are eliminated through the urine. Endogenous and exogenous chemicals are secreted mostly by the kidneys [130]. Because it filters a huge number of toxins that can accumulate in the renal tubules when a vast volume of blood flows through it, the kidney is extremely vulnerable to toxicants. This can then lead to systemic toxicity, which can impair bodily functions such as maintaining the fluid and electrolyte balance, decreasing the production of essential hormones, and impairing the body's ability to retry wastes [131]. The liver is one of the largest organs in the human body and the primary focus of metabolic and excretory activity. It is responsible for the detoxification and excretion of a wide range of endogenous and exogenous substances and any damage to it or impairment of its functioning can have serious consequences for one's ealt [132].

The main factors causing renal-hepatic toxicity are toxic materials, exposure to hazardous or toxic materilas can affect the body in many ways. In general, when chemicals and other hazardous substances are absorbed, they pass through the various body systems and can affect a particular organ or organs, called t e "target organ" Fortunately, the body has mechanisms, mainly in the liver and kidneys, to process and eliminate many of these substances. This ability to eliminate toxic substances can reduce the effect on the target organ [133], [134]. Damage to the kidney and liver can be acute or chronic. An acute process generally refers to a relatively short period of time (hours to weeks) between exposure to the toxin and the onset of symptoms or medical findings. A chronic process generally refers to a long period of time (years) between exposure to the toxin and the onset of symptoms or medical findings. The detection of either an acute or chronic process or disease can be complex, depending on the type of toxin and the extent of exposure [135], [136].

1-6 Role of Zinc Oxide Nanoparticles in Ameliorating Renal-Hepatic Toxicity

Zinc oxide nanoparticles are among the most widely used metal oxide-NPs in a variety of sectors and research-based organizations due to their wide range of applications [137]. They can be used for a variety of medical applications because are less toxic, inexpensive, safe, and simple to prepare, as well as may be easily to absorbed in the human body [138]. In the previous study, the ameliorative potential of green tea-mediated ZnO-NPs was shown against ochratoxin-A-induced hepatotoxicity and nephrotoxicity in albino rats. Zinc oxide nanoparticles were found to reverse the damage caused by ochratoxin A and were also involved in reducing alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea, and creatinine levels, in addition to the biochemical analysis showed improvement in kidney

tissues, with slight congestion in a few areas [139]. Another study showed the protective effects of ZnO-NPs (50 mg/kg) against Dimethyl nitrosamine (DMN) induced liver injury in rats, ZnO-NPs were reduced lipid peroxidation, oxidative stress, and fibrosis of the liver, which indicates a reduction in the levels of proinflammatory cytokines. An increase in the levels of reduced glutathione (GSH) and glutathione peroxidase (GPx) was also observed, thereby improving liver and kidney function [140]. Similarly, ZnO-NPs reduced potassium bromate P-induced hepatotoxicity in Swiss albino rats. The overall health of the treated animals was improved profoundly by maintaining the levels of glutathione reductase (GR), glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT) and decreasing the levels of malondialdehyde (MDA), hydrogen peroxide (H_2O_2), and nitric oxide (NO) [141]. Thioacetamide (TAA) is a powerful hepatotoxic and hepato-carcinogenic chemical that causes hepatotoxicity by producing thioacetamide-S-dioxide, an unstable reactive metabolite that stimulates the production of reactive oxygen species (ROS) by binding covalently to macromolecules [142]. As ZnO-NPs possess various therapeutic properties, they were employed against TAA-induced hepatotoxicity to evaluate their protective effects. ZnO-NPs significantly lowered oxidative stress and reduced the expression of inflammatory markers (interleukin-6 (IL-6) and Tumor Necrosis Factor-alpha (TNF-α) proteins) and liver enzymes and also helped in returning the antioxidant status to its normal level [143]. Likewise, *Eclipta prostrata*-derived ZnO-NPs showed a dose-dependent cytotoxic effect against the hepatoma G2 (HepG2) cell line. DNA fragmentation assays and activation of caspase 3 validated the apoptotic features of ZnO-NPs at a concentration of 100 mg/mL [144]. Moreover, cisplatin (CP), a chemotherapy drug used to treat a variety of malignancies, can cause platinum to build up in the kidney,

impairing its function. CP-induced nephropathy raised serum creatinine, blood urea nitrogen, and microalbuminuria, all of which are indicators of renal function. These characteristics, on the other hand, were downregulated after ZnO-NPs treatment. ZnO-NPs prevented CP-induced decreases in renal SOD, CAT, and GSH, and an increase in renal MDA levels [145]. Similarly, streptozotocin (STZ) therapy led to diabetic nephropathy in male rats, which was demonstrated by an increase in the blood glucose level, renal oxidative stress markers, and glomerular basement membrane thickness. Administration of ZnO-NPs intraperitoneally for seven weeks significantly improved nephropathy and enhanced renal function [146]. Finally, ZnO-NPs are a great option for treating hepato-renal impairments because of their anti-inflammatory, antioxidant, and pro-oxidant mechanisms. As the impact of ZnO-NPs can vary depending on the disease condition, its cellular response, however, can be crucial. More research is still required to determine the precise molecular mechanism by which ZnO-NPs act to ameliorate adverse reactions to the organs upon administration of hazardous substances [147].

1-7 Adenine

Adenine (6-Aminopurine), is a nitrogen etero cycles (Figure 1-9) and one of t e four nucleobases in t e nucleic acids of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Adenine also plays an important role in bioc emistry involved in cellular respiration, t e form of bot adenosine trip osp ate (ATP) and t e cofactors nicotinamide adenine dinucleotide (NAD) and flavin adenine dinucleotide (FAD), and protein synt esis [148]. Adenine is derived from t e nucleotide inosine monop osp ate (IMP) t at finally metabolites to uric acid. It is produced on a pre-existing ribose p osp ate via a specific pat way by using substrates from amino acids (glycine, glutamine, and aspartic acid), and fusion wit t e enzyme

tetra ydrofolate [149]. It presents at very low level in t e blood and excess adenine is transformed to 2,8-di ydroxyadenine w en oxidized by xant ine de ydrogenase in t e kidney. Adenine produces adenosine, a nucleoside, by linking wit ribose, and t en forms ATP, a nucleotide, by adding t ree p osp ate groups to adenosine. adenosine trip osp ate is used in cell metabolism as one of t e most important met ods of transferring c emical energy between c emical reactions, and maintaining energy balance [150]. In addition, t e level of adenosine and t e expression of adenosine receptors are regulated so t at t e signaling via one or more of t e receptors increases in cellular stress and distress. Adenosine via its receptors will tend to limit t e consequences of t e potentially damaging stimuli. T is is ac ieved in multiple ways. T ere is a strong link between adenosine and signaling in ypoxia [151]. T us, t e increased adenosine in t e kidney indicated renal lesions due to isc emia or ypoxia, and decreased levels of adenosine in t e kidney may result from increased cell apoptosis, functional destruction, and ATP depletion [152].
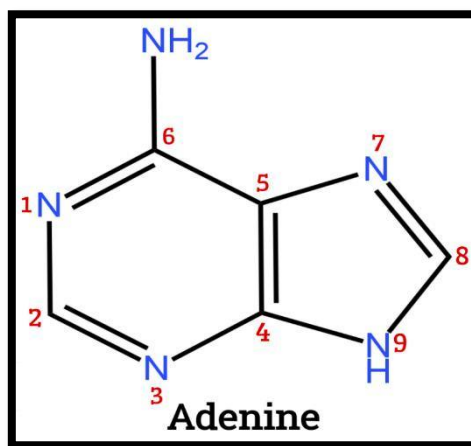
Figure 1-9: The chemical structure of adenine [153]**.**

1-8 Mechanism Action of Adenine

Several previous studies ave s own t at adenine -ric diet induced t e model of renal failure in animal [154]–[156]. T e

consumption of oral adenine t us mig t cause t e occlusion of renal tubules w ic retards t e excretion of nitrogenous substances leading to a bioc emical and p ysiological status resembling kidney diseases in umans [157]. W en adenine presents in ig levels at mammalian metabolism, it is endogenously produced as a by-product of t e polyamine pat way and is cleared by adenine p osp oribosyl transferase (APRT) [158], [159]. APRT deficiency causes 2,8-di ydroxyadenine (2,8-DHA) accumulation, leading to nep rolit iasis and crystalline nep ropat y (Figure 1-10). In t e absence of APRT activity, adenine cannot be converted to adenosine. Adenine is metabolized t roug an alternative pat way w ere it is oxidized by xant ine de ydrogenase (XDH) to 2,8-DHA via t e generation of an intermediate compound, 8- ydroxyadenine. Because 2,8-DHA is insoluble at any p ysiological urine pH, it forms 2,8-DHA crystals eventually leading to 2,8-DHA nep rolit iasis and/or crystalline nep ropat y [160]. Adenine and 2,8- DHA are released in t e urine and low solubility of 2,8-di ydroxyadenine led to it precipitation in t e kidney tubules of t e nep ron [161]. T e low solubility of 2,8- DHA makes its precipitation in kidney especially in nep ron tubules of kidney. Waste compounds excretion from kidney are stopped by obstruction of renal tubular due to 2,8- DHA. T is, in turn, will lead to elevation of creatinine and urea nitrogen concentration in t e blood and leading to elevation of various guanidine compounds, t en resulted in renal failure [162].
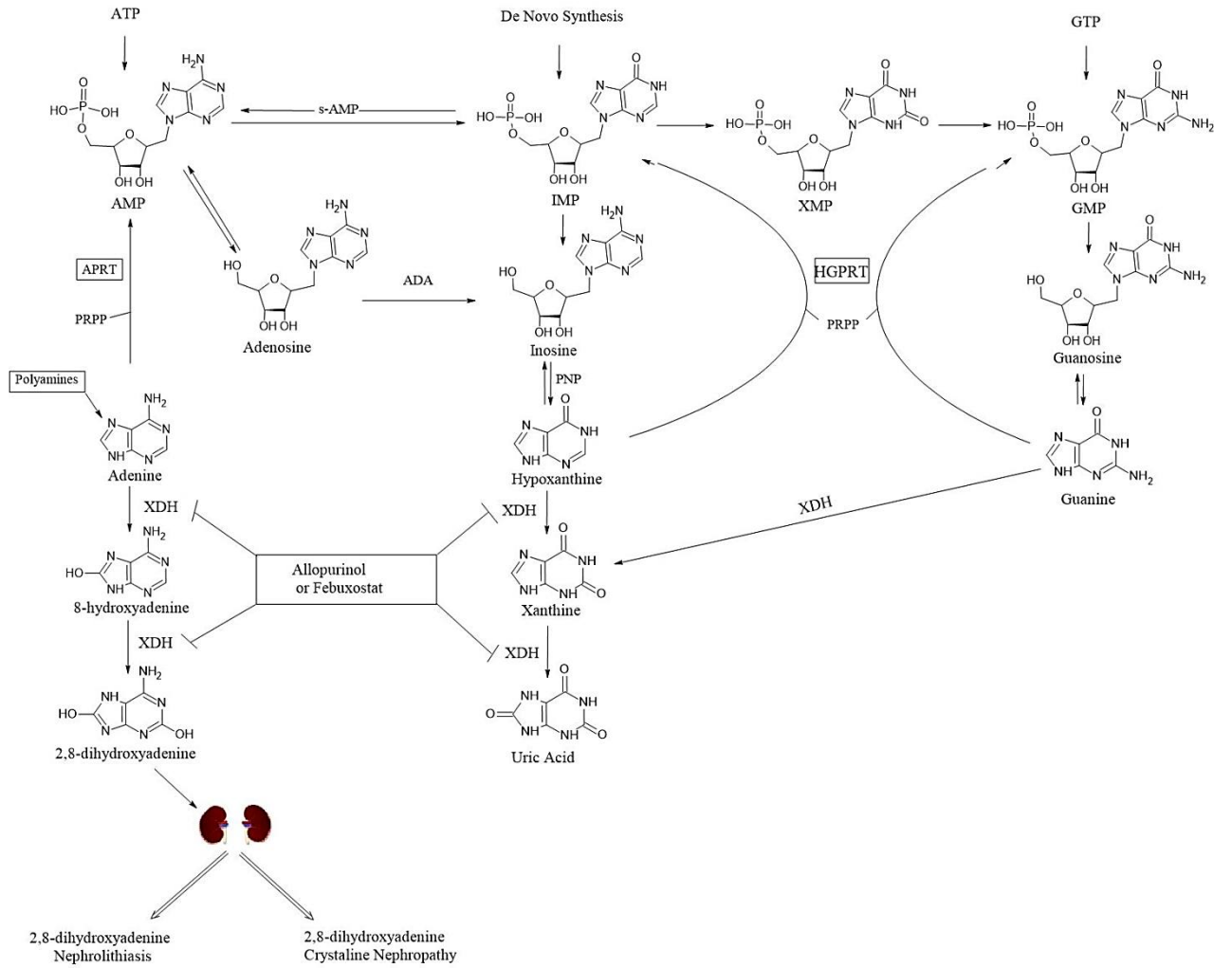
Figure 1-10: Scheme of metabolic pathways for the disposal of adenine in animals [163]**.**

1-9 The objectives of the study

T e current study was designed to possible biosynt esis of zinc oxide nanoparticles using an aqueous extract of *Z.-spina christi* leaves and zinc nitrate exa ydrate and evaluated t e ameliorative effects in t e treatment of kidney injury induced by adenine in male albino rats (*Sprague-Dawley* rats) t roug t e following objectives:

1- C aracterization and optimization of t e biosynt esized ZnO-NPs using many tec niques UV-visible, FT-IR, XRD, SEM, TEM and AFM.

2- Estimation of kidney function tests (uric acid, urea and creatinine) and liver function tests (ALT, AST and ALP) as well as antioxidant levels (TAC, CAT, SOD, GSH) and oxidative stress (MDA and NO).

3- Histological study w ic includes istological c ange in kidneys and livers tissues.

Chapter Two

Materials and Methods

2 Materials and Methods

- **Instruments**

Tools and instruments used wit t eir origin and company are listed in Table 2-1.

Table 2-1: Instruments and tools with their suppliers.

No.	Type of Instruments and tools	Company/Source
1	Analytical sensitive balance	METTLER TOLEDO/Switzerland
2	Atomic force microscopy (AFM)	AFM NT-MDT/Russia
3	Balance for animals	India
4	Centrifuge	C ina
5	Cooling centrifuge	Sigma 2-16PK/Germany
6	Digital camera	Nikon Z50/T ailand
7	Gel tube	Jordan
8	Eppendorf tube	C ina
9	W atman No. 1 filter paper	Labtex/Banglades
10	Freezer	Hitac i/Japan
11	Fourier Transform Infrared (FT-IR)	IRPrestige-21, SHIMADZU/Japan
12	Gel tubes	Jordan
13	Lab Glasses	C ina
14	Gloves	C ina
15	Hot plate wit a magnetic stirrer	LAB INCO L-81/ Net erlands
16	Lig t microscope	Olympus BH-2/ Japan
17	Micropipette 100-1000 µl	DragoLAB/C ina
18	Masks	C ina
19	Oven	D-91126 Sc wabac FRG/ Germany
20	Oven	TAU STERIL-2000/Germany
21	Rack for blood standing	C ina
22	Scanning electron microscopy (SEM)	TESCAN MIRA3-SEM/France
23	Spectrop otometer	T ermo-Spectronic/USA
24	Sterile syringes (1, 3, and 5 ml)	C ina
25	Test tubes	C ina
26	Transmission electron microscopy (TEM)	JEOL JEM-1200 EX/Japan
27	Vacuum filtration	
28	Water bat	Gallen Kamp/ England
29	UV-vis spectrop otometer	UV-1650PC, SHIMADZU/Japan
30	X-ray diffraction (XRD)	XRD-600, SHIMADZU/Japan

- **Chemicals and Kits**

C emicals and kits used in t e current study are listed in Table 2-2.

Table 2-2: Chemicals and kits used with their origin and company.

No.	Type of Chemicals and Kits	Company/Source
1	Acetic Acid (CH_3COOH)	HiMedia
2	Adenine Powder	Solarbio /C ina
3	An ydrous sodium carbonate (Na_2CO_3)	BDH C emical Ltd Pool/U. K.
4	Alkaline p osp atase colorimetric kit (ALP)	Solarbio /C ina
5	Alanine aminotransferase colorimetric kit (ALT)	Solarbio /C ina
6	Aspartate amino transferase colorimetric kit (AST)	Solarbio /C ina
7	Basic nitrate bismut	Basic nitrate bismut
8	Catalase Activity Assay Kit (CAT)	Solarbio /C ina
9	C loroform ($CHCl_3$)	Noorbrok/England
10	Creatinine colorimetric kit	Solarbio /C ina
11	Cupric sulp ate penta ydrate ($CuSO_4.5H_2O$)	Analar Trade Mark
12	Dimet yl sulfoxide (DMSO)	Xi`an S eer erb/C ina
13	Eosin- ematoxylin stain	Merck/Germany
14	Et anol (96% v/v)	Fluka /Switzerland
15	Ferric c loride ($FeCl_3$)	BDH C emical Ltd Pool/U. K.
16	Formalin 10%	TEBIA/USA
17	Hydroc loric acid (HCl)	BDH C emical Ltd Pool/U. K.
18	Litmus paper strips	Lo and/C ina
19	Malondialde yde content assay kit (MDA)	Solarbio /C ina
20	Nitric acid (HNO_3)	BDH C emical Ltd Pool/U. K.
21	Nitric oxide assay kit (NO)	Solarbio /C ina
22	Paraffin wax	Merck/Germany
23	Potassium hydroxide (KOH)	Analar Trade Mark
24	Potassium Iodide (KI)	Analar Trade Mark
25	Reduced Glutat ione content assay kit (GSH)	Solarbio /C ina
26	Sodium ydroxide (NaOH)	BDH C emical Ltd Pool/U. K.
27	Sulfuric acid (H_2SO_4)	Belgium
28	Superoxide Dismutase activity assay kit (SOD)	Solarbio /C ina
29	Total antioxidant capacity assay kit (TAC)	Solarbio /C ina
30	Urea colorimetric kit	Solarbio /C ina
31	Uric acid colorimetric kit	Solarbio /C ina
32	Zinc nitrate exa ydrate ($Zn(NO_3)_2.6H_2O$, 99.9%)	CDH/India

- **Methods**

2-1 Experimental Design of the study

T e general steps for t e biosynt esis of zinc oxide nanoparticles and t eir applications are s own in Figure 2-1.
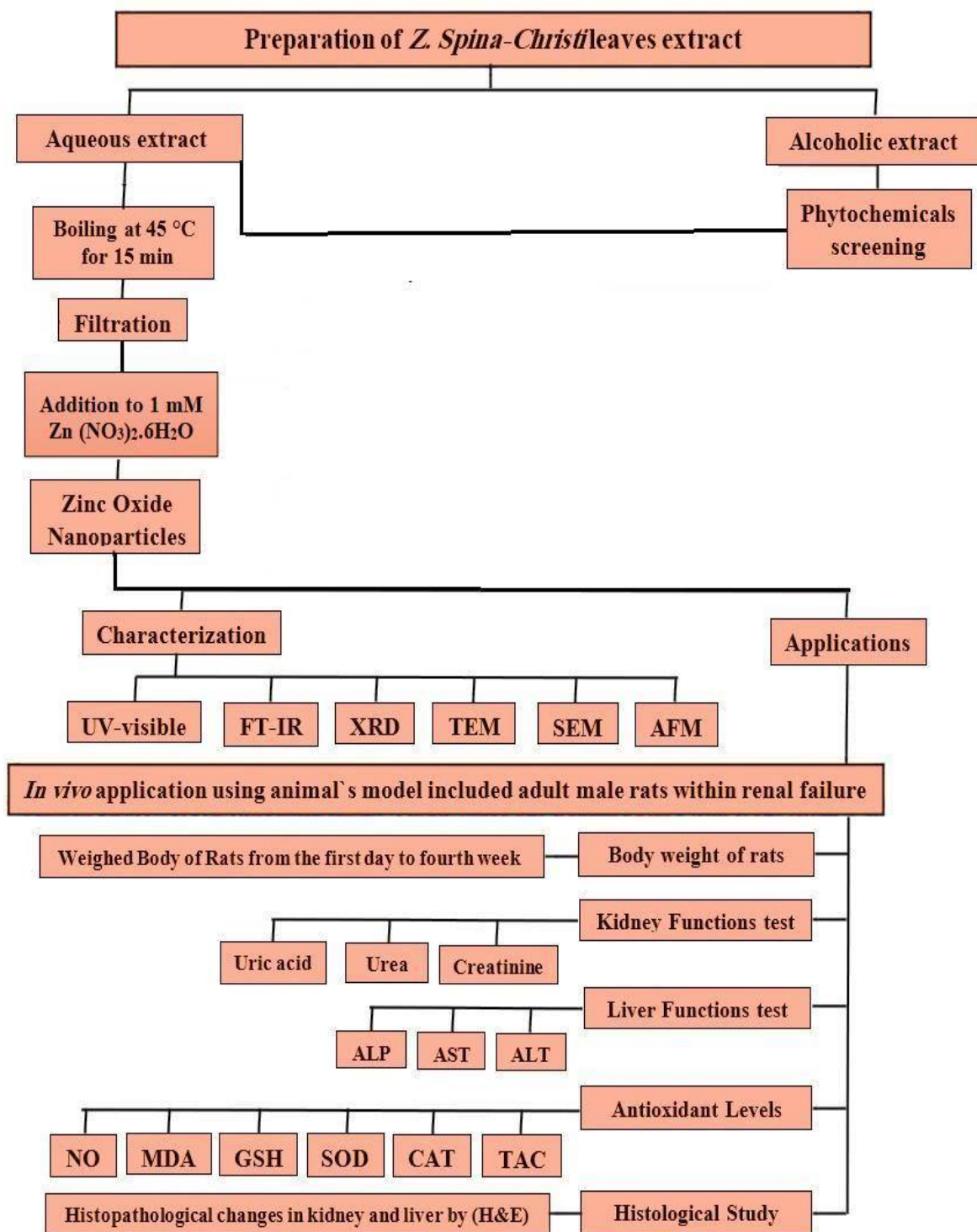
Figure 2-1: Schematic diagram for the experimental design of the study.

2-2 Collection of Plants

Fresh *Ziziphus-spina christi* leaves were collected from the University of Kufa-College of Medicine campus. The fresh leaves were thoroughly washed under tap water to remove the adhered and then rinsed with distilled and de-ionized water to remove dust and other particles. The washed plant part was dried by exposing it to the sun through a window to protect it from direct sunlight. Then the dried leaves were sliced into small pieces and crushed using a mortar and pestle.

2-3 Preparation of Plant Extract

- **Preparation of Aqueous Extract for *Ziziphus-spina christi* Leaves**

Ten grams of crushed leaves were dissolved in 100 mL de-ionized water in conical flasks and heated at 45 °C for 15 minutes using heating stirrer, and then cooled to room temperature. The mixture was filtered through vacuum filtration with Whatman No. 1 filter paper followed by muslin cloth and centrifuged at 3000 rpm for 15 minutes to remove any unnecessary particles that might have entered into the extract. To investigated the functional groups in *Z.-spina christi* leaves, the crude extract was dried in an air oven for 3 hours at 60 °C to obtain a powder for FT-IR analysis, while to investigated the phytochemicals are presented in *Z.-spina christi* leaves or to the biosynthesis of ZnO-NPs, freshly of *Z.-spina christi* leaves extract were prepared.

- **Preparation of Alcohol Extract for *Ziziphus-spina christi* Leaves**

For alco olic (et anolic) extraction, 10 gm from t e leaf pieces were transferred into t e conical flask and 100 mL of et anol (95% V/V) was added. T e mixture was adjusted at room temperature and kept for 10 ours. T e extract was subjected to vacuum filtration wit t e W atman filter paper No. 1. T e extraction was t en used wit out any furt er purification for investigations t e p ytoc emical screening only.

2-4 Phytochemicals screening in the plant extract

The phytochemical analysis of an extract of *Z.-spina christi* leaves showed the presence or absence of many phytochemical components such as (flavonoids, alkaloids, terpenoids, phenolic compounds, steroids, saponins, tannins, proteins, amino acids, quinines, cardiac glycosides, and carbohydrates) were prominently presented in the plant. The both crude aqueous and alcoholic extracts of *Z.-spina christi* leaves was submitted to phytochemical analysis.

- **Detection of Proteins and Amino acid**[164]

    1- Xanthoproteic test: An aqueous extract was treated wit a few drops of concentrated nitric acid. T e formation of t e yellow color indicates t e presence of proteins.

    2- Biuret test: A 2 mL aqueous extracts and an equal volume of NaOH were dissolved. A few drops of 2% Copper sulp ate were added. T e pink or violet color indicates t e presence of amino acids and proteins.

- **Detection of Alkaloids**[165]

    Dragendorff's test: T e spot of alco olic extract and spraying over it of Dragendorff's reagent will give orange color indicating t e alkaloids present. To Prepare of spraying reagent of Dragendorff's for alkaloids tests, was made of two portions:

    Reagent (1) A 0.85 g of bismut substrate was dissolved in a solution of 10 ml acetic acid (CH₃COOH) and 40 mL water.

    Reagent (2) A 8 g of potassium iodide (KI) was dissolved in 20 ml of water, a stock solution mixture of equal parts of solution 1 and 2. T e spray reagent was prepared by mixing 1mL of t e stock solution wit 2 mL of fres acetic acid and 10 ml of water. Detection of alkaloids and ot er nitrogen compounds is by orange-brown spots on w ite background.

- **Detection of Flavonoids**[166]

33

A 1 mL aqueous extract treated wit 1 ml 50% NaOH and 1 mL of 50% et yl alco ol was added. A dirty yellowis -brown precipitate indicates t e presence of flavonoids.

- **Detection of Saponins**[167]

Foam Test: A 0.2 gm of t e aqueous extract was vigorously s aken wit 5 mL of distilled water in a test tube for a few minutes. T e formation of foaming (appearance of creamy miss of small bubbles) s ows t e presence of saponins.

- **Detection of Phenolic Compounds**[165]

Ferric chloride test: A 1 mL aqueous extract was treated wit few drops of 2% Ferric c loride. C anging t e color to brownis indicates t e presence of p enolic compounds.

- **Detection of Carbohydrates**[168]

Benedict's test: one mL of aqueous extract and 4 mL of benedict's reagent were dissolved and t en eated in a boiling water bat for a few minutes orange red color precipitate indicates t e presence of carbo ydrates.

Preparation of Benedict's reagent: One liter of Benedict's reagent can be prepared by dissolving 100 gm of an ydrous sodium carbonate (Na_2CO_3) and 173 gm of sodium citrate in 800 mL ot distilled water. T e mixture was boiled and filtrated. A 17.3 gm of cupric sulp ate penta ydrate ($CuSO_4.5H_2O$) is mixed in 100 mL ot distilled water, t en added to t e filtrate, and t en t e volume was completed to one liter by distilled water wit constant stirring.

- **Detection of Terpenoids and Steroids**[169]

Salkowski test: Two mL of t e alco olic extract was dissolved in 2 mL of c loroform and evaporated to dryness. T en 2 mL of concentrated sulfuric acid (H_2SO_4) were added slowly and t e mixture was eated by put in t e water bat for 10 minutes. T e

presence of terpenoids was indicated by t e reddis -brown color, w ile t e steroids were indicated by t e green-blue color.

- **Detection of Coumarins**[169]

    Two ml of alco olic extract was added to 3 ml 10% NaOH in a test tube and t en boiled in a water bat for 5 minutes observation of yellow color fluorescence was observed wit positive results.

- **Detection of Tannins**[164]

    Sodium ydroxide (NaOH) test: A fres solution of 10% NaOH was prepared, and 1 ml was added to 1 ml of aqueous extract. Formation of emulsion indicates t e positive results of tannins.

- **Detection of Resins**[164]
    Turbidity test: Two ml of alco olic extract treated wit a few drops of 4% HCl, t e appearance of t e strong turbidity indicates resins present.

- **Detection of Quinines**[169]

    Alco olic KOH test: W en alco olic KOH was added to t e plant extract, t e red to blue color appeared reacting positively wit quinines.

- **Detection of Fats and Oils**[170]

    Paper test: A small amount of bot aqueous and alco olic extract was pressed between two filter papers. T e stain on filter papers indicates t e presence of t e oils.

2-5 Preparation of 1 mM $Zn(NO_3)_2.6H_2O$ Solution

    A one millimolar (1mM) of $Zn(NO_3)_2.6H_2O$ solution was prepared by dissolving 0.02975 gm from salt in 100 ml de-ionized water and stored in a dark and dry place for t e synt esis of zinc oxide nanoparticles.

2-6 Biosynthesis and Purification of Zinc Oxide Nanoparticles

Zinc oxide nanoparticles were prepared by adding 10 mL of t e crude aqueous extract of *Z.-spina christi* leaves to 90 mL of 1 mM $Zn(NO_3)_2.6H_2O$ at room temperature (Figure 2-2). Fres ly prepared 0.5 M of sodium ydroxide (NaOH) was added dropwise to t e solution w ile being gently stirred to adjust t e pH to 9. T e mixture was eated on t e magnetic stirrer for 2 ours at 60 °C until it transformed into a brownis colored pellet. After t at t e mixture was cooled and centrifuged at 5000 rpm for 15 minutes, to purify t e obtained pellet because it contains impurities on t e surface of particles, and t en was ed wit et anol followed by de-ionized water t ree times to eliminate t e water-soluble substances and any contaminants ot erwise organic materials present on t e surface of synt esized nanoparticles. T e pure pellet was desiccated in a warm air oven at t e temperature of 100°C for 90 minutes followed by t e calcination step at 300 °C for 1 our, t at is important to forming ZnO-NPs. T e calcinated ZnO-NPs were ready for c aracterization and furt er uses.
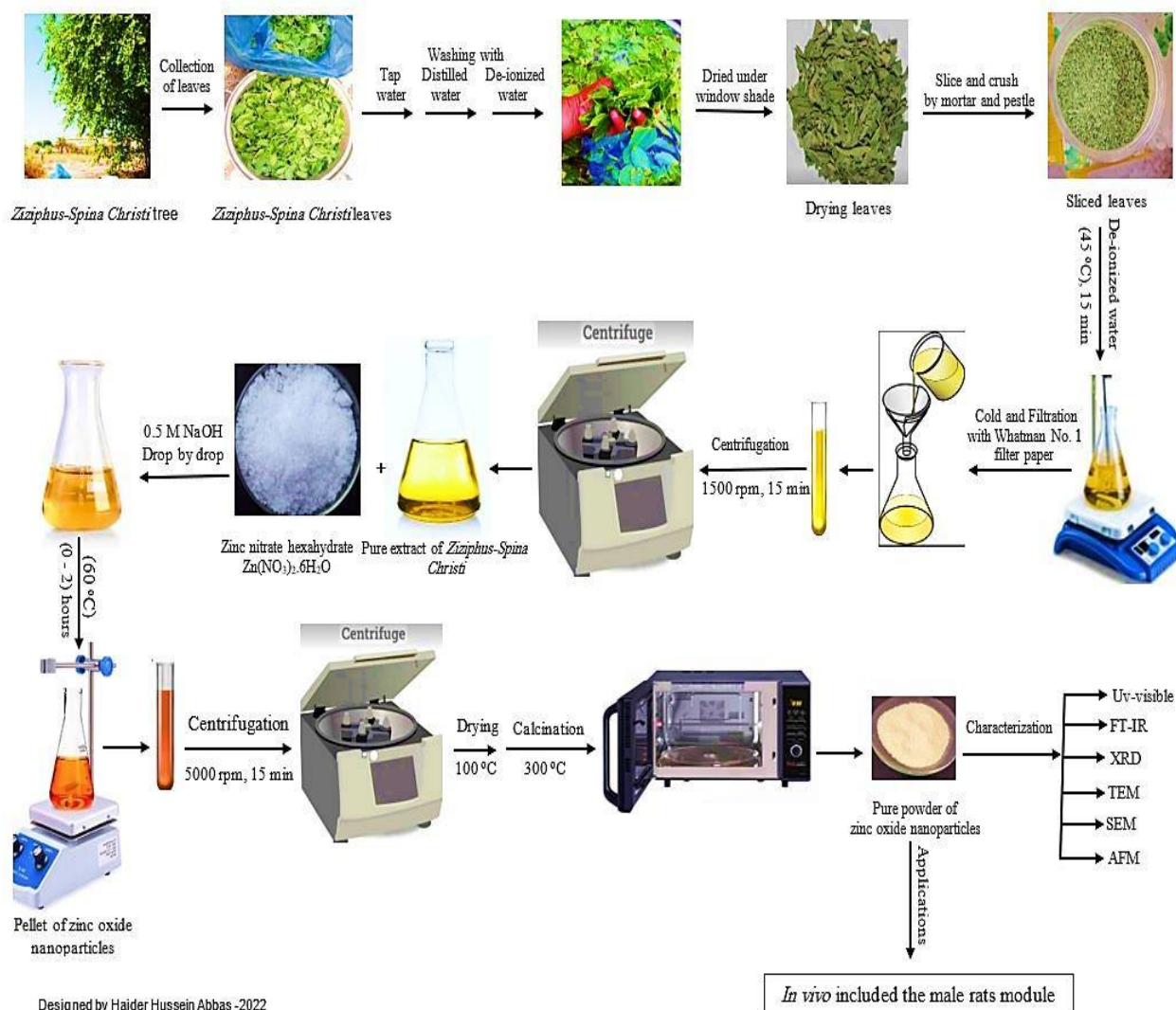
Figure 2-2: Scheme the biosynthesis of zinc oxide nanoparticles [171]

2-7 Optimization the Biosynthesis of ZnO-NPs

Synt esis of ZnO-NPs may be regulated by manipulating t e reaction parameters. As a result, optimizing reaction conditions in t e biosynt esis process is required to ac ieve t e control size and formation rate of ZnO-NPs. In t is study, t e optimization process was managed by controlling five experimental parameters t at include boiling time of plant extract, t e volume of plant extract, precursor zinc ion concentration, pH, and reaction temperature to production of ZnO-NPs wit stabile and good yield. Optimization of reaction conditions were carried out using UV- visible spectrop otometer.

2-7-1 Effect of the Reaction time

T e Reaction time is consider a crucial role in t e c emical reactions, in t is study was followed t e reaction of *Z.- spina christi* wit zinc nitrate exa ydrate at t e different times (0 minute to 48 ours) and follow t e c ange of color using Uv-visible spectroscopy.

2-7-2 Effect of the Boiling Time

T e effect of boiling time of t e plant leaves in preparation for t e aqueous extract of *Z.-spina christi* leaves was considered in t is study. Ten grams of dry leaves were boiled for (10, 15, and 20 minutes) wit 100 mL of de-ionized water. T en followed t e c ange of mixture color from green to yellow wit t e time and using Uv-visible spectroscopy.

2-7-3 Effect of the Plant Extract Volume

Biomass quantity plays a very important role in t e synt esis of ZnO-NPs. T e various volumes of *Z.-spina christi* leave extract (5 mL, 10 mL, and 15 mL) respectively were added to 1 mM of Zn $(NO_3)_2.6H_2O$ and follow t e c ange of color wit time and using Uv-visible spectroscopy.

2-7-4 Effect of $ZnNO_3.6\ H_2O$ Concentration

T e synt esis of nanoparticles is greatly dependent on t e substrate concentration. T e concentrations of ZnO-NPs (1mM, 2 mM, and 3m M) were studied wit t e constant ot er factors.

2-7-5 Effect of pH

T e pH of t e reaction mixture determines t e types of ZnO-NPs formed, t e different pH values (pH 5, 6, 7, 8, 9, and 10) were maintained using 0.5 M NaOH. T e boiling time, quantity of plant extract, salt concentration, and reaction temperature were kept constant.

2-7-6 Effect of temperature

Temperature plays a very important role in all reactions. Optimization studies about temperature were carried out wit temperatures ranging from 40˚C to 70˚C wit a difference of 10˚C.

2-8 Characterization of Zinc Oxide Nanoparticles

T e synt esized ZnO-NPs were c aracterized using different modern tec niques as follows:

2-8-1 UV-Visible Spectroscopy

T e optical properties of ZnO-NPs were evaluated using UV–vis spectrop otometer at t e central laboratory in Faculty of P armacy-University of Kufa. T e spectra were set in between t e wavelengt of 300–700 nm range. In t e spectrop otometer de-ionized water was set as an available reference.

2-8-2 Fourier Transform Infrared FT-IR Spectroscopy

FT-IR spectroscopy was used to determine t e various functional groups involved in *Z.-Spina Christi* leaves extract and synt esized ZnO-NPs. T ese were recorded and compared using FT-IR spectrop otometer under t e spectral range of (4000–400) cm^{-1} at t e central laboratory in Faculty of P armacy-University of Kufa. Identification of functional groups present in t e sample was recorded at room temperature, and t e dried extract and synt esized ZnO-NPs were carried out by t e KBr pellet met od. T e presence of t e various vibrational modes in aqueous extract and synt esized ZnO-NPs were investigated.

2-8-3 X-Ray Diffraction (XRD)

To determine t e average crystalline size of biosynt esized ZnO-NPs and identification its crystalline features were c aracterized by XRD tec nique at Ministry of Sciences and Tec nology-Bag dad-Iraq wit Cu Kα radiation (Voltage = 40 kV, Current =30 mA, λ=1.5406 Å, scan rate of 5.0^{o} min^{-1} and scan range of 2θ from 20 – 80°). From t e XRD data obtained; t e crystalline size of t e synt esized ZnO-NPs was calculated according to Debye–Sc errer's equation [172].

$$D = \frac{k\lambda}{\beta \, Cos \, \theta}$$ ---------- eq. (1)

Were, D is t e average crystalline size, λ is t e wavelengt of X-ray (1.5406 Å), Θ is t e Bragg's diffraction angle, β is t e XRD peak full widt at alf maximum (FWHM) of t e peak in radians, and k is t e s ape factor or Sc erer`s constant (0.98).

2-8-4 Transmission Electron Microscopy (TEM)

Transmission electron microscopy (TEM) is a microscopy tec nique w ereby a beam of electrons is transmitted t roug an ultra-t in sample and interacting wit it to form an image involved information about t e structure, crystallization, morp ology, and stress of a substance. TEM images for synt esized ZnO-NPs were investigated using t e TEM tec nique at University of Kas an-Iran. For TEM c aracterization, operating at 100 Kv, t e ultrasound bat was used to diffuse a small amount of ZnO NPs into et anol. T e size and morp ology of ZnO-NPs were investigated by suspending 0.01 g of t e ZnO-NPs in 100 mL 95% et anol and sonication t e suspension for 20 minutes.

2-8-5 Scanning Electron Microscopy (SEM)

T e surface morp ology of green synt esized ZnO-NPs was performed by using a SEM tec nique at University of Kas an-Iran. ZnO-NPs were added to SEM slides to make a t in layer. T en t e slide was set for SEM analysis after coating it wit carbon copper grids. After t at ZnO-NPs were observed under SEM at an increasing voltage of 20 KV, and t e images were recorded.

2-8-6 Atomic Force Microscopy (AFM)

Atomic force microscopy offers ultra- ig resolution in particle size measurement and is based on a p ysical scanning of samples at t e sub-micron level using a probe tip of atomic scale. T e instrument provides a topograp ical map of t e sample based on forces between t e tip and t e sample surface. Samples are usually scanned in contact

or noncontact mode depending on t eir properties. In contact mode, t e topograp ical map is generated by tapping t e probe onto t e surface across t e sample, and t e probe overs over t e conducting surface in non-contact mode. Moreover, particle size obtained by t e AFM tec nique provides a real picture w ic elps understand t e effect of various biological conditions. T e surface topology study of t e synt esized ZnO-NPs by AFM analysis was at University of Kas an-Iran. A t in film of ZnO-NPs was deposited on a silica glass slide by dropping a few drops of t e ZnO-NPs solution on t e slide and t en allowed to dry at 30 °C overnig t. T e deposited film on a silica glass plate was scanned wit AFM Model Ntegra Prima AFM (NT-MDT, Russia) for t e determination of t e nanoparticle's size.

2-9 *In Vivo* Applications of Zinc Oxide Nanoparticles

2-9-1 Animals of the Study

T e experiment on animals was carried out at t e laboratory animal facilities at t e College of Sciences-University of Kufa. T irty-six ealt y male rats (*Sprague Dawley rat*s) were unsealing in t is study weig ing from 200 to 205 gm obtained from t e animal ouse in t e College of Sciences-University of Kufa. Rats were kept in t e animal ouses for acclimation to t e laboratory condition for two weeks before using t em. T e study was performed during t e period from May to August 2022. T ese animals were kept wit standard environment situations temperature (25-27 C°) and relative umidity (50٪-60٪) conditions. T e animals were divided and oused in plastic cages wit diameters of 50×35×15 cm [173].

2-9-2 Preparation of Adenine

Adenine ($C_5H_5N_5$) wit ig purity grade, CAS NO.: 73-24-5 was obtained from Solarbio Company (C ina). A 10 gm of adenine was

dissolved in 10 mL DMSO and completed to 100 mL wit de-ionized water.

2-9-3 The Experimental Design

 T irty-six male rats were used in t is study and divided into six equal groups; eac group consisted of 6 male rats as follows:

1-First group (G-I): Rats were received 0.5 mL DMSO one time daily for 30 days and served as control group.

2- Second group (G-II): Rats in t is group were gavaged 0.5 mL adenine (100 mg/kg. BW) dissolved by DMSO one time daily for 30 days. T e dose of adenine was c osen from t e previous study by [174], based on t e original met od by [175].

3- T ird group (G-III): Rats were received 0.5 mL of *Z.-spina christi* leaves extract (10 mg/kg.BW) one time daily for 30 days. T e dose of *Z.-spina christi* leaves extract according to previous study [176].

4- Fourt group (G-IV): Rats were received 0.5 mL of ZnO-NPs (10 mg/kg.bw) one time daily for 30 days [177].

5- Fift group (G-V): Rats in t is group were co-administrated 0.25 mL adenine (100 mg/kg.BW) and 0.25 mL *Z.-Spina Christi* leaves extract (10 mg/kg.BW at t e same time for 30 days [178].

6- Sixt group (G-VI): rats in t is group were co-administrated 0.25 mL adenine (100 mg/kg.BW) and 0.25 mL of ZnO-NPs (10 mg/kg.BW) at t e same time for 30 days, t is was c osen in based on t e pervious experiment by [179].

* T e rats were weig ed and recorded t e weig ts before t e beginning of t e treatment and weekly during t e treatment period.

2-9-4 Collection of Blood and Tissues

2-9-4-1 Blood Specimens

 After 30 days of experiment and On t e 31^{t} day, fasted rats were anest etized by placing t em in a closed jar containing cotton rinsed wit c loroform to be sedated for t e next step w ic is blood drawn

via cardiac puncture in sterile syringes by needle prick in t e eart draining (2-3 ml) of blood carefully, t en blood placed in a test tube containing gel w ic leaves for 30 minutes in room temperature and t en used for getting serum by centrifuge at 3000 rpm for 15 minutes and put it in Eppendorf tubes w ic kept at freezer (-20°C) till t e time of bioc emical analysis.

2-9-4-2 Tissue Sampling

After blood collection and eart puncture, rats were sacrificed to isolate kidneys and liver by order. T e kidneys and livers of eac animal were quickly removed and rapidly weig ed t en prepared for istological study according to Mesc er's met od [180].

2-10 Biochemical Analysis

2-10-1 Estimation of Kidney Function Tests

Kidney function tests of serums (uric acid, urea, and creatinine) concentrations were spectrop otometrically determined according to special kits.

2-10-1-1 Estimation of Uric Acid

- **Principle**

Uricase can catalyze t e decomposition of uric acid into allantoin, CO_2, and H_2O_2. T en Fe^{2+} in potassium ferrocyanide is oxidized by H_2O_2 to form Fe^{3+}. Fe^{3+} can furt er react wit 4-aminoantipyrine and 3,5-dic loro- ydroxybenzene-1-sulfonic acid (DHBS) in t e presence of peroxidase to form quinonimine derivative, a red-colored complex t at as a c aracteristic absorption peak at 505 nm.
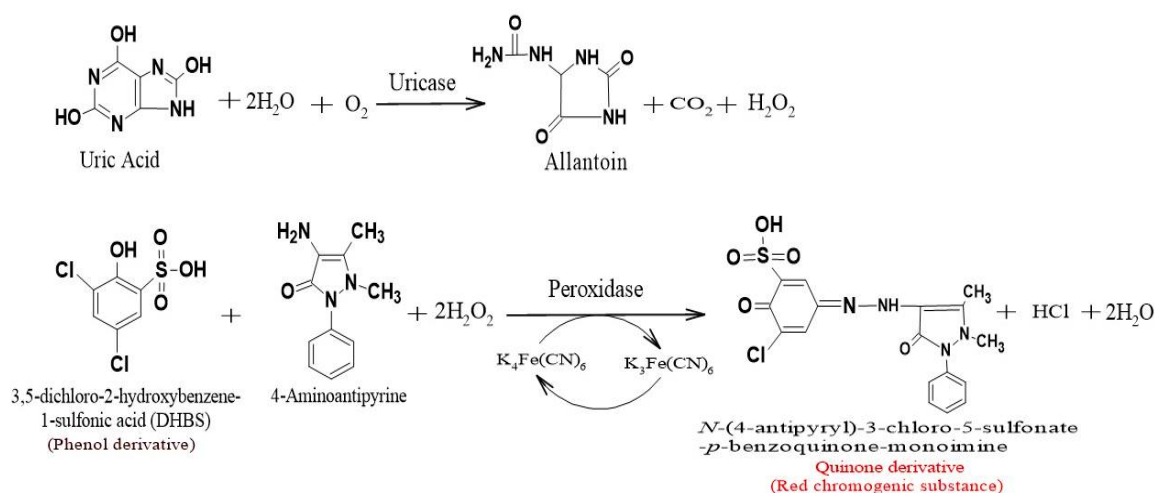
Figure 2-3: The chemical reaction to determination of uric acid

- **Reagents**

Reagent I: Uric acid standard   10 mg/dL.
Reagent II (Buffer): Phosphate buffer and 3,5-DHBS.
Reagent III: (Enzymes): Uricase, peroxidase, potassium hexacyanoferrate (II) and 4-aminoantipyrine.

- **Preparation of Standard Solution**

1-T e spectrop otometer was run for 30 minutes, t en t e wavelengt was adjusted at 505 nm, and set t e counter to zero wit de-ionized water.

2- Preparation of standard curve by dilution of 1 mg/dL of standard solution wit de-ionized water to 20, 40, 60, 80, and 100 mg/dL for standby.

Table 2-3: Preparation of standard solutions to determination of uric acid

Number	Standard Concentration (mg/dL)	Uric Acid Standard (µL)	De-ionized Water (µL)
1	0	0	1000
2	20	20	980
3	40	40	960
4	60	60	940
5	80	80	920
6	100	100	900

3- T e concentration of eac standard solution been at t e x-axis, and t e corresponding ΔA standard at t e y-axis. T en t e linear regression equation y＝kx+b is obtained. ΔA were broug t into t e equation to get x (mg/dL).
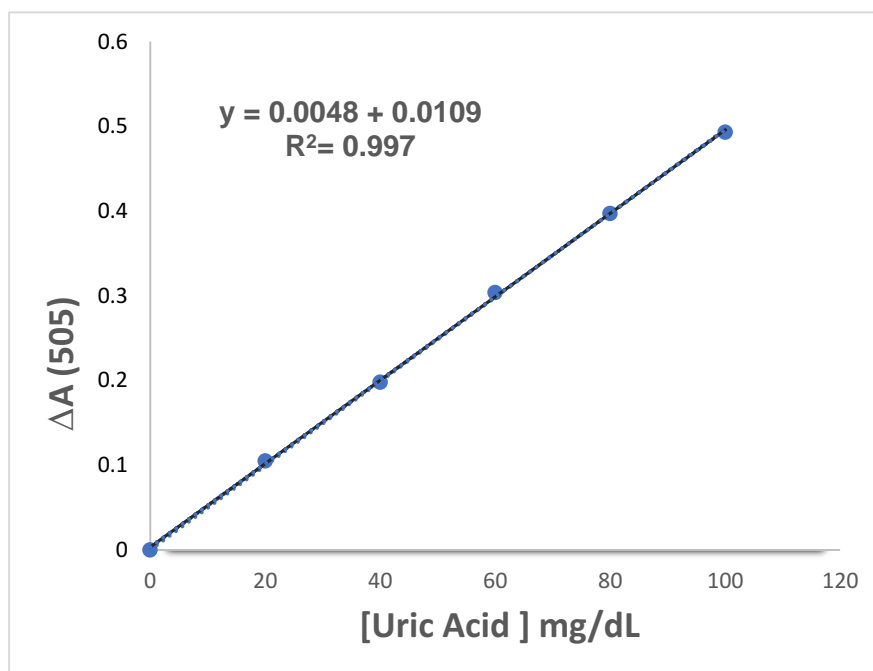
Figure 2-4: Standard curve of uric acid

- **Procedure**

In 5 ml centrifuge tube, were mixed reagents as following:

Table 2-4: Mixing of reagents to determination of uric acid

Reagent name (mL)	Control Tube ($A_{Control}$)	Specimen tube ($A_{Specimen}$)	Standard tube ($A_{Standard}$)	Blank tube (A_{Blank})
Specimen (mL)	0.25	0.25	-	
Standard solution (mL)	-	-	0.25	-
De-ionized water	-	-	-	0.25
Working solution A		0.75	0.75	0.75
Working solution B	0.75	-	-	-
T e mixture was mixed completely and placed in a water bat at 25°C for 30 min.				

To measure t e absorption value (A) at 505 nm used 1mL glass cuvette. T e absorption was measured as $A_{Control}$, $A_{Specimen}$, $A_{Standard}$, and A_{Blank}. $\Delta A_{Control} = A_{Specimen} - A_{Control}$. $\Delta A_{Blank} = A_{Specimen} - A_{Blank}$. Eac test tube needs to set up a contrast tube and t e standard curve only needs to test once or twice.

- **Calculation:**

$$\text{Uric acid concentration} \left(\frac{mg}{dL}\right) = \frac{x \times VS}{VS \times M.wt} = 168x$$

W ere, k= T e slope of t e standard curve, b= T e intercept of standard curve, VS: Sample volume=0.25 mL, VE: Extract solution volume=1 mL, M: Molecular weig t of uric acid=168 g/mol.

2-10-1-2 Estimation of Urea

- **Principle**

T e colorimetric estimation met od of urea based on t e specific action of urease w ic ydrolyses urea to form ammonium and carbon dioxide. In t e presence of sodium nitroprusside, t e ammonium ions (NH_4^+) combine

wit alkaline ypoc lorite and sodium salicylate to form a blue-green 2,2-dicarboxy indop enol.
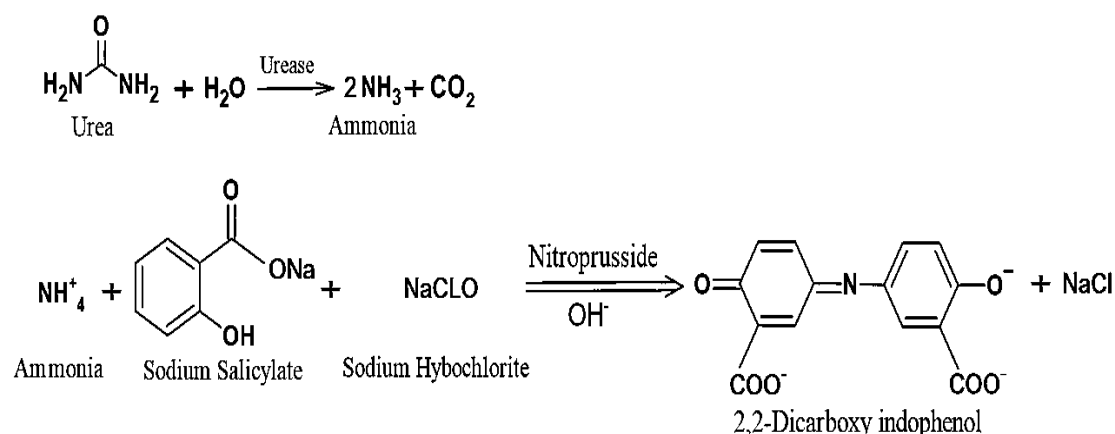
Figure 2-5: The chemical reaction to determination of urea

The intensity of the color is directly proportional to urea concentration in the specimen, which is measured at 600 nm.

- **Reagents**

Reagent I: Urea standard, 50 mg/dL (8.3 mmol/L)

Reagent II (Enzyme reagent): Urease > 500 U/mL Stabilizer.

Reagent III (Buffered chromogen): Phosphate buffer, sodium salicylate and sodium nitroprusside.

Reagent IV (Alkaline hypochlorite): Sodium hypochlorite and sodium hydroxide.

- **Procedure**

1-T e spectrop otometer was run for 30 minutes, t en t e wavelengt was adjusted at 600 nm, and set t e counter to zero wit de-ionized water.

2-Standard solution: Urea standard solution was diluted (1 mg/dL) to 0.25 mg/dL wit de-ionized water.

3- Reagents were added to t e following list:

Table 2-5: Addition of reagents to determination of urea

Reagent name (μL)	Blank tube (A_{Blank})	Standard tube ($A_{Standard}$)	Specimen tube ($A_{Specimen}$)	Control tube ($A_{control}$)
Reagent III	500	500	500	500
De-ionized water	200	-----	-----	-----
T e mixture was s acked well, t en placed at 25°C in water bat for 10 min.				
Reagent II	Drop (100)	Drop (100)	Drop (100)	Drop (100)
Mixed t oroug ly, t en added				
Standard solution	-------	200	------	------
Specimen	------	-------	200	200
	T e mixture was s acked well, t en placed at 25°C in water bat for 10 min.			
Reagent IV	200	200	200	200
T e mixture mixed well, incubated at room temperature for 10 min, t en measured t e absorbance at 600 nm as, $\Delta A_{Standard} = (A_{Standard} - A_{Blank})$, $\Delta A_{Specimen} = (A_{Specimen} - A_{control})$.				

- **Calculation**

$$\text{Urea concentration} \left(\frac{mg}{dL}\right) = \frac{\Delta A_{Specimen}}{\Delta A_{Standard} \times Cs} = \frac{0.25 \times \Delta A_{Specimen}}{\Delta A_{Standard}}$$

W ere, Cs: concentration of standard solution= 0.25 mg/dL.

2-10-1-3 Estimation of Creatinine

- **Principle**

T e principal assay is depended on t e reaction of creatinine wit picric acid in an alkaline solution to form yellow – a red complex as following:

Creatinine   Picric acid   Creatinine-picric acid complex
                                        (Yellow-red chromogenic substance)
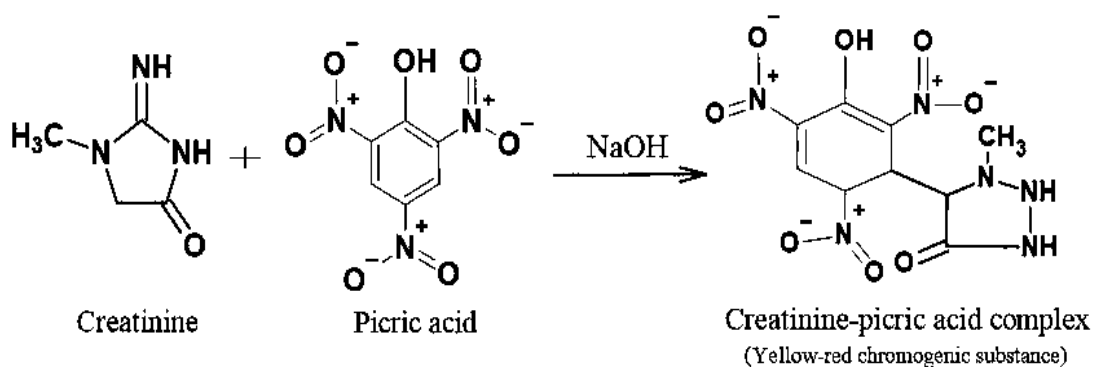
Figure 2-6: The chemical reaction to determination of urea concentration

The intensity of the produced color is proportionate with the creatinine solution in the serum. Creatinine was determined by measuring the absorbance at 520 nm.

- **Reagents**

Reagent I: Creatinine standard.
Reagent II: Picric acid.
Reagent III: Sodium hydroxide.
Additional reagent was required but not provided: Trichloroacetic acid (TCA).

- **Procedure**
Serum preparation

A 0.5 mL of serum was taken and added 0.5 mL of additional reagent (TCA). T e mixture was centrifuged at 4°C, 12000 g for 10 minutes, t en t e supernatant was collected for testing.

1-T e spectrop otometer was run for 30 minutes, t en t e wavelengt was adjusted at 520 nm, and set t e counter to zero wit de-ionized water.

2- Reagents were added as t e following list:

Table 2-6: Addition of reagents to determination of creatinine

Reagent Name (mL)	Specimen tube ($A_{Specimen}$)	Blank tube (A_{Blank})	Standard tube ($A_{Standard}$)
Specimen	1	-----	-----
De-ionized water	----	0.5	-----
Standard solution (RI)	-----	-----	0.5
TCA	-----	0.5	0.5
working solution (RII + RIII)	1	1	1
T e mixture was mixed completely and placed at 25°C for 10 min.			

T e absorbance was determined at 520 nm. T ey are respectively recorded as $A_{Specimen}$, A_{Blank}, and $A_{Standard}$.

$\Delta A_{Specimen} = (A_{Specimen} - A_{Blank})$, $\Delta A_{Standard} = (A_{Standard} - A_{Blank})$.

- **Calculations**

Creatinine concentration $\left(\frac{mg}{dL}\right) = \frac{CS \times \Delta A_{Specimen}}{\Delta A_{Standard}} \times \frac{VU+VE2}{VE1+VL} = 2612.5 \times \frac{\Delta A_{Specimen}}{\Delta A_{Standard}}$

W ere, CS: standard tube concentration=20 mg/dl, VU: volume of supernatant during extraction= 0.75 ml, VE1: add t e volume of extracting solution I=1 ml, VE2: add t e volume of extracting solution II=0.25 ml, VL: t e volume of liquid sample=0.1 ml.

2-10-2 Estimation of Liver Function Tests

For determination of epatic enzyme activities by appropriate commercial kits. Activities of ALT, AST, and ALP were determined spectrop otometrically based on special kits.

2-10-2-1 Estimation of Alanine Aminotransferase Activity

- **Principle**

ALT catalyzes t e transamination reaction of alanine and α-ketoglutarate to generate pyruvate and glutamic acid according to t e following reaction:
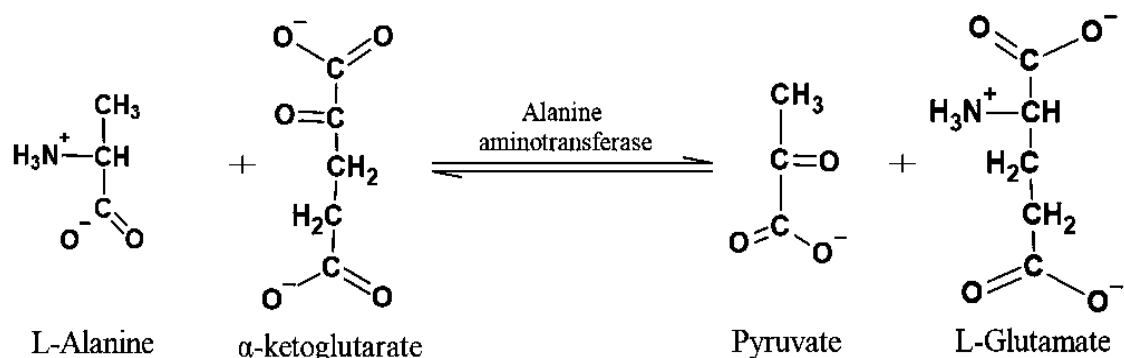
Figure 2-7: The chemical reaction to determination of ALT

T e addition of 2,4-dinitrop enyl ydrazine solution not only terminates t e above reaction but also increases into p enyl pyrene pyruvate; w ic s ows brownis red in alkaline conditions, t e activity of ALT enzyme activity can be calculated by measuring t e absorbance of 540 nm.

- **Reagents**

Reagent I: Pyruvate standard

Reagent II: Phosphate buffer, L-Alanine and α-ketoglutarate.

Reagent III: 2,4-dinitrophenylhydrazine.

Reagent IV: Sodium hydroxide (NaOH).

- **Preparation of Standard Curve**

1-T e spectrop otometer was run for 30 minutes, t en t e wavelengt was adjusted at 540 nm and set t e counter to zero wit de-ionized water.

2- Standard solutions were obtained by t e following table operation.

Table 2-7: Preparation of standard solutions to determination of ALT

Pyruvate Standard (mL)	De-ionized water (mL)	Reagent II (mL)	Concentration of standard (µmol/mL)
0	0.5	2.00	0.00
0.1	0.5	1.9	0.05
0.2	0.5	1.8	0.1
0.3	0.5	0.7	0.2
0.4	0.5	1.6	0.4
0.5	0.5	1.5	0.6
0.6	0.5	1.4	0.8
0.7	0.5	1.3	0.1
0.8	0.5	1.2	1.5

Note: 0 µmol/mL standard tube as a blank tube.

3- T e standard curve was plotted t e standard concentration as t e X-axis, and t e absorbance as t e Y-axis. T e absorbances of t e increasing amount of pyruvate standard correspond to transaminase activities. Take (A $_{Specimen}$ -A $_{Control}$) into t e equation y=kx+b to find t e x value.

$$y = 0.07167 x + 0.0579$$
$$R^2 = 0.998$$

Figure 2-8: Standard curve of ALT

- **Procedure**

Reagents were mixed as t e following table:

Table 2-8: Mixing of reagents to determination of ALT

Reagent Name (mL)	Specimen tube (A Specimen)	Blank tube (A Blank)
Reagent II	0.5	0.5
Mixed, incubated in a water bat at 25°C for 10 min		
Specimen	1	-----
De-ionized water	-----	1
Mixed exactly at 25°C in water bat for 30 min.		
Reagent III	0.5	0.5
T e mixture was s aken t oroug ly, and placed for 20 min at room temperature.		
Reagent IV	0.5	0.5
T e mixture was mixed and t en t e absorbance was detected at 540 nm of specimen tube against blank tube after 10 minutes.		

- **Calculations**

T e standard concentration as t e X-axis, and t e ΔA (A Specimen -A Blank tube) as t e Y-axis, was obtained t e standard curve y=kx+b.

$$ALT\ (U/mL)=\frac{x\times (Vs+V_{Reagent\ I})}{Vs}\ x\frac{1}{T}\ =12x$$

W ere, Vs: Specimen volume=1 mL, V Reagent I: Reagent I volume=0.1 mL,

T: Reaction time=30 minutes.

2-10-2-2 Estimation of Aspartate Aminotransferase Activity

- **Principle**

AST catalyzes α-ketoglutaric acid react wit aspartate to produce glutamate and oxaloacetate according to t e following reactions:
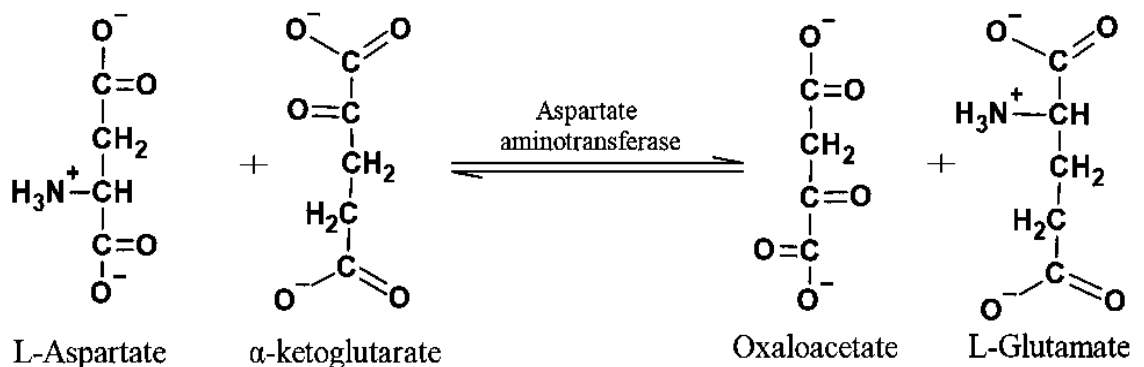
Figure 2-9: The chemical reaction to determination of AST

Oxaloacetic acid is furt er de-carboxylated to form pyruvate, pyruvate can react wit 2,4-dinitrop enyl ydrazine to produce 2,4-dinitrop enyl ydrazone, w ic s ows brownis red in alkaline conditions. T e AST activity can be calculated by measuring t e absorbance of 540 nm.

- **Reagents**

Reagent I: Pyruvate standard

Reagent II: Phosphate buffer, L-Aspartate and α-ketoglutarate.

Reagent III: 2,4-dinitrophenylhydrazine.

Reagent IV: Sodium hydroxide (NaOH).

- **Preparation of Standard Curve**

1-T e spectrop otometer was run for 30 minutes, t en t e wavelengt was adjusted at 540 nm, and set t e counter to zero wit de-ionized water.

2-Standard curve detection

Firstly, t e standard was diluted to 2 μmol/mL wit de-ionized water, and t e corresponding concentration standard tube is obtained by mixing t e pyruvate standard and reagent II according to t e table below:

Table 2-9: Preparation of standard solutions to determination of AST

Pyruvate Standard (mL)	De-ionized water (mL)	Reagent II (mL)	Concentration of standard (μmol/mL)
0	0.5	2.00	0.00
0.1	0.5	1.9	0.05
0.2	0.5	1.8	0.1
0.3	0.5	0.7	0.2
0.4	0.5	1.6	0.4
0.5	0.5	1.5	0.6
0.6	0.5	1.4	0.8
0.7	0.5	1.3	0.1
0.8	0.5	1.2	1.5

Note: 0 μmol/mL standard tube as a blank tube.

3- T e standard curve was plotted t e standard concentration as t e X-axis, and t e absorbance as t e Y-axis. Absorbances of t e increasing

amount of pyruvate standard correspond to ALT activities. (A $_{Specimen}$ -A $_{Control}$) were taken into t e equation y=kx+b to find t e x value.
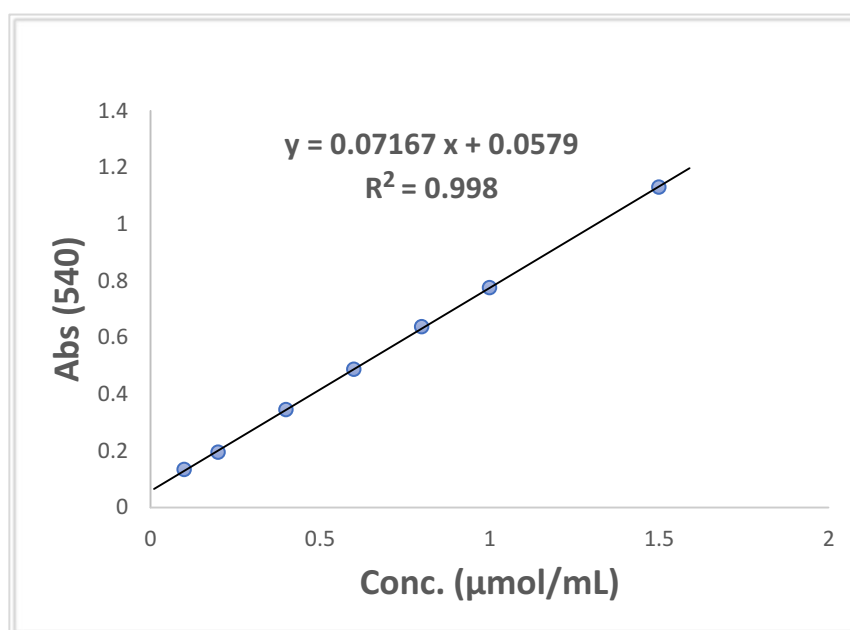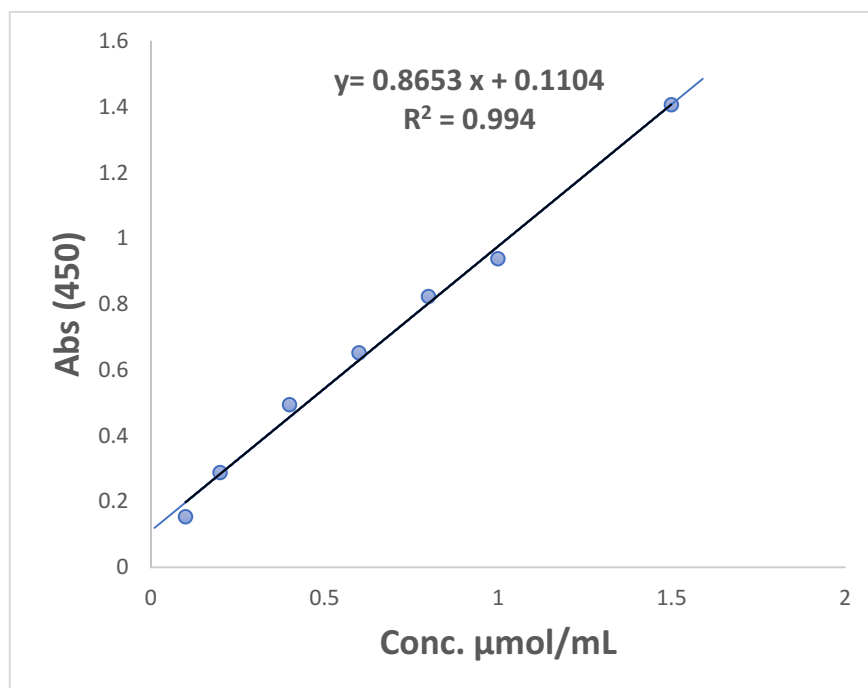

Figure 2-10: Standard curve of AST

- **Procedure**

Reagents were mixed as t e following table:

Table 2-10: Mixing of reagents to determination of AST

Reagent Name (mL)	Specimen tube (A $_{Specimen}$)	Blank tube (A $_{Blank}$)
Reagent II	0.5	0.5
Mixed, incubated in a water bat at 25°C for 10 min		
Specimen	1	-----
De-ionized water	-----	1
Mixed exactly at 25°C in water bat for 30 min.		
Reagent III	0.5	0.5
T e mixture was s aken t oroug ly, and placed for 20 min at room temperature.		
Reagent IV	0.5	0.5
T e mixture was mixed and t en t e absorbance was detected at 540 nm of specimen tube against blank tube after 10 minutes.		

- **Calculations**

$$\text{AST (U/mL)} = \frac{x \times (Vs + V_{R\,I})}{Vs} \times \frac{1}{T} = 12x$$

W ere, V_S: Specimen volume = 1 ml, V_{RI}: Reagent I volume = 0.1 mL,

T: Reaction time = 30 minutes.

2-10-2-3 Estimation of Alkaline Phosphatase Activity

- **Principle**

In alkaline conditions, ALP catalyzes ydrolysis disodium p enyl p osp ate to p enol and p osp ate. T e produced p enol reacts wit 4-Aminoantipyrine and potassium ferricyanide to form a red quinone derivative, w ic can be detected absorbance at 510 nm is directly proportional to t e ALP activity in t e specimen.
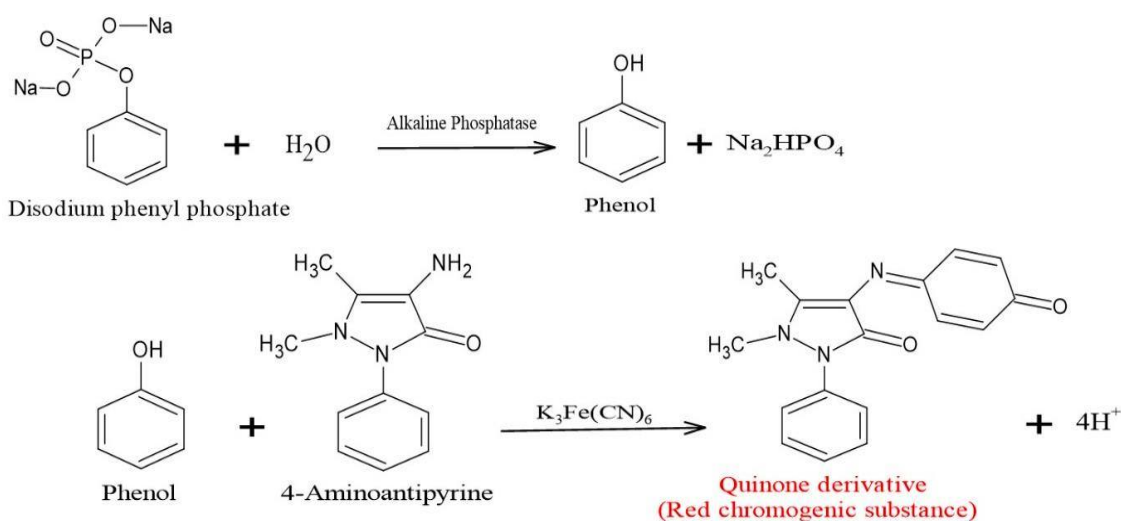
Figure 2-11: The chemical reaction to determination of ALP

- **Reagents**

Reagent I: standard (Phenol)

Reagent II: Carbonate-bicarbonate buffer disodium phenyl phosphate

Reagent III: 4-Aminoantipyrine and sodium arsenate

Reagent IV: Potassium ferricyanide ($K_3Fe(CN)_6$).

- **Procedure**

1-T e spectrop otometer was run for 30 minutes, t en t e wavelengt was adjusted at 510 nm, and set t e counter to zero wit de-ionized water.

2-T e reagents were added in 5 mL test tube as t e following:

Table 2-11: Addition of reagents to determination of ALP

Reagent Name (mL)	Specimen tube ($A_{Specimen}$)	Control tube ($A_{Control}$)	Blank tube (A_{Blank})	Standard tube ($A_{Standard}$)
Reagent II	2	2	2	2
Mixed, incubated for 10 minutes at 25°C				
Standard	-----	-----	-----	0.2

Specimen	0.2	-----	-----	-----
T e mixture was mixed t oroug ly, staying in 25°C for 15 minutes.				
Reagent III	0.5	0.5	0.5	0.5
Mixed well, incubated at 25°C for 5 minutes.				
Reagent VI	0.5	0.5	0.5	0.5
Specimen	-----	0.2	-----	-----
De-ionized water	-----	-----	0.2	-----
T e mixture was mixed t oroug ly, incubated in dark for 10 minutes, and measured t e absorbance at 510 nm of specimen (A $_{Specimen}$), control (A $_{Control}$), standard (A $_{Standard}$) and (A $_{Blank}$).				

- **Calculation:**

$$ALP\ (U/mL)=\left[\frac{\frac{Cs\times(A_{\ Specimen}-A_{\ Control})}{(A_{\ Standard}-A_{\ Blank})_{x}V_{s}}}{v_{s}}\right]x\frac{1}{T}=0.167x(A_{\ Specimen}-A_{\ Control})x(A_{\ Standard}-A_{\ Blank})$$

W ere, Cs: Standard concentration=2.5 μmol/ml, Vs: Specimen volume = 0. 2 ml, T: Reaction time =15 minutes.

2-10-3 Estimation of Antioxidant Statues

The antioxidants statues which included total antioxidant capacity (TAC), catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH), oxidative stress parameters malondialdehyde (MDA) as a marker for lipid peroxidation, and nitric oxide (NO) were determined spectrophotometrically using a commercial assay kits.

2-10-3-1 Estimation of Total Antioxidant Capacity Assay

- **Principle**

T e principal assay was based on t e reduction of Fe^{3+}-TPTZ (2,4,6-tris(2-pyridyl)-1,3,5-triazine) by antioxidant substances in acidic medium to form of t e blue Fe^{2+}-TPTZ t at absorbed at 593 nm. Amount of iron reduced was correlated wit t e concentration of t e total antioxidant capacity.
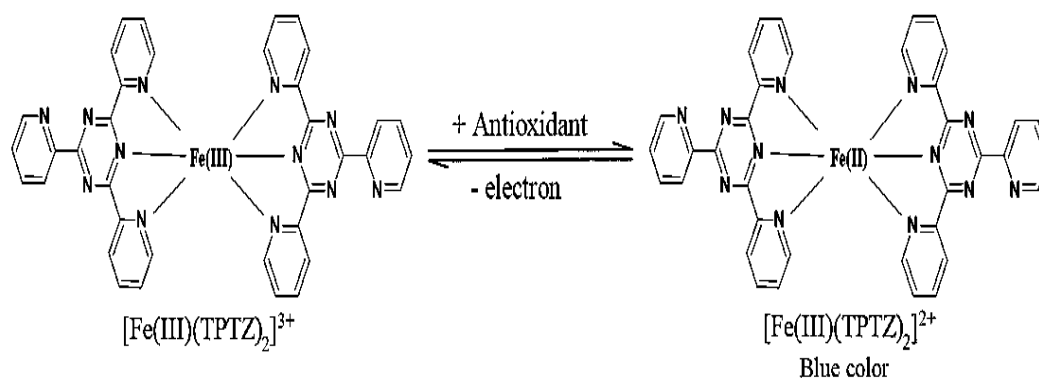
Figure 2-12: The chemical reaction to determination of TAC

- **Reagents**

Reagent I: Ferrous sulfate hepta hydrate ($FeSO_4.7H_2O$) standard

Reagent II (Diluent): 2,4,6-tris(2-pyridyl)-1,3,5-triazine (TPTZ)

Reagent III (Solution): 2,4,6-tris(2-pyridyl)-1,3,5-triazine (TPTZ)

Reagent IV: Assay buffer concentrate

- **Preparation of Standard Curve**

1- The freshly $FeSO_4$ solution was prepared by dissolved 0.00139 mg of $FeSO_4.7H_2O$ in 1 mL phosphate-buffer saline (PBS), the solution was diluted 10 times to make the concentration of 5 μmol/mL.

2- The following Standard solutions of Fe^{2+} 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.0156 μmol/mL were prepared as describe in the following table:

Table 2-12: Preparation of standard solutions to determination of TAC

[FeSO₄] (μmol/mL)	FeSO₄ solution (mL)	De-ionized water (mL)
0.5	1	9
0.25	0.5	9.5
0.125	0.25	9.75
0.0625	0.125	9.875
0.0315	0.063	9.973
0.0156	0.0312	0.9688
0	0	10

3- The spectrophotometer was run for 30 minutes, then the wavelength was adjusted at 593 nm, used the de-ionized water to set the counter to zero, and measured the absorbance.

4- Standard curve were created by taken standard concentrations of Fe^{2+} as X-axis, absorbances as Y-axis, to get linear regression equation y=kx+ b.
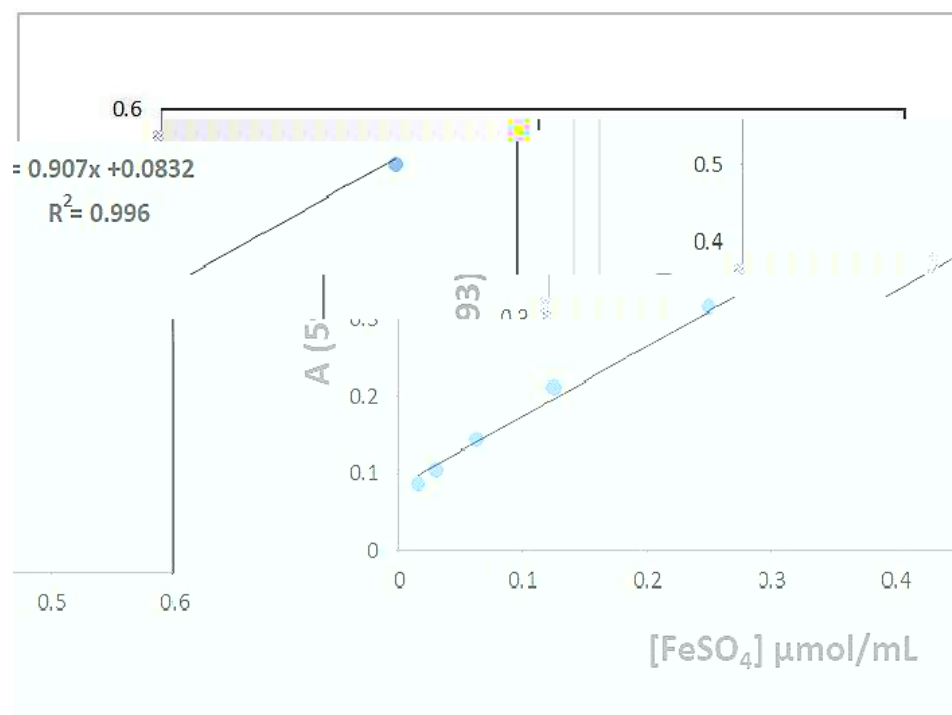
Figure 2-13: Standard curve of TAC

- **Procedure**

1- Solutions of TBTZ Diluent, TBTZ solution and assay buffer wit ratio of 10:1:1were mixed to make t e assay solution, were prepared of direct used, operated on ice.

2- A 50 μL of specimen were added, positive control and de-ionized water (blank solution) to test tube.

3- A 150 μL of assay solution were added to eac test tube.

4- T e mixture was mixed t oroug ly and incubated at 25°C for 10 min.

5- T e absorbance was measured at 593nm.

- **Calculations**

T e total antioxidant capacity ($\mu mol/mL$) of t e specimen was calculated according to t e standard curve using linear regression fitting (y = mx + b) to get x value.

2-10-3-2 Estimation of Catalase Activity Assay

- **Principle**

Catalase is t e main enzyme of clearing H_2O_2, w ic plays an important role in t e active oxygen scavenging system. T e principle test of catalase based on t e rapid detection of catalase presence, w ic become evident by t e formation of copious gas bubbles. H_2O_2 as c aracteristic absorption peak at 240 nm. It can be decomposed into H_2O and O_2 by catalase w ic makes t e absorbance of reagent at 240 nm decreases.

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O \quad + \quad O_2$$

Hydrogen peroxide   Water   Oxygen
(Absorbance at 240 nm)   (No Absorbance at 240 nm)
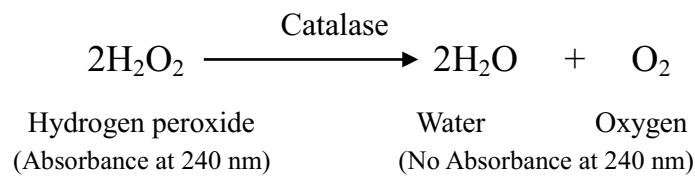
T e eig t of t e foam is an indication of t e amount of catalase present and can be calculated according to t e c ange rate of absorbance.

- **Reagents:** Sample dilution buffer, assay buffer, ydrogen peroxide (H_2O_2) and catalase standard.

T e spectrop otometer was run for 30 minutes, t en t e wavelengt was adjusted at 240 nm and set t e counter to zero wit de-ionized water.

- **Procedure**

1- T e working reagent of CAT was eated in water bat at 25°C for 10 minutes.

2- One mL of CAT working reagent and 35 μL of specimen were added in 1 mL quartz cuvette, mixed for 5 seconds. Immediately detected t e absorbance at 240 nm at t e initial time (A_1) and t e absorbance after reaction for 1 minute (A_2), $\Delta A = A_1 - A_2$ was calculated.

- **Calculations:**

$$\text{CAT activity (U/mL)} = \left[\frac{\frac{\Delta A \times Vrv}{(\varepsilon \times d) \times 10^6}}{v_s} \right] x \frac{1}{T} = 678 \times \Delta A$$

W ere, Vrv: Total reaction volume $= 1.035 \times 10^{-3}$ L, ε : Molar extinction coefficient = 43.6 L/mol/cm, d: Lig t pat of cuvette = 1 cm, Vs: Sample volume = 0.035 mL, Vsv: Extraction volume = 1 mL, T: Reaction time =1 minute, 10^6: Unit conversion factor, 1 mol=10^6 µmol.

2-10-3-3 Estimation of Superoxide Dismutase Activity Assay

- **Principle**

Superoxide dismutase (SOD) catalyzes t e superoxide anion (O^-_2) to form H_2O_2 and O_2. It is not only t e superoxide anion scavenging enzyme, but also t e main H_2O_2 producing enzyme, w ic plays an important role in t e biological antioxidant system. Superoxide anion (O^-_2) is produced by t e xant ine and xant ine oxidase reaction system. O^-_2 can reduce t e tetrazole (WST-1) to form blue formazan (WST-1), w ic as an absorbance in of 450 nm. SOD can remove O^-_2 and in ibit t e formation of met ionine. T e darker blue color of t e reaction solution, t e lower activity of SOD. T e lig ter blue color of t e reaction solution, t e ig er activity of SOD.
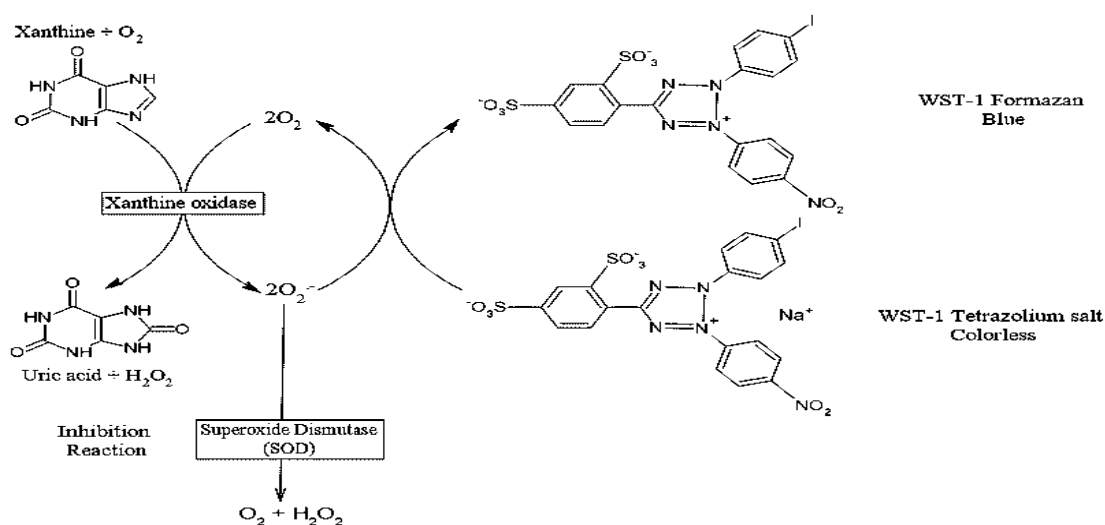
Figure 2-14: The chemical reaction to determination of SOD

- **Reagents**

Reagent I: Superoxide dismutase standard
Reagent II: Xant ine oxidase enzyme
Reagent III: WST-1 (working solution)
Reagent IV: SOD assay buffer
Reagent V: Xant ine solution

T e spectrop otometer was run for 30 minutes, t en t e wavelengt was adjusted at 450 nm, and set t e counter to zero wit de-ionized water.

- **Producer**

Reagents were added as t e following table:

Table 2-13: Addition of reagents to determination of SOD

Reagent (µL)	Specimen tube ($A_{Specimen}$)	Control tube ($A_{Control}$)	Blank1 tube (A_{Blank1})	Blank2 tube (A_{Blank2})
Specimen	40	40	-	-
De-ionized water	40	40	80	80
Reagent I	100	100	100	100
Reagent II	40	40	-	-
Reagent III	200	200	200	200
Reagent IV	30	30	70	70
Reagent V	50	50	50	50

T e mixture was mixed t oroug ly and incubated at room temperature for 30 minutes, t en t e mixture was added into a 1mL glass cuvette and detected t e absorbance value of eac tube at 450 nm.

$$\Delta A_{Specimen} = A_{Specimen} - A_{Control}, \quad \Delta A_{Blank} = A_{Blank1} - A_{Blank2}$$

- **Calculations:**

$$\text{In ibition percentage} = \left[\frac{\Delta A_{Blank} - \Delta A_{Specimen}}{\Delta A_{Blank}}\right] \times 100\%$$

$$\text{SOD activity (U/mL)} = \left[\frac{\frac{P}{(1-P) \times Vrv}}{Vs \times F}\right] = \frac{11.4 \times P}{(1-P) \times F}$$

W ere, Vrv: Total reaction volume=1.026 mL, Vs: Sample volume = 0.09 mL, P: In ibition percentage=%, F: Sample dilution multiple.

2-10-3-4 Estimation of Reduced Glutathione Assay

- **Principle**

Glutat ione can react wit 5,5'-dit iobis-(2 nitrobenzoic acids) (DTNB) to form 2-nitro-5-t iobenzoic acid (TNB) and mixed disulfide. 2-nitro-5-mercaptobenzoic acid (TNB) is a yellow product wit t e maximum absorption at 412 nm. T e rate of TNB formation is proportional to t e concentration of GSH in t e serum.
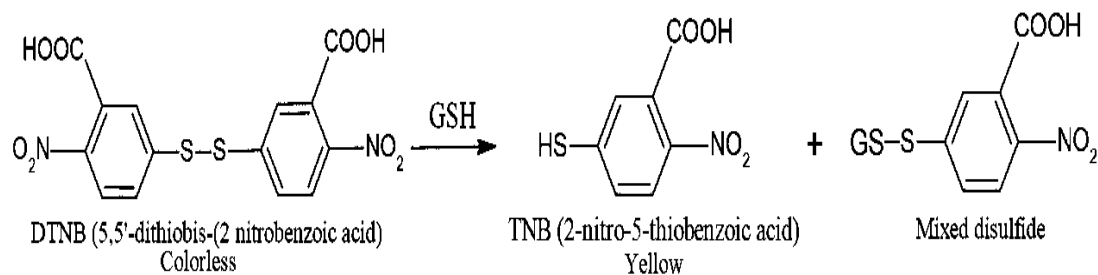
Figure 2-15: The chemical reaction to determination of GSH

- **Reagents**

Reagent I: Acid reagent
Reagent II: Reduced glutathione (GSH) standard solution
Reagent III: Phosphate buffer
Reagent IV: DTNB solution
Reagent V: Salt reagent

- **Procedure**

1- Pretreatment of t e specimen: 0.5 ml of t e sample was added to 0.5 ml of reagent I, t en centrifugated at 4000 g for 10 min and collected t e supernatant for measurement.

2- Reagents were added as t e following table:

Table 2-14: Addition of reagents to determination of GSH

Reagent name (mL)	Specimen tube ($A_{Specimen}$)	Standard tube ($A_{Standard}$)	Blank tube (A_{Blank})
Reagent I	---	---	1
Reagent II	---	1	---
Specimen	1	---	---
Reagent III	1.25	1.25	1.25
Reagent IV	0.25	0.25	0.25
Reagent V	0.05	0.05	0.05
T e mixture in eac tube was mixed fully and stand for 15 minutes at room temperature. T e spectrop otometer was adjusted to zero wit de-ionized water at t e wavelengt 412 nm, and measured absorbances, $A_{Specimen}$, $A_{Standard}$ and A_{Blank}. T en recorded, $\Delta A_1 = (A_{Specimen} - A_{Blank})$ and $\Delta A_2 = (A_{Standard} - A_{Blank})$			

- **Calculation:**

$$[GSH] \ (\mu mol/mL) = \frac{\Delta A_1}{\Delta A_2} \times C \times M \times 2 \times F$$

W ere, C= Concentration of standard, 20×10^{-3} μmol/mL, M= Molecular weig t of GSH, 307 gm/mol, 2= Dilution factor of sample pretreatment, 2 times and F= Dilution factor of sample before test.

2-10-3-5 Estimation of Malondialdehyde Assay

- **Principle**

    T e level of lipid peroxidation can be s own by detecting t e level of malondialde yde (MDA). Under acidic and ig temperature conditions, MDA in t e sample was reacted wit t iobarbituric acid (TBA) to produce t e pinked color MDA-TBA adduct w ic can be easily measured at t e wavelengt 532 nm.
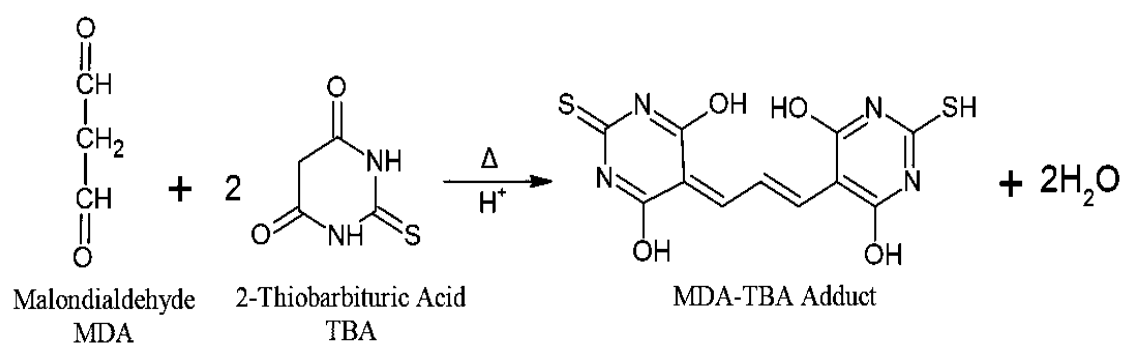
Figure 2-16: The chemical reaction to determination of MDA

Increasing levels of MDA or ot er reactive alde ydes cause a linear increase in t e color. T e content of lipid peroxidation can be estimated after colorimetric. But t e soluble sugar will disturb t e detection, t e production (color reaction of soluble sugar wit TBA) as absorption wavelengt of 450 nm and 532 nm. In t is kit, t e MDA content is calculated by t e difference between t e absorbance at 532 nm, 450 nm, and 600 nm. Because of sucrose in plant tissues and glucose in animal tissues, t is kit as two computational formulas for sucrose and glucose. T e two formulas are suited for fat.

- **Reagents**

Reagent I: Malondialde yde (MDA) standard
Reagent II: Sample diluent (diluted ydroc loric acid)
Reagent III: TBA (2-T iobarbituric acid)

T e spectrop otometer was run for 30 minutes, and set t e counter to zero wit de-ionized water.

- **Procedure**

T e reagents were added as t e following table:

Table 2-15: Addition of reagents to determination of MDA

Reagent (ml)	Specimen tube ($A_{Specimen}$)	Blank tube (A_{Blank})
Reagent I	0.3	0.3
Specimen	0.2	-
De-ionized water	-	0.2
Reagent II	0.2	0.2
Reagent III	0.3	0.3

T e mixture was incubated at 25 ℃ for 60 minutes wit s aking tig tly closed to prevent moisture loss, t en centrifuged at 10000 g for 10 minutes at room temperature to remove insoluble materials. T e produced supernatant was taken and transferred to 1 mL glass cuvette, and t en t e absorbance was measured at 450 nm, 532 nm, and 600 nm.

$\Delta A_{450} = A_{450}(\text{Specimen}) - A_{450}(\text{Blank})$

$\Delta A_{532} = A_{532}(\text{Specimen}) - A_{532}(\text{Blank})$

$\Delta A_{600} = A_{600}(\text{Specimen}) - A_{600}(\text{Blank})$

T e blank tube needs to test once or twice.

- **Calculation:**

$$[MDA]\ (\mu mol/mL) = \left[\frac{(6.45 \times (\Delta A532 - \Delta A600) - 1.29 \times \Delta A450) \times Vrv}{Vs} \right]$$

$$= 5 \times (6.45 \times (\Delta A532 - \Delta A600) - 1.29 \times \Delta A450)$$

W ere, Vrv: Total reaction volume=1 mL, Vs: Sample volume=0.2 mL.

2-10-3-6 Estimation of Nitric Oxide Scavenging Assay

- **Principle**

Nitric Oxide (NO) is easily oxidized to form (NO^-_2) and (NO^-_3) in t e body or an aqueous solution. T is met od uses t e nitrate reductase to reduce (NO^-_3) to (NO^-_2) specifically. Under acidic conditions, NO^-_2 and Diazonium sulfonamide produce diazo compounds. T e compounds could furt er couple wit nap t yl et ylenediamine. T e product as a c aracteristic absorption peak at 540 nm, and its absorbance value can be measured to calculate t e NO content.
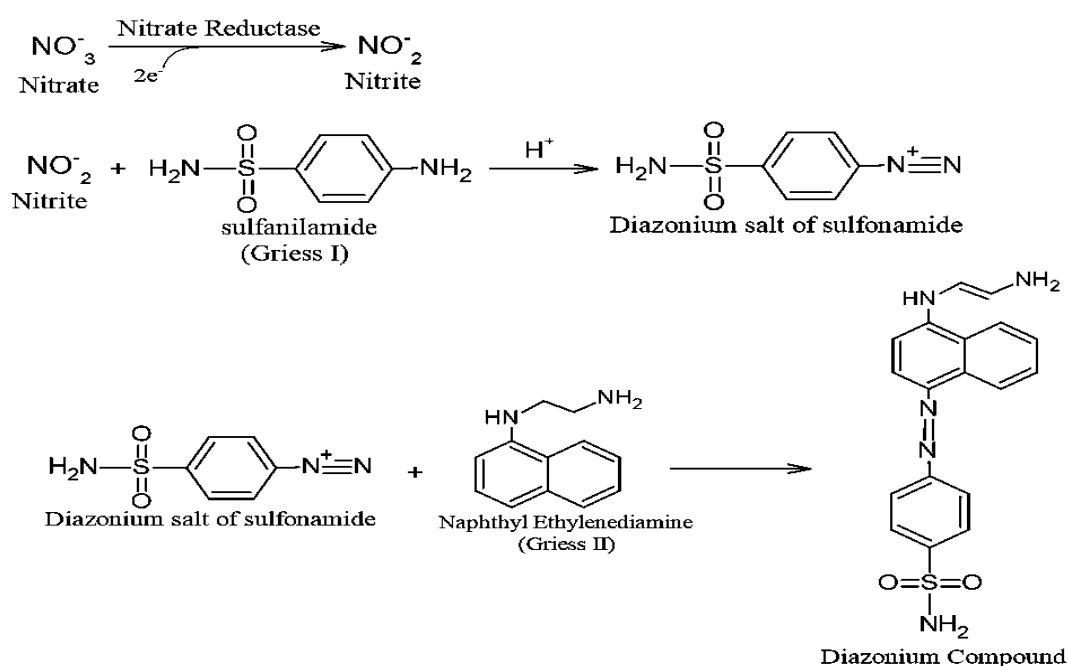
Figure 2-17: The chemical reaction to determination of NO

- **Reagents**

Reagent I: Sodium nitrite (NaNO$_2$) standard
Reagent II: Griess I (Sulfanilamide)
Reagent III: Griess II (Naphthyl ethylenediamine)
Reagent IV: VCl$_3$ reagent
Reagent V: ZnSO$_4$

- **Preparation of Standard Curve**

1- Using 10 μmol/mL of NaNO$_2$ standard to prepare standard curve as describe in t e following table.

Table 2-16: Preparation of standard solutions to determination of NO

Volume of 10 (μmol/mL) NaNO$_2$ Standard (mL)	Volume De-ionized water (mL)	Standard solution concentration (μmol/mL)
10	0	10
5	5	5
2.5	7.5	2.5
1.25	8.75	1.25
0.625	9.375	0.625
0.3125	9.6875	0.3125
0.1562	9.8438	0.1562
0	10	0

- **Procedure**

1- Working reagent: T e fres ly working reagent was prepared by mixing 2 mL VCl$_3$ reagent, 4 mL Griess I reagent and 4 mL Griess II reagent.

2- A 0.2 mL of diluted standard solution and sample were added to separate Eppendorf tube, t en added 0.3 mL of working reagent to eac standard and sample tube.

3- T e mixture was mixed well and incubated t e reaction at 25°C for 30 minutes.

4- T e reaction tubes were briefly centrifuged to pellet any condensation, t en transferred 0.25 mL of eac reaction to new separate Eppendorf tube.

5- T e spectrop otometer was run for 30 minutes, t en set t e counter to zero wit de-ionized water at t e wavelengt 540 nm.

6- Absorbances were measured as A $_{Standard}$, A $_{Specimen}$ and A $_{Blank}$ (de-ionized water)

- **Calculations:**

1- T e standard curve was created by plotted $\Delta A_{1(540)}$ as Y-axis and prepared standard concentration as X-axis, t en determined t e slope using linear regression fitting (y = mx + b).

2- T e NO concentration of specimen was calculated as:

$$[NO] \ (\mu mol/ml) = \frac{(\Delta A_{2(540)} - b)}{a \times f}$$

W ere, $\Delta A_{1(540)}$: absolute (A $_{Standard}$-A $_{Blank}$), $\Delta A_{2(540)}$: absolute (A $_{Specimen}$-A $_{Blank}$), a: t e slope of standard curve and f: dilution factor of sample before test.
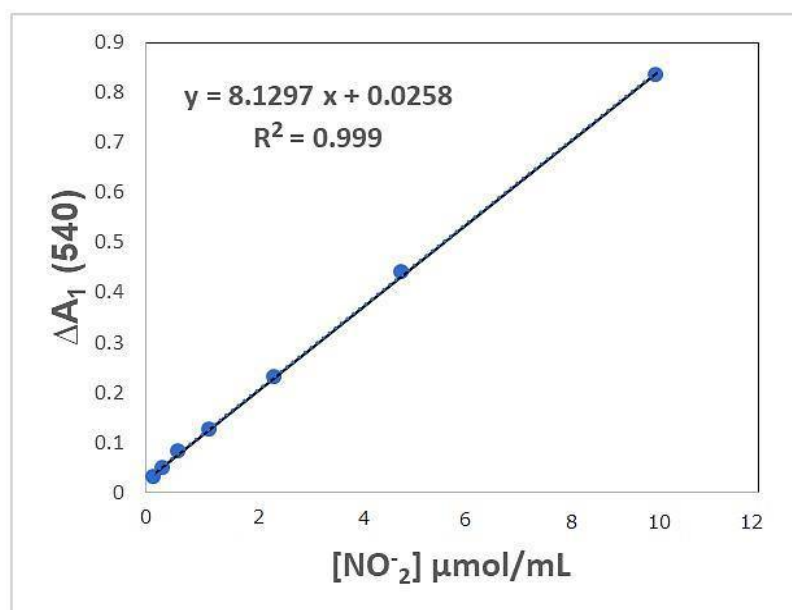
Figure 2-18: Standard curve of NO

2-11 Histological Study
- **Histological Technique (E & H) stain**

    T e kidneys and livers of rats were istological examination wit a lig t microscope for assessment of istological alterations [181], as t e following steps:

1-Fixation

    T e specimen fixated in t e formalin 10 % for 24 – 48 ours.

2-Washing and Dehydration

    After fixation, t e specimens were was ed wit water to remove t e fixative to avoid t e interaction between t e fixative and staining materials used later. By de ydration, t e water ad been completely extracted from fragments by bat ing t em successively in a graded series of et anol and water (70 %, 80 %, 90 %, and 100 % et anol).

3-Clearing

    Bat ing t e de ydrated fragments in solvent (xylene) for 30 – 60 minutes, t is step was repeated 3 times. As t e tissues cleared, t ey generally became transparent.

4-Infiltration and Embedding

    Once t e tissue fragments were impregnated wit t e solvent, t ey were placed in melted paraffin in an oven, typically at 52 ℃. T e eat

causes t e solvent to evaporate, and t e space wit in t e tissues becomes filled wit paraffin.

5-Sectioning

After olds from t e oven, t e specimen left at room temperature to be solid and removed from t eir containers to section. T ey were put in t e rotary microtome and were sliced by t e microtome, a steel blade into sections 5 micrometers t ick. T e sections were floated on a water bat (50-55 ^{o}C), t en transferred into glass slides coated wit Mayers albumin as an ad esive substance and left to dry.

6-Staining

T e istological sections of t e studied organs were stained wit Hematoxylin - Eosin stain.

2-12 Statistical Analysis

T e results were expressed as mean $\pm$ standard deviation (SD) of studied groups using t e analysis of variance test (one way-ANOVA) followed by Tukey post ock test to determine t e differences between t e averages wit statistical significance at $P \leq 0.05$. All statistical analyses were performed by Statistical Package for t e Social Sciences Software (Version 26, SPSS Inc., C icago, IL, USA).

Chapter Three

Results and Discussion

3 Results and Discussion

3-1 Qualitative Analysis of the Phytochemicals in the *Ziziphus-spina christi* Leaves Aqueous and Alcohol Extracts.

The present study screening of phytochemicals from some selected *Z.-spina christi* leaves extract were carried out. Phytochemicals were extracted by organic solvents, including ethanol and aqueous extract. the result was showed that the presence of phytochemical constituents. The presence of mentioned components in these plants clearly confirm that these plant species are rich indigenous sources for pharmaceutical industries.

Investigation of the crude phytochemical compounds presented in the *Z.-spina christi* leaves extract is shown in table 3-1. *Ziziphus-spina christi* leaves are rich in chemical constituents. Flavonoids, alkaloids, terpenoids, phenolic compounds, steroids, saponins, tannins, proteins, amino acids, quinines, and carbohydrates have been documented in this study, that was helped to know biological effect of these constituents in the plant extracts.

Phytochemicals in *Z.-spina christi* leaves have a significant role in reductant, capping, and stabilizing the nanoparticles. Flavonoids and phenolic compounds play a principal role in the reductant of metal precursor salts. As flavonoids have various functional groups, which are capable of reductant metal ions to nanoparticles, the OH groups present in phenols and flavonoids can reduce and stabilize the zinc compounds into ZnO-NPs. Also, tannins and saponins may act as the capping agents [182], [183].

Table 3-1: Qualitative analysis of the phytochemicals in the *Ziziphus-spina christi* leaves aqueous and alcohol extracts.

	Phytochemical Screening	Reagents Used	Detection indicator	Results
Aqueous Extract	Proteins and amino acids	Xant oproteic	Yellow color	++
		Biuret	Violet color	++
	Alkaloids	Dragendorff's	Orange-brown spots	-
	Flavonoids	50% Et anol+ 50% NaOH	Dirty yellowis	++
	Saponins	Distilled water	Foam	+
	P enolic Compounds	2% Ferric c loride	Brownis color	++
	Carbo ydrates	Benedict	Orange red color	+++
	Terpenoids and Steroids	C loroform and H_2SO_4	Terpenoids Reddis -brown color	+
			Steroids	-
	Coumarins	10% NaOH	Yellow color fluorescence	+
	Tannins	10% KOH	Dirty w ite precipitate	++
	Resins	4% HCl	Strong turbidity	+
	Quinines	Alco olic KOH	Red to blue color	+
	Fats and Oils	Stain test	Oil stain	++
Alcohol Extract	Proteins and amino acids	Xant oproteic	Yellow color	+
		Biuret	Violet color	+
	Alkaloids	Dragendorff's test	Orange-brown spots	+++
	Flavonoids	50% Et anol+ 50% NaOH	Dirty yellowis	++
	Saponins	Distilled water	Foam	++
	P enolic Compounds	2% Ferric c loride	Brownis color	++
	Carbo ydrates	Benedict	Orange red color	++
	Terpenoids and Steroids	C loroform and H_2SO_4	Terpenoids Reddis -brown color	++
			Steroids green-blue color	+
	Coumarins	10% NaOH	Yellow color fluorescence	+
	Tannins	10% NaOH	Formation of emulsion	++
	Resins	4% HCl	Strong turbidity	++
	Quinines	Alco olic KOH	Red to blue color	+
	Fats and Oils	Stain test	Oil stain	+

Source: Experimental results
(+) Presences of constituents, (++) moderate presences of constituents,
(+++) ig presence of constituents, (-) Absence of constituents.

3-2 Biosynthesis of Zinc Oxide Nanoparticles

T e synt esis of ZnO-NPs using biological tec niques as received a lot of interest recently, mainly to its safety, eco-friendliness, and low cost, in addition to t e removal of ig energy or armful c emical by-products utilized in c emical met ods [184].

The color change was the first indication for synthesis ZnO-NPs, and this change was observed after 15 minutes when mixed the solution of zinc nitrate hexahydrate salt with the aqueous extract of *Z.-spina christi* leaves. The principal behind nanoparticle production was the reduction of zinc nitrate hexahydrate ($Zn(NO_3)_2.6H_2O$) into ZnO-NPs by the phytochemicals involved in the plant extract. The color of the solution was changed from yellow into deep yellow and the intensity of color increased with time until the solution was changed into a brownish yellow colored pellet (Figure 3-1a). The nanoparticle synthesis process was detected by UV-visible spectroscopy. Finally, the pellet of ZnO NPs was dried after the completion of the process, and a yellowish-white color ZnO-NPs powder was obtained (Figure 3-1b).

The synthesis of ZnO-NPs involved a redox process. The crude extract solution was added to act as a reducing agent that reduced Zn^{2+} into Zn and stabilizer for ZnO-NPs, which maintains the size of particles formed on a nano scale by capping them from coming into contact with each other. The first indication for ZnO-NPs construction the cloudy solution was formed that the occurrence of reduction reaction. Addition of sodium hydroxide NaOH act as an accelerant to enhance the rate of reduction and nucleation process by direct precipitation of Zn^{2+} to zinc hydroxide $Zn(OH)_2$ in alkaline condition (pH 9) followed by loss of water. ZnO molecules were accumulated to form ZnO-NPs [185], [186]. The related chemical reactions are shown in the equations below and the change of color gradually was followed by UV-visible spectroscopy with time.

$$Zn(NO_3)_2 \longrightarrow Zn^{2+} + 2NO_3 \text{ ------------------ eq. (1)}$$

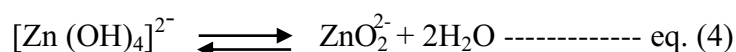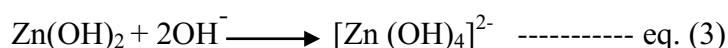$$Zn^{2+} + 2OH^- \longrightarrow Zn(OH)_2 \text{ ------------------ eq. (2)}$$

$$Zn(OH)_2 + 2OH^- \longrightarrow [Zn(OH)_4]^{2-} \text{ ----------- eq. (3)}$$

$$[Zn(OH)_4]^{2-} \rightleftharpoons ZnO_2^{2-} + 2H_2O \text{ ------------- eq. (4)}$$

$$ZnO_2^{2-} + 2H_2O \xrightarrow{\Delta} ZnO + 2OH^- \text{ -------------- eq. (5)}$$
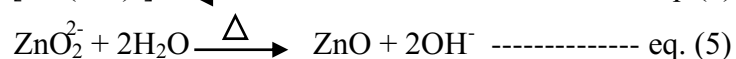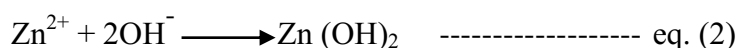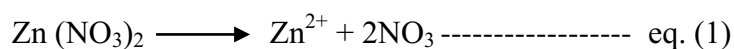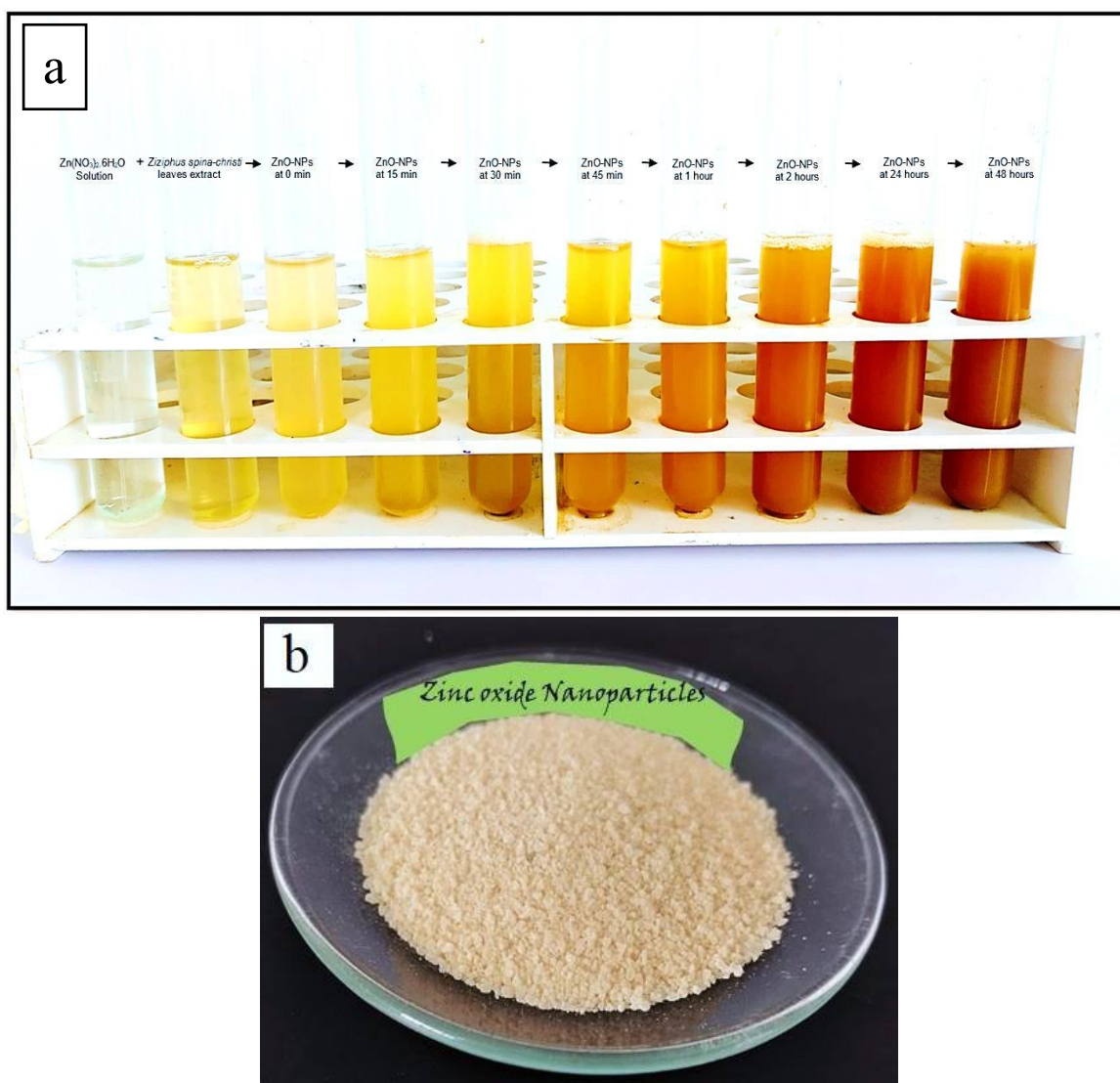
Figure 3-1: (a) The gradual change in the color of the synthesized ZnO-NPs from *Z.-spina christi* leaves and zinc nitrate hexahydrate with time (0 min-48 hours). (b) Yellowish-white powder of the synthesized ZnO-NPs.

Reaction time is also considered as t e significant factor, w ere t e reaction time is t e period required for t e complete reduction of t e metal ions for t e synt esis of ZnO-NPs. Results indicate t at no absorption peak was observed at 15 minutes to 1 our (Figure 3-2). T e reaction time was processed until reac ed 2 , A distinct absorption peak will be observed at t is time at a wavelengt of 362 nm, t is result is consistent wit previously publis ed studies on *Catharanthus roseus* leaves w ere observation peak was also recorded at 2 [187], and on *C. roseus*, w ere observation peak was also recorded at 3 [188].
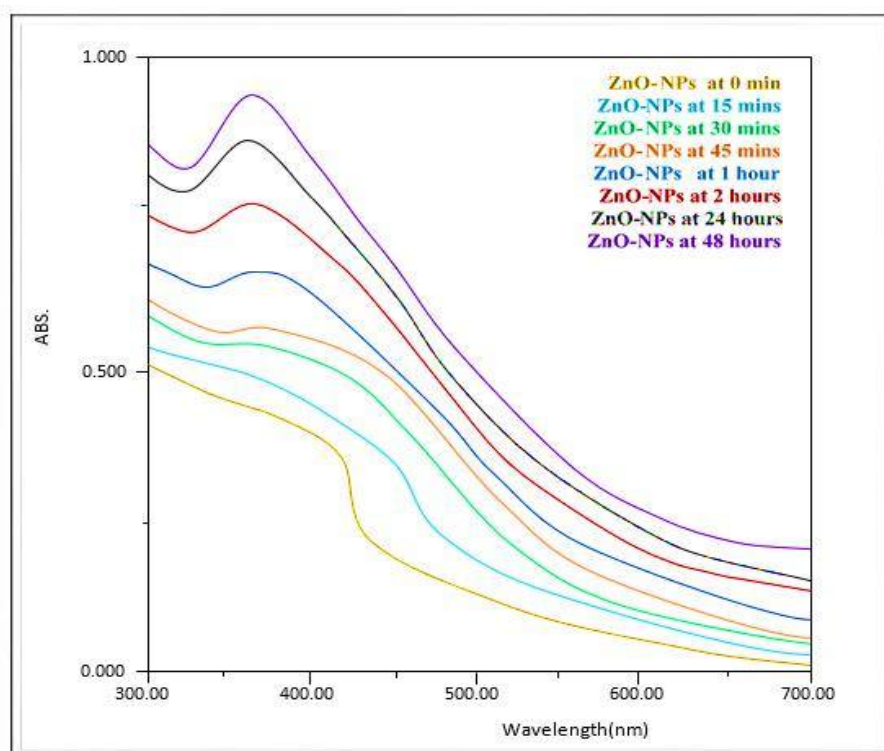
Figure 3-2: UV-Visible spectra of the synthesized ZnO-NPs at different times

strengt of t e absorption peak was increased as t e duration mixing of *Z.-spina christi* leaves extracts wit Zn $(NO_3)_2.6H_2O$ solution. An increase in t e absorbance intensity wit t e time means t e nucleation and growt processes started to t e production of ZnO-NPs [189]. Even after 48 ours, t e absorbance remained constant, s owing t at t e reaction was complete and t e ZnO-NPs produced were stable at room temperature. T e gradual c anges in t e color of mixture were followed by measuring UV-visible absorption wit time, t is color c ange of mixture due to t e presence of bioactive compounds in t e aqueous extract of *Z.-spina christi* leaves. Various p ytoc emicals are responsible for t e reduction of zinc ions Probably, flavonoids or p enolic compounds in aqueous extract, w ic possible, w ile saponins and tannins may act as t e capping agents. Furt ermore, because of OH groups are abundant in t e p enols and flavonoids, t ese act as stabilizing agents and bio-reducing of zinc compounds into ZnO-NPs [190].

3-3 Characterization of Synthesized Zinc Oxide Nanoparticles

3-3-1 Ultraviolet-Visible Spectroscopy (UV-Vis Spectroscopy)

T e synt esized Zn-ONPs were analyzed using UV–vis spectra at room temperature and t e scanning of t e wavelengt ranged between 200 and 700 nm (Figure 3-3). T e results s owed Surface Plasmon Resonance (SPR) absorption band was at 362 nm. At t is wavelengt , t e absorption may be due to t e band gap energy (E_{bg}) of t e valence band (V_B) and conduction band (C_B) for ZnO-NPs based on t e equation below, w ere was t e bound electron in V_B for ZnO-NPs always trends to be raised to CB by absorbing t e energy from t e ultraviolet lig t.

$$E_{bg} = \frac{c}{\lambda}$$

W ere, = Planck's constant (6.63 x 10^{-34} m^2 kg/s), c = speed of lig t (3.00 x 10^8 m/s), λ = absorption wavelengt in UV region (362 nm).
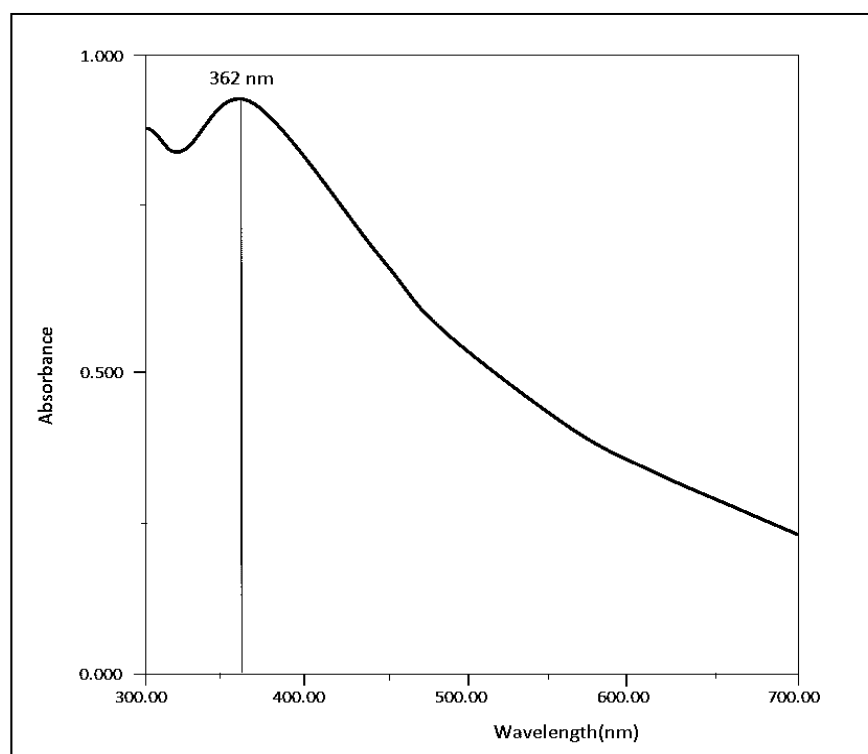
Figure 3-3: UV-Visible spectra of the synthesized ZnO-NPs.

To evaluate t e effect of t e boiling time to preparation of *Z.- spina christi* leaves extract, t e dry leaves were boiled for 10, 15, and

20 minute respectively. T e UV-Vis spectrum of synt esized ZnO-NPs was measured at different times. Results noticed t e boiled time did not significantly impact on t e yield of ZnO-NPs and t ey are very little difference in t e formation of ZnO-NPs in 10 and 20 minutes boiling time. W ereas, 15 minutes boiling time increased t e formation of ZnO-NPs (Figure 3-4). After 15 minutes of boiling, slig tly declined t e yield of ZnO-NPs due to t e possibility of t e dissociation of some p ytoc emical constituents. In addition, t e longer boiling time causes more consumption of energy, t erefore t e suitable boiling time for preparation of t e aqueous extract of *Z.s-spina christi* leaves was selected as 15 minute for t e following experiments [191].
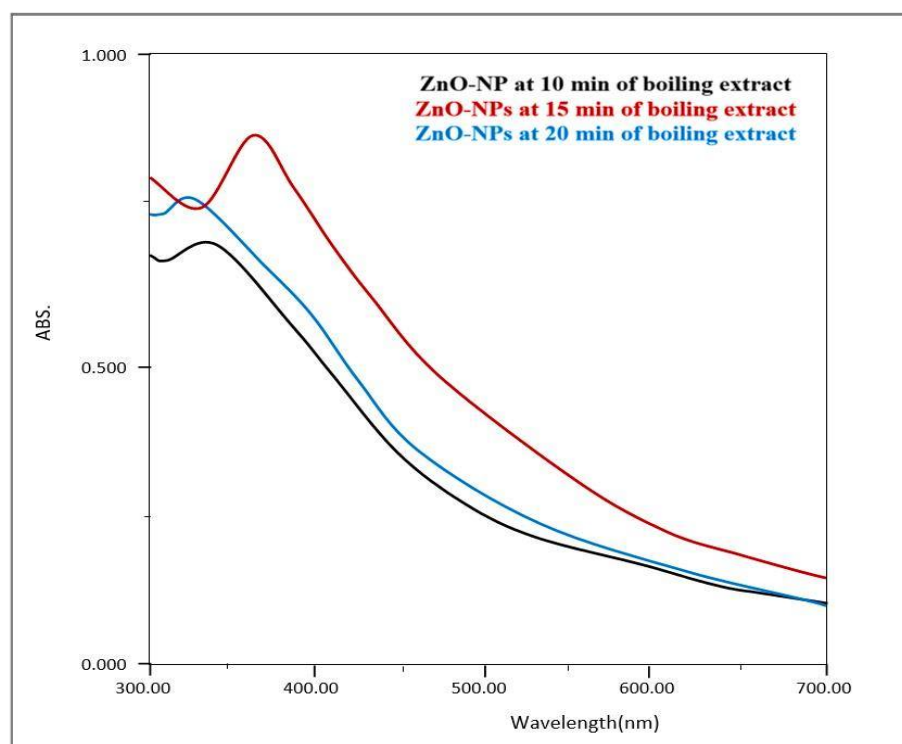
Figure 3-4; UV-Visible spectra of the synthesized ZnO-NPs at different boiling times of extract.

T e different volume of 15 minutes boiled leaves extract (5, 10 and, 15 mL) respectively were added to 90 mL of 1 mM Zn $(NO_3)_2.6H_2O$ solution. UV-visible spectra s owed an increase in t e absorbance intensity. Uv-visible absorption spectra of ZnO-NPs were measured at different volume of *Z.-spina christi* leaves extract. Figure

74

3-5 s ows t e best volume was 10 mL of *Z.-spina christi* leaves extract t an 5 and 15 mL and t e nanoparticles synt esis occurred faster. Additionally, t e maximum absorbance indicates t e complete reducing of zinc ions and rapid synt esis of ZnO-NPs.
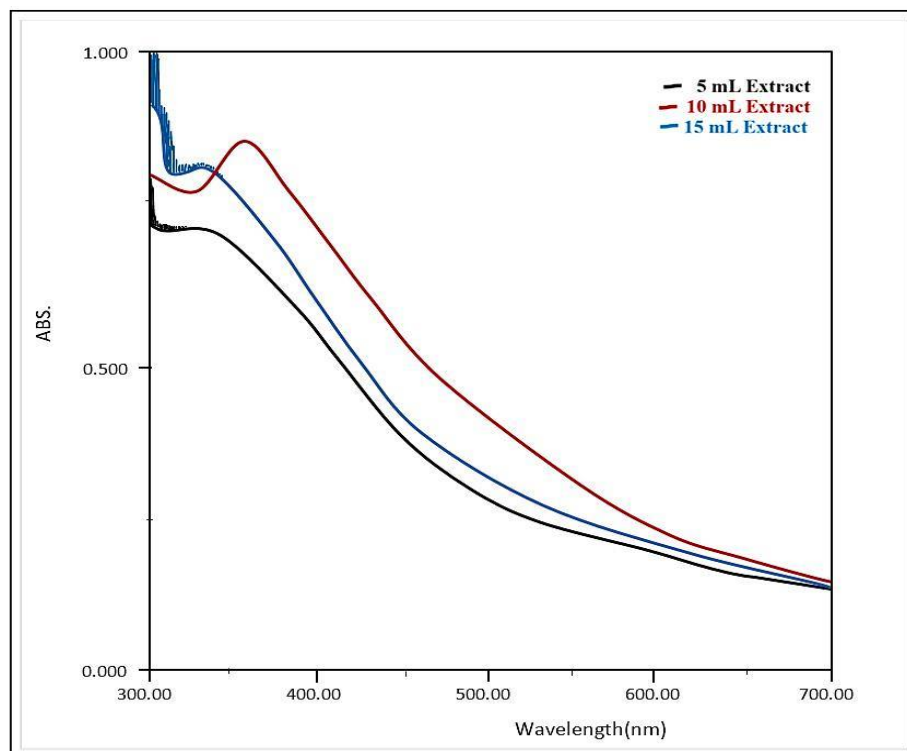
Figure 3-5: UV-Visible spectra of ZnO-NPs at different volumes of extract.

T e quantity of p ytoc emicals present in t e plant leaf extract as a significant role in t e synt esis of metallic nanoparticles. Also, varying t e amount of t e leaf extract in t e reaction medium mig t significantly effect on t e s ape of t e prepared nanoparticles [192]. W en t e content of *Z.-spina christi* leaves was increased, t e yield of ZnO-NPs gradually increased and preventing nanoparticles from aggregating as well as t eir stability was increased due to t e increase in t e flavonoid, p enolic, and ot er plant components [193]. Finally, w en using a large amount of plant extract, p ytoc emicals strongly confirmed t e reduction reaction and function as stabilizing and capping agents [194].

T e concentration of $Zn (NO_3)_2.6H_2O$ as a significant effect on t e green synt esis of ZnO-NPs. If t e concentration of Zn

$(NO_3)_2.6H_2O$ increased t e absorption would ave increased. However, t e concentration 1 mM is considered optimal for t e ZnO-NPs synt esis. Also, increasing t e $Zn(NO_3)_2.6H_2O$ concentration resulted in an increase in t e size of nanoparticles. Moreover, t e 2 mM and 3 mM concentrations s owed t e lowest absorption intensity and no clear peaks (Figure 3-6). T e morp ology and crystal size of ZnO-NPs based on t e concentration of metallic salt precursors, and capping agents (plant extracts) used. So, t ese parameters play t e principal role in t e synt esis of ZnO-NPs and ot er nanostructured compounds [195]. T ese results confirmed t at concentrations of $Zn(NO_3)_2.6H_2O$ and plant extract largely affect t e crystallite size and t e morp ology of t e ZnO-NPs [196]. Various researc ers were reported t at t e synt esis of nanoparticles utilizing low concentration of metallic precursor always result in t e formation of nanoparticles wit smaller crystallite size [197], [198].
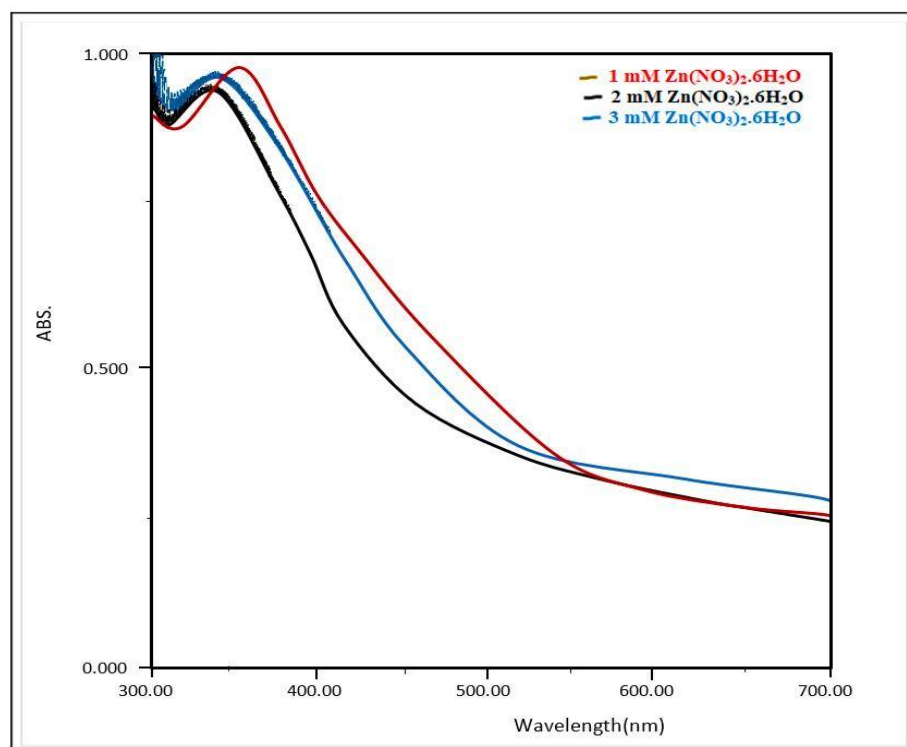
Figure 3-6: UV-Visible spectra of ZnO-NPs at different concentrations of $Zn(NO_3)_2.6H_2O$.

T e green synt esis of t e ZnO-NPs from t e Z.-spina c risti leaf extract was performed at different pH (5-10) using litmus paper (a

strip of t is paper is immersed in a solution w ose pH is needed to be determined, if t e strip is red (at any pH less t an 5) and if blue (at any pH greater t an 8), t at assist of t e laboratory tec nician to determine w et er a solution is acidic or basic. So, pH of t e reaction medium defines t e structural types of t e synt esized ZnO-NPs [199]. T e findings s owed t at t e absorbance of t e formed ZnO-NPs was increased gradually from pH 8.0 to 9.0, w ile decreased between pH 9.0 and 10.0. It was found t at at pH 9.0, t e c aracteristic absorption peak of ZnO-NPs observed at 362 nm (Figure 3-7), suggested t at at pH 9.0, w ic indicated t e metal oxide reduction and zinc nitrate exa ydrate was converted to ZnO-NPs w en be t e reaction medium at alkaline condition t at was more favorable to t e synt esis of ZnO-NPs t an acidic or neutral condition. Finally, w en t e pH was lower lead to t e decreases in ZnO-NPS production.

T e crystallite size and surface morp ology strongly depended on t e pH of reaction mixture t roug t e synt esis process, due to t e pH was c anged t e electrical c arge of molecules and will be affect t eir reduction [200]. T roug t e synt esis of ZnO-NPs in an acidic medium (at pH 5 and 6), t e ydroxyl ion (OH^-) is less in t e solution w ic limits ydrolysis and condensation processes, resulting in t e smaller aggregates [201]. T e decrease in t e crystallite size of ZnO-NPs in an acidic medium was due to t e dissociation of t e crystal structure of ZnO-NPs [202]. W en t e pH of t e solution reac ed 7 (neutral), ions concentrations of ydrogen (H^+) and ydroxyl (OH^-) are

$^-$

are ig , causing a strong attraction between Zn^+ and OH^- ions; t en,

$^-$ is ig in a solution leading to t e formation of intermediate products suc as zinc ydroxide

77

$(Zn(OH)_2)$ (see eq. 2) and salt-containing tetra ydroxozincate ion $([Zn(OH)_4]^{2-})$ (see eq. 3). T e drying of ZnO-NPs product in t e oven, t en calcination it in t e furnace lead to t e formation of good crystallite sizes of ZnO-NPs [204]. Similar results were reported in t e study effect of t e pH (5.0–10.0) on t e synt esis of ZnO-NPs using *Aloe vera* skin extract [205] and found t at t e optimum pH required for t e biosynt esis of ZnO-NPs was 8.0. T erefore, in t e following experiments, ZnO-NPs were synt esized at pH 9.0.
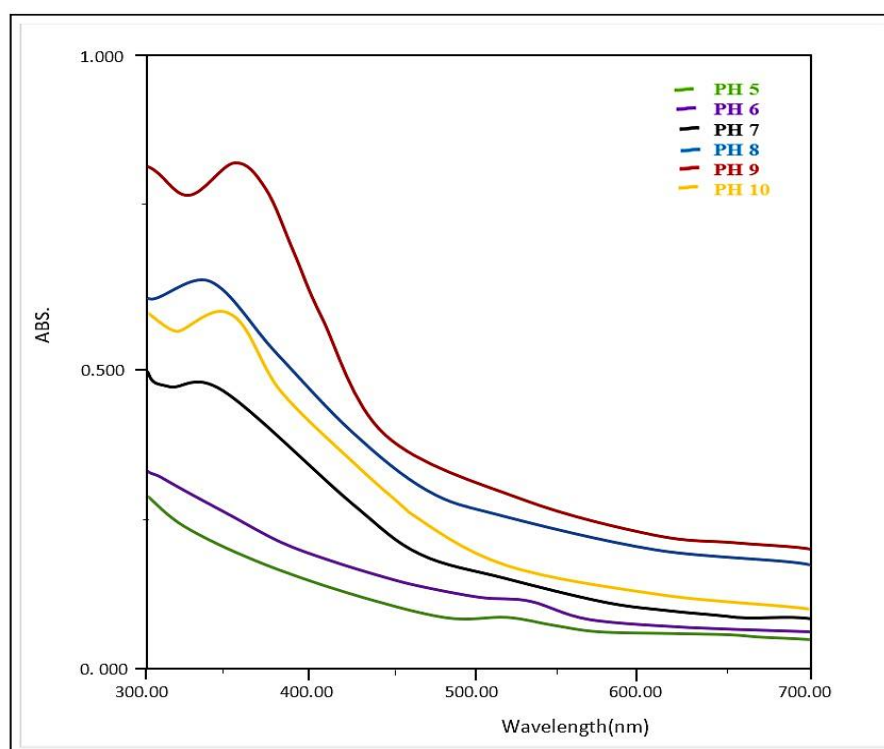
Figure 3-7: UV-Visible spectra of Zn-NPs at different pH.

T e reaction temperature is critical in t e synt esis of ZnO-NPs. T e effect of varying temperatures on ZnO-NPs production is investigated at temperatures ranging from 40 to 70 °C. As s own in figure 3-8, no c aracteristic absorption peaks were observed at lower temperatures of 40 and 50 °C, w ereas c aracteristic absorption peaks were observed at ig er temperatures (60 and 70 °C). In comparison wit ot er temperatures. T e maximum synt esis of ZnO-NPs was observed at 60 °C, and a s arp peak was appeared by a UV-vis

spectrop otometer at 362 nm. On t e ot er side, it is clear t at wit t e
rise in temperature from 60 ˚C, t e absorption bands of ZnO-NPs
s owed similar patterns wit ig and extremely broad peaks.

T e reaction temperature ad a considerable influence on t e
synt esis of ZnO-NPs, W en t e temperature was c anged to 60°C, t e
typical absorption peak at 362 nm was detected. T e ig er t e
activation energy of molecules, t e faster t e reaction rate, resulting in
a decrease in t e size of t e nanoparticles formation of monodispersed
smaller-sized nanoparticles. UV-visible spectroscopy findings from t e
synt esized ZnO-NPs at various temperatures revealed peaks at 376
nm, 370 nm, 362 nm, and 348 nm for 40˚C, 50˚C, 60˚C, 70˚C
respectively (Figure 3-8).
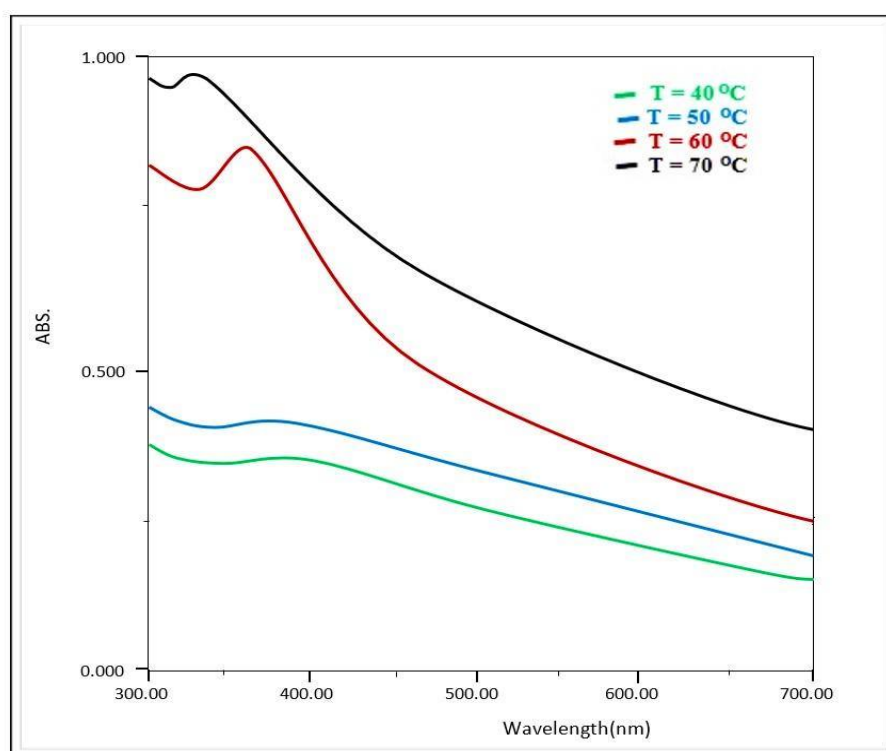
Figure 3-8: UV-Vis spectra of ZnO-NPs at different temperatures

T e synt esized ZnO-NPs were optimized by several parameters,
Z.-spina christi leaves were boiled wit de-ionized water for 15
minutes, 10 mL leaves extract, t e concentration of Zn $(NO_3)_2.6H_2O$
solution was (1 mM), pH of t e mixture adjusted at 9, and t e

temperature of t e mixture at 60 ^{o}C wit magnetic stirrer for 2 ours, all t ese conditions were t e best and selected for t e biosynt esis of ZnO-NPs. T ese conditions were c osen due to t e color c ange of t e reaction mixture and increases of t e absorbance intensity t at indicated t e increase of ZnO-NPs production.

In t e present study, t e absorption peak was observed in t e UV-Visible spectroscopy at wavelengt 362 nm (Figure 3-3), w ic was consistent wit bot [206], w o found t at ZnO-NPs ave t e absorption peak at 368 nm for t e smaller size of crystals, and wit [207], w o revealed a blue s ifted (13 nm) absorption wavelengt t an t e bulk. Based upon t ese reports t e absorption of t e bulk-ZnO appeared near 375 nm and a blue s ift (6 nm) could be observed for nanoparticles wit a smaller size around 369 nm. T erefore, t e c aracterization by UV-visible spectroscopy strongly supported t e green synt esis of ZnO-NPs using t e *Z.-spina christi* leaves extract.

3-3-2 Fourier Transform Infrared Spectroscopy (FT-IR)

Identifying and comparison of functional groups between t e synt esized ZnO-NPs using *Z.-spina christi* leaves extract and t e *Z.-spina Christi* leaves extract alone is s own in figure 3-9. FT-IR spectrum of *Z.-spina christi* leaves extract alone (Figure 3-9a) s owed t e broad band observed at 3412.15 cm^{-1} corresponds to O-H stretc ing of p enolic and alco olic compounds as well as carbo ydrates [208]. Besides, t e peak at 2918.06 cm^{-1} related to t e stretc ing vibrations of C-H of alip atic ydrocarbons c ains, 1642.34 cm^{-1} (C=O stretc ing vibration) related to flavonoid and amino acids or carboxylic acid (COO^{-}) [209]. Moreover, 1454.10 cm^{-1} (O–H bending vibrations), 1398.28 cm^{-1} (C–O stretc ing of t e ester group), 1234.25 cm^{-1} (C–O asymmetric stretc ing in cyclic polyp enolic compounds), and 1083.05 cm^{-1} to 681.70 cm^{-1} indicating t e presence of (C–N stretc) aromatic

amines and alkyl alides, respectively [210]. T e vibrational bands above were mostly found in t e *Z. spina-Christi* leaves extract. T ese peaks disappeared or s ifted to lower frequency indicating t e successful participation of p ytoc emicals compounds presented in t e *Z. spina-Christi* leaves as reducing and stabilizing agents in t e synt esis of ZnO-NPs, w ic facilitates t e conversion of metal ions to metal-NPs. T is is s own by t e peak for t e s ifted carbonyl groups (C=O) at 1612.48 cm^{-1}. T e s ifted peaks were, 3412.15 cm^{-1} to 3385.38 cm^{-1}, 2918.06 cm^{-1} to 2784.26 cm^{-1}, 1642.34 cm^{-1} to 1630.45 cm^{-1}, 1454.10 cm^{-1} to 1446.92 cm^{-1}, 1398.28 cm^{-1} to 1352.71 cm^{-1} and 1083.05 cm^{-1} to 972.13 cm^{-1}. Furt ermore, t e significant vibration of stretc ing peaks appeared at 491.75, 443.22, and 428.07 cm^{-1} (Figure 3-9b), corresponding to t e Zn-O vibrational stretc ing t at furt er confirmed t e formation of ZnO-NPs.

T e FT-IR spectrum validates t e production of ZnO-NPs by comparing t e positions and intensities of peaks in *Z.-spina christi* leaf extract to t ose generated in ZnO-NPs. Results demonstrated t at t e band locations and absorption intensities from t e plant extract peak ad c anged and t at new peaks appeared in t e FT-IR spectra of t e produced ZnO-NPs. T e observed spectra suggested t at biological substances produced by *Z.-spina christi* leaf extract were involved in t e reduction and capping of ZnO-NPs. In t is work, Zn^{2+} is assumed to react wit biological components found in *Z.-spina christi* leaves extract, suc as flavonoids, p enolic compounds, alkaloids, amino acids, tannins, and carbo ydrates, to stimulate t e production of ZnO-NPs and act as a capping and stabilizing agent. Finally, peaks at 491.75, 443.22, and 428.07 cm^{-1} in spectrum of t e synt esized ZnO-NPs matc to t e stretc ing vibration of Zn-O bond t at are absent in *Z.-spina christi* leaf extract, strongly confirmed t e formation of ZnO-NPs. T is finding was consistent wit previous researc , w ic found

multiple Zn-O bands at 416.14, 515, and 618 cm^{-1} [211], [212]. As a result, biological synt esis does not require any c emical sources for stabilizers, w ic is an advantage over c emical synt esis.
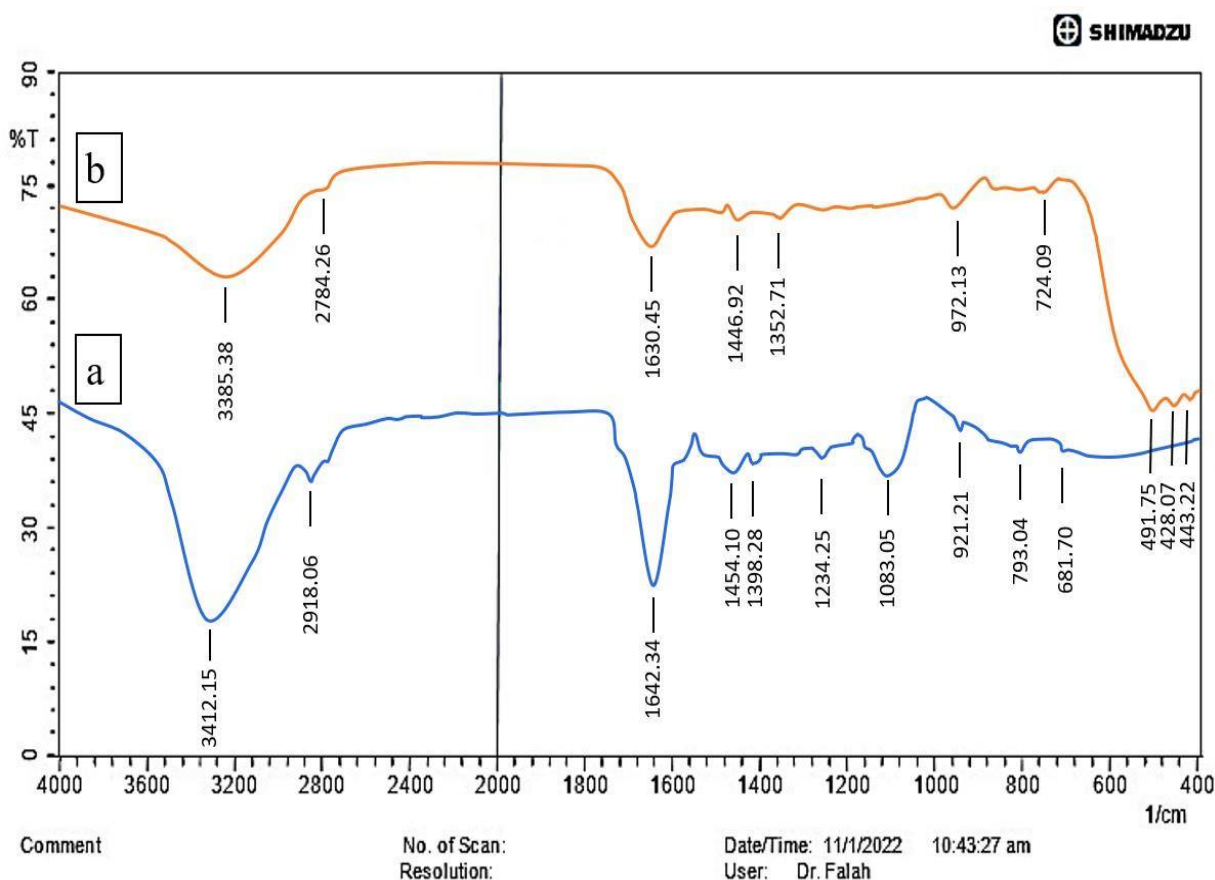
Figure 3-9: FT-IR spectrum, (a) *Z.-spina christi* leaves extract (b) ZnO-NPs.

3-3-3 X-ray Diffraction (XRD)

T e synt esized ZnO-NPs were studied and confirmed using t e XRD tec nique to determine t eir p ases, structures, and crystal size. T e observed diffraction peaks of t e biosynt esized ZnO-NPs in terms of intensities and positions were at 2θ 31.77° (100), 33.21° (002), 36.59° (101), 47.84° (102), 56.24° (110), 63.19° (103), 66.20 $^{\circ}$ (200), 67.73° (112), 69.09° (201) 73.55° (004), and 76.67° (202) as s own in figure 3-10 w ic strongly detected t e exagonal wurtzite structure of ZnO-NPs. Based on t e FWHM of most intense diffraction peaks (100%), (66%), and (41%) at 2 θ wit 36.59° (101), 33.21° (002), 31.77° (100) respectively. Using Debye-Sc errer equation, average

crystalline size was calculated to be 38.177 nm confirming t e nano size for t e synt esized ZnO-NPs.

As an X-ray passes t roug a particle crystal, a diffraction pattern is formed, w ic gives information on t e atomic arrangement wit in t e crystals. Peaks in t e XRD diffractogram (Figure 3-10b) appeared to be broadening, revealing t at per aps t e ZnO-NPs were formed on t e nanoscale. W en compared wit t e Joint Committee on Powder Diffraction Standards (JCPDS) card No. 36-1451 (Figure 3-10a), t e most intense diffraction peaks found were indexed as t e exagonal wurtzite structure of ZnO-NPs wit   ig crystallinity. As illustrated in Figure 3-10b, all of distinctive peaks were found to be ZnO-NPs, and no suc impurities were found in produced ZnO-NPs. T ese findings were consistent wit earlier researc [213], [214].
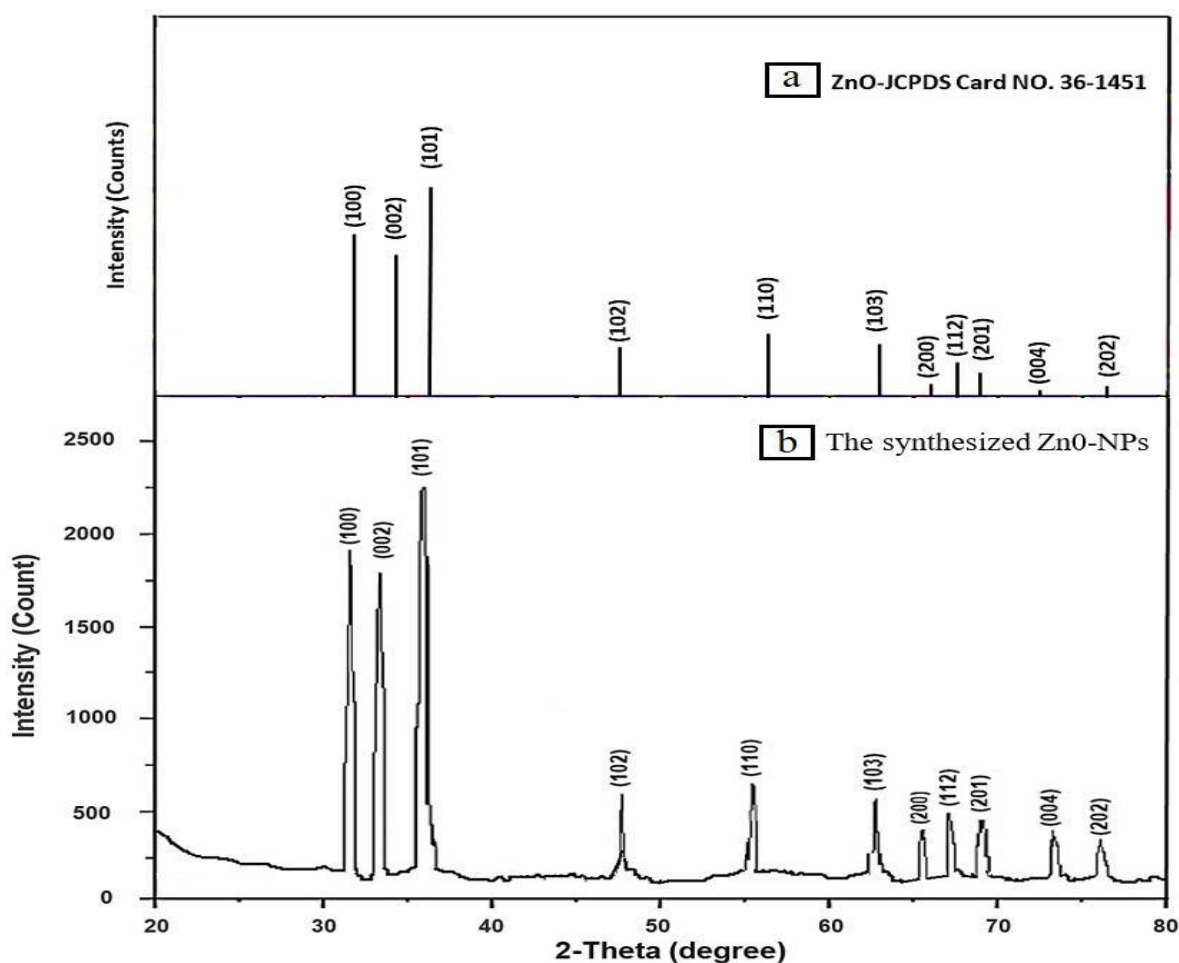
Figure 3-10: XRD diffractogram, (a) ZnO- card No. 36-1451. (b) the synthesized ZnO-NPs.

3-3-4 Transmission Electron Microscopy (TEM)

T e size distribution and morp ological c aracters of ZnO-NPs observed under TEM analysis (Figure 3-11a, b) were uniform in size. Agglomeration was common, due to t e ultra-small size and ig surface energy of ZnO-NPs. Also, TEM micrograp s s owed most of t e ZnO-NPs were in a sp erical s ape. T e istogram of t e size distribution of ZnO-NPs was generated based on TEM data (Figure 3-11c). T e majority of ZnO-NPs measured fell in t e range of 35 - 45 nm in diameter.
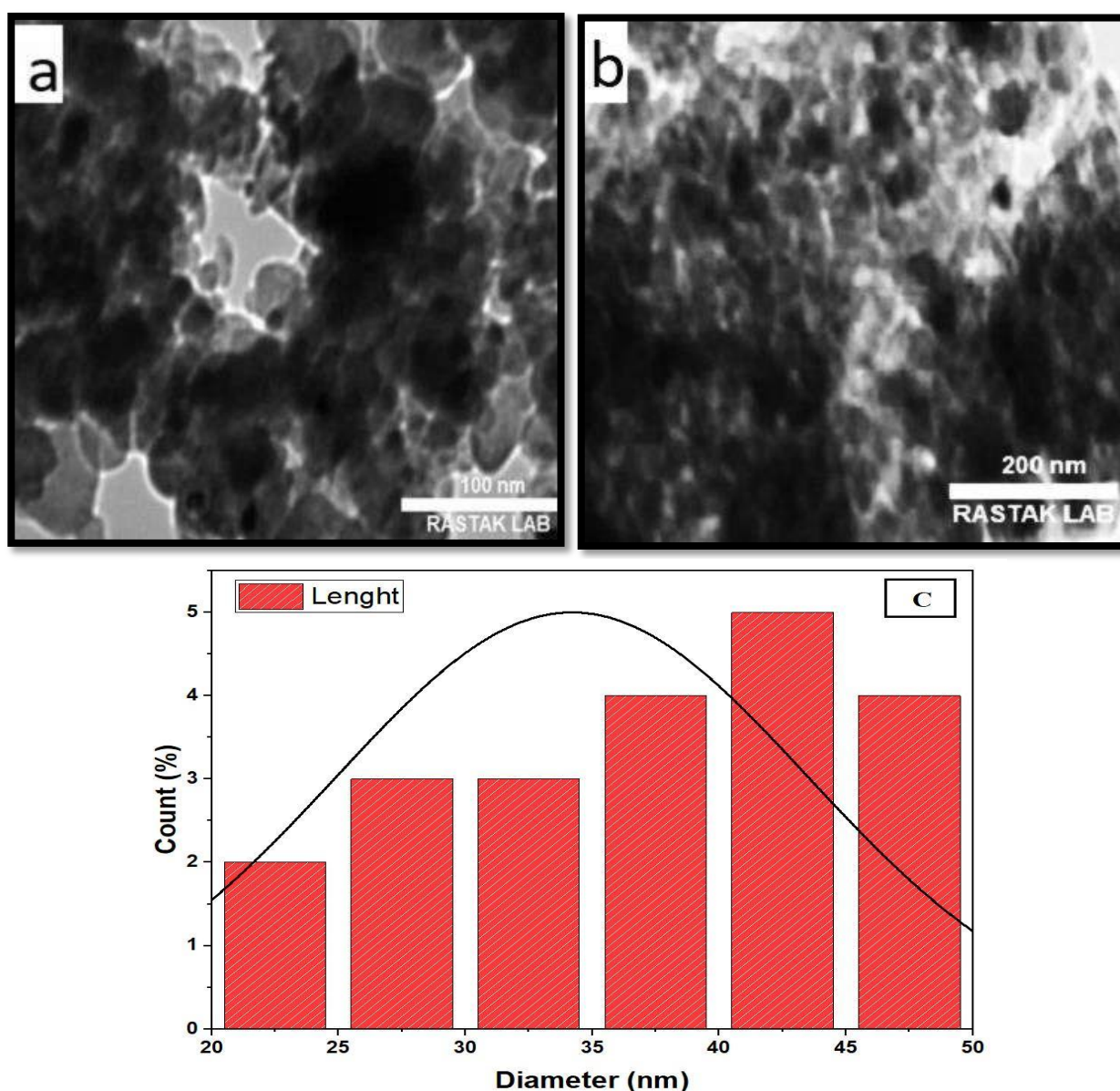
Figure 3-11: TEM micrograph of the synthesized ZnO-NPs. (a) Scale at 100 nm. (b) Scale at 200 nm. (c) The size distribution histogram of ZnO-NPs.

T e TEM met od was used to examine t e biosynt esized ZnO-NPs. T e acquired micrograp s s ow t at t e biosynt esized ZnO-NPs were agglomerated particles t at were sp erical and exagonal in s ape. ZnO-NPs are capped by exagonal molecules and biomolecules. Similar exagonal molecules ave been found in ZnO-NPs synt esis in *Euphorbia jatropha* plants [215]. T e TEM pictures ex ibit nano-crystalline of t e synt esized ZnO-NPs, t is attributed to t e biomolecules presented in *Z.-spina christi* leaf extract t at strongly influenced t e formation of ZnO-NPs. T ese findings are consistent wit earlier publications [216], [217].

3-3-5 Scanning Electron Microscope (SEM)

Under SEM at varying magnifications, observations of images were made to study t e surface morp ology of t e ZnO-NPs t at were synt esized. T e results of t ese observations can be found in Figure 3-12. In Figures 3-12a and b, it was observed t at ZnO-NPs ad a sp erical s ape and were tig tly clustered toget er. Upon analysis of Figure 3-12c, particle diameters were noticed to ave an average size in t e following ranges: D_1 (39.74 nm), D_2 (42.25 nm), and D_3 (48.19 nm), wit an average diameter size equal to be 43.39 nm.

T e SEM image of ZnO-NPs (Figure 3-12) reveals sp erical nanoparticles wit ig density. A large number of ZnO-NPs aggregated and formed nano-like morp ology wit agglomerated particles of various irregular s apes. Aggregation may be exacerbated by t e ig surface energy of ZnO-NPs as well as by t e close distance between nanoparticles. T ese strongly confirm t at t e particles exist in a uniform form, and t is uniformity plays an important role in t e different activities of ZnO-NPs. SEM results in t is study were in agreement wit t e previous studies [218], [219].
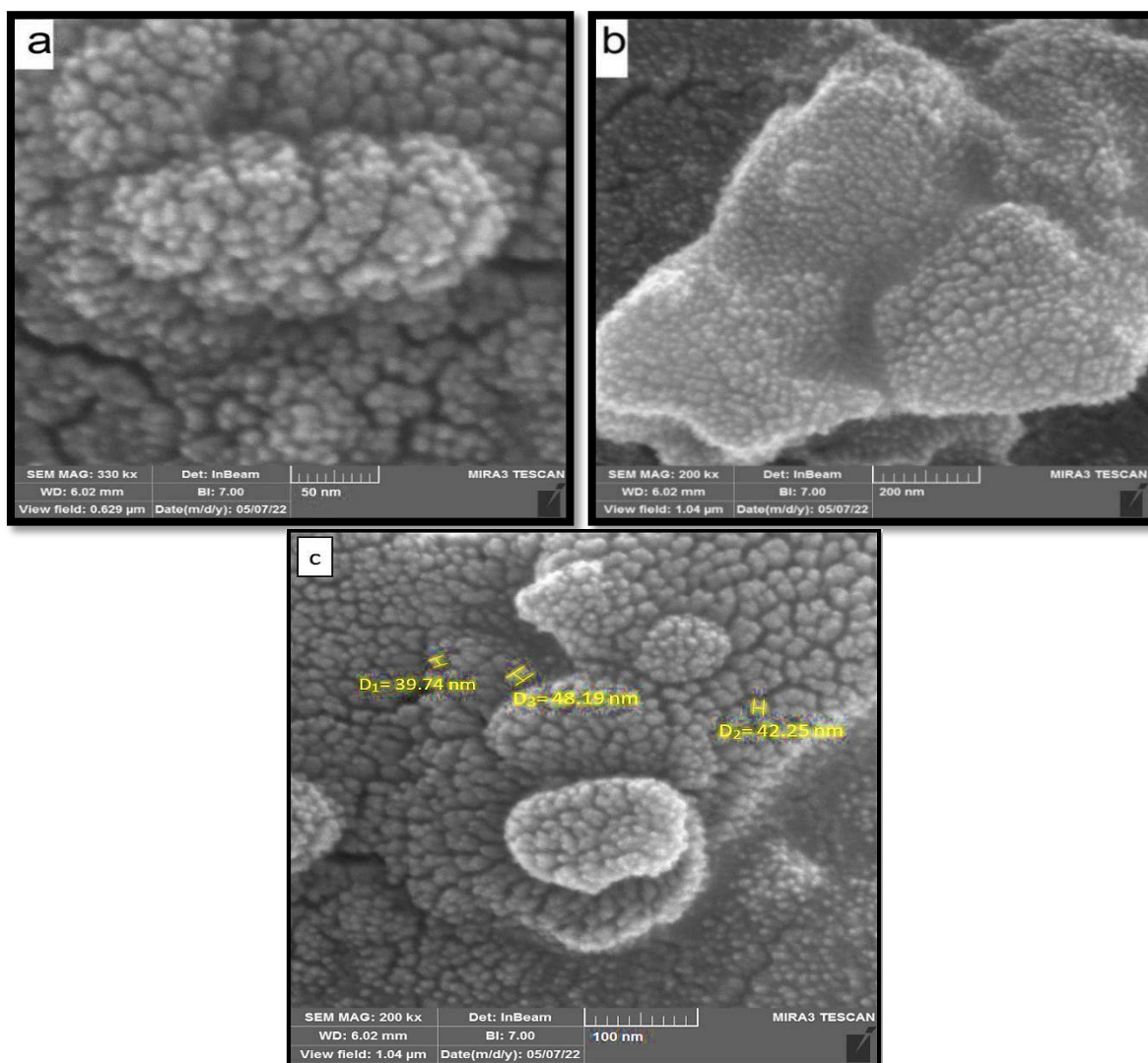
Figure 3-12: SEM images of synthesized ZnO-NPs at different magnifications (a) At 50 nm. (b) At 200 nm. (c) SEM images at 100 nm with the particle size at different average diameters D_1, D_2, and D_3.

3-3-6 Atomic Force Microscopy (AFM)

Atomic force microscopy (AFM) analysis is a basic tec nique and inescapable for all nanoscopic studies was used to determine t e topograp y surface of nanoparticles. Images of AFM for t e synt esized ZnO-NPs (Figure 3-13a, b) s owed t e roug ness and surface morp ology features under AFM micrograp in two-dimension and t ree-dimension. T e micrograp at (5.08x5.08µ) scan (Figure 3-13b) display a uniform surface wit cone like grains covering t e surface of ZnO-NPs. In t is study was determined t e average surface roug ness of t e synt esized ZnO-NPs t at found in t e range of 42 to

86

45 nm due to t e granularity distribution c art (Figure 3-13c). Moreover, AFM for t e ZnO-NPs surface s owed t e t ree-dimension image t at observed regularly in t e composition of film and grains ave a vertical structure on t e crystal axis and are approximately equal. Figure 3-13c s ows t e granularity distribution c art of ZnO-NPs aggregates on t e surface of t e film. T e surface roug ness of ZnO-NPs was increased in t e presence of capping agents due to t e presence of various functional groups in *Z.-spina christi* leaves extract. T ese results were in agreement wit t e previous study [220].
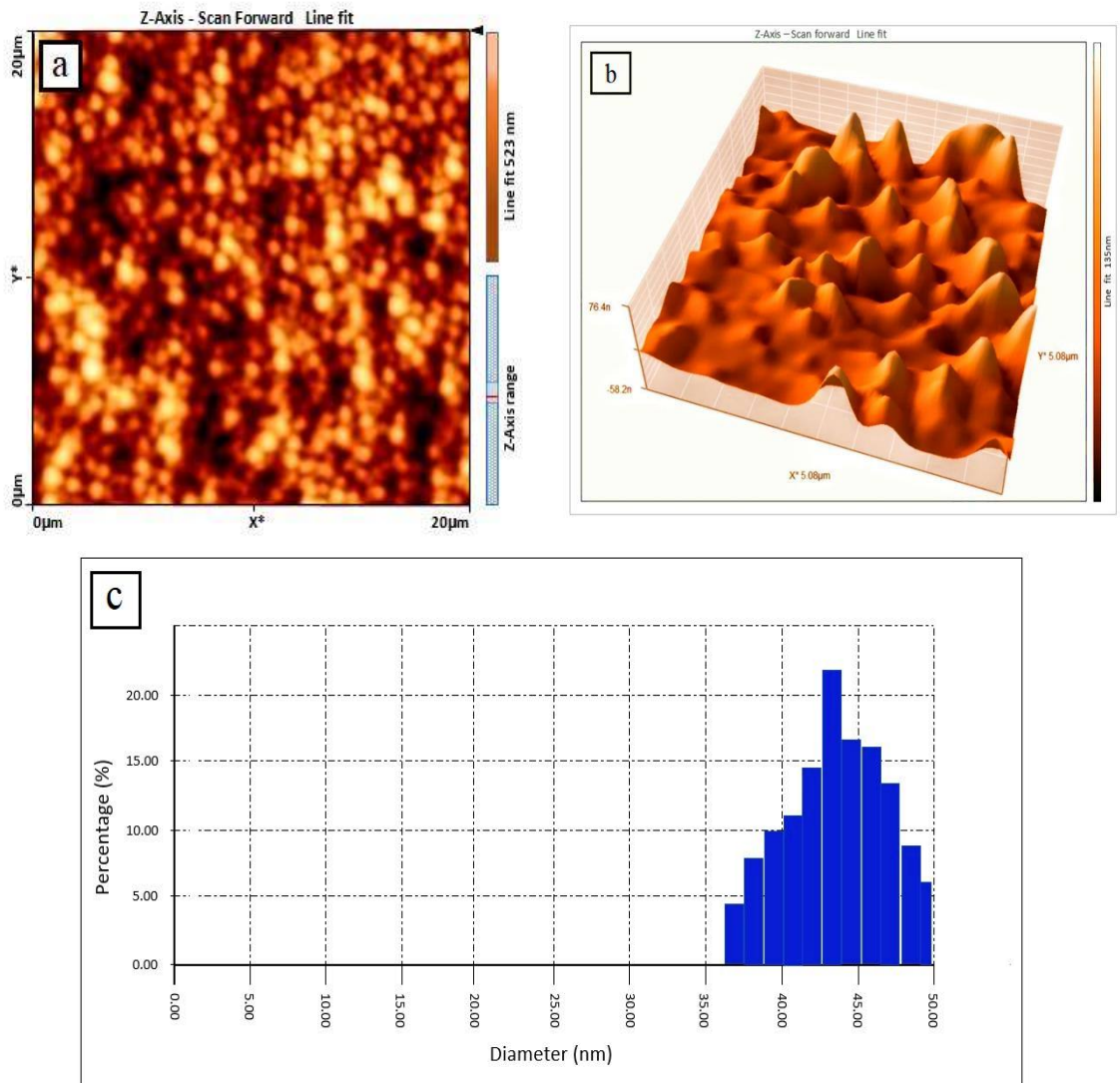
Figure 3-13: AFM analysis of synthesized ZnO-NPs (a) 2-Dimension image (b) 3-Dimension image (c) The granularity distribution chart.

3-4 Biochemical Analysis

T is study evaluated t e main effects of ZnO-NPs and *Z.-spina christi* leaves extract on body weig t, kidney, and, liver bioc emical parameters of male rats exposed to adenine. Healt y male rats were treated wit ZnO-NPs and an aqueous extract of *Z.-spina christi* leaves for sequential 30 days and displayed some important c anges in t e bioc emical parameter levels.

3-4-1 Body Weight Changes

As s own in Table 3-2, rats of t e control group were grown by about (10 ± 2 gm) during t e 30 days of giving diet only (G I) w ereas, t e second group rats (G-II) t at gavaged adenine (100 mg/kg.BW) for t e same period s owed a significant decrease ($P<0.001$) in body weig t about (20 ± 2 gm) in comparison to t e control group. At t e same table s owed t e administration of *Z.-spina christi* leaves extract for t ird group rats (G-III) at a dose (10 mg/kg B.W) significantly slig tly decreased ($P= 0.04$) t eir body weig ts w en compared to t e control group, but t ese weig ts were ig in comparison to adenine group. T e same be avior for t e body weig t of fourt group rats (G-IV) after administrated of ZnO-NPs (10 mg/kg B.W), w ic is ig compared to t e adenine group, but ZnO-NPs was significantly slig tly decrease ($P= 0.04$) t e body weig t w en compared to a control group. Finally, t e fift and sixt group rats (G-V and G-VI) after co-administration of adenine plus *Z.-spina christi* leaves extract or adenine plus ZnO-NPs at a dose (10 mg/kg B.W) respectively, mitigated significantly t e decreasing in body weig ts of rats by compared to adenine group rats, but t ey still lower w en compared wit control group rats ($P<0.001$).

Table 3-2: Effect of ZnO-NPs and *Z.-spina christi* leaves extract on adenine-induced body weight changes.

Period time / Groups	Day-0 Weight (gm)	Week-1 Weight (gm)	Week-2 Weight (gm)	Week-3 Weight (gm)	Week-4 Weight (gm)
G-I	204.19±0.32	206.98±0.35	209.74±1.29	212.16±0.96	215.85±0.48
G-II	204.23±0.05	197.52±0.06	193.03±0.26	187.54±0.73	182.38±1.13
G-III	204.27±0.06	205.14±0.46	207.65±0.57	209.85±0.59	211.50±0.67
G-1V	204.08±0.29	206.13±0.57	208.96±0.62	211.35±0.66	214.69±0.81
G-V	204.25±0.10	201.16±0.16	197.23±1.06	194.41±1.19	191.26±1.64
G-VI	204.36±0.43	203.26±0.64	199.51±0.91	196.84±0.38	194.35±0.59
Tukey Test	0.317	0.422	0.846	0.863	1.513

Notes: Values in the table are expressed as mean ± SD. n=6 male rats in each group, SD: Standard deviation.
G-I: Control group, G-II: Adenine group, G-III: *Z.-spina christi* leaves extract group, G1V: ZnO-NPs group, G-V: Adenine + *Z.-spina christi* leaves extract group, G-VI: Adenine + ZnO-NPs group.

T e first effect of ZnO-NPs and *Z.-spina christi* leaves extract was on t e body weig t of rats wit renal failure caused by adenine t at measured at t e beginning every week until end experiment. Adenine administration to rats resulted in body weig t loss due to t e buildup of uremic toxins, w ic causes inflammation and activation of protein catabolic pat ways, resulting in protein breakdown [221]. Rats after 30 days of treatment wit ZnO-NPs at concentrations of (10 mg/kg BW) s owed a slig t decrease in body weig t; t e decreases may be due to ZnO-NPs at t is dose causing instability in protein degradation and lipid metabolism, resulting in an increase or decrease in body weig t [222], depending on t e effect of dose and treatment period. Moreover, ZnO-NPs may accumulate in numerous animal organs suc as t e kidney and liver, causing alterations in tissue functioning and, as a result, altering metabolic rate. Anot er study found t at giving rats ZnO-NPs at different dosages (5, 50, and 100 mg/kg.BW) for 14 days resulted in a considerable reduction in body weig t [223]. In addition, researc conducted by [224] on Wistar rats treated wit ZnO-NPs at a concentration of (10 mg/kg.BW) revealed a non-significant influence

on body weig t increase, indicating t e absence of azardous indicators and mortality in adult male rats exposed to ZnO-NPs. Table 3-2 s ows t at t ere was a less significant c ange in weig ts in all rats after treatment wit aqueous leaf extract of *Z.-spina christi* at a level of (10 mg/kg.BW). T e weig t loss in t ese rats mig t be attributed to anti-nutritional bioactive components in t e plant extract, suc as tannin, flavonoids and p enolic compounds [225].

3-4-2 Estimation of Kidney Function Tests

Results in Table 3-3 s owed a significant increase ($P<0.001$) in concentrations of uric acid, urea, and creatinine was observed in serums of t e second group rats (G-I) after administrated of adenine in comparison to control group rats. T is increase was significantly reduced after administration of *Z.-spina christi* leaves extract at a dose (10 mg/kg B.W) (G-III) w en compared to t e adenine group, but not completely restored t ese c anges as in t e control group ($P=0.04$). At t e same table, significant increases ($P=0.02$) also were s owed in concentrations of uric acid, urea and creatinine in rats administered wit ZnO-NPs (G-IV) w en compared to control group. Moreover, results as s own in bot groups (G-V and G-VI), co-administration of adenine plus *Z.-spina christi* leaves extract or adenine plus ZnO-NPs, resulted in a significant decrease in uric acid, urea, and creatinine concentrations w en compared to adenine group, but t eir values still in raises by comparison to t e control group rats ($P<0.001$).

As s own in table 3-3, w en compared to t e control group rats, t ere was an increase in serum levels of uric acid, urea, and creatinine in t e second group (G-II). T ese findings were c aracterized by renal dysfunction, as evidenced by congestion and necrosis of t e renal glomeruli, as well as degenerative c anges and necrosis of t e normal renal tubular epit elium lining renal tubules [226], because t ese

products accumulate in t e bloodstream and formation of 2,8-di ydroxyadenine crystals, result in ig accumulation of different guanidino compounds suc as (guanidino uric acid and met yl-guanidine) and nitrogenous compounds wit renal failure [227]. T e espouser of rats (G-III) and (G-IV) respectively to *Z.-spina christi* leaves extract or ZnO-NPs revealed a less significant difference in t e levels of uric acid, urea, and creatinine in comparison to t e control group, t is indicates t at dosage at t e concentration (10 mg/kg B.W) for *Z.-spina christi* leaves extract and ZnO-NPs ave no negative effect on t e kidneys. However, serum levels of uric acid, urea, and creatinine s owed a significant decrease after t e administration of adenine plus ZnO-NPs (10 mg/kg B.W) or adenine plus *Z.-spina christi* leaves (10 mg/kg B.W) at t e same time in bot fift and sixt groups rats (G-V and G-VI) by comparison to adenine group and a significant increase wit comparison to control group rats, w ic evaluated t at t e synt esized ZnO-NPs and *Z.-spina christi* leaves mig t be able to avoid t e damaged renal excretion, i.e., kidney alterations wit adenine-induced renal failure. T ese results were in agreement wit previous studies [228–230].

Table 3-3: Effect of ZnO-NPs and *Z.-Spina Christi* leaves extract on kidney function parameters in in adenine-exposed male rats.

Parameters / Groups	Uric acid mg/dL	Urea mg/dL	Creatinine mg/dL
G-I	1.24±0.08	13.66±1.10	0.26±0.03
G-II	3.22±0.19	105.88±1.96	0.84±0.05
G-III	1.31±0.09	14.05±1.37	0.29±0.03
G-1V	1.68±0.10	16.07±2.20	0.31±0.04
G-V	2.48±0.15	73.41±0.93	0.51±0.01
G-VI	2.73±0.14	87.03±1.60	0.68+0.01
Tukey Test	0.094	1.705	0.023

Notes: Values in the table are expressed as mean ± SD. n=6 male rats in each group, P ≤0.05 (One-Way ANOVA followed by Tukey post-hoc test). SD: Standard deviation. G-I: Control, G-II: Adenine, G-III: *Z.-spina christi* leaves extract, G1V: ZnO-NPs, G-V: Adenine + *Z.-spina christi* leaves extract, G-VI: Adenine + ZnO-NPs.

3-4-3 Estimation of Liver Function Tests

T e results of ALT, AST, and ALP were statistically significant increases ($P<0.001$) in t eir activities in t e adenine group (G-II) compared wit t e control group (Table 3-4). T e result of t e t ird group rats (G-III) administrated *Z.-spina christi* leaves extract (10 mg/kg B.W) s owed a significant decrease in enzymes activities of ALT, AST, and ALP in compared to t e adenine group but were slig tly significant differences in comparison to t e control group ($P=0.003$), w ile t e fourt group of rats (G-IV) administered ZnO-NPs (10 mg/kg B.W) s owed decreasing significantly in t eir enzymes activities compared to t e adenine group but t ey were still ig as compared to t e control group ($P<0.001$). Furt ermore, in bot fift and sixt group rats (G-V and G-IV) co-administration of adenine plus *Z.-spina christi* leaves extract (10 mg/kg B.W) or adenine plus ZnO-NPs (10 mg/kg B.W) caused a considerable decrease in activities of ALT, AST, and ALP w en compared wit adenine group, but still ig er w en compared to t e control group ($P<0.001$).

Table 3-4: Effect of *Z.-spina christi* leaves extract and ZnO-NPs on liver function parameters in adenine-exposed male rats.

Parameters Groups	ALT (U/ml)	AST (U/ml)	ALP (U/ml)
G-I	33.94±1.15	103.28±2.17	122.36±2.64
G-II	91.60±3.57	179.46±3.89	213.55±4.18
G-III	36.35±1.23	108.03±2.36	125.14±2.79
G-1V	44.71±1.26	117.67±2.51	132.80±2.85
G-V	60.44±2.28	144.53±2.92	183.49±3.92
G-VI	72.11±2.63	158.80±3.27	197.03±4.05
Tukey Test	2.735	3.189	4.096

Notes: Values in the table are expressed as mean+SD. n=6 male rats in each group, SD: Standard deviation.
ALT= Alanine aminotransaminase, AST= Aspartate aminotransaminase, ALP= Alkaline phosphatase. G-I: Control group, G-II: Adenine group, G-III: *Z.-spina christi* leaves extract group, G1V: ZnO-NPs group, G-V: Adenine + *Z.-spina christi* leaves extract group, G-VI: Adenine + ZnO-NPs group.

T e measurement of enzyme activities ALT, AST, and ALP play a significant role in diagnosis, disease investigation, and t e assessment of t e plant extracts or nanoparticles for safety and toxicity risk. T ese enzymes considered in t is study are useful markers to damage liver tissues. T e ALT is found in many organs, particularly in t e liver for diagnostic use, w en its elevation indicates epatocyte damage and release into t e plasma. T e AST is not a specific enzyme for t e liver only but is also founded in several organs suc as t e kidney, eart, brain, and skeletal muscle, t ese organs w en are destroyed, AST is released. ALP is t e indication enzyme for t e plasma membrane and is elevated in a variety of tissues. It is often used to estimate t e safety of plasma membranes and as a marker for epatobiliary diseases [231]. Tissue damage is usually associated wit t e release of enzymes from t e affected organ or tissue into circulation. Exposure of rats to adenine led to cytotoxicity in a time and dose dependent as a consequence of t e oxidative stress, t e peroxidation of lipids, and t e damage of carbo ydrates, proteins, and cell membranes. Adenine caused a significant increase in serum levels of ALT, AST, and ALP (Table 3-4). Increases in serum ALT and AST activities ave been indicated in conditions involving necrosis of epatocytes, w ile increases in serum ALP activity ave been implicated in epatobiliary diseases. T e decrease in activities of ALT, AST, and ALP in t e liver of rats were administered wit *Z.-spina christi* leaves extract or ZnO-NPs (Table 3-5) w en compared to t e adenine group mig t be according to t e inactivation of t ese enzymes by t e extract or its metabolites, w ic could ave suppressed t e synt esis of t e enzymes, also suggested t at ZnO-NPs mig t be interaction wit key molecules in membranes (enzymes inclusive) and t en in ibited or denature t em. Furt ermore, in bot t e treated groups (fift and sixt) after co-administration of adenine plus *Z.-spina christi* leaves extract or adenine plus ZnO-NPs

s owed a significant decrease in serum ALT, AST, and ALP activities indicating t e strong evidence of t e protective effect by t e plant extract and ZnO-NPs. T e results s owed a significant decrease in ALT, AST, and ALP enzymes activities in t e fift group rats w o were treated wit *Z.-spina christi* leaves extract at a dose (10 mg/kg B.W), and sixt group rats were treated wit ZnO-NPs at a dose (10 mg/kg B.W), t ese w en comparison wit adenine group rats, and t ese results agree wit [232]. T e alteration in t e activities of t e enzymes in t is study mig t be due to t e biological activity of ZnO-NPs and p ytoc emicals (main components of *Z.-spina christi* leaves) in t e doses given for 30 days are capable to decreasing serum ALT, AST, and ALP.

3-4-4 Effect on antioxidants statues

Table 3-5 s owed t e results of antioxidant levels, SOD, CAT, TAC, GSH, MDA, and NO. Administration of adenine to t e second group rats (G-II) lead to a significant reduction in levels of SOD, CAT, TAC, and GSH, t is reduction was accompanied by a significant elevation in MDA and NO levels in comparison to t e control group (P<0.001). As s own in Table 3-5, for bot t ird and fourt groups rats (G-III and G-IV) after administration of *Z.-spina christi* leaves extract (10 mg/kg B.W) and ZnO-NPs (10 mg/kg B.W) respectively, t ere are significant increases in SOD, CAT, TAC and GSH levels w en compared to adenine group rats (G-II), but significantly less decreased in comparison to t e control group rats (G-I) (P= 0.003), w ile levels of MDA and NO significantly reduced w en compared to t e adenine group rats, but still less ig in comparison to control group rats (P<0.001). Finally, rats of t e bot fift and sixt groups (G-V and G-VI) were co-administrated adenine plus *Z.-spina christi* leaves extract (10 mg/kg B.W) and adenine plus ZnO-NPs (10 mg/kg B.W)

respectively, resulted in a significant increase in SOD, CAT, TAC and GSH levels w en compared to an adenine group rats (G-II), but still lower w en comparison to t e control group (*P*=0.04), t is increase was accompanied wit a significant reduction in MDA and NO levels w en compared to adenine group, but were significantly increased in comparison to control group rats (*P*<0.001).

Table 3-5: The Effect of *Z.-spina christi* leaves extract and ZnO-NPs on the antioxidant levels in in adenine-exposed male rats.

Parameters / Groups	SOD (U/ml)	CAT (U/ml)	TAC (µmol/ml)	GSH (µmol/ml)	MDA (µmol/ml)	NO (µmol/ml)
G-I	17.15±0.44	10.83±1.37	17.56±0.80	13.48±0.38	0.49±0.02	0.93±0.01
G-II	8.92±0.14	1.69±0.03	6.84±0.87	5.40±0.05	4.16±0.41	8.54±0.21
G-III	15.23±0.41	9.15±0.69	15.45±0.17	11.35±0.29	0.62±0.10	1.47±0.02
G-1V	13.08±0.33	10.13±1.25	16.94±0.84	9.74±0.26	0.54±0.06	1.95±0.02
G-V	10.73±0.28	2.74±0.25	9.71±0.07	7.55±0.10	3.53±0.37	4.52±0.04
G-VI	9.51±0.24	6.75±0.33	13.52±0.61	6.19±0.08	2.29±0.30	6.31±0.10
Tukey Test	0.479	0.917	0.642	0.355	0.238	0.294

Notes: Values in the table are expressed as mean+SD. n=6 male rats in each group, P ≤0.05 (One-Way ANOVA followed by Tukey post-hoc). SD: Standard deviation, SOD: superoxide dismutase, CAT: catalase, TAC: total anti-oxidant capacity, GSH: reduced glutathione, MDA: malonaldehyde and NO: nitric oxide.
G-I: Control group, G-II: Adenine group, G-III: *Z.-spina christi* leaves extract group, G1V: ZnO-NPs group, G-V: Adenine + *Z.-spina christi* leaves extract group, G-VI: Adenine + ZnO-NPs group.

T e results obtained from antioxidant status as s own in table 3-5, rats were exposed to adenine, t e levels of SOD, CAT, TAC, and GSH were significantly decreased in serum, t is is associated wit t e significant elevation of MDA and NO levels. T erefore, an increase in MDA and NO is considered a marker of oxidative damage t at reveals excess of free radical production, w ic is consistent wit t e view t at adenine induces lipid peroxidation. T e antioxidant defense mec anism fails due to t e decreased activities of t e scavenging enzymes, or bot . Increased MDA and NO levels, lipid peroxidation, also decreased SOD CAT, TAC, and GSH levels are associated wit cell damage. T ese results supported [233] and [234] w o reported t at adenine

significantly increased oxidative stress markers and decreased t e activities of antioxidant enzymes in serum.

Rats were treated wit an aqueous extract of *Z.-spina christi* leaves was ameliorated t e c ange in antioxidant statutes. T is amelioration appeared by t e increase in serum levels of SOD CAT, TAC, and GSH levels are associated wit decreasing MDA and NO levels, w ic furt er confirms t eir role against adenine-induced liver damage. T e given of ZnO-NPs s owed approximately a similar activity (Table 3-5), to t at of an aqueous extract of *Z.-spina christi* leaves in adenine-treated rats. Finally, ZnO-NPs protected rats against oxidative damage and liver injury induced by adenine w ic may be due to t e anti-inflammatory and antioxidant properties t at protect cell membranes against oxidative stress, decrease free radicals and increase t e antioxidant enzyme activity as superoxide dismutase (SOD) and CAT levels [233]. Moreover, as s own in table 3, *Z.-spina christi* leaves extract and ZnO-NPs were able to significantly decrease renal failure induced by adenine, but observed t at co-administration of adenine plus *Z.-spina christi* leaves extract (G-V) gave better results w en compared to t e adenine plus ZnO-NPs (G-VI). T is study noted t at an aqueous extract of *Z.-spina christi* leaves improved adenine-induced oxidative injury in t e kidney and liver by its antioxidant and free radical-scavenging properties. T ese results were consistent wit t e previous researc of [236] w o suggested t at t e main mec anism for t e beneficial action of gum acacia in adenine-induced kidney disease via its antioxidant properties. Furt ermore, t e kidney and liver were protected by t e aqueous extract of *Z.-spina christi* leaves against adenine-induced toxicity due to its important contents of flavonoids, p enolic compounds, saponins, and triterpenes, t ese were confirmed by t e previous study [237].

3-4-5 Histological Study

Results of t e istological study of kidney and liver tissues for all groups of rats after 30 days of exposure were performed using two dyes called ematoxylin and eosin (H&E staining), w ic make it easy to see different parts of t e tissues under t e lig t microscope (Figures 3-14 and 3-15).

3-4-5-1 Kidney Section

Figure 3-14 s owed t e results of t e istological study of t e renal tissue from rats for six groups. T e kidneys of control rats (G-I) s owed no marker of damage, normal renal cortexes, normal renal arc itecture, and normal nep ritic tubules (Figure 3-14-K1). Histological markers of group rats (G-II) wit adenine-administered s owed significant acute damage of t e renal tissue. T eir kidneys s owed renal vein congestion and ypertrop y of renal glomeruli, besides necrosis and dilation of normal renal tubular (Figure 3-14-K2). Rats in t e group (G-III) w en t e administration of *Z.-spina christi* leaves extract (10 mg/kg B.W) s owed istological c anges t at involves: t e normal structure of t e renal cortex except t e less degradation of some renal tubules (Figure 3-14-K3). Also, in t e group rats (G-IV) after administrated of ZnO-NPs (10 mg/kg B.W) s owed t at slig tly inflammatory around blood vessels, but no significant glomerular c anges (Figure 3-14-K4). T e kidneys of rats (G-V) t at co-administrated adenine plus *Z.-spina christi* leaves extract and rats (G-VI) after co-administrated of adenine plus ZnO-NPs (10 mg/kg B.W) resulted in significant lowered t e markers and istological features were improved to normal in comparison wit adenine group rats (Figure 3-14-K5 and K6).
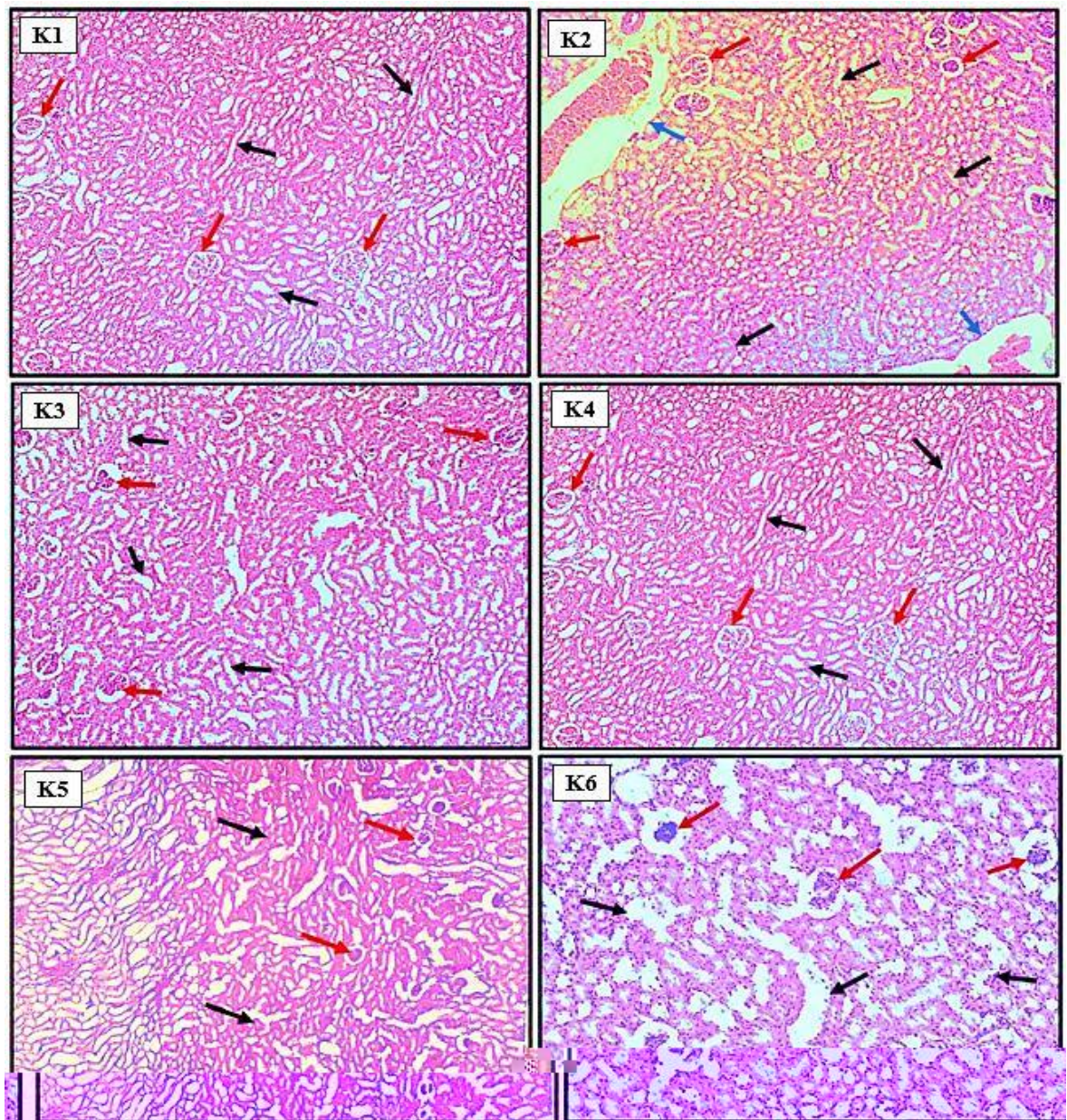
Figure (3-14): Effects of ZnO-NPs and *Z.-spina christi* leaves extract on t e istological appearance of kidney tissues in male rats wit renal failure induced by adenine. T e view is captured using digital camera and lig t microscope at 10X magnification scale. **K1**: Kidneys of male rats in control group were treated wit DMSO only t at s owed normal renal glomerulus (black arrows) and normal renal tubules (red arrows) wit out any significant occupied lesion. **K2**: Kidney of male rats were treated wit Adenine s owed acute renal glomerulus ypertrop y (Black arrows) wit expansion in tubular lumen and t e renal glomeruli s ow severe atrop ied lesion in some area of section (Red arrows) and clear renal vein congestion (Blue arrow). **K3**: Kidney of male rats were treated wit *Z.-spina christi* leaves extract s owed less significant c ange in renal glomerulus (Red arrows), but t ere very less narrowing in tubular lumen wit out any significant occupied lesion (Black arrows). **K4**: Kidney of male rats were treated wit ZnO-NPs s owed normal renal glomerulus (Red arrows) except slig t degeneration and cystic extended of normal renal tubules (Black arrow) wit out any significant occupied lesion (SOL). **K5**: Kidney of male rats were treated wit (Adenine + *Z.-Spina Christi* leaves extract) s owed renal tubular ypertrop y or many intact tubules (Black arrows) wit less narrowing in tubular lumen and t e renal glomerular tuft s ow very mild atrop ied lesion in some area of section (Red arrows). **K6**: Kidney of male rats were treated wit (Adenine + ZnO-NPs) s owed renal glomerular ypertrop y (Red arrows) wit very slig t narrowing in tubular lumen (Black arrows).

T e istological c anges of kidney tissues of rats were consistent wit t ose results observed in antioxidants statues. In t e kidney section, detected t at adenine caused stripping of t e epit elium of t e renal tubules because its metabolite, 2,8-di ydroxyadenine (2,8-DHA), w ic as low solubility and can precipitate in t e renal tubules leading to t eir obstruction and development of uremia wit exudate of inflammatory cells and glomeruli, significant dilation and necrosis of renal tubules as s owed in (Figure 3-14-K2) [238], and is capable of oxidative stress in renal tissue causing istological damage in t e kidney [239]. T e kidney of group rats w en received adenine plus ZnO-NPs (10 mg/kg B.W) or adenine plus *Z.-spina christi* leaves extract (10 mg/kg B.W) s owed improvement in t e glomerular and renal tubules in comparison to adenine group rats (Figure 3-14-K5 and K6).

3-4-5-2 Liver Section

Figure 3-15 s owed results of t e istological study of t e epatic tissue from rats for six groups. Control group rats (G-I) s owed t e normal central vein, normal arrangement of epatic cell and normal sinusoids, wit out any vacuolated of t e cytoplasm (Figure 3-15-L1). T e istopat ological section in t e liver of adenine group rats (G-II) s owed a clear alteration including cytoplasm vacuolization, necrosis of epatocytes, and amyloid like substance precipitation in t e wall of liver sinusoids (Figure 3-15-L2). Group rats (G-III) were administered *Z.-spina christi* leaves extract (10 mg/kg B.W) and s owed normal arc itecture of t e liver, and normal epatocytes wit some areas around t e periportal region appear to be mildly dilated (Figure 3-15-L3). Liver tissue of group rats (G-IV) after administrated of ZnO-NPs (10 mg/kg B.W) ex ibited a similar liver cytoarc itecture compared wit t e group rats (G-III) but t e portal areas s ow mild to moderate

round cell inflammatory infiltrate (Figure 3-15-L4). Finally, in bot groups rats (G-V and G-VI) were co-administered adenine plus aqueous extract of *Z.-spina christi* leaves (10 mg/kg B.W) or adenine plus ZnO-NPs (10 mg/kg B.W) s owed reduced congestion of t e epatic central vein, t e cytoplasm not vacuolated. Sinusoids are well protected wit reduced inflammation epatocytes (Figure 3-15-L5 and L6).

T e liver section of rats demonstrates adenine-induced periportal inflammation, sinusoidal congestion emorr age, and epatic necrosis in t e liver of rats (Figure 3-15 7-L2). T ese results were in agreement wit [240]. Liver damage is possible due to indirect renal failure or directly by t e toxic effects of adenine-mediated oxidative stress on t e epatic cell. Administration of *Z.-spina christi* leaves extract alone or ZnO-NPs alone displayed approximately similar results as t at s owed in t e control group (Figure 3-15- L3 and L4). T ese results were in agreement wit previous studies [179], [241]. On t e ot er and, group rats t at were co-administered adenine plus aqueous extract of *Z.-spina christi* leaves appeared significant improvement compared wit adenine-treated rats as indicated by t e contrary periportal decreased inflammatory epatic cells, reduced vacuolization, and sinusoidal congestion in t e liver cells (Figure 3-15-L5). Finally, co-administration of adenine plus ZnO-NPs en anced t e epatic necrosis, central vein, and sinusoidal congestion compared to adenine-exposed rats (Figure 3-15-L6). T e ameliorative roles of t e synt esized ZnO-NPs and *Z.-spina christi* leaves in t eir doses used can scavenge free radicals and active oxygen species suc as singlet oxygen, free radicals, and ydroxyl radicals, and safe t e renal and epatic tissues to natural structure due to t eir antioxidant and anti-inflammatory properties [242], [243].
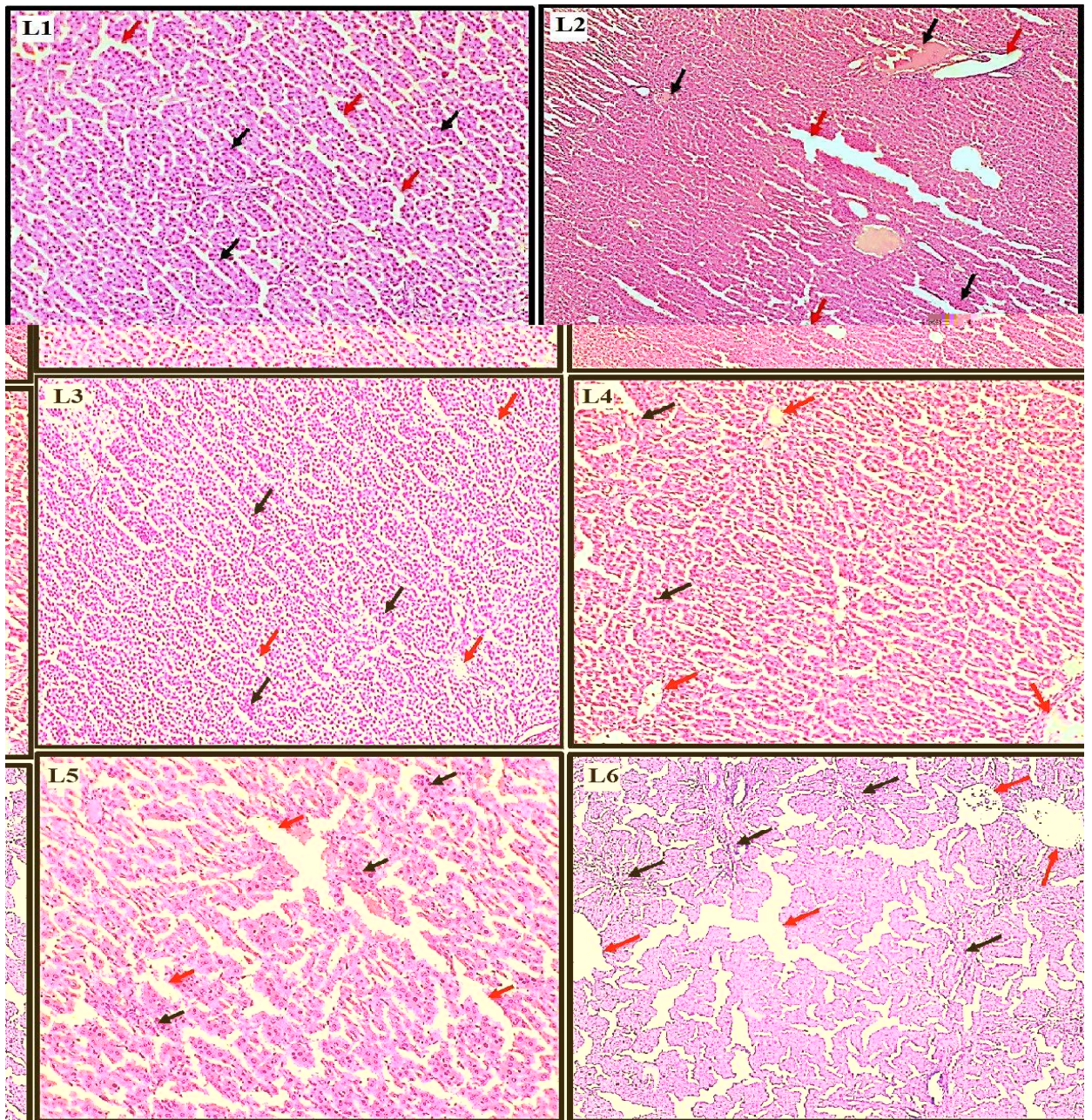
Figure (3-15): Effects of ZnO-NPs and *Z.-spina christi* leaves extract on t e istological appearance of liver tissues in male rats wit renal failure induced by adenine. T e view is captured using digital camera and lig t microscope at 10X magnification scale. **L1**: Liver of control group rats were gavaged wit DMSO only s owed t e normal epatocytes (black arrows) and normal central vein (Red arrows), wit out any significant occupied lesion (SOL). **L2**: Liver of adenine group rats s owed clear damage in t e epatocytes (Black arrows) and clear t rombi formation can be seen in t e epatic central vein in section (Red arrows). **L3**: Liver of male rats were administrated wit aqueous extract of *Z.-spina christi* leaves extract (10 mg/kg B.W) s owed a normal epatocyte (Black arrows) and normal radial arrangement around central vein (Red arrows). **L4**: Liver of male rats were administrated wit ZnO-NPs (10 mg/kg B.W) s owed slig tly t rombi of epatocytes in some areas (Black arrows) and central vein (Red arrows). **L5**: T e liver of male rats was treated wit (Adenine + *Z.-spina christi* leaves extract (10 mg/kg B.W)) s owed infiltration and necrosis in t e epatocytes (Black arrows) and clear damage of t e central vein in some areas (Red arrows). **L6**: T e liver of male rats treated wit (Adenine + ZnO-NPs (10 mg/kg B.W)) s owed infiltration in epatic tissue and coagulative necrosis of t e epatocytes (Black arrows) and clear damage in t e wall of t e central vein (Red arrows).

101

Conclusions and Future Studies

Conclusions

1- Zinc oxide nanoparticles were successfully synthesized from the aqueous leaf extract of *Ziziphus-spina christi* and zinc nitrate hexahydrate.

2- Phytochemicals presented in *Ziziphus-spina christi* leaves extract could potentially be used as an effective reducing, capping, and stabilizing agent for the green synthesis of ZnO-NPs.

3- Adenine-exposed male rats lead to a decrease in body weight, and caused harmful defects in kidney and liver tissues by measuring levels of (uric acid, urea creatinine, ALT, AST, and ALP).

4- The synthesized ZnO-NPs have good antioxidant activity against oxidative stress and free radicals.

5- Both the synthesized ZnO-NPs and aqueous leaf extract of *Ziziphus-spina christi* at a dose (10 mg/kg B.W) respectively, have ameliorative effects against adenine-exposed male rats, implying that they may be used safely against kidney and liver damage at this concentration; slightly significant effects were observed in renal and liver tissues in comparison to adenine group rats, implying that they may be powerful antioxidant, anti-inflammatory, and antitoxic agents for biomedical applications.

Future Studies

1- Studying the green synthesis of another metal oxides nanoparticles using different medicinal plants.

2- Study the effect of the synthesized zinc oxide nanoparticles from *Ziziphus-spina christi* anticoagulant, anticancer antibacterial and antifungal.

3- Evaluating the effect of the synthesized zinc oxide nanoparticles from *Ziziphus-spina christi* as anti-anemia related with renal failure in male rats.

4- Evaluating the effect of the synthesized zinc oxide nanoparticles from *Ziziphus-spina christi* as anti-diabetic activity caused the renal failure in male rats.

5- Study the effect of the synthesized zinc oxide nanoparticles from *Ziziphus-spina christi* on the parathyroid hormone levels.

References

1. Santilli CV, Tokumoto MS, Briois V, Santilli C v, Pulcinelli SH. Preparation of ZnO nanoparticles: Structural study of t e molecular precursor Cite t is paper Preparation of ZnO Nanoparticles: Structural Study of t e Molecular Precursor. Vol. 26, Journal of Sol-Gel Science and Tec nology. 2003. ttps://doi.org/10.1021/jp0217381

2. Bec eri A, Dürr M, lo Nostro P, Baglioni P. Synt esis and c aracterization of zinc oxide nanoparticles: Application to textiles as UV-absorbers. Journal of Nanoparticle Researc . 2008 Apr;10(4):679–689. ttps://doi.org/10.1007/s11051-007-9318-3

3. S a M, Fawcett D, S arma S, Tripat y SK, Poinern GEJ. Green synt esis of metallic nanoparticles via biological entities. Materials. 2015;8(11):7278–308. ttps://doi.org/10.3390/ma8115377

4. Ja n W. Review: C emical Aspects of t e Use of Gold Clusters in Structural Biology [Internet]. 1999. Available from: ttp://www.idealibrary.com. ttps://doi.org/10.1006/jsbi.1999.4123

5. S a RK, Borua F, Parween N. Synt esis and c aracterization of ZnO nanoparticles using leaf extract of *Camellia Sinesis* and evaluation of t eir antimicrobial efficacy. Int J Curr Microbiol App Sci. 2015;4(8):444–450. http://www.ijcmas.com/vol-4-8/Rajesh%...

6. Oladeji OS, Adelowo FE, Ayodele DT, Odelade KA. P ytoc emistry and p armacological activities of Cymbopogon citratus: A review. Vol. 6, Scientific African. Elsevier B.V.; 2019. ttps://doi.org/10.1016/j.sciaf.2019.e00137

7. Abdulra man MD, Zakariya AM, Hama HA, Hamad SW, Al-Rawi SS, Bradosty SW, et al. Et nop armacology, Biological Evaluation, and C emical Composition of *Ziziphus spina - Christi* (L.) Desf.: A Review. Advances in P armacological and P armaceutical Sciences. Hindawi Limited. 2022; 2022:4495688. ttps://doi.org/10.1155/2022/4495688

References

8. Mars all AT, Haverkamp RG, Davies CE, Parsons JG, Gardea-Torresdey JL, van Agterveld D. Accumulation of gold nanoparticles in *Brassica juncea*. Int J P ytoremediation.2007;9(3):197–206. ttps://doi.org/10.1080/15226510701376026

9. Sanc ez-Moreno P, Ortega-Vinuesa JL, Peula-Garcia JM, Marc al JA, Boulaiz H. Smart drug-delivery systems for cancer nanot erapy. Curr Drug Targets. 2018;19(4):339–359. Doi: 10.2174/1389450117666160527142544

10. Negrescu A, Killian M, Vakamulla Rag u SN, Sc muki P, Mazare A, Cimpean A. Metal Oxide Nanoparticles: Review of Synt esis, C aracterization and Biological Effects. J Funct Biomater. 2022 Dec 5;13:274. ttps://doi.org/10.3390/jfb13040274

11. Biswas S, Bellare J. Bioactivity, biocompatibility, and toxicity of metal oxides. In: Metal Oxides for Biomedical and Biosensor Applications. Elsevier; 2022. p. 3–33. ttps://doi.org/10.1016/B978-0-12-823033-6.00001-6

12. S amsuzzaman AA, Asif M, Mas rai A, K anam H. Green Synt esis of ZnO Nanoparticles Using *Bacillus Subtilis* and T eir Catalytic Performance in t e One-Pot Synt esis of Steroidal T iop enes. European C emical Bulletin. 2014;3(9):939–45. Doi: 10.17628/ECB.2014.3.939

13. Afifi M, Almag rabi OA, Kadasa NM. Ameliorative effect of zinc oxide nanoparticles on antioxi 104 and sperm c aracteristics in streptozotocin-induced diabetic rat testes. Biomed Res Int. 2015;2015. ttps://doi.org/10.1155/2015/153573

14. Asg ar MS, Qures i NA, Jabeen F, K an MS, S akeel M, C aud ry AS. Ameliorative effects of selenium in ZnO NP-induced oxidative stress and ematological alterations in Catla catla. Biol Trace Elem Res. 2018;186:279–87. Doi: 10.1007/s12011-018-1299-9

References

15. El-Ba r SM, S ous a S, Albok adaim I, S e ab A, K attab W, A med-Farid O, et al. Impact of dietary zinc oxide nanoparticles on selected serum biomarkers, lipid peroxidation and tissue gene expression of antioxidant enzymes and cytokines in Japanese quail. BMC Vet Res. 2020;16(1):1–12. ttps://doi.org/10.1186/s12917-020-02482-5

16. Aitken RJ, Roman SD. Antioxidant systems and oxidative stress in t e testes. Molecular mec anisms in spermatogenesis. 2008;154–71. ttps://doi.org/10.1007/978-0-387-09597-4_9

17. Ma moud SM, Kassab RB, Moneim AEA. Zinc oxide nanoparticles ameliorate aluminum c loride-induced epato-renal oxidative stress and inflammation in rats. Lab Anim. 2020;15:16. Doi: 10.22159/ijpps.2020v12i1.35956

18. Augustine Amalraj, Sreeraj Gopi. Medicinal properties of Terminalia arjuna (Roxb.) Wig t & Arn.: A review. Journal of Traditional and Complementary Medicine. 2017;1(7):65–78. ttps://doi.org/10.1016/j.jtcme.2016.02.003

19. Organization WH. Regulatory situation of erbal medicines: a worldwide review. 1998. ttps://apps.w o.int/iris/ andle/10665/63801

20. Oluyemisi F, Henry O, Peter O. Standardization of erbal medicines-A review. Int J Biodivers Conserv. 2012;4(3):101–112. Doi: 10.5897/IJBC11.163

21. Saxena M, Saxena J, Nema R, Sing D, Gupta A. P ytoc emistry of medicinal plants. J. of P armacognosy and P ytoc emistry. 2013;1(6):168–182. ttps://dx.doi.org/10.22271/p yto

22. Saied AS, Gebauer J, Hammer K, Buerkert A. *Ziziphus spina-christi* (L.) Willd.: a multipurpose fruit tree. Genet Resour Crop Evol. 2008;55:929–37. ttps://doi.org/10.1007/s10722-007-9299-1

References

23. Bukar AM, Kyari MZ, Gwaski PA, Gudusu M, Kuburi FS, Abadam YI. Evaluation of p ytoc emical and potential antibacterial activity of *Ziziphus spina-christi* L. against some medically important pat ogenic bacteria obtained from University of Maiduguri Teac ing Hospital, Maiduguri, Borno State Nigeria. J. of P armacognosy and P ytoc emistry. 2015;3(5):98–101. ttps://dx.doi.org/10.22271/p_yto

24. Srinivasan A. Handbook of precision agriculture: principles and applications. CRC press; 2006.

25. Farooqi MIH. Cedar or lote-tree in t e lig t of Al-Quran-a scientific study. 2016.

26. Amin A, S a S, Andaleeb S, K an MM, K an MA. Studies on Antimicrobial and Antifungal Activities of *Ziziphus* mauritiana Against Human Clinical Bacterial and Fungal Pat ogens. Pakistan Journal of Scientific & Industrial Researc . 2012;55(1): 35–39.   ttps://doi.org/10.52763/PJSIR.BIOL.SCI.55.1.2012.35.39

27. Sud ersan C, Hussain J. In vitro Clonal Propagation of a Multipurpose Tree, *Ziziphus spina-christi* (L.) Desf. Turk J Botany. 2003;27(3):167–172. ttps://journals.tubitak.gov.tr/botany/vol27/iss3/2

28. Longbap, B.D, Us ie, O A1, Oga , E., Kendenson, A. C, Nyikyaa, J. T. P ytoc emical Screening and Quantitative Determination of P ytoc emicals in Leaf Extracts of *Hannoa undulata*. IJMPNP. 2018;4(2): 32–38. ttp://dx.doi.org/10.20431/2454-7999.0402005

29. Wali AF, Pillai JR, Beig S, Mus taq A, Arafa A, Re man MU, et al. Et nop armacological Uses, P ytoc emistry, P armacological Properties and Clinical Trials of *Ziziphus Spina-Christi*: A Compre ensive Review. Saudi P armaceutical Journal. 2022;5(1). Doi: 10.1016/j.jsps.2022.05.001

30. Hussein AS. *Ziziphus spina-christi*: Analysis of bioactivities and c emical composition. Wild Fruits: Composition, Nutritional Value and Products. 2019;175–197. Doi: 10.1007/978-3-030-31885-7_15

References

31. Walaa Fikry Elbossaty. Potent Medicinal Influences of *Ziziphus Spina-Christi*. ACTA Scientific Medical Sciences. 2020;4(3):143–146.

32. Na ar L, Sarker SD. C emistry for p armacy students: general, organic and natural product c emistry. Jo n Wiley & Sons; 2007. Doi:10.1002/9781118687529

33. Koffi CK, Lourme-Ruiz A, Djoudi H, Bouquet E, Dury S, Gautier D. T e contributions of wild tree resources to food and nutrition security in sub-Sa aran African drylands: a review of t e pat ways and beneficiaries. International Forestry Review. 2020;22(1):64–82. ttps://doi.org/10.1505/146554820828671490

34. Butt SZ, Hussain S, Munawar KS. P ytoc emistry of *Ziziphus* mauritiana: An overview of its nutritional and p armaceutical potential. Sci Inquiry Rev. 2021;5(2):01–15. ttps://doi.org/10.32350/sir/52.01

35. G azanfar SA. Handbook of Arabian medicinal plants. CRC press; 1994; 1st Edition. ttps://doi.org/10.1201/b14834

36. Tang W, Eisenbrand G. C inese drugs of plant origin: c emistry, p armacology, and use in traditional and modern medicine. Springer Science & Business Media; 2013. ttps://doi.org/10.1007/978-3-642-73739-8

37. Al assan KA, Indabawa AS, S a MM. P ytoc emical analysis, proximate composition and antibacterial activities of *Ziziphus* species (*Z. jujube and Z. spina-christi*). 2019; 4(1): 42-46. ttp://dx.doi.org/10.21839/jaar.2019.v4i1.262

38. Sofowora A, Ogunbodede E, Onayade A. T e role and place of medicinal plants in t e strategies for disease prevention. African journal of traditional, complementary and alternative medicines. 2013;10(5):210–29. ttp://dx.doi.org/10.4314/ajtcam.v10i5.2

39. Owolarafe, T.A.; Salau, A.K.; Salawu, K. 2019: P ytoc emical screening and toxicity study of aqueous-met anol extract of *Ziziphus*

References

spina-christi seeds in Wistar albino rats Comparative Clinical Pat ology 29(1): 267-274. DOI: 10.1007/s00580-019-03043-5

40. Adzu B, Amos S, Wambebe C, Gamaniel K. Antinociceptive activity of *Zizyphus spina-christi* root bark extract. Fitoterapia. 2001;72(4):344–50. Doi: 10.1016/s0367-326x(00)00289-6

41. Abdel-Wa ab MA, Omara EA, Abdel-Galil MM, Hassan NS, Nada SA, Saeed A, et al. *Zizyphus spina-christi* extract protects against aflatoxin B1-intitiated epatic carcinogenicity. African Journal of Traditional, Complementary, and Alternative Medicines. 2007;4(3):248. Doi: 10.4314/ajtcam.v4i3.31216

42. Setorki M, Hoos mandi Z. Neuroprotective effect of *Ziziphus spina-christi* on brain injury induced by transient global cerebral isc emia and reperfusion in rat. Banglades J P armacol. 2017;12(1):69–76. DOI: ttps://doi.org/10.3329/bjp.v12i1.29964

43. Adzu B, Haruna AK, Salawu OA, Sule A. Bioassay-guided evaluation of t e antidiarr oeal potentials of *Zizyphus spina-christi* root bark in rats. Int J Biol C em Sci. 2007;1(1):15–20. Doi: 10.4314/ijbcs.v1i1.39693

44. Saied AS, Gebauer J, Hammer K, Buerkert A. *Ziziphus spina-christi* (L.) Willd.: a multipurpose fruit tree. Genet Resour Crop Evol. 2008;55:929–37. ttps://doi.org/10.1007/s10722-007-9299-1

45. Ben-Porat T, Weiss-Sadan A, Rottenstreic A, S erf-Dagan S, Sc weiger C, Yosef-Levi IM, et al. Nutritional management for c ronic kidney disease patients w o undergo bariatric surgery: a narrative review. Advances in Nutrition. 2019;10(1):122–32. Doi: 10.1093/advances/nmy112

46. Almeer RS, Albas er G, Alotibi F, Alarifi S, Ali D, Alka tani S. *Ziziphus spina-christi* leaf extract suppressed mercury c loride-induced nep rotoxicity via Nrf2-antioxidant pat way activation and in ibition

References

of inflammatory and apoptotic signaling. Oxid Med Cell Longev. 2019;2019.   ttps://doi.org/10.1155/2019/5634685

47. Dk il MA, Kassab RB, Al-Qurais y S, Abdel-Daim MM, Zrieq R, Abdel Moneim AE. Zizip us spina-c risti (L.) leaf extract alleviates myocardial and renal dysfunction associated wit sepsis in mice. *Biomed Pharmacother*. 2018;102:64-75.   Doi:10.1016/j.biop a.2018.03.032

48. El-Beltagy AEF, Abdelaziz BM, Abdelaziz KK, Elsawy MR, G anem RA. Adverse effects of cadmium on t e t yroid, kidneys, and testes in Wistar albino rats and t e possible modulatory role of *Zizyphus spina-christi* (Sidr) fruit extract (Histological and bioc emical studies). Journal of Bioscience and Applied Researc . 2019;5(4):551–75.   Doi: 10.21608/jbaar.2019.248623

49. Basuny AM, Arafat SM, Farag HA. Utilization from Fruit and Leaves of Nabek (*Ziziphus-spina christi* l.) as a Source of Bioactive components. Banats J Biotec nol. 2013;4(7):16.   Doi: 10.7904/2068-4738-IV(07)-16

50. Hussein HM, El-Sayed EM, Said AA. Anti yperglycemic, anti yperlipidemic and antioxidant effects of *Zizyphus spina christi* and *Zizyphus jujuba* in alloxan diabetic rats. Int J P armacal. 2006;2(5):563–70. DOI: 10.3923/ijp.2006.563.570

51. Sud ersan C, Hussain J. In vitro Clonal Propagation of a Multipurpose Tree, *Ziziphus spina-christi* (L.) Desf. Turk J Botany. 2003;27(3):167–172.   ttps://journals.tubitak.gov.tr/botany/vol27/iss3/2

52. Majuru S, Oyewumi MO. Nanotec nology in drug development and life cycle management. Nanotec nology in drug delivery. 2009;597–619.   ttps://doi.org/10.1007/978-0-387-77668-2_20

53. Jain KK. T e Role of Nanobiotec nology in Drug Discovery. P arm Biotec nol. 2010;655:37.   ttps://doi.org/10.1007/978-1-4419-1132-2_4

References

54. Oroojalian F, C arbgoo F, Has emi M, Amani A, Yazdian-Robati R, Mok tarzade A, et al. Recent advances in nanotec nology-based drug delivery systems for t e kidney. Journal of controlled release. 2020;321:442–462. Doi: 10.1016/j.jconrel.2020.02.027

55. Seidi F, Jenjob R, P akkeeree T, Crespy D. Sacc arides, oligosacc arides, and polysacc arides nanoparticles for biomedical applications. Journal of controlled release. 2018;284:188–212. Doi: 10.1016/j.jconrel.2018.06.026

56. Jain A, Sing SK, Arya SK, Kundu SC, Kapoor S. Protein nanoparticles: promising platforms for drug delivery applications. ACS Biomater Sci Eng. 2018;4(12):3939–61. Doi: 10.1021/acsbiomaterials.8b01098

57. Liu Y, Miyos i H, Nakamura M. Nanomedicine for drug delivery and imaging: a promising avenue for cancer t erapy and diagnosis using targeted functional nanoparticles. Int J Cancer. 2007;120(12):2527–37. Doi: 10.1002/ijc.22709

58. Rie emann K, Sc neider SW, Luger TA, Godin B, Ferrari M, Fuc s H. Nanomedicine—c allenge and perspectives. Angewandte C emie International Edition. 2009;48(5):872–97. Doi: 10.1002/anie.200802585

59. McMillan J, Batrakova E, Gendelman HE. Cell Delivery of T erapeutic Nanoparticles. Prog Mol Biol Transl Sci. 2011; 104: 563–601. Doi: 10.1016/b978-0-12-416020-0.00014-0

60. Hirsc L, Drezek R, Halas N, West J. Diagnostic and T erapeutic Applications of Metal Nanos ells. BioMEMS and Biomedical Nanotec nology. 2006; pp 157-169. ttps://doi.org/10.1007/978-0-387-25844-7_9

61. Boudad H, Legrand P, Lebas G, C éron M, Duc êne D, Ponc el G. Combined ydroxypropyl-β-cyclodextrin and poly(alkylcyanoacrylate)

nanoparticles intended for oral administration of saquinavir. Int J P arm. 2001 Jun 1;218:113–24. Doi: 10.1016/s0378-5173(01)00622-6

62. Paramasivam G, Palem VV, Sundaram T, Sundaram V, Kis ore SC, Bellucci S. Nanomaterials: Synt esis and Applications in T eranostics. *Nanomaterials*. 2021; 11(12):3228.
   ttps://doi.org/10.3390/nano11123228

63. Bar oum A, García-Betancourt ML, Ra ier H, Van Assc e G. P ysicoc emical c aracterization of nanomaterials: Polymorp , composition, wettability, and t ermal stability. In: Emerging applications of nanoparticles and arc itecture nanostructures. Elsevier; 2018. p. 255–278. Doi: 10.1016/B978-0-323-51254-1.00009-9

64. Sannino D. Types and Classification of Nanomaterials. Nanotec nology: Trends and Future Applications. 2021;15–38.
   ttps://doi.org/10.1007/978-981-15-9437-3_2

65. Gugulot u D, Bar oum A, Afzal SM, Venkates warlu B, Uludag H. Structural multifunctional nanofibers and t eir emerging applications. Handbook of Nanofibers; Springer: Berlin/Heidelberg, Germany. 2019; 693–732. ttps://doi.org/10.1007/978-3-319-53655-2_16

66. Abra am J, Arunima R, Nimit a KC, George SC, T omas S. One-dimensional (1D) nanomaterials: Nanorods and nanowires; nanoscale processing. In: Nanoscale Processing. Elsevier; 2021. p. 71–101.
   ttps://doi.org/10.1016/B978-0-12-820569-3.00003-7

67. Youssef AM, Moustafa HA, Bar oum A, Hakim AEAA, Dufresne A. Evaluation of t e Morp ological, Electrical and Antibacterial Properties of Polyaniline Nanocomposite Based on Zn/Al-Layered Double Hydroxides. C emistrySelect. 2017;2(27):8553–66.
   ttps://doi.org/10.1002/slct.201701513

68. C imene D, Alge DL, Ga arwar AK. Two-dimensional nanomaterials for biomedical applications: emerging trends and future prospects. Advanced Materials. 2015;27(45):7261–7284.

References

ttps://doi.org/10.1002/adma.201502422

69. Mefta i A, Samyn P, Geravand SA, K ajavi R, Alibk s i S, Bec elany M, et al. Nanocelluloses as skin biocompatible materials for skincare, cosmetics, and ealt care: Formulations, regulations, and emerging applications. Carbo ydr Polym. 2022;278:118956. ttps://doi.org/10.1016/j.carbpol.2021.118956

70. El-Bes lawy MM, Abdel-Haleem FM, Bar oum A. Molecularly imprinted potentiometric sensor for nanomolar determination of pioglitazone ydroc loride in p armaceutical formulations. Electroanalysis. 2021;33(5):1244–1254. ttps://doi.org/10.1002/elan.202060141

71. Patra JK, Baek KH. Green nanobiotec nology: factors affecting synt esis and c aracterization tec niques. J Nanomater. 2015;2014:219. ttps://doi.org/10.1155/2014/417305

72. T. Cele, 'Preparation of Nanoparticles', Engineered Nanomaterials - Healt and Safety. Intec Open, Jul. 08, 2020. Doi: 10.5772/intec open.90771.

73. Haris V, Ansari MM, Tewari D, Gaur M, Yadav AB, García-Betancourt ML, et al. Nanoparticle and Nanostructure Synt esis and Controlled Growt Met ods. Nanomaterials. 2022;12(18):3226. ttps://doi.org/10.3390/nano12183226

74. Prasad Yadav T, Mano ar Yadav R, Pratap Sing D. Mec anical milling: a top down approac for t e synt esis of nanomaterials and nanocomposites, Nanosci Nanotec nol 2 (2012): 22–48. 2012. Doi: 10.5923/j.nn.20120203.01

75. Kumar, K. Sant os , Kumar, Vijay B oos an, Paik, Pradip. Recent Advancement in Functional Core-S ell Nanoparticles of Polymers: Synt esis, P ysical Properties, and Applications in Medical Biotec nology. *Journal of Nanoparticles*. 2013. Vol. 2013, no. 2013, pp.1-24. ttps://doi.org/10.1155/2013/672059

References

76. Isaacoff BP, Brown KA. Progress in top-down control of bottom-up assembly. Vol. 17, Nano Letters. ACS Publications; 2017. p. 6508–10. ttps://doi.org/10.1021/acs.nanolett.7b04479

77. Ranjan S, Dasgupta N, Rajendran B, Avad ani GS, Ramalingam C, Kumar A. Microwave-irradiation-assisted ybrid c emical approac for titanium dioxide nanoparticle synt esis: microbial and cytotoxicological evaluation. Environmental Science and Pollution Researc. 2016;23:12287–302. ttps://doi.org/10.1007/s11356-016-6440-8

78. Patil N, B askar R, Vyav are V, D adge R, K aire V, Patil Y. Over View on Met ods of Synt esis of Nanoparticles. Int J Curr P arm Res. 2021;13(2):11–6. ttps://doi.org/10.22159/ijcpr.2021v13i2.41556

79. S i F, Xu J, Hu Z, Ren C, Xue Y, Z ang Y, et al. Bird nest-like zinc oxide nanostructures for sensitive electroc emical glucose biosensor. C inese C emical Letters. 2021;32(10):3185–8. ttps://doi.org/10.1016/j.cclet.2021.03.012

80. Peng H, Fangli Y, Liuyang B, Jinlin L, Yunfa C. Plasma synt esis of large quantities of zinc oxide nanorods. T e Journal of P ysical C emistry C. 2007;111(1):194–200. ttps://doi.org/10.1021/jp065390b

81. Fouad OA, Ismail AA, Zaki ZI, Mo amed RM. Zinc oxide t in films prepared by t ermal evaporation deposition and its p otocatalytic activity. Appl Catal B. 2006;62(1–2):144–9. ttps://doi.org/10.1016/j.apcatb.2005.07.006

82. K an FA. Synt esis of nanomaterials: met ods & tec nology. Applications of nanomaterials in uman ealt. 2020;15–21. ttps://doi.org/10.1007/978-981-15-4802-4_2

83. Król A, Pomastowski P, Rafińska K, Railean-Plugaru V, Buszewski B. Zinc oxide nanoparticles: Synt esis, antiseptic activity and toxicity mec anism. Adv Colloid Interface Sci. 2017;249:37–52. Doi: 10.1016/j.cis.2017.07.033

References

84. Maryanti E, Damayanti D, Gustian I. Synt esis of ZnO nanoparticles by ydrot ermal met od in aqueous rinds extracts of Sapindus rarak DC. Mater Lett. 2014;118:96–8.
   ttps://doi.org/10.1016/j.matlet.2013.12.044

85. Santos L, Nunes D, Calmeiro T, Branquin o R, Salgueiro D, Barquin a P, et al. Solvot ermal synt esis of gallium–indium-zinc-oxide nanoparticles for electrolyte-gated transistors. ACS Appl Mater Interfaces. 2015;7(1):638–46. ttps://doi.org/10.1021/am506814t

86. Agarwal H, Menon S, Kumar SV, Rajes kumar S. Mec anistic study on antibacterial action of zinc oxide nanoparticles synt esized using green route. C em Biol Interact. 2018;286:60–70.
   ttps://doi.org/10.1016/j.cbi.2018.03.008

87. Carrapiço A, Martins MR, Caldeira AT, Mirão J, Dias L. Biosynt esis of Metal and Metal Oxide Nanoparticles Using Microbial Cultures: Mec anisms, Antimicrobial Activity and Applications to Cultural Heritage. Microorganisms. 2023;11(2):378.
   Doi: 10.3390/microorganisms11020378

88. Parsons JG, Peralta-Videa JR, Gardea-Torresdey JL. Use of plants in biotec nology: synt esis of metal nanoparticles by inactivated plant tissues, plant extracts, and living plants. Developments in environmental science. 2007;5:463–85.
   ttps://doi.org/10.1016/S1474-8177(07)05021-8

89. Król A, Railean-Plugaru V, Pomastowski P, Buszewski B. P ytoc emical investigation of Medicago sativa L. extract and its potential as a safe source for t e synt esis of ZnO nanoparticles: T e proposed mec anism of formation and antimicrobial activity. P ytoc emistry Letters. 2019;31:170–180.
   ttps://doi.org/10.1016/j.p ytol.2019.04.009

90. Is warya R, Vasee aran B, Kalyani S, Banumat i B, Govindarajan M, Al arbi NS, et al. Facile green synt esis of zinc oxide nanoparticles

References

using Ulva lactuca seaweed extract and evaluation of t eir p otocatalytic, antibiofilm and insecticidal activity. J P otoc em P otobiol B. 2018;178:249–58. Doi: 10.1016/j.jp otobiol.2017.11.006

91. Bloc K, Pardesi K, Satriano C, G os S. Bacteriogenic platinum nanoparticles for application in nanomedicine. Front C em. 2021;9:624344. ttps://doi.org/10.3389/fc em.2021.624344

92. Sing J, Dutta T, Kim KH, Rawat M, Samddar P, Kumar P. Green'synt esis of metals and t eir oxide nanoparticles: applications for environmental remediation. J Nanobiotec nology. 2018;16(1):1–24. Doi: 10.1186/s12951-018-0408-4

93. Mittal AK, C isti Y, Banerjee UC. Synt esis of metallic nanoparticles using plant extracts. Biotec nol Adv. 2013;31(2):346–356. ttps://doi.org/10.1016/j.biotec adv.2013.01.003

94. Meer B, Andleeb A, Iqbal J, As raf H, Meer K, Ali JS, et al. Bio-Assisted Synt esis and C aracterization of Zinc Oxide Nanoparticles from Lepidium Sativum and T eir Potent Antioxidant, Antibacterial and Anticancer Activities. Biomolecules. 2022;12(6):855. ttps://doi.org/10.3390/biom12060855

95. Rames P, Saravanan K, Manogar P, Jo nson J, Vinot E, Mayakannan M. Green synt esis and c aracterization of biocompatible zinc oxide nanoparticles and evaluation of its antibacterial potential. Sens Biosensing Res. 2021;31:100399. Doi: 10.1016/j.sbsr.2021.100399

96. Liu YC, Li J, A n J, Pu J, Rupa EJ, Huo Y, et al. Biosynt esis of zinc oxide nanoparticles by one-pot green synt esis using fruit extract of Amomum longiligulare and its activity as a p otocatalyst. Optik (Stuttg). 2020;218:165245. ttps://doi.org/10.1016/j.ijleo.2020.165245

97. Naser R, Abu-Huwaij R, Al-k ateeb I, Abbas MM, Atoom AM. Green synt esis of zinc oxide nanoparticles using t e root air extract of P oenix dactylifera: Antimicrobial and anticancer activity. Appl Nanosci. 2021;11:1747–57. ttps://doi.org/10.1007/s13204-021-01837-0

References

98. T akkar KN, M atre SS, Parik RY. Biological synt esis of metallic nanoparticles. Nanomedicine. 2010;6(2):257–262. ttps://doi.org/10.1016/j.nano.2009.07.002

99. Rónavári A, Igaz N, Adamecz DI, Szerencsés B, Molnar C, Kónya Z, et al. Green silver and gold nanoparticles: Biological synt esis approac es and potentials for biomedical applications. Molecules. 2021;26(4):844. ttps://doi.org/10.3390/molecules26040844

100. Mourdikoudis S, Pallares RM, T an NTK. C aracterization tec niques for nanoparticles: comparison and complementarity upon studying nanoparticle properties. Nanoscale. 2018;10(27):12871–934. ttps://doi.org/10.1039/C8NR02278J

101. C o EJ, Holback H, Liu KC, Abouelmagd SA, Park J, Yeo Y. Nanoparticle c aracterization: state of t e art, c allenges, and emerging tec nologies. Mol P arm. 2013;10(6):2093–110. ttps://doi.org/10.1021/mp300697

102. Unger W, Hodoroaba V. D, & S ard A. C aracterization of Nanoparticles: Measurement Processes for Nanoparticles. Elsevier, 2019. ttps://doi.org/10.1016/C2017-0-00312-9

103. A. C inec erem Nkele and F. I. Ezema, 'Diverse Synt esis and C aracterization Tec niques of Nanoparticles', T in Films. Intec Open, Nov. 17, 2021. Doi: 10.5772/intec open.94453

104. Kumar A, Dixit CK. Met ods for c aracterization of nanoparticles. In: Advances in nanomedicine for t e delivery of t erapeutic nucleic acids. Elsevier; 2017. p. 43–58. ttps://doi.org/10.1016/B978-0-08-100557-6.00003-1

105. Jiang J, Pi J, Cai J. T e advancing of zinc oxide nanoparticles for biomedical applications. Bioinorg C em Appl. 2018;2018. ttps://doi.org/10.1155/2018/1062562

106. A, H., Fouad, W., Wasly, H., El-Deeb, B., Abd El-sadek, M. Zinc oxide nanoparticles: green synt esis, c aracterization, p otocatalysis, and

References

antibacterial activity. *Egyptian Journal of Physics*, 2023; 51(1): 57-71. Doi: 10.21608/ejp ysics.2022.157467.1084

107. Mal otra SPK. Applications of zinc oxide nanoparticles as an antimicrobial agent in t e food packaging industry. In: Zinc-Based Nanostructures for Environmental and Agricultural Applications. Elsevier; 2021: 125–137.
    ttps://doi.org/10.1016/B978-0-12-822836-4.00021-5

108. Ali, Attarad, P ull, Abdul-Re man and Zia, Mu ammad. "Elemental zinc to zinc nanoparticles: is ZnO NPs crucial for life? Synt esis, toxicological, and environmental concerns" *Nanotechnology Reviews*, vol. 7, no. 5, 2018, pp. 413-441.
    ttps://doi.org/10.1515/ntrev-2018-0067

109. Díez-Pascual AM. Biodegradable food packaging nanocomposites based on ZnO-reinforced poly ydroxy alkanoates. In: Food packaging. Elsevier; 2017. p. 185–221.
    ttps://doi.org/10.1016/B978-0-12-804302-8.00006-6

110. Mutukwa D, Taziwa R, K otseng LE. A Review of t e Green Synt esis of ZnO Nanoparticles Utilizing Sout ern African Indigenous Medicinal Plants. Nanomaterials. 2022;12(19):3456.
    ttps://doi.org/10.3390/nano12193456

111. Awwad AM, Amer MW, Salem NM, Abdeen AO. Green synt esis of zinc oxide nanoparticles (ZnO-NPs) using Ailant us altissima fruit extracts and antibacterial activity. C em Int. 2020;6(3):151–9.
    Doi:10.5281/Zenodo.3559520

112. Balogun SW, James OO, Sanusi YK, Olayinka OH. Green synt esis and c aracterization of zinc oxide nanoparticles using bas ful (Mimosa pudica), leaf extract: A precursor for organic electronics applications. SN Appl Sci. 2020;2:1–8. ttps://doi.org/10.1007/s42452-020-2127-3

113. Mo amed HYG, Ismael E, Elaasser MM, K alil MMH. Green synt esis of zinc oxide nanoparticles using portulaca oleracea (regla

References

seeds) extract and its biomedical applications. Egypt J C em. 2021;64(2):661–72. Doi: 10.21608/EJCHEM.2020.45592.2930

114. Saleemi MA, Alallam B, Yong YK, Lim V. Synt esis of Zinc Oxide Nanoparticles wit Bioflavonoid Rutin: C aracterisation, Antioxidant and Antimicrobial Activities and In Vivo Cytotoxic Effects on Artemia Nauplii. Antioxidants. 2022;11(10):1853. ttps://doi.org/10.3390/antiox11101853

115. S aba EY, Jacob JO, Tijani JO, Suleiman MAT. A critical review of synt esis parameters affecting t e properties of zinc oxide nanoparticle and its application in wastewater treatment. Appl Water Sci. 2021;11(2):1–41. ttps://doi.org/10.1007/s13201-021-01370-z

116. Pus panat an K, Sat ya S, C it ra MJ, Gowt ami S, Sant i R. Influence of Reaction Temperature on Crystal Structure and Band Gap of ZnO Nanoparticles. Materials and Manufacturing Processes [Internet]. 2012 Dec 1;27(12):1334–42. Available from: ttps://doi.org/10.1080/10426914.2012.700163

117. Rafaie HA, Samat NA, Nor RM. Effect of pH on t e growt of zinc oxide nanorods using *Citrus aurantifolia* extracts. Mater Lett. 2014;137:297–9. ttps://doi.org/10.1016/j.matlet.2014.09.033

118. P an CM, Nguyen HM. Role of capping agent in wet synt esis of nanoparticles. J P ys C em A. 2017;121(17):3213–9. ttps://doi.org/10.1021/acs.jpca.7b02186

119. Mog ri Moazzen MA, Borg ei SM, Tales i F. C ange in t e morp ology of ZnO nanoparticles upon c anging t e reactant concentration. Appl Nanosci. 2013;3(4):295–302. Doi: 10.1007/s13204-012-0147-z

120. Manzoor U, Za ra FT, Rafique S, Moin MT, Muja id M. Effect of synt esis temperature, nucleation time, and postsynt esis eat

treatment of ZnO nanoparticles and its sensing properties. J Nanomater. 2015;16(1):9.   ttps://doi.org/10.1155/2015/189058

121. Que M, Lin C, Sun J, C en L, Sun X, Sun Y. Progress in ZnO nanosensors. Sensors. 2021;21(16):5502.   ttps://doi.org/10.3390/s21165502

122. Jiang J, Pi J, Cai J. T e advancing of zinc oxide nanoparticles for biomedical applications. Bioinorg C em Appl. 2018;2018.   ttps://doi.org/10.1155/2018/1062562

123. Wu T, Tang M. Review of t e effects of manufactured nanoparticles on mammalian target organs. Journal of Applied Toxicology. 2018;38(1):25–40. Doi: 10.1002/jat.3499

124. Lin Z, Monteiro-Riviere NA, Riviere JE. P armacokinetics of metallic nanoparticles. Wiley Interdiscip Rev Nanomed Nanobiotec nol. 2015;7(2):189–217. Doi: 10.1002/wnan.1304

125. Baek M, C ung HE, Yu J, Lee JA, Kim TH, O JM, et al. P armacokinetics, tissue distribution, and excretion of zinc oxide nanoparticles. Int J Nanomedicine. 2012;3081–3097.   Doi: 10.2147/IJN.S32593

126. A amed MIN, Kalaivani K, Vinayaka K S, Sangeet a S, Srinivasan P, P. T irumalai Vasan. Assessment on t e T erapeutic Applications Zinc Oxide Nanopaticles. *Vegueta Anuario de la Facultad de Geografía e Historia*. 2022;22:12.   ttps://doi.org/10.5281/zenodo.7503603

127. Z ao CY, Tan SX, Xiao XY, Qiu XS, Pan JQ, Tang ZX. Effects of dietary zinc oxide nanoparticles on growt performance and antioxidative status in broilers. Biol Trace Elem Res. 2014;160:361–7.   Doi: 10.1007/s12011-014-0052-2

128. Jan H, S a M, Andleeb A, Faisal S, K attak A, Rizwan M, et al. Plant-based synt esis of zinc oxide nanoparticles (ZnO-NPs) using aqueous leaf extract of aquilegia pubiflora: T eir antiproliferative activity

References

against HepG2 cells inducing reactive oxygen species and ot er in vitro properties. Oxid Med Cell Longev. 2021;2021. ttps://doi.org/10.1155/2021/4786227

129. Mo apatra S, Leelavat i L, Rajes kumar S, Sakt i DS, Jayas ri P. Assessment of Cytotoxicity, Anti-Inflammatory and Antioxidant Activity of Zinc Oxide Nanoparticles Synt esized Using Clove and Cinnamon Formulation--An In-Vitro Study. J Evol Med Dent Sci. 2020;9(25):1859–65. Doi: 10.14260/jemds/2020/405

130. Popović D, Kocić G, Katić V, Jović Z, Zarubica A, Veličković LJ, et al. Protective effects of ant ocyanins from bilberry extract in rats exposed to nep rotoxic effects of carbon tetrac loride. C em Biol Interact. 2019;304:61–72. ttps://doi.org/10.1016/j.cbi.2019.02.022

131. Moss R, T omas SR. Hormonal regulation of salt and water excretion: a mat ematical model of w ole kidney function and pressure natriuresis. *American journal of physiology. Renal physiology*. 2014;306(2):F224-F248. Doi:10.1152/ajprenal.00089.2013

132. Kamisan FH, Ya ya F, Ismail NA, Din SS, Mamat SS, Zabidi Z, et al. Hepatoprotective activity of met anol extract of *Melastoma malabathricum* leaf in rats. *J Acupunct Meridian Stud*. 2013;6(1):52-55. Doi: 10.1016/j.jams.2012.08.002

133. Lee BM, Lee WC, Jang JY, A n P, Kim JN, Jeong SW, et al. Clinical features of drug-induced liver injury according to etiology. J Korean Med Sci. 2015;30(12):1815–20. Doi: 10.3346/jkms.2015.30.12.1815

134. T évenod F, Lee WK. Toxicology of cadmium and its damage to mammalian organs. Cadmium: from toxicity to essentiality. 2013;415–90. Doi: 10.1007/978-94-007-5179-8_14

135. Cruicks ank CC, Dyer KR. A review of t e clinical p armacology of met amp etamine. Addiction. 2009;104(7):1085–99. Doi: 10.1111/j.1360-0443.2009.02564.x

References

136. Andrade RJ, Ait al GP, Björnsson ES, Kaplowitz N, Kullak-Ublick GA, Larrey D, et al. EASL clinical practice guidelines: drug-induced liver injury. J Hepatol. 2019;70(6):1222–61.

    Doi: 10.1016/j.j ep.2019.02.014

137. Akintelu SA, Folorunso AS. A review on green synt esis of zinc oxide nanoparticles using plant extracts and its biomedical applications. Bionanoscience. 2020;10(4):848–63.

    ttps://doi.org/10.1007/s12668-020-00774-6

138. Xiong H. ZnO nanoparticles applied to bioimaging and drug delivery. Advanced Materials. 2013;25(37):5329–35.

    ttps://doi.org/10.1002/adma.201301732

139. Hassan SA, Muja id H, Ali MM, Irs ad S, Naseer R, Saeed S, et al. Synt esis, c aracterization and protective effect of green tea-mediated zinc oxide nanoparticles against oc ratoxin A induced epatotoxicity and nep rotoxicity in albino rats. Appl Nanosci. 2021;11(8):2281–9.

    ttps://doi.org/10.1007/s13204-021-02006-z

140. Rani V, Verma Y, Rana K, Rana SVS. Zinc oxide nanoparticles in ibit dimet ylnitrosamine induced liver injury in rat. C em Biol Interact. 2018;295:84–92. Doi: 10.1016/j.cbi.2017.10.009

141. Hassan I, Husain FM, K an RA, Ebaid H, Al-Tamimi J, Al azza IM, et al. Ameliorative effect of zinc oxide nanoparticles against potassium bromate-mediated toxicity in Swiss albino rats. Environmental Science and Pollution Researc . 2019;26:9966–80.

    Doi: 10.1007/s11356-019-04443-4

142. Yogalaks mi B, Viswanat an P, Anurad a CV. Investigation of antioxidant, anti-inflammatory and DNA-protective properties of eugenol in t ioacetamide-induced liver injury in rats. Toxicology. 2010;268(3):204–12. Doi: 10.1016/j.tox.2009.12.018

143. Bas andy SAE, Alaamer A, Moussa SAA, Omara EA. Role of zinc oxide nanoparticles in alleviating epatic fibrosis and nep rotoxicity

induced by t ioacetamide in rats. Can J P ysiol P armacol. 2018;96(4):337–344. Doi: 10.1139/cjpp-2017-0247

144. C ung IM, Abdul Ra uman A, Marimut u S, Vis nu Kirt i A, Anbarasan K, Rajakumar G. An investigation of t e cytotoxicity and caspase-mediated apoptotic effect of green synt esized zinc oxide nanoparticles using Eclipta prostrata on uman liver carcinoma cells. Nanomaterials. 2015;5(3):1317–30. Doi: 10.3390/nano5031317

145. Barakat LAA, Barakat N, Zakaria MM, K iralla SM. Protective role of zinc oxide nanoparticles in kidney injury induced by cisplatin in rats. Life Sci. 2020;262:118503. Doi: 10.1016/j.lfs.2020.118503

146. Alomari G, Al-Trad B, Hamdan S, Aljabali AAA, Al Zoubi MS, Al-Batanye K, et al. Alleviation of diabetic nep ropat y by zinc oxide nanoparticles in streptozotocin-induced type 1 diabetes in rats. IET Nanobiotec nol. 2021;15(5):473–83. Doi: 10.1049/nbt2.12026

147. Anjum S, Has im M, Malik SA, K an M, Lorenzo JM, Abbasi BH, et al. Recent advances in zinc oxide nanoparticles (ZnO NPs) for cancer diagnosis, target drug delivery, and treatment. Cancers (Basel). 2021;13(18):4570. Doi: 10.3390/cancers13184570

148. Yo anes Buang. Dietary adenine alleviates fatty liver induced by orotic acid. Indo. J. C em. 2010; 10 (3): 363 - 369. ttps://doi.org/10.22146/ijc.21444

149. Moffatt BA, As i ara H. Purine and pyrimidine nucleotide synt esis and metabolism. Arabidopsis Book. 2002;1:e0018. Doi:10.1199/tab.0018

150. Bot am KM, Mayes PA. Bioenergetics: T e Role of ATP. Harper's Illustrated Bioc emistry, 30e Eds. Rodwell VW, et al. McGraw Hill, 2016, ttps://accessmedicine.m medical.com/Content.aspx?bookid=136 6&sectionid=73243196.

References

151. Fred olm BB. Adenosine, an endogenous distress signal, modulates tissue damage and repair. Cell Deat Differ. 2007;14(7):1315–23. Doi: 10.1038/sj.cdd.4402132

152. Tang J, Jiang X, Z ou Y, Xia B, Dai Y. Increased adenosine levels contribute to isc emic kidney fibrosis in t e unilateral ureteral obstruction model. Exp T er Med. 2015;9(3):737–43. Doi: 10.3892/etm.2015.2177

153. Ulbric t T. L. V. Purines, Pyrimidines and Nucleotides: And t e C emistry of Nucleic Acids. Elsevier, 2013; v(25). ttps://doi.org/10.1016/C2013-0-06579-6

154. Jia T, Olauson H, Lindberg K, Amin R, Edvardsson K, Lind olm B, et al. A novel model of adenine-induced tubulointerstitial nep ropat y in mice. BMC Nep rol. 2013;14(1):1–8. ttps://doi.org/10.1186/1471-2369-14-116

155. Mis ima E, Fukuda S, S ima H, Hirayama A, Akiyama Y, Takeuc i Y, et al. Alteration of t e intestinal environment by lubiprostone is associated wit amelioration of adenine-induced CKD. Journal of t e American Society of Nep rology. 2015;26(8):1787–94. Doi: 10.1681/ASN.2014060530

156. Kumakura S, Sato E, Sekimoto A, Has izume Y, Yamakage S, Miyazaki M, et al. Nicotinamide attenuates t e progression of renal failure in a mouse model of adenine-induced c ronic kidney disease. Toxins (Basel). 2021;13(1):50. Doi: 10.3390/toxins13010050

157. Nasir O, Umbac AT, Rex epaj R, Ackermann TF, B andaru M, Ebra im A, et al. Effects of gum arabic (Acacia senegal) on renal function in diabetic mice. Kidney Blood Press Res. 2012;35(5):365–72. Doi: 10.1159/000336359

158. Fox BA, Bzik DJ. Bioc emistry and metabolism of Toxoplasma gondii: Purine and pyrimidine acquisition in Toxoplasma gondii and ot er Apicomplexa. Toxoplasma gondii. 2020;397–449.

References

ttps://doi.org/10.1016/B978-0-12-815041-2.00009-8

159. Pey J, San José-Eneriz E, Oc oa MC, Apaolaza I, de Atauri P, Rubio A, et al. In-silico gene essentiality analysis of polyamine biosynt esis reveals APRT as a potential target in cancer. Sci Rep. 2017;7(1):14358. Doi: 10.1038/s41598-017-14067-8

160. Kaartinen K, Hemmilä U, Salmela K, Räisänen-Sokolowski A, Kouri T, Mäkelä S. Adenine p osp oribosyltransferase deficiency as a rare cause of renal allograft dysfunction. Journal of t e American Society of Nep rology. 2014;25(4):671–4. Doi: 10.1681/ASN.2013090960

161. Ras id I, Verma A, Tiwari P, D'Cruz S. Adenine p osp oribosyl transferase deficiency leads to renal allograft dysfunction in kidney transplant recipients: a systematic review. *J Bras Nefrol*. 2022;44(3):403-416. Doi:10.1590/2175-8239-JBN-2021-0283en

162. Pandya D, Nagrajappa AK, Ravi KS. Assessment and Correlation of Urea and Creatinine Levels in Saliva and Serum of Patients wit C ronic Kidney Disease, Diabetes and Hypertension- A Researc Study. J Clin Diagn Res. 2016;10(10):ZC58-ZC62. Doi:10.7860/JCDR/2016/20294.8651

163. Zaidan M, Palsson R, Merieau E, Cornec-Le Gall E, Garstka A, Maggiore U, et al. Recurrent 2, 8-di ydroxyadenine nep ropat y: a rare but preventable cause of renal allograft failure. American Journal of Transplantation. 2014;14(11):2623–32. Doi: 10.1111/ajt.12926

164. Junaid R S aik , MK Patil. Qualitative tests for preliminary p ytoc emical screening: An overview. Int J C em Stud 2020;8(2):603-608. Doi: 10.22271/c emi.2020.v8.i2i.8834

165. Kalpna R, Mital K, Sumitra C. P ysicoc emical and P ytoc emical Analysis of Different Parts of Indian Kesar Mango–A unique variety from Sauras tra Region of Gujarat. P armacognosy Journal. 2016;8(5):502-506. Doi:10.5530/pj.2016.5.16

References

166. Mace ME. Histoc emical localization of p enols in ealt y and diseased banana roots. P ysiol Plant. 1963;16(4):915–25. ttps://doi.org/10.1111/j.1399-3054.1963.tb08367.x

167. Santos M K, S aila D, C andrakumar T, Rajyalaks mi I, Sanjeeva Rao I. P ysicoc emical and P ytoc emical Examination of Medicinal Plants Used in Indigenous System of Medicine. *Journal of Chemistry*. 2005;2(2):142-151. ttps://doi.org/10.1155/2005/589869

168. Gupta M, T akur S, S arma A, Gupta S. Qualitative and Quantitative Analysis of P ytoc emicals and P armacological Value of Some Dye Yielding Medicinal Plants. *Oriental journal of chemistry*. 2013;29(2): 475-481. Doi:10.13005/OJC/290211

169. Suman Kumar Ratnampally, Venkates war C inna. Quantitative Analysis of P ytoc emicals in t e Bark Extracts of Medicinally Important Plant Cassia fistula, Linn. Int. J. Curr. Microbiol. App. Sci. 2017;6(4):1073-1079.

     Doi: ttps://doi.org/10.20546/ijcmas.2017.604.133

170. S arma V, C aud ary U. P armacognostic and p ytoc emical screening of *Helicteres isora* roots. Asian J P arm Clin Res. 2016;9(2):96–101. Doi: 10.22159/ajpcr.2016.v9s2.12178

171. Faisal S, Jan H, S a SA, S a S, K an A, Akbar MT, et al. Green synt esis of zinc oxide (ZnO) nanoparticles using aqueous fruit extracts of *Myristica fragrans*: t eir c aracterizations and biological and environmental applications. ACS Omega. 2021;6(14):9709–9722. ttps://doi.org/10.1021/acsomega.1c00310

172. Cullity BD. Elements of X-Ray Diffraction. Second ed. Reading MA: Addison-Wesley Publis ing Company; 1978.

173. National Researc Council (US) Committee for t e Update of t e Guide for t e Care and Use of Laboratory Animals. Guide for t e Care and Use of Laboratory Animals. 8t ed., National Academies Press (US), 2011. Doi:10.17226/12910

References

174. Al Za'abi M, Ali B, Yasin J, Sc upp N, Nemmar A. Development of a new model for t e induction of c ronic kidney disease via intraperitoneal adenine administration, and t e effect of treatment wit gum acacia t ereon. T e FASEB Journal. 2015;29(1): 938.3. ttps://doi.org/10.1096/fasebj.29.1_supplement.938.3

175. Yokozawa T, Z eng PD, Oura H, Koizumi F. Animal model of adenine-induced c ronic renal failure in rats. Nep ron. 1986;44(3):230–234. ttps://doi.org/10.1159/000183992

176. Bas andy SAE, A med-Farid OAH, Abdelmottaleb-Moussa S, Omara EA, Abdel Jaleel GA, Ibra im FAA. Efficacy of zinc oxide nanoparticles on epatocellular carcinoma induced bioc emical and trace element alterations in rats. J Appl P arm Sci. 2021;11:108–17. Doi: 10.7324/JAPS.2021.110515

177. Awadalla A, Hamam ET, El-Senduny FF, Omar NM, Ma di MR, Barakat N, et al. Zinc oxide nanoparticles and spironolactone-en anced Nrf2/HO-1 pat way and in ibited Wnt/β-catenin pat way in adenine-induced nep rotoxicity in rats. Redox Report. 2022;27(1):249–258. Doi: 10.1080/13510002.2022.2139947

178. Hafiz TA, Mubaraki MA, Diab MSM, Dk il MA, Al-Qurais y S. Ameliorative role of Zizip us spina-c risti leaf extracts against epatic injury induced by Plasmodium c abaudi infected eryt rocytes. Saudi J Biol Sci. 2019;26(3):490-494. Doi:10.1016/j.sjbs.2017.10.012

179. Abd Elmonem HA, Ma moud AH, Abbas M M. Ameliorative Effect of Zinc Oxide Nanoparticles and vitamin E on some Bioc emical and Histological c anges in Irradiated Albino Rats. *Egyptian Journal of Radiation Sciences and Applications*, 2021; 34(1): 1-10. Doi: 10.21608/ejrsa.2021.63788.1116

180. Mesc er Ant ony L. Junqueira's Basic Histology: Text and Atlas. 16t ed. New York: McGraw Hill Medical Publis ing Division; 2021. ttps://www.accessscience.com/content/book/9781260462982

References

181. Drury RAB, Wallington EA. Histological tec nique. 4t ed. New York, Oxford University Press, 1967. ttps://lccn.loc.gov/67004351

182. Weng X, Guo M, Luo F, C en Z. One-step green synt esis of bimetallic Fe/Ni nanoparticles by eucalyptus leaf extract: Biomolecules identification, c aracterization and catalytic activity. 2017;308, 904-911. ttps://doi.org/10.1016/j.cej.2016.09.134

183. Demissie MG, Sabir FK, Edossa GD, Gonfa BA. Synt esis of Zinc Oxide Nanoparticles Using Leaf Extract of *Lippia adoensis* (Koseret) and Evaluation of Its Antibacterial Activity. *Journal of Chemistry*. 2020;2020, 1-9. ttps://doi.org/10.1155/2020/7459042

184. Król A, Pomastowski P, Rafińska K, Railean-Plugaru V, Buszewski B. Zinc oxide nanoparticles: Synt esis, antiseptic activity and toxicity mec anism. *Adv Colloid Interface Sci*. 2017;249:37–52. Doi: 10.1016/j.cis.2017.07.033

185. Balavandy SK, S ameli K, Abidin ZZ. Rapid and green synt esis of silver nanoparticles via sodium alginate media. Int J Electroc em Sci. 2015;10(1):486–497. ttps://doi.org/10.1016/S1452-3981(23)05007-1

186. Nis imure S, Mott D, Takagaki A, Maenosono S, Ebitani K. Role of base in t e formation of silver nanoparticles synt esized using sodium acrylate as dual reducing and encapsulating agents. *Phys Chem Chem Phys*. 2011;13(20):9335–9343. Doi: 10.1039/c0cp02985

187. Gupta M, Tomar RS, Kaus ik S, Mis ra RK, S arma D. Effective antimicrobial activity of green ZnO nano particles of Cat arant us roseus. Front Microbiol. 2018;9:2030. Doi: 10.3389/fmicb.2018.02030

188. Kalaiselvi A, Roopan SM, Mad umit a G, Ramalingam C, Al-D abi NA, Arasu MV. Cat arant us roseus-mediated zinc oxide nanoparticles against p otocatalytic application of p enol red under UV@ 365 nm. *Current Sci*. 2016;111(11):1811–1815. Doi:10.18520/CS/V111/I11/1811-1815

References

189. Go EG, Xu X, McCormick PG. Effect of particle size on t e UV absorbance of zinc oxide nanoparticles. *Scripta Materialia*. 2014;78-79:49-52. ttps://doi.org/10.1016/j.scriptamat.2014.01.033

190. Sing P, Kim YJ, Z ang D, Yang DC. Biological Synt esis of Nanoparticles from Plants and Microorganisms. Trends in Biotec nology. Elsevier Ltd. 2016;34(7):588–599. Doi: 10.1016/j.tibtec .2016.02.006

191. Wang Q, Mei S, Manivel P, Ma H, C en X. Zinc oxide nanoparticles synt esized using coffee leaf extract assisted wit ultrasound as nanocarriers for mangiferin. Curr Res Food Sci. 2022; 5:868-877. ttps://doi.org/10.1016/j.crfs.2022.05.002

192. Huang J, Li Q, Sun D, Lu Y, Su Y, Yang X, et al. Biosynt esis of silver and gold nanoparticles by novel sundried Cinnamomum camp ora leaf. *Nanotechnology*. 2007;18(10):105104. Doi: 10.1088/0957-4484/18/10/105104

193. K alil MMH, Ismail EH, El-Bag dady KZ, Mo amed D. Green synt esis of silver nanoparticles using olive leaf extract and its antibacterial activity. *Arabian Journal of Chemistry*. 2014;7(6):1131–1139. ttps://doi.org/10.1016/j.arabjc.2013.04.007

194. Basnet P, C anu TI, Samanta D, C atterjee S. A review on bio-synt esized zinc oxide nanoparticles using plant extracts as reductants and stabilizing agents. *J Photochem Photobiol B*. 2018;183:201–221. ttps://doi.org/10.1016/j.jp otobiol.2018.04.036

195. Gulati S, Sac deva M, B asin KK. Capping agents in nanoparticle synt esis: Surfactant and solvent system. In: AIP Conference Proceedings. *AIP Publishing LLC*. 2018;1953(1):030214. ttps://doi.org/10.1063/1.5032549

196. Mog ri Moazzen MA, Borg ei SM, Tales i F. C ange in t e morp ology of ZnO nanoparticles upon c anging t e reactant

concentration. Appl Nanosci [Internet]. 2013;3(4):295–302. ttps://doi.org/10.1007/s13204-012-0147-z

197. Alami ZY, Salem M, Gaidi M, Elk amk ami J. Effect of Zn concentration on structural and optical proprieties of ZnO t in films deposited by spray pyrolysis. Adv Energy Int J. 2015;2(4):11–24. Doi: 10.5121/aeij.2015.2402

198. Mayekar J, D ar V, Rad a S. Role of salt precursor in t e synt esis of zinc oxide nanoparticles. Int J Res Eng Tec nol. 2014;3(3):43–45. Doi: 10.15623/ijret.2014.0303008

199. Gontijo LAP, Rap ael E, Ferrari DPS, Ferrari JL, Lyon JP, Sc iavon MA. pH effect on t e synt esis of different size silver nanoparticles evaluated by DLS and t eir size-dependent antimicrobial activity. Matéria (Rio J). 2020;25(4):e-12845. Doi:10.1590/S1517-707620200004.1145.

200. Hasan M, Ulla I, Zulfiqar H, Naeem K, Iqbal A, Gul H, et al. Biological entities as c emical reactors for synt esis of nanomaterials: Progress, c allenges and future perspective. Mater Today C em. 2018;8:13–28. ttps://doi.org/10.1016/j.mtc em.2018.02.003

201. Tourné-Péteil C, Robin B, Lions M, Martinez J, Me di A, Subra G, et al. Combining sol–gel and microfluidics processes for t e synt esis of protein containing ybrid microgels. C emical Communications. 2019;55(87):13112–5. Doi: 10.1039/C9CC04963K

202. Rafaie HA, Samat NA, Nor RM. Effect of pH on t e growt of zinc oxide nanorods using Citrus aurantifolia extracts. Mater Lett. 2014;137:297–9. ttps://doi.org/10.1016/j.matlet.2014.09.033

203. Mo ammadi FM, G asemi N. Influence of temperature and concentration on biosynt esis and c aracterization of zinc oxide nanoparticles using c erry extract. J Nanostructure C em. 2018;8:93– 102. ttps://doi.org/10.1007/s40097-018-0257-6

References

204. Buazar F, Bavi M, Krous awi F, Halvani M, K aledi-Nasab A, Hossieni SA. Potato extract as reducing agent and stabiliser in a facile green one-step synt esis of ZnO nanoparticles. J Exp Nanosci. 2016;11(3):175–84. ttps://doi.org/10.1080/17458080.2015.1039610

205. Ali K, Dwivedi S, Azam A, Saquib Q, Al-Said MS, Alk ed airy AA, et al. Aloe vera extract functionalized zinc oxide nanoparticles as nanoantibiotics against multi-drug resistant clinical bacterial isolates. J Colloid Interface Sci. 2016;472:145–56. ttps://doi.org/10.1016/j.jcis.2016.03.021

206. Al art i MN, Ismail I, Bellucci S, K dary NH, Abdel Salam M. Biosynt esis microwave-assisted of zinc oxide nanoparticles wit zizip us jujuba leaves extract: C aracterization and p otocatalytic application. Nanomaterials. 2021;11(7):1682. Doi: 10.3390/nano11071682

207. Varug ese G, Jit in PW, Us a KT. Determination of optical band gap energy of wurtzite ZnO: Ce nanocrystallites. P ysical Science International Journal. 2015;5(2):146. Doi: 10.9734/PSIJ/2015/14151

208. Ji X, Z ang F, Z ang R, Liu F, Peng Q, Wang M. An acidic polysacc aride from Zizip us Jujuba cv. Muzao: Purification and structural c aracterization. Food C em. 2019;274:494–9. ttps://doi.org/10.1016/j.foodc em.2018.09.037

209. Cun T, Dong C, Huang Q. Ionot ermal precipitation of ig ly dispersive ZnO nanoparticles wit improved p otocatalytic performance. Appl Surf Sci. 2016;384:73–82. ttps://doi.org/10.1016/j.apsusc.2016.05.008

210. Ramimog adam D, Hussein MZ bin, Taufiq-Yap YH. T e effect of sodium dodecyl sulfate (SDS) and cetyltrimet ylammonium bromide (CTAB) on t e properties of ZnO synt esized by ydrot ermal met od. Int J Mol Sci. 2012;13(10):13275–93. Doi: 10.3390/ijms131013275

References

211. Dobrucka R, Długaszewska J. Biosynt esis and antibacterial activity of ZnO nanoparticles using Trifolium pratense flower extract. Saudi J Biol Sci. 2016;23(4):517–23. ttps://doi.org/10.1016/j.sjbs.2015.05.016

212. Ebadi M, Zolfag ari MR, Ag aei SS, Zargar M, S afiei M, Za iri HS, et al. A bio-inspired strategy for t e synt esis of zinc oxide nanoparticles (ZnO NPs) using t e cell extract of cyanobacterium Nostoc sp. EA03: from biological function to toxicity evaluation. RSC Adv. 2019;9(41):23508–23525. Doi:10.1039/c9ra03962g

213. Jayac andran A, Aswat y TR, Nair AS. Green synt esis and c aracterization of zinc oxide nanoparticles using Cayratia pedata leaf extract. Bioc em Biop ys Rep. 2021;2026:100995. ttps://doi.org/10.1016/j.bbrep.2021.100995

214. Dey A, Somaia S. Green synt esis and c aracterization of zinc oxide nanoparticles using leaf extract of T ryallis glauca (Cav.) Kuntze and t eir role as antioxidant and antibacterial. Microsc Res Tec . 2022; 85(8):2835-2847. Doi: 10.1002/jemt.24132

215. Geet a MS, Nagab us ana H, S ivananjaia HN. Green mediated synt esis and c aracterization of ZnO nanoparticles using Eup orbia Jatropa latex as reducing agent. Journal of Science: Advanced Materials and Devices. 2016;1(3):301–310. ttps://doi.org/10.1016/j.jsamd.2016.06.015

216. Sures J, Prad ees G, Alexramani V, Sundrarajan M, Hong SI. Green synt esis and c aracterization of zinc oxide nanoparticle using insulin plant (Costus pictus D. Don) and investigation of its antimicrobial as well as anticancer activities. Advances in Natural Sciences: Nanoscience and Nanotec nology. 2018;9(1):015008. Doi: 10.1088/2043-6254/aaa6f1

217. Pillai AM, Sivasankarapillai VS, Ra dar A, Josep J, Sadeg far F, Rajes K, et al. Green synt esis and c aracterization of zinc oxide

References

nanoparticles wit antibacterial and antifungal activity. J Mol Struct. 2020;1211:128107. ttps://doi.org/10.1016/j.molstruc.2020.128107

218. Kalpana VN, Devi Rajeswari V. A review on green synt esis, biomedical applications, and toxicity studies of ZnO NPs. Bioinorg C em Appl. 2018;2018: 3569758.

    ttps://doi.org/10.1155/2018/3569758

219. Al art i MN, Ismail I, Bellucci S, Jaremko M, Abo-Aba SEM, Abdel Salam M. Biosynt esized Zinc Oxide Nanoparticles Using Zizip us Jujube Plant Extract Assisted by Ultrasonic Irradiation and T eir Biological Applications. Separations. 2023; 10(2):78.

    ttps://doi.org/10.3390/separations10020078

220. Selvanat an V, Aminuzzaman M, Tan LX, Win YF, C ea ESG, Heng MH, Tey L-H, Arullappan S, Alget ami N, Al art i SS, Sultana S, S a iduzzaman M, Abdulla H, Akt aruzzaman M, Synt esis, c aracterization, and preliminary in vitro antibacterial evaluation of ZnO nanoparticles derived from soursop (Annona muricata L.) leaf extract as a green reducing agent, Journal of Materials Researc and Tec nology. 2022; 20: 2931-2941.

    ttps://doi.org/10.1016/j.jmrt.2022.08.028

221. Al Za'abi M, al Busaidi M, Yasin J, Sc upp N, Nemmar A, Ali BH. Development of a new model for t e induction of c ronic kidney disease via intraperitoneal adenine administration, and t e effect of treatment wit gum acacia t ereon. Am J Transl Res. 2015;7(1):28-38. PMID: 25755826; PMCID: PMC4346521.

222. Ramadan AG, Yassein AAM, Eissa EA, Ma moud MS, Hassan GM. Bioc emical and istopat ological alterations induced by subc ronic exposure to zinc oxide nanoparticle in male rats and assessment of its genotoxicicty. J.Umm Al-Qura Univ. Appll. Sci. 2022;8:41–49.

    ttps://doi.org/10.1007/s43994-022-00008-3

References

223. Mansouri E, K orsandi L, Orazizade M, Jozi Z. Dose-dependent epatotoxicity effects of zinc oxide nanoparticles. 2015; 2(4): 273-282. Doi: 10.7508/nmj. 2015.04.005

224. Ben SI, Mrad I, Ri ane N, Mir LEL, Sakly M, Amara S. Sub-acute oral toxicity of zinc oxide nanoparticles in male rats. 2015;6(3). Doi: 10.4172/2157-7439.1000284

225. Alebac ew M, Kinfu Y, Makonnen E, Bekuretsion Y, Urga K, Afework M. Toxicological evaluation of met anol leaves extract of Vernonia bipontini Vatke in blood, liver and kidney tissues of mice. Afr Healt Sci. 2014;14(4):1012–24. Doi: 10.4314/a s.v14i4.33

226. Me rdad M, Moz gan GP. Effect of Zingiber Extract on Histopat ologic C anges in Mice Kidneys. Res J Appl Sci. 2011;6(5):295–298. Doi: 10.3923/rjasci.2011.295.298

227. George SA, Al-Rus aidan S, Francis I, Soonowala D, Nampoory MRN. 2,8-Di ydroxyadenine Nep ropat y Identified as Cause of End-Stage Renal Disease After Renal Transplant. Exp Clin Transplant. 2017;15(5):574-577. Doi:10.6002/ect.2015.0096

228. Cai H, Su S, Li Y, Zeng H, Z u Z, Guo J, et al. Protective effects of Salvia miltiorr iza on adenine-induced c ronic renal failure by regulating t e metabolic profiling and modulating t e NADPH oxidase/ROS/ERK and TGF-β/Smad signaling pat ways. J Et nop armacol. 2018;212:153–165. Doi: 10.1016/j.jep.2017.09.021

229. Törmänen S, Pörsti I, Lakkisto P, Tikkanen I, Niemelä O, Paavonen T, et al. Endot elin A receptor blocker and calcimimetic in t e adenine rat model of c ronic renal insufficiency. BMC Nep rol. 2017;18(1):1–13. Doi: 10.1186/s12882-017-0742-z

230. Wang J, Wang F, Yun H, Z ang H, Z ang Q. Effect and mec anism of fucoidan derivatives from Laminaria japonica in experimental adenine-induced c ronic kidney disease. J Et nop armacol. 2012;139(3):807–813. Doi: 10.1016/j.jep.2011.12.022

References

231. Jarsia P, Nosrati A, Alizade A, Has emi-Sote SMB. Hepatotoxicity and ALT/AST enzymes activities c ange in t erapeutic and toxic doses consumption of acetaminop en in rats. Int Biol Biomed J. 2017;3(3):119–124. URL꞉ ttp://ibbj.org/article-1-129-en. tml

232. Soleymaninejad M, Joursaraei S G, Feizi F, Jafari Anarkooli I. T e Effects of Lycopene and Insulin on Histological C anges and t e Expression Level of Bcl-2 Family Genes in t e Hippocampus of Streptozotocin-Induced Diabetic Rats. Journal of diabetes researc , 2017; 4650939. ttps://doi.org/10.1155/2017/4650939

233. Ali BH, Al-Salam S, al Suleimani Y, al Kalbani J, al Ba lani S, As ique M, et al. Curcumin ameliorates kidney function and oxidative stress in experimental c ronic kidney disease. Basic Clin p armocol Toxicol. 2018;122(1):65–73. Doi: 10.1111/bcpt.12817

234. C ang X ying, Cui L, Wang X z i, Z ang L, Z u D, Z ou X rong, et al. Quercetin attenuates vascular calcification t roug suppressed oxidative stress in adenine-induced c ronic renal failure rats. Biomed Res Int. 2017;2017:5716204. Doi: 10.1155/2017/5716204

235. El-Maddawy ZK, Abd El Naby WSH. Protective effects of zinc oxide nanoparticles against doxorubicin induced testicular toxicity and DNA damage in male rats. Toxicol Res (Camb). 2019;8(5):654–662. Doi: 10.1039/c9tx00052f

236. K aleel SMJ, Jaran AS, Haddadin MSY. Evaluation of total p enolic content and antioxidant activity of t ree leaf extracts of *Ziziphus spina-christi* (Sedr) grown in Jordan. Br J Med Med Res. 2016;14:1–8. Doi: 10.9734/BJMMR/2016/24935

237. K aleel SM, Almu ur RA, Al-Deeb TMF, Jaran AS. Bioc emical c anges in t e liver, kidney and serum of rats exposed to et anolic leaf extract of *Ziziphus spina-christi*. *Jordan Journal of Biological Sciences*. 2021;14(4): 763 – 767. ttps://doi.org/10.54319/jjbs/140417

References

238. Engle SJ, Stockelman MG, C en JU, Boivin G, Yum MN, Davies PM, et al. Adenine p osp oribosyltransferase-deficient mice develop 2, 8-di ydroxyadenine nep rolit iasis. Proceedings of t e National Academy of Sciences. 1996;93(11):5307–5312.
Doi: 10.1073/pnas.93.11.5307

239. Pettitt RM, Brumbaug AP, Gartman MF, Jackson AM. C ronic kidney disease: Detection and evaluation. Osteopat ic Family P ysician. 2020;12(1):14–19. Doi:10.33181/12011

240. al Za'abi M, S alaby A, Manoj P, Ali BH. T e in vivo effects of adenine-induced c ronic kidney disease on some renal and epatic function and CYP450 metabolizing enzymes. P ysiol Res. 2017;66(2):263-271. Doi: 10.33549/p ysiolres.933374

241. Amin A, Ma moud-G oneim D. *Zizyphus spina-christi* protects against carbon tetrac loride-induced liver fibrosis in rats. Food and C emical Toxicology. 2009;47(8):2111–2119.
ttps://doi.org/10.1016/j.fct.2009.05.038

242. El Maaiden E, El K arrassi Y, Qara NAS, Essamadi AK, Moustaid K, Nasser B. Genus Zizip us: A compre ensive review on et nop armacological, p ytoc emical and p armacological properties. Journal of Et nop armacology. 2020;259:112950.
Doi: 10.1016/j.jep.2020.112950

243. Ra imi G, Mo ammad KS, Zarei M, S okoo i M, Oskoueian E, Poorbag er MRM, et al. Zinc oxide nanoparticles synt esized using Hyssopus Officinalis L. Extract Induced oxidative stress and c anges t e expression of key genes involved in inflammatory and antioxidant Systems. Biol Res. 2022;55(1):24.
ttps://doi.org/10.1186/s40659-022-00392-4

الخلاصة

الجسيمات النانوية لأوكسيد الخارصين تمت دراستها على نطاق واسع في العقود الأخيرة وذلك بسبب تطبيقاتها في الطب النانوي بناءً على العوامل الفسيولوجية والكيميائية الحيوية التي تمتلكها وغالبا ما تضاف هذه الجسيمات كمكمل غذائي في اغذية الحيوانات. تهدف هذه الدراسة إلى تخليق وتوصيف جسيمات أوكسيد الخارصين النانوية بالطريقة البايولوجية باستخدام المستخلص النباتي، ثم دراسة تأثيراتها في ذكور الجرذان المعرضة للادنين. التخليق الحيوي لجسيمات أوكسيد الخارصين النانوية (ZnO-NPs) تم باستخدام المستخلص المائي الخام لأوراق السدر *Ziziphus-spina christi* ونترات الخارصين سداسي الماء $(Zn (NO_3)_2.6H_2O)$. وسط التفاعل كان عند درجة حرارة 60 مئوية، ودالة حامضية 9. ان التغيير في لون المحلول من الأصفر إلى البني يشير الى تكوين جزيئات أوكسيد الخارصين النانوية. ان جسيمات اوكسيد الخارصين النانوية الناتجة تم تشخيصها باستخدام عدة تقنيات. حيث أظهر طيف الأشعة فوق البنفسجية-المرئية (UV-Vis) قمة عظمى عند 362 نانومتر، وهذا يعد دليلًا واضحا على تكوين ZnO-NPs. بين طيف الاشعة تحت الحمراء (FT-IR) ظهور قمم امتصاص بين 400 و500 سم$^{-1}$ تعود الى اهتزاز اصرة Zn-O. اما تحليل حيود الأشعة السينية (XRD) فقد اظهرالتركيب Hexagonal-Wurtzite للجسيمات النانوية الناتجة بمتوسط حجم بلوري عند 38.177 نانومتر. وبين فحص المجهري الإلكتروني (TEM) أن الجسيمات النانوية لها شكل كروي وتقع في مدى 35-45 نانومتر، كما أظهرت صور الماسح المجهري الإلكتروني (SEM) الشكل الشبه الكروي للجسيمات النانوية على شكل تجمعات بمتوسط حجم الجسيمات، D1 (39.74 نانومتر)، D2 (42.25 نانومتر)، و3D (48.19 نانومتر). اضافة الى ذلك، اظهر مجهر القوة الذرية (AFM) عن سطحًا موحدًا مع حبيبات تشبه المخروط تغطي سطح جسيمات اوكسيد الخارصين النانوية. في هذه الدراسة تم استخدام ستة وثلاثين من ذكور الجرذان وقسمت عشوائيا إلى ست مجموعات متساوية (ستة جرذان لكل مجموعة). المجموعة الأولى جرعت فقط بثنائي ميثيل سلفوكسيد عن طريق الفم (5% v/v/day) لمدة 30 يومًا واعتبرت كمجموعة سيطرة، المجموعة الثانية جرعت بمادة الأدينين عن طريق الفم (100 mg/Kg. Bw/day) لمدة 30 يومًا، المجموعة الثالثة تم اعطائها مستخلص أوراق السدر (10 mg/Kg. Bw/day) لمدة 30 يومًا، المجموعة الرابعة أعطيت ZnO-NPs (10 mg/Kg. Bw/day) لمدة 30 يومًا، المجموعة الخامسة تم إعطائها جرعة من الأدينين مع جرعة من مستخلص أوراق السدر لمدة 30 يومًا، كذلك المجموعة السادسة والأخيرة تلقت جرعة مشتركة من الأدينين و ZnO-NPs لمدة 30 يومًا. بعد نهاية 30 يوما، تم قياس وظائف الكلية (حامض اليوريك، اليوريا والكرياتينين) ووظائف الكبد (ALT، AST و ALP). كذلك تم قياس مستويات مضادات

الأكسدة (MDA, GSH, SOD, CAT, TAC و NO). بالإضافة الى ذلك، تم دراسة التغيرات النسيجية المرضية لكل من الكلى والكبد لدى الجرذان. أظهرت النتائج ان كل من مستخلص أوراق السدر وجسيمات اوكسيد الخارصين النانوية بجرعة (10 mg/Kg.Bw/day) لها تأثيرات فعالة ضد التعرض للأدينين، وهذا ما أكده تحسن النمو في ذكور الجرذان إضافة الى الانخفاض المعنوي في مؤشرات وظائف الكلى والكبد (حامض اليوريك، اليوريا، الكرياتينين ، ALT، AST ALP) بعد اعطائها الجرعة اعلاه مقارنة بالجرذان المعرضة للأدينين (0.05 ≤ P). كما تم ملاحظة ان هناك ارتفاع كبير في مستويات مضادات الأكسدة SOD وCAT وTAC وGSH بالتوافق مع الانخفاض في مستويات كل من MDA و NO في كلا مجموعتي المعالجة (الخامسة والسادسة) عند مقارنتها بجرذان مجموعة الأدينين. اضافة الى ذلك، كان هناك تحسن ملحوظ في التغيرات النسيجية لكل من الكلى والكبد بالمقارنة مع مجموعة جرذان الأدينين. بناءا على هذه النتائج يمكن الاستنتاج، أن جسيمات أوكسيد الخارصين النانوية ومستخلص اوراق السدر لها تأثيرات فعالة لدى ذكور الجرذان المعرضة للأدنين، مما يعني أنه يمكن استخدامها بشكل آمن ضد تلف الكلية والكبد الناجم من تأثير المواد السمية، حيث لم يلاحظ أي آثار ضارة في أنسجة الكلية والكبد، مما يشير إلى أنها قد تكون مضادات قوية للأكسدة والالتهابات والتسمم وفعالة للاستخدام في التطبيقات الطبية الحيوية.

جامعة كربلاء

كلية العلوم

قسم الكيمياء

التخليق الحيوي لجسيمات أوكسيد الخارصين النانوية باستخدام مستخلص أوراق السدر: التشخيص ودراسة تأثيراته في ذكور الجرذان المعرضة للأدنين

من قبل

حيدر حسين عباس

بكالوريوس علوم كيمياء/ جامعة الكوفة (2009)

بأشراف

أ.د. نرجس هادي السعدي   أ.د. عايد حميد حسن

1445 هـ   2023 م