CAPITAL UNIVERSITY OF SCIENCE AND TECHNOLOGY, ISLAMABAD

Evaluation of *In vivo* Toxicity of Green Synthesized Silica Nanoparticles using *Ficus carica* L. Leaf Extract on *Sprague dawley* Rat Model

by

Aqsa Zafar

A thesis submitted in partial fulfillment for the degree of Master of Science

in the

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Thanks to Allah Almighty, we proudly dedicate this project to our loved ones and friends; without their support and love, this would not have been possible. Most importantly, our parents and friends who supported us both morally and financially, if it were not for them our goals would be unattainable.

CERTIFICATE OF APPROVAL

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(MBS223002)

THESIS EXAMINING COMMITTEE

S. No.	Examiner	Name	Organization
(a)	External Examiner	Dr. Muhammad Ali	UMT, Lahore
(b)	Internal Examiner	Dr. Rizwan Ur Rehman	CUST, Islamabad
(c)	Supervisor	Dr. Sania Riaz	CUST, Islamabad

Dr. Sania Riaz
Thesis Supervisor
September, 2024

Dr. Syeda Mariam Bakhtiar
Head
Dept. of Bioinfo. and Biosciences
September, 2024

Dr. Sahar Fazal
Dean
Faculty of Health and Life Sciences
September, 2024

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(Aqsa Zafar)

Abstract

Silica nanoparticles (SiNPs) have gained attention to get used in numerous fields like drug delivery system, cancer therapy, biosensors and electronic chips and even in production of solar cells with nano sized silica due to their porous and non-porous nature. Besides these useful aspects of SiNPs, there are some drawbacks regarding the use of SiNPs particularly in living organisms causing damage to cells or DNA as they are inorganic particles. There is need to reduce the toxicity of these particles in order to use them for biological treatments. Synthesis methods of NPs play vital role in this regard. Biologically synthesized nanoparticles induce less toxicity than chemically synthesized. Aim of this study was to synthesize Silica nanoparticles using green synthesis method to reduce their toxicity, enhance biocompatibility and targeting ability. Synthesis of NPs was done by using aqueous leaf extract of *Ficus carica* (common fig) and characterizations were done by UV-Vis, FTIR, XRD, SEM and EDX techniques. These characterization techniques showed that the synthesized NPs were from 19-33nm in size. After characterization and confirmation of NPs synthesis, effect of low dose (7.5mg) and high dose (22.5mg) of SiNPs was checked on Rat Model (*Sprague Dawley*). Hematological, biochemical and histological parameters were compared. Overall slight difference in WBCs, RBCs, HB, MCV, Platelets, ALT level, AST level, Urea, Creatinine, CRP and ESR levels was reported when compared with the standard control. In histological investigations, hepatocytes with minimum changes were observed in low dose group and liver cells with cellular morphology and fatty changes were observed in high dose group. From this study, it is concluded that NPs can be further optimized for further biocompatible use for various purposes.

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Abbreviations

AFM	Atomic Force Microscopy
AgNPs	Silver Nanoparticles
AuNPs	Gold Nanoparticles
BET	Brunaur, Emmett and Teller Method
CVD	Chemical Vapor Deposition
DLS	Dynamic Light Scattering
EDX	Energy Dispersive X-ray
ENMs	Engineered Nanomaterials
Gd	Gadolinium
HTFs	Human Tenon's Fibroblast
IRS	Infrared Spectroscopy
LDH	Lactate Dehydrogenase
LSPR	Localized Surface Plasmon Resonance
MOFs	Metal Organic Frameworks
MSN	Mesoporous Silica Nanoparticles
NPs	Nanoparticles
NiO2NPs	Nickel Oxide Nanoparticles
ROS	Reactive Oxygen Species
SEM	Scanning Electron Microscopy
SiNPs	Silica Nanoparticles
TEM	Tunneling Electron Microscopy
XPS	X-ray Photoelectron Spectroscopy
XRD	X-Ray Diffraction Analysis

Symbols

D	Crystal size
Θ	Diffraction angle
λ	X-ray wavelength
B	Full width at half maximum

Chapter 1

Introduction

1.1 Background

It is fascinating to learn that some of the early uses of Nanotechnology can be dated as far back as 4th century in the Roman Empire. This can be attested in fashioning of the Lycurgus Cup which is a glasswork that holds the ability to change color [1]. Nanoparticles are extremely small particles usually having a size between one and 100 nanometers (nm) in diameter. Due to their small size, nanoparticles have attributes that distinguish them from other scaled-up matters or substances. These peculiarities of the structure and state make nanoparticles applicable for a specific range of objects and purposes such as in medicine, technology, and industry [2].

There are different materials used in the manufacturing of nanoparticles, for instance, metal and non-metal, polymer and semiconductor, and bio molecules among others. The specific characteristics of a nanoparticle will thus be a function of size, shape, chemical composition and surface chemistry. For instance, gold NPs can be red or blue depending on the size of the NP. This is due to its undeniable antimicrobial characteristics that would make it useful in or wound dressings and other related medical uses. Cantilevers made of carbon nanotubes are extremely strong and can be used for making light-weight high strength composites [2].

One of the preserved approaches of applying drugs into the body is through Nanoparticles, and this has proven to be an innovation in the medical field. These systems employ nanoparticle, particles with at least one dimension being in the nanometer scale (1-100 nm) as micro carriers for drugs [1][3]. This technology has immense potential in overcoming some of the barriers that are associated with the conventional drug deliveries. Here's a glimpse into the key advantages of nanoparticle-based systems: Thus, cellular and tissue barriers can be penetrated by nanoparticles because of their small size and action can be aimed at target cells or tissues [4][5]. This makes it less invasive to the body which may reduce the impact to healthy cells and thus less side effects. Most of the therapeutic drugs have low solubility in water and hence experience low absorption when administrated in the body. These drugs can be incorporated into nanoparticles which can help in increasing the solubility and bioavailability of the said drugs [2].

They are prepared and synthesized in different ways which partly define their nature, characteristics, toxicity and uses. Two main approaches that are fundamental strategies for the synthesis and fabrication of nanoparticles are: Top-down approach and the bottom-up approach which has its strengths and weaknesses. Fragmentation means the creation of nanoparticles through the reduction of larger materials: this is the top down approach. This method normally employs approaches like lithography, milling, and etching to either cut or wear down mass materials to nanoparticles. On the other hand, bottom-up approach uses the formation of nanoparticles from atomic or molecular levels. This method is based on the use of chemical reactions, processes of self-assembly or occurs in living organisms to create nanoparticles from small parts [4].

Metals NPs have interest of researcher from different field due to their characteristics and multiple uses and application. Another interesting feature of metallic particles is the ability to control their chemical and physical characteristics because of the possession of nano-scaled size and high ratio of surface area to volume. These characteristics allowed for numerous applications that span any from the catalysis and sensing on to biomedicine and environment remediation [5]. Nevertheless, the application of metallic nanoparticles is facing numerous problems. Due to the controversies on their possible toxicity and effects on the environment,

there is the need to conduct a risk assessment and a general hazard analysis to guard against any bare acknowledged susceptibilities on safety and sustainability [6].

Silica nanoparticles (SiO2) NPs have attained considerable recognition and acceptances as a base for different multifaceted therapeutic applications because of these distinctive quality. SiO2 NPs has a very large surface area that enables it to effectively interact with and encapsulate therapeutic drugs, genes or imaging agents [6]. The release of the therapeutic cargo can be adjusted depending of the surface characteristics or porosity of the NPs, so that it will be reached only at the desired tissue [7]. Depending on the type of silica NPs, it is possible to state that they possess, to a significant extent, biocompatibility and that may eliminate the problem of toxicity [8].

SiO2 NPs are a good substrate for delivering contrast media for these diagnostic procedures such as the Magnetic Resonance Imaging (MRI) or computed tomography (CT) [8]. This helps in better visualization of the diseased tissues. Antibacterial molecules can be anchored on the surface of the silica NPs where desired. Some of these NPs can kill bacteria selectively and may provide a solution in preventing antibiotic resistance [9]. Due to the high surface area, biocompatibility and biodegradability of SiO2 NPs they act as good scaffolds for tissue engineering applications. These scaffolds could also help tissue cells to grow and regenerate [10]. Some types of SiO2 NPs are thermosensitive that means they are capable to produce heat in response to light or magnetic fields. Therefore, the localized heating effect can be applied to cancer treatment with attempts aiming at killing the cancerous cells [11].

Thus the common fig (*Ficus carica*) belongs to the two set of chromosomes family of mulberry (Moraceae). It has been cultured for centuries, and it is classified into over 1400 varieties, which are in about thirty seven groups. Common fig species have been a human source of food for several centuries now mostly through the figs and the leaves. In the past few years, there has been growing research concern in the medical uses of different organs of the fig tree. It has also been reported that the common fig operates against viruses, bacteria, fungi and also reduce blood sugar

level. These attributes may be attributed to the existence of certain chemicals known as secondary metabolites that include the flavonoids, phenolic compounds, phytosterols and fatty acids. Out of these phytoconstituents, phenolic compounds, show redox property and hence act as reducing agent in solution [12].

1.2 Problem Statement

Conventional drug delivery systems suffer from limitations like non-specific and poor bioavailability, and toxicity to healthy cells of the human body. If using nanoparticles as targeted drug delivery agent, chemically synthesized nanoparticles induce hyper toxicity and cellular damage as compared with the ecofriendly green synthesized nanoparticles used in different conjugated drugs.

1.3 Gap Analysis

The information regarding the potential use of *Ficus carica* based Silica nanoparticles synthesis is lacking and their impact on hepato and renal toxicity is also not been reported earlier.

1.4 Hypothesis

Green synthesized Silica nanoparticles with *Ficus carica* may show minimum to no toxicity in animal model.

1.5 Aim and Objectives

The aim of the study is to investigate the in-vivo toxicity of biosynthesized silica nanoparticles with *Ficus carica L.* leaf extract.

- To synthesize SiO2 NPs using *Ficus carica* leaf extract.
- To characterize the SiO2.
- To evaluate the toxicity of the biosynthesized Silica NPs in animal model.

Chapter 2

Literature Review

2.1 Nanoparticles Overview

Nanoparticles or NPs are a large class of materials which contain particles with at least one dimension – less than 100 nm in size. These particles can be of assorted shapes that would give them dimensions of zero (0D), one (1D), two (2D), or three dimensions (3D). A characteristic feature is that the size of nanoparticles substantially affects their external and internal properties, such as optical ones. For instance, 20nm gold nanoparticles have a wine red color, platinum nanoparticles yellowish-gray, silver nanoparticles black and palladium nanoparticles dark black [13].

2.1.1 Classification

NPs can be divided into various forms depending on their shape, size, and composition. The physical and chemical attributes of some common types of NPs are reported in different studies [13]. Carbon Based NPs, two vital types of nanoparticles (NPs) include fullerenes and carbon nanotubes - CNTs (Figure 2. 1 A and B). These materials have multiple applications; as fillers, gas absorbents for environmental remediation purposes, and as supportive matrix for homogenous and heterogeneous inorganic/organic catalysts Metal NPs, NPs of metals are made of

only metal atoms. In the definition of metal nanoparticles, the words metal and atoms connect in ways that require definition. These atoms are derived from metal precursor compounds that is used in the synthesis process. Metallic nanoparticles (MNPs) are characterized by tunable optical phenomena, which makes them useful in various scientific disciplines. One common application that can be described is in the deposition of Gold NPs (AuNPs) on samples to be analyzed by SEM. This layer prevents formations of thin oxide layers on the surface and enhances the flow of electrons leading to a better SEM picture [13].

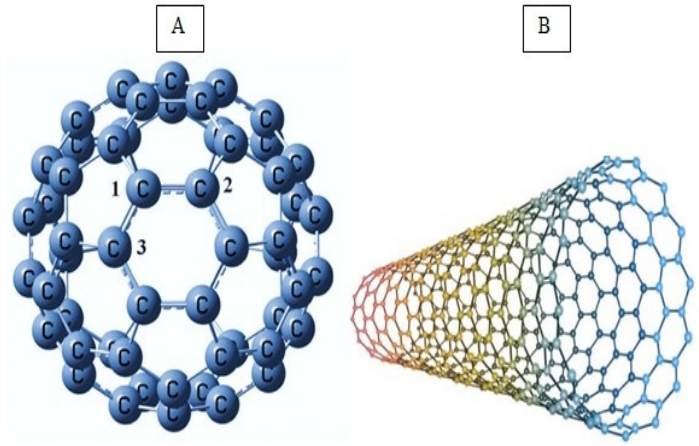

FIGURE 2.1: (A) Fullerene structure C60 and (B) Carbon Nanotube CNT [13]

While the regular ceramics are made through a series of heating and forming processes, the ceramic nanoparticles are made essentially at a nanoscale, these are objects that range in size between 0.1 and 0.3um. They may be amorphous that is they do not have a regular crystal structure, polycrystalline- consisting of many tiny crystals, dense- devoid of many pores, porous- full of many tiny holes or even hollow. Because of these characteristics, there is rising interest of researchers in the application of ceramic nanoparticles [13]. Polymeric NPs, these nanoparticles are generally organic in nature. They often come in two main shapes: These are; the nano spheres and the nano capsules. Nanospheres are a set of solid particles in which other molecules are adsorbed on the outer surface. They again, surround the entire bulk of the solid material inside their shells [13]. Lipid-based NPs are described as those NPs that encapsulate or conjugate fatty molecules (lipid moieties) and have been well exemplified in biomedical uses. The practices of NPs

for numerous usages are under research such as, drug delivery and release, and RNA in cancer treatment [13].

2.1.2 Physiochemical Properties of Nanoparticles

This section explores how some of the most important physicochemical properties change when materials are reduced to the nanoscale [14].

2.1.2.1 Mechanical Properties

In case of conventional materials, mechanical properties include generally ten characteristics which are brittleness, toughness, elasticity, strength, hardness, ductility, rigidity, plasticity, fatigue, and yield stress. It should be noted that the specific characteristics of NPs are due to factors such as the area/volume ratio and quantum effects. These effects lead to mechanical behaviors of NPs that are away from the behaviors of bulk materials [14]. In the lubricated contacts, whether the nanoparticles are indented the softer surface or, the NP itself is plastically deformed mainly depends on the relative stiffness between the nanoparticles and the contact surface. It is useful for analyzing how NP performs in these circumstances. In other words, it becomes critical to control the mechanical properties of NPs and their behaviors with different surfaces in order to obtain favorable surface quality and efficient material removal as claimed [13].

2.1.2.2 Thermal Properties

The size of nanoparticles (NPs) significantly affects their electrical and thermal conductivity. As NPs are formed, the ratio of their surface area to volume increases dramatically. This enlarged surface area plays a crucial role in heat transfer. Because heat conduction partly relies on electron movement, the abundance of electrons near the surface of NPs enhances their ability to conduct heat compared to bulk materials [14]. Compared to fluids in solid form (like ice), metals in nanoparticle form typically demonstrate much higher thermal conductivity. This

translates to a significant difference in heat transfer efficiency. For instance, copper nanoparticles at room temperature can conduct heat roughly 700 times better than water ice and around 3,000 times better than engine oil. In order to maximize heat transfer efficiency, particles with a larger total surface area are preferred. This is because a larger surface area allows for greater contact between the particles and the surrounding environment, facilitating heat exchange. Additionally, a larger surface area contributes to the stability of the suspension by preventing the particles from clumping together or settling out [13].

2.1.2.3 Magnetic Properties

The mentioned elements are also diamagnetic in a mass state, including palladium (Pd), gold (Au), and silver (Ag). However, these clusters do not always behave in the same manner and this is very noticeable and in fact can shift markedly at the nanoscale. For these elements when they are made into nanoparticles (NPs) some of them will possess magnetism since the electron distribution within the particles is not symmetrical. The magnetic properties of bulk materials are primarily determined by four factors: the type of atoms involved (constituents), the manner in which these atoms are grouped into a characteristic pattern (as a crystal lattice), the application's inclination for the material to align in a single direction (magnetic partial preference), and the existence of sites lack atoms (vacancies). At the nanoscale, however, two additional factors become crucial: with the size and the shape of the nanoparticles [14]. Thus, the final characteristics of these materials depend on the method employed to produce them. Some common synthetic methods are solvothermal, coprecipitation, micro emulsion synthesis, and thermal decomposition, and flame spray synthesis [13].

2.1.2.4 Electronic and Optical Properties

Nanoparticles (NPs) display different properties of both optics and electronics, and the two areas are still inextricably connected. One rather vivid example of this can be observed in noble metal NPs. Unlike the bulk materials, these materials have

distinctive optical characteristics that are dependent on size of the nanoparticles. This size dependence becomes show by a strong absorption band in the ultraviolet and visible (UV-Vis) region of the electromagnetic spectrum. This band which is missing in the bulk metals appears because of a phenomenon called Localized Surface Plasmon Resonance or LSPR. LSPR is characterized by the detection of the nanoparticle's capability to interact with light by using a spectrophotometer when the wavelength of the incoming light corresponds with the resonance of the conduction electrons in the nanoparticle. There are many works that have clearly demonstrated that the specific color of light that get absorbed in the d end-users (represented by the LSPR spectrum peak) depends on the size, shape, and interparticle spacing of the nanoparticles [13].

2.1.3 Synthesis Approaches

There are two main approaches for synthesizing NPs:

- Bottom-up approach
- Top-down approach

2.1.3.1 Top Down Approach

This method uses a breakdown approach. It starts with large molecules, which are broken down into smaller components using various methods (Fig: The following strategies are proposed for the implementation of this part of the research: 2. 2). They are then ground into the required smaller particle sizes which can be in the nano sized particles range [13]. This method helps researchers to divide large material into much smaller and actually nano-level parts. Focusing on the top-down process has limitations of creating complicated patterns or shapes of the particles or specific sizes of the particles. One of the major issues that need to be addressed by this method is the ability to produce the nanoparticles of the correct size and shape. Common methods applied to make nanoparticles to include mechanical breakdown, photolithography, laser vaporization, sputtering and thermal cracking [15].

FIGURE 2.2: Top-Down Approach [16]

2.1.3.2 Bottom Up Approach

In nanofabrication, a method called bottom-up or self-assembly builds functional structures by harnessing chemical and physical forces at the nanoscale (Fig: In this case, the opinions are as follows: 2. 3). This approach works on the principle of putting in place elaborate structures through the compilation of small elements. Bottom-up techniques utilize principles of biological systems where like forces are used to build everything that is required by life. Scientists are emulating this by developing concepts of atomic clusters that self-assemble into different forms of structures. Among the most popular techniques for the nanoparticle synthesis that can be attributed to bottom-up approach, it is possible to distinguish sol-gel, spinning, chemical vapor deposition (CVD), pyrolysis, and biosynthesis [15].

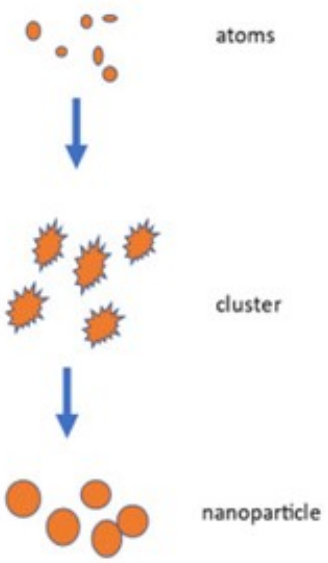

FIGURE 2.3: Bottom-Up Approach [16]

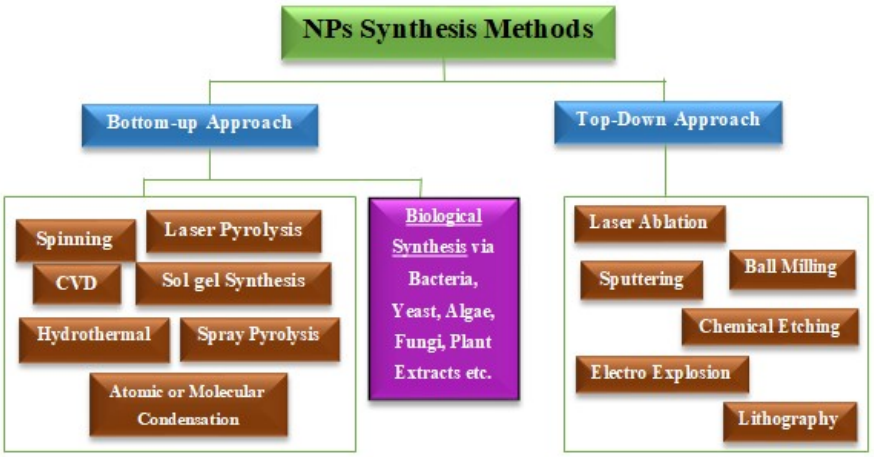

FIGURE 2.4: Flowchart of Synthesis Approaches of NPs

2.1.4 Characterization of NPs

Understanding the physicochemical properties of nanoparticles (NPs) is crucial for determining their potential applications. Therefore, scientists employ a variety of methods and techniques to analyze and characterize these properties [14].

2.1.4.1 Morphological Characterizations

Shape and structure or morphological characteristics of nanoparticles (NPs) are aspects of research that have significant consequences. This is because shape defines many of a nanoparticle's characteristics, indeed, morphology has a strong influence on a nanoparticle's properties. There are many methods for the morphological characterization of NPs But use of techniques like scanning electron microscopy (SEM) and tunneling electron microscopy (TEM) are widely used in morphological characterization of NPs. SEM is a technique that usually based on the scanning electron principle, Several microscopy techniques can be employed in SEM including type transmission electron microscopy and scanning tunneling microscope amongst others. It gives information concerning particles at the nanoscale. First of all, It gives us the information of nanoparticles not only morphology of the particles but also the dispersion of nanoparticles in matrices or bulk. Scanning electron microscopy (SEM) also used to investigate the morphology of Gold nanoparticles (Au nanospheres) shown in Fig.2.5. Fig.2.6 insights into the dispersion of ZnO nanoparticles and the overall morphologies of the MOFs synthesized under various reaction conditions [13].

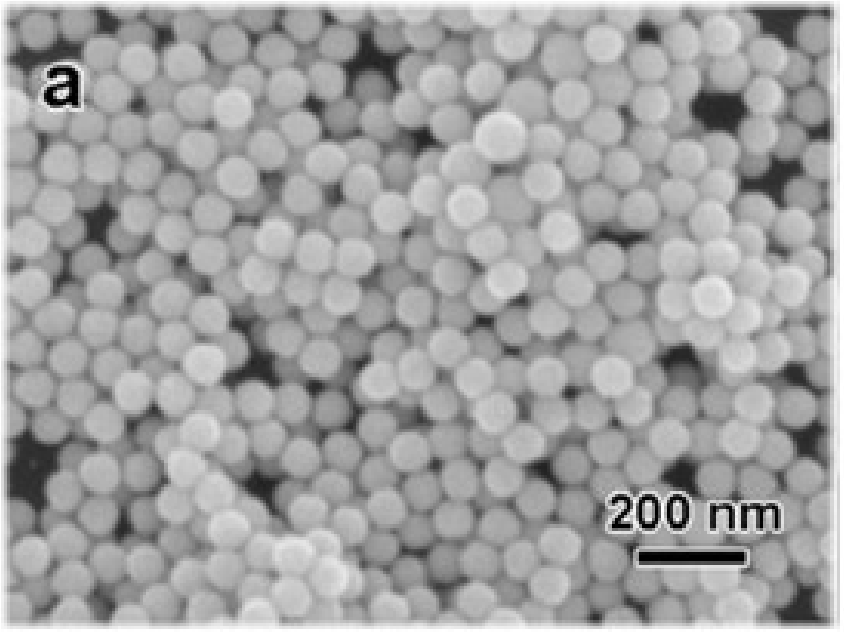

FIGURE 2.5: SEM of Gold (Au) Nanospheres [13].

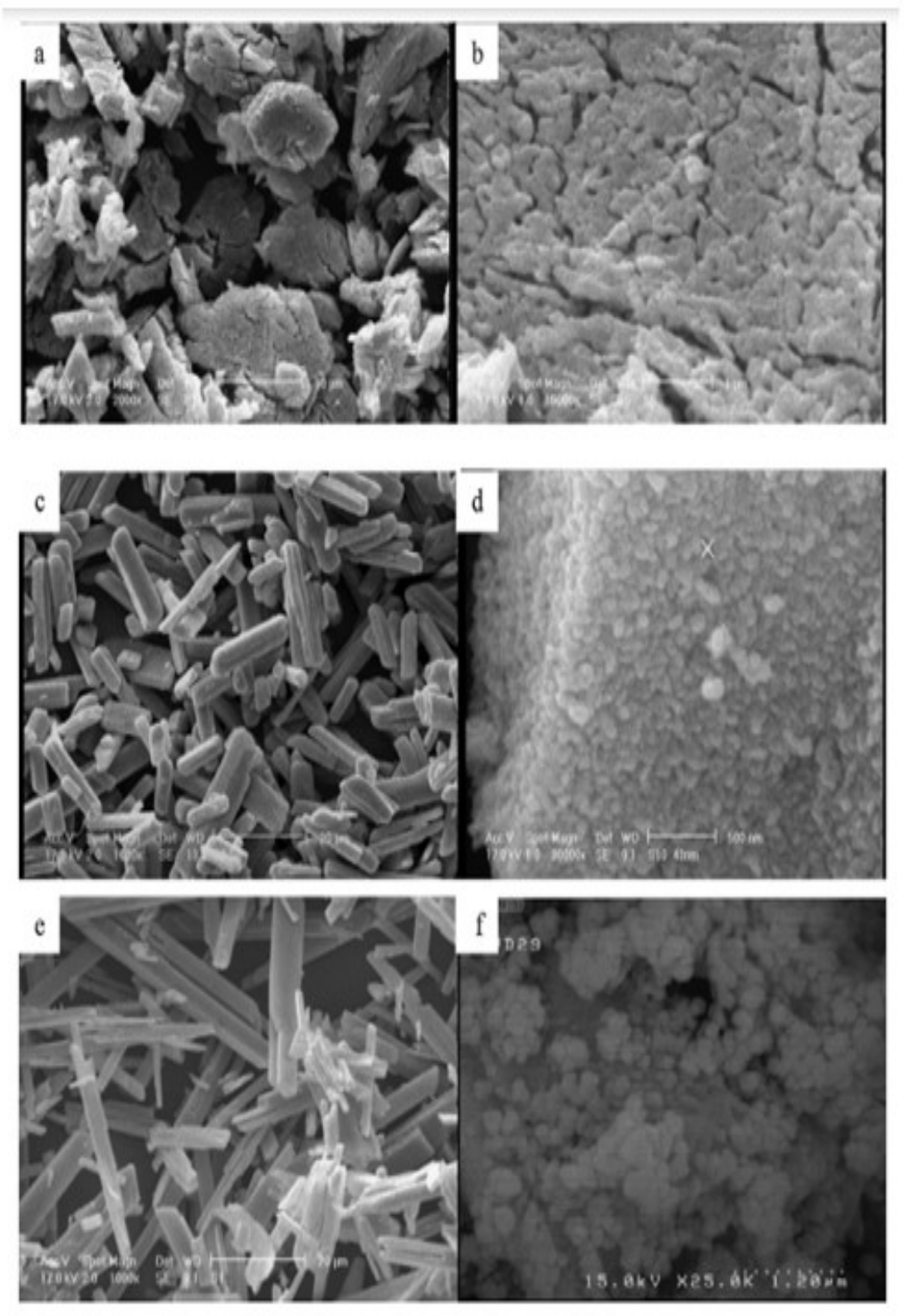

FIGURE 2.6: SEM images of ZnO NPs at different temperatures [13].

Transmission electron microscopy (TEM) utilizes the interaction of electrons with a sample to create high-resolution images. This allows TEM to reveal details of a material across a wide range of magnifications, from very low to high. By examining the sample through TEM, researchers can analyze the various morphologies,

or shapes and structures, of gold nanoparticles. Fig: 2.7 shows some of the Au nanoparticles graphs that were prepared using various methods [13].

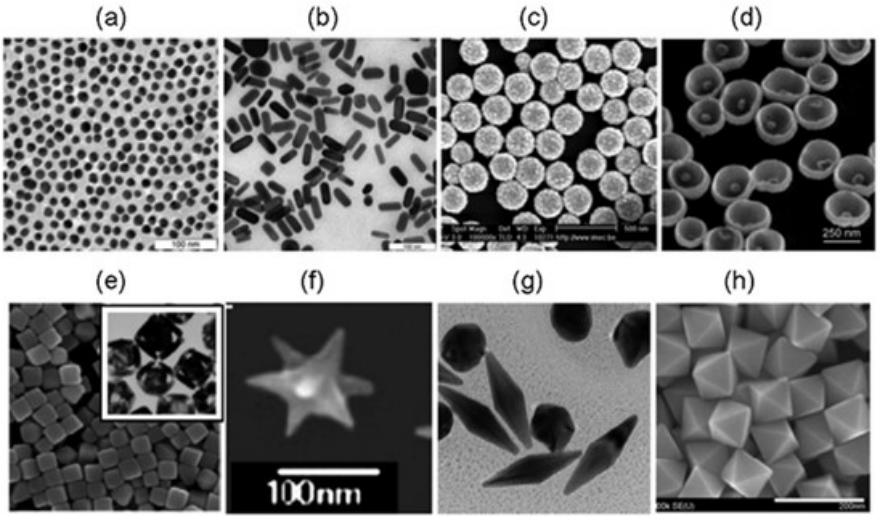

FIGURE 2.7: Different TEM graphs of Au nanoparticles prepared by different techniques [13].

2.1.4.2 Structural Characterizations

Knowledge of the structural parameters of nanoparticles can therefore be very important in terms of determining the nature of the atoms in the nanoparticles as well as the chemical bonds within the nanoparticles. They affect several features of the material. XRD, EDX, XPS, IR spectroscopy, Raman spectroscopy, surface area analyzer by BET method, and particle size by Zetasizer are expected to be employed to study the structural parameters of nanoparticles [13].

There are several techniques used for the structural characterization of NPs out of which x-ray diffraction (XRD) is one of the most powerful techniques. They help to show information pertaining to the NPs' crystallinity, which enables one to determine the particular crystal phase in their sample. Furthermore, XRD analysis with help of the Debye-Scherer formula can be applied to give the average size of the particles. This technique is general for the single phase NPs as well as multi-phase NPs. Thus, one could use a double check of structural arrangement of atoms in bimetallic NPs by comparing the computer generated model with the experimental data, namely the XRD one [13]. Fig. 2.8 showing X-ray diffraction pattern of Silver (Au) Nanoparticles pattern [19].

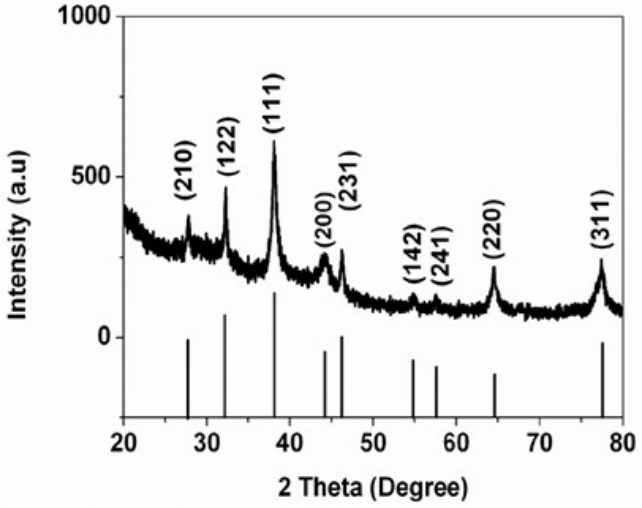

FIGURE 2.8: Ag nanoparticles RRD pattern [19]

Energy dispersive x-ray (EDX) is another method that is applied together with primarily TEM or SEM to understand the elemental composition of nanoparticles. In different elements, nanoparticles is made of. If an electron beam is applied to each element, then each of them will emit X-radiation with specific energies. In that paper it is stated that intensity of a specific X-ray signal depends from concentration of this element in the nanoparticle. I discovered that this method called Energy Dispersive X-ray Spectroscopy (EDX) is very useful for researchers. It may be employed in combination with other techniques like Scanning Electron Microscopy (SEM), in order to validate the materials' elemental constituents [13]. Energy Dispersive X-Ray of Silver (Ag) are displayed in Fig.29 [20].

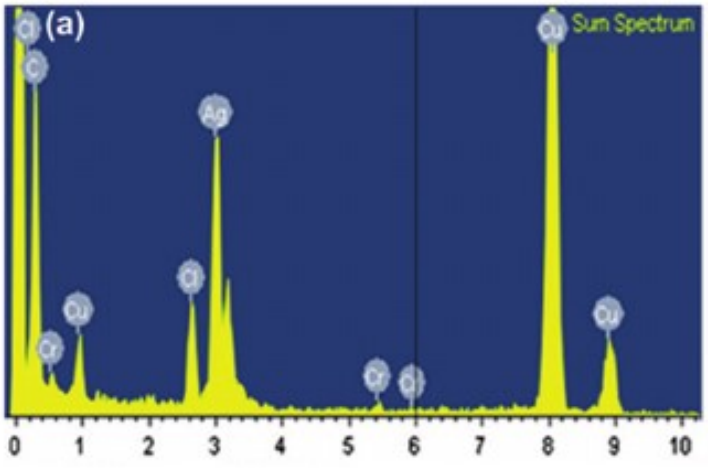

FIGURE 2.9: EDX spectrum of Silver (Ag) NPs [20]

Among various characterization techniques, X-ray photoelectron spectroscopy (XPS) stands out for its exceptional sensitivity. It is a popular tool for researchers to analyze the elemental makeup of nanoparticle (NP) materials with high accuracy. XPS not only reveals the exact type and ratio of elements present, but also provides valuable information about their chemical bonding states.

XPS not only reveals the exact type and ratio of elements present, but also provides valuable information about their chemical bonding states. Due to its surface sensitivity, XPS is particularly useful for investigating the outermost layers of NPs. Due to its surface sensitivity, XPS is particularly useful for investigating the outermost layers of NPs. Additionally, it can be combined with depth profiling techniques to create a compositional map, revealing how the elemental makeup varies beneath the surface [13].

2.1.4.3 Particle Size and Surface Area Characterization

There are various techniques used to estimate the size of the Nanoparticles. Techniques like XRD, SEM, AFM, TEM and Dynamic Light Scattering (DLS) are used for this purpose. Among these techniques, DLS and XRD are the best techniques that can be used to get the size information even at a lowest level. One of the newest and rather promising methods of the investigation of biological samples including proteins and DNA is nanoparticle tracking analysis or NTA. Scientists can apply using the NNTA to directly observe and quantify the nanoparticles (NPs) in suspension.

One of the newest and rather promising methods of the investigation of biological samples including proteins and DNA is nanoparticle tracking analysis or NTA. Scientists can apply using the NNTA to directly observe and quantify the nanoparticles (NPs) in suspension.

NTA analyzes the movement of these NPs, called Brownian motion, to determine their size. This technique is useful for characterizing the size distribution of NPs in liquids, with a size range typically between 10 and 1000 nanometers (nm) [13]. NTA Analysis of proteinaceous particles have showed in Fig: 2.10 [21].

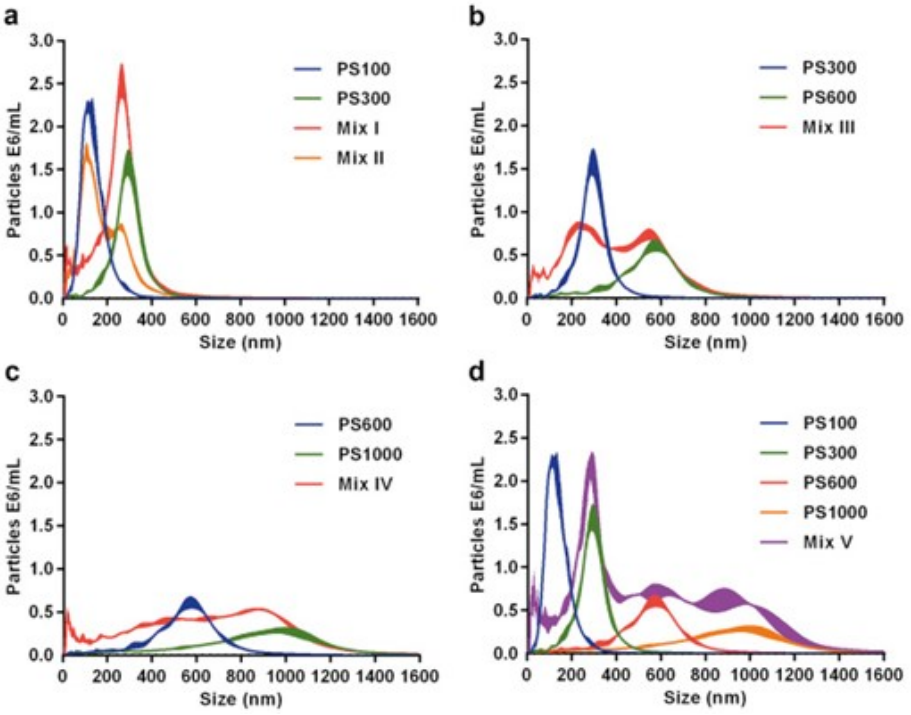

FIGURE 2.10: Nanoparticle Tracking Analysis (NTA) of Proteinaceous Submicron Particles [21].

2.1.4.4 Optical Characterizations

Optical properties of a material is important when it comes to the use of photo catalysis. Photographers have thus endeavored to understand these properties through research on photo chemists' understandings of such reactions. Optical characterization techniques can also be used because they can tell us about the behavior of light with nanoparticles , absorption, reflectance, luminescence and phosphorescence of the NPs. Specifically, metallic and semiconductor NPs, which are characterized by various colors, are used in processes related to light. Knowledge of how these materials work in terms of light absorption and reflection is central to the dissection of different photo-related applications [21].

2.2 Drug Delivery System

Medication transport systems are methods used to get drugs into or around the body. These systems can involve different administration methods, like swallowing

a tablet or getting a shot with a vaccine, and depends on the way that drugs are packaged. Packaging can include microscopic carriers such as micelles or nanoparticles, or even implantable devices. The fundamental intentions of a drug transport system in body are to: Deliver a therapeutic amount of medication to the target site in the body, improve the safety and efficacy of a pharmaceutical product with regulated release kinetics, and improve patient compliance by making it easier or more convenient to take medication [17].

2.2.1 Conventional Drug Delivery System

These routes can be classified further as oral, parenteral, topical including transdermal, nasal, ocular, pulmonary, rectal and intrathecal, IN which substances are injected into the spinal canal [22]. There are set ways through which medication is given which include preparing specific forms of medication such as tablets which are meant to be swallowed or intravenous injections. These techniques, however, are not without their demerits. They include the need to use higher concentrations of the active agents, substandard efficacy, toxicity as well as undesired side effects. (Fig: 2.11) [23].

FIGURE 2.11: Drawbacks of Conventional Drug Delivery Systems.

2.2.2 Targeted Drug Delivery Systems

The concept of drug delivery systems (DDS) has in fact enhanced the world of modern medicine through facilitating the methods of transporting therapeutic agents with a high level of accuracy in the human body. The primary emphasis is placed on methods for the approach to the drug for the activation of certain cells or organs; such a procedure increases violence and reduces the toxic influence of any drugs. Also, it has been discussed different carrier systems, such as liposomes, nanoparticles, and micelles focusing on their capacity to shield the drugs from degradation and their controlled release. (Fig: 2.12) [18].

FIGURE 2.12: Advantages of TDDS.

As a contrast in targeted drug delivery there is an objective to deliver medicine more accurately. This is the concept of getting the drug to areas that it is required while at the same time reducing its concentration in other tissues which are perfectly healthy. Think of a delivery system avoiding the body's immune system and not getting locked up in the liver or spleen. This results in a higher concentration of the drug at the target site since the active transport is now lowering its concentrations further away from this area [24].

2.3 Pathogenic Bacteria in Human Health

Bacteria that make us sick are termed as pathogenic bacteria. Our bodies have natural defense system against these harmful and pathogenic bacteria. Thousands of bacterial species live in our gut. About hundreds of these pathogenic species have been studies so far in humans [25].

2.3.1 Types of Microflora

Normal gut flora, contains a diverse community of microbes, including beneficial bacteria like Lactobacillus and Bifidobacterium. Some, like Helicobacter pylori, can be potential pathogens under certain conditions. These gut bacteria can be categorized based on their oxygen needs, with some like Bacteroides being anaerobic (thriving without oxygen). Streptococcus spp. is a normal flora of the respirational tract. These Gram-positive, sphere-shaped bacteria cause a variety of diseases, including strep throat, pneumonia, wound skin, heart valve, and circulatory diseases. Corynebacterium diphtheria bacteria can cause a serious infection called diphtheria. This infection produces a toxin that can lead to difficulty breathing, heart failure, paralysis, and even death [26].

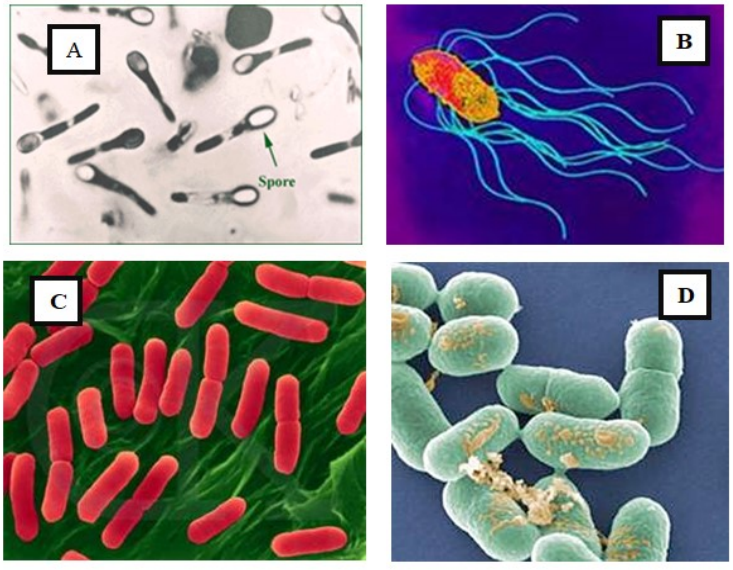

FIGURE 2.13: Different Pathogenic Bacteria for Human Health. A: Clostridium, B: Salmonella, C: E. coli, D: Listeria

2.3.2 Modes of Transmission

There are various modes of transmission for microbials from reservoirs to other living organism bodies. The mode of spread is said to be airborne transmission if the infectious agents such as bacteria, fungi etc. get into other living organisms' bodies through aerosols, the dispersion forms of the bacteria that is in the air. Here, the pathogens remain in the air for some time thus contributing to the easy spread of diseases. That is why if a person has the flu, one can easily contact the disease simply breathing in the air inside for a room [27].

This disease can fly, from person to person via droplets that are thrown up by ill persons' respiratory systems. It can occur through exposure to other people's coughs or sneezes or during a simple conversation.

But despite this, the risk of getting infected is higher when you are close to the infected person; however, it is not restricted to being right next to this person. The medical affection can occur through inhaling of the droplets containing the germs or through a direct contact with the infected surface and, consequently, the face [27].

Diseases can spread through objects that carry germs. Skin-to-skin contact or contact between mucous membranes (like eyes, mouth, or genitals) can spread germs. Sharing contaminated blood or blood products can transmit certain bacterial infections [28].

Hospital acquired infections, which are a type of Healthcare infections, are infections that a patient acquires while in the hospital and can be caused by hard to kill germs. Patients can fall sick with infections in the various institutions through acquiring other illnesses in the healthcare facilities. These types of infections which are acquired during the stay in care facilities are usually referred to as nosocomial or hospital-acquired infections. Other familiar causes are contaminated by germs that are not killed by many antibiotics, which is usually transmitted through medical procedures that are poorly filtered or untreated antibiotic abuse [29].

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2.4 Nanoparticles-Based Targeted Drug Delivery System

Normal/general drug delivery system is not capable of this while nanoparticles have the ability and proclivity to home in on the precise biological cell or tissue. This increased targeting capacity is achieved through alteration of a nanoparticle's surface so that it can engage only specific cellular receptors or biomarkers, thereby increasing drug delivery at the specific site and reducing side effects [30].

The active agent is embedded into a nano carrier system through different techniques. Such methods can produce nanoparticles, nanospheres and or Nano capsules; all of which have properties that affect the drug delivery and release. This approach allows for the optimization of the carrier system to best suit the specific needs of the drug [31].

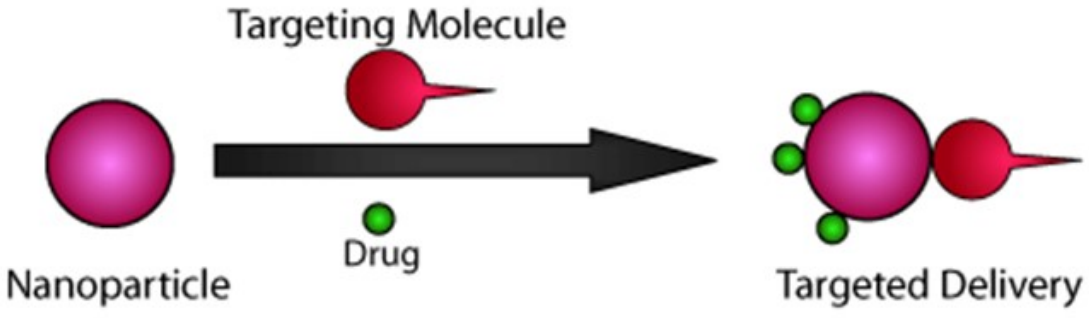

FIGURE 2.14: NPs based Targeted Drug Delivery System.

TABLE 2.1: Evolution of nanoparticles from recent years (2015-2021).

Year	**Types of NPs**	**Drug Delivery Approach**	**Disease**	**Applications**	**References**
2015	Polyamidoamine NPs	Worked as Nano drug carrier, delivered malarial drug to its targeted site.	Malaria	Increased drug solubility, bioavailability, reduced its toxicity, enhanced efficacy and targeting.	32
2016	Solid-Lipid Nanoparticles	Nanocarrier used in drug delivery. Loaded with cyanine and flavonoids.	Colorectal carcinoma (CRC)	Enhanced delivery of therapeutics using electroporation.	32
2017	Nano phage (Bacteriophage NPs)	Drug delivery and gene transfer by chemical or genetic designing.	Viral or bacterial infections treatments.	Filamentous phage bacter can be designed to deliver medicines, antibodies or peptides.	32
2018	Mesoporous Silica Nanoparticles (MSNPs)	MSNPs laden with siRNA via electrostatic absorption.	No	Non-viral vectors offer faster development, lower toxicity, and affordability compared to other methods.	32

Year	Types of NPs	Drug Delivery Approach	Disease	Applications	References
2019	Chitin NPs	Loaded drug delivery systems of all kinds.	No	Cancer treatment, vaccine transfer, gene transfer, ocular and buccal medicine transfer.	32
2020	Mesoporous Silica NPs loaded with folic acid	pH based drug delivery system based on folic acid. H based drug delivery system based on folic acid.	Cancer treatment	Branched polymer (HBP) act as lid over drug in MSNPs and keep it lasting.	32
2021	Silver Nanoparticles (AGNPs)	DNA or RNA transfer in body for protein production to enhance immune response.	Severe Acute Respiratory Syndrome Coronavirus 2	Nucleic acid vaccines hold promise against SARS-CoV-2 and other diseases.	32
2021	Lipid-based, metal and metal oxide nanoparticles	Effective against covid-19 due to their distinct properties.	Viral diseases like sars-cov 2, covid-19 etc.	Such NPs offer preventive benefits in face masks, sensors, and coatings.	32

2.5 Nanoparticles as Antimicrobial Agents

Thus, nanoparticles are being used extensively in the treatment of bacterial infections with references to antibiotics. Hence, owing to the versatility that is characteristic of nano-biotechnology, the approach has a great potential in the treatment of bacterial infections as indicated [33]. It is assessed that more than 70% of bacterial diseases can emerging resistance against commonly used antimicrobials which is in practice situation and around 79% bacteria can develop resistance against multiple number of antibiotics [34].

Nanotechnology for the eradication of bacterial infections has a great potential for the solution of major drawbacks of the antibiotics. Thus, the use of metals such as copper, silver or zinc with antimicrobial properties in infection control in modern medicine has been practiced for many years.

At present, these metals utilize a new concept in the form of nanometals, which are the enhanced metallic nanoparticles, metal oxides, and combinations of layers of the metals. Recent innovation in nanobiotechnology results to functional nanomaterials with distinct peculiarity of the physical and chemical attributes exhibiting promising outcomes as antimicrobial agents [35]. Different types of nanoparticles used as antimicrobials are as follows:

2.5.1 Silver (Ag) Based Nanoparticles

These nanoparticles have inherent antimicrobial activity and are used in the applications of cosmetics, healthcare products, textile materials, wound dressings, and coatings. They are also applied in medical operations involving chronic ulcers, resistant diabetic wounds and burns among others.

Also, these nanoparticles increase the formation of the collagen material when used in wound healing which increases the rate of the healing and decreases inflammatory conditions that are associated with wounds [36].

2.5.2 Gold (Au) Based Nanoparticles

We can particularly design different nanostructures of gold such as rods and shells for their antibacterial characteristics. When the properties of these ligands themselves are changed or when other compounds are incorporated the effectiveness of this is increased. It has been reported that even conjugating of the gold nanoparticles with either the antibodies or the antibiotics develops very formidable weapons against different kinds of bacterial infections including the antibiotic resistant ones [37]. Gold nanoparticles (AuNPs) have comfortable tendency in HIV treatment, malaria treatment, microbial infections treatment and arthritis treatment. It also uses in gene therapy, drug delivery and diagnose also [38].

2.5.3 Titanium Dioxide (TiO2) Nanoparticles

In the case of photocatalytic applications aiming at bacteria, titanium dioxide is a very practical photoactive material to work with because of its inherent stability, availability, and cost [39]. The comparative non-specific antibacterial effect of TiO2 nanoparticles with different types of fungi and bacteria including MDR has stipulated the applicability of this material for the process of disinfection. Besides, nanocomposite effected disinfection benefits from the property that, unlike many other disinfectants, TiO2-based nanocomposites do not release nanoparticles into water because they possess non-contact biocidal effect and are eco-friendly [40].

2.6 Nanoparticles Toxicity in Animal Models

Toxicity induced due to engineered nanoparticles in animals is termed as Nanotoxicity. Scientists use a variety of methods to assess the potential toxicity of nanomaterials, including: cell cultures in both two and three dimensions, microfluidic organ-on-a-chip systems, and studies using isolated organs or tissues. In recent years, significant focus has been placed on developing requirements and approaches to address the dangers posed by emerging NPs. A number of studies have been done on toxicity assessment of engineered nanomaterials (ENMs). To

study the in-vivo toxicity of ENMs, some researchers focuses on either one organ or organ system like respiratory systems, nervous system, cardiovascular system, excretory system, reproductive system etc [41].

2.6.1 Aluminum Based Nanoparticles

Metal oxide based nanoparticles, especially aluminum-based nanoparticles are now being sought after for its usefulness in fuel cell technology, polymers, paints and many more [42]. Several studies have shown that with aluminum oxide nanoparticle exposure, cell health, mitochondria, and the tight junctions of the blood-brain barrier are affected alongside raising the level of oxidative stress. More investigation employing mouse lymphoma cells also shows that aluminum oxide nanoparticles could cause DNA damage without the formation of mutations [43].

2.6.2 Copper Oxide (CuO) Nanoparticles

CuO NPs have uses in semiconductors, antimicrobial agents, HTFs and IUCDs. According to the current studies exposure of CuO NPs is toxic to the liver and the kidney [44]. Ingesting copper nanoparticles, the substance dissolves in the stomach fluids releasing copper ions which are highly reactive. These ions then build up in the kidneys of exposed animals. In a separate laboratory study, copper oxide nanoparticles were found to damage genes and cells, disrupt cell membrane function, and create oxidative stress [45].

2.6.3 Silver Nanoparticles

Reports show that the application of silver as a microbial agent is traceable back in history. Now it is even tinier i. e. Silver nanoparticles (NPs), have sneaked their way into a seemingly endless array of everyday use products. This is in everything from wound dressings to coating of surgical instruments and prosthetics due to its demonstrated ability to kill bacteria [46]. These NPs displayed higher cytotoxicity

than the other NPs; thus, they brought a significant decrease in cell viability, increased ROS generation, and LDH leakage [47].

2.6.4 Carbon-Based Nanomaterials

Within the application area, carbon nanotubes, fullerenes, single-wall nanotubes, and multi-wall nanotubes are at the forefront in nanomaterials development because of their tunable characteristics [48]. From the earlier research, it has been found that the size of carbon nanotubes determines how toxic they are. Multi-walled carbon nanotubes administered stereotypically into the abdominal cavity have been proven to display the feature of carcinogenicity similar to those of asbestos in animal models. This was distinctly different from single-walled carbon nanotubes that got readily phagocytosed by macrophages [49]. C60 Buck-minster fullerenes in their native form, meaning that their surfaces are not chemically modified in any way are capable of circulation in the human body and tend to concentrate in the organs and tissues with time; such organs and tissues include liver, kidneys, bones, and spleen. The in vitro studies on fullerenes carried out at the laboratory level show that these materials may pose genotoxic effects in the sense of DNA strand breaks, chromosomal mutations, and micronuclei. This was noticed when the tested cell types include human and animal cells such as Chinese hamster ovary cells, human epidermoid carcinoma cells, and human embryonic kidney cells (HEK293) which were treated with fullerenes at a concentration of 1 ng/mL for 80 days [50].

2.7 Role of Silica Nanoparticles (SiNPs) in Health Sciences

Silica or silicon also referred to as silicon dioxide SiO2 is a plentiful compound in Earth crust. Nanoparticles of silica are frequently utilized in different fields: food industry, medicine including as drug delivery agents and diagnostic tools, and chemical industry due to the peculiarities of their structure [51].

2.7.1 Silica Nanoparticles in Targeted Drug Delivery System

Meso-porous silica nanoparticles are preferred in controlled and target delivery of drugs since they are biocompatible and can load high amounts of drugs. Silica is readily available in the nature and appears to have better biocompatibility compared to other metal oxides abounding in nanomedicine like titanium and iron. MSNs, which are based on silica, possess inherent stability because of a highly stable bond Si-O. These figures give them an advantage over niosomes, liposomes, and dendrimers, making them hard to degrade as well as withstand mechanical stress. Consequently, MSNs do not always need post-synthesis stabilization processes to be carried out [6]. Silica nanoparticles appear to have various uses in cancer, microbial infections, and theranostic utilities attributing to the different functions which are observed in the delivery of small biological molecules including small interference RNA, DNA, peptide molecules, Abs, antigens etc to the site of interest within the living organisms. Tumor environments have been acidic, compared to healthy tissues. Researchers seized this opportunity by creating a pH-sensitive peptide (RGDFFFFC) that functions as a doorkeeper of a MSN drug delivery system. In the acidic micro-environment of the tumor, the peptide uncovers the targeting ligand to facilitate enthrance of the MSN into the tumour cells. Within the cells, these proteins with high glutathione results in the liberation of the drugs. SNPs are ideal for housing antimicrobial agents because they can be designed to be stimuli responsive with regard to controlled release. This specific targeting reduces the doses that the healthy tissues receive hence reducing the chances of detrimental side effects. MRI contrast agents are currently under the gadolinium-based compound, which cause toxicity issues. However, there are problems related to low solubility and bioavailability of the above compounds; still, the inclusion of SNPs can be an effective solution. SNPs could provide high resolution MRI at comparatively lower doses of Gd and at the same time restrict the liberation of Gd3+ into the bloodstream [52]. Various categories of clinical approved silica nanoparticles which are in use in different fields are presented in Fig; 2.14 [53].

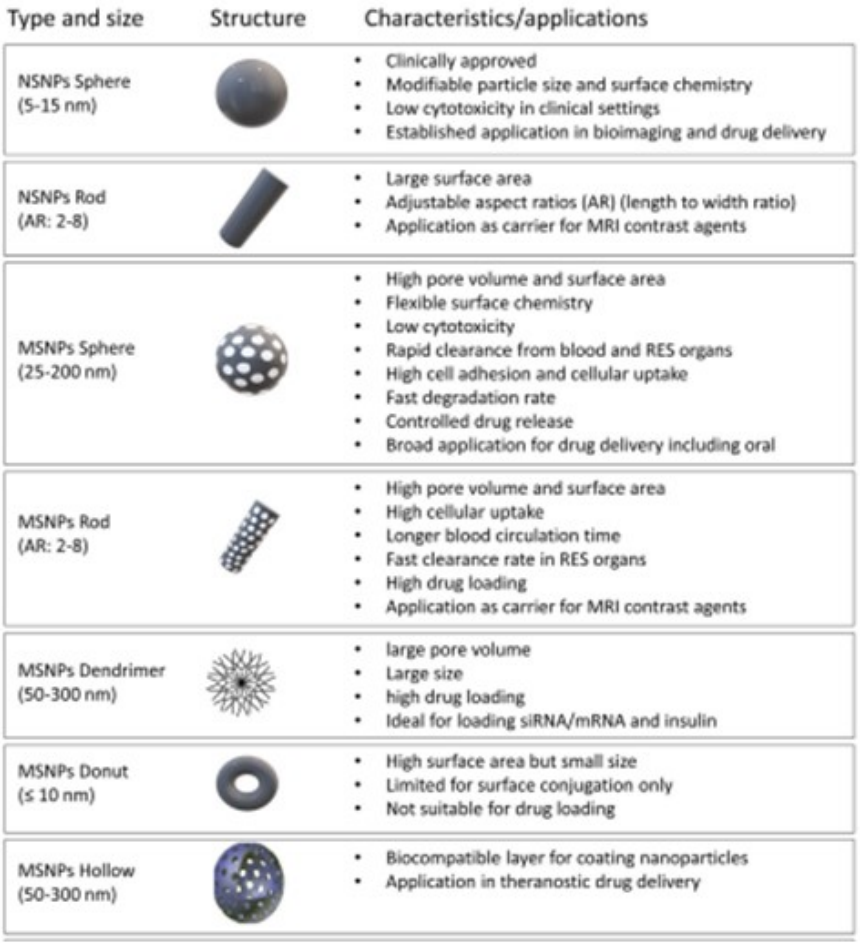

FIGURE 2.15: Types of Silica nanoparticles.

2.7.2 Silica Nanoparticles as Antimicrobial Agents

For these reasons, and there are many, silica nanoparticles (SiNPs) have been widely used in drug delivery mainly because they have a large surface area, are easy to functionalize and are biocompatible. Specifically, MSNs as a type of SiNPs with porous structures have the advantages of tunable pore size, and pore volume which can provide a high drug loading. In this regard, efforts are made to enhance the efficacy of antibiotics by either increasing the dose and/or administration of the second course of the antibiotic. However, this approach is known to exaggerate the side effects of the antibiotics and toxicity as well as promote the development of antibiotic-resistant bacteria. Thus, it was expected that SiNPs exhibit potent antimicrobial activity against biofilms, especially concerning novel therapies and the increasing problem of antimicrobial resistance. Thus, SiNPs have the ability

to prove quite lethal in the case of pathogens through their mere presence as they employ physical membrane disruption, ROS generation and endosomal overload to ensure a potent antimicrobial effect regardless of the payload they are loaded with. This is a multi-faceted approach which greatly limits the chances of bacteria mutating into some kind of resistance. Studies revealed that Silicon nanoparticles (SiNPs) have possibilities for functionalization by antimicrobial agents such as antibiotics, peptides and other functional materials. These material can be bonded through covalent or non-covalent bonding depending on the bonding agents. This strategy can successfully overcome some of the issues, for example AMR, limitations associated with enzymes or serum proteins, and any other issues in relation to a drug substance such as solubility and toxicity [54].

2.8 Synthesis of Silica Nanoparticles

There are generally two methods that are used to synthesize silica nanoparticles i.e. chemical synthesis method green synthesis method.

2.8.1 Chemical Synthesis Method

Synthesis of nanoparticles by chemical methods is one of the most effective and common techniques to create nanoscale particles intended for different uses. It vests in the processes of catalysis by which precursors are converted chemically into nanoparticles of specific size, shape, chemical composition and architecture. Many approaches have been developed for the synthesis of nanoparticles and give possibilities to produce various nanomaterials. Some of the known techniques of synthesis include precipitation, sol-gel method, micro-emulsion, hydrothermal synthesis and electrochemical synthesis. In the precipitation method nanotarticles are prepared by process of reduction of precursor salt solutions with the help of reducing agents. Sol-gel method this process is characterized by conversion of precursor solution to gel and then to nanoparticles by chemical reactions. Micro emulsion technique hence work by encapsulating the chemical reactions in a stable

emulsions meanng that the chemical reactions will only take place in nanoscale droplets. Nanoparticles are produced and designed by hydrothermal synthetic techniques which actually employ hot and high-pressure water to catalyze reactions. Electrochemical synthesis comprises the deposition of nanoparticles by applying a potential in a solution [55].

Thus, the technique of chemical synthesis of nanoparticles has a number of advantages, but, at the same time, it also has several disadvantages and challenges. Some of them are instability of size and shape, possibility of contamination, problems in up-scaling from laboratory to pilot scale, environment and safety issues linked with the use of certain chemicals and energy consumption, incapability of controlling the surface properties, and compatibility of the materials. All these challenges indicate that more research needs to be conducted to optimize the synthesis processes and to overcome the environmental and safety issues connected to the use of nanoparticles [55].

2.8.2 Green Synthesis Method

Recently, people have begun to pay more attention to the functional application of metal oxide nanoparticles (NPs). On the other hand, issues on the negative impact of nanomaterials emerged as the primary area of interest. In this regard, one of the potential solutions is the development of environment friendly methods for synthesis of these particles so that they pose lesser threats and hence are equally suitable for application to various products that are in use today. Among the former, there is the biological synthesis of metal oxide NPs using microorganisms and plant extracts. This approach has the potential of coming up with particles that are bio compatible at a cheaper cost making this approach suitable for fields that are closely related to health sectors. One important area, which should be mentioned is cancer treatment, as nanotechnology applications in this field are designed to improve the conventional approaches to the disease's management. Green synthesis of nanoparticles as mentioned earlier has got the following advantages. First of all, it serves the purpose of environmental sustainability due to the use of natural resources and processes. Secondly, it frequently produces

biocompatible nanoparticles which can then be used in the healthcare industry and consumer goods. Thirdly, it is generally carried out more energy efficiently and is associated with minimal toxic side products than the conventional synthesis processes. In general, green synthesis is a green way and hence economic as far as the synthesizing of nanoparticles for so many utilizations is concerned [56].

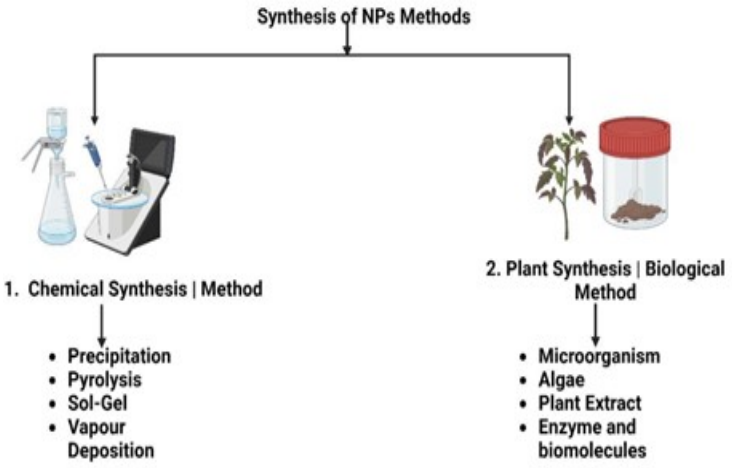

FIGURE 2.16: Synthesis methods of Nanoparticles

2.9 *Ficus carica* Phytoconstituents

The leaves of Fig 2.16 *Ficus carica* are source of bioactive constituents which can be simply defined as natural health promoting agents. Fig A presents the list of the bioactive components which are present in the fig leaves. Phenolics, a large group of compounds that ranges from flavonoids, phenolic acid and coumarins. The predominant flavonoids include luteolin, quercetin and apigenine, among these flavonoids luteolin is the most dominant with several properties such as; antioxidant, anti-inflammatory and anticancer. Some of the organic acids are; malic, citric and oxalic acids. It is involved in giving the somewhat sharp taste to fig leaves and has some health benefits like helping in digestion. Carotenoids are pigments that give fig leaves a slightly yellowish hue. They have antioxidant properties and may help protect cells from damage. Other bioactive compounds that fig leaves contain are triterpenoids, phytosterols, and fatty acids [57]. Some of the useful features of fig plant and its parts in food industry are given below in Table 2.2 [58].

TABLE 2.2: Role of Fig in food industry [58]

Industry	Food Product	Additives	Functions
Food Industry	Fig powder and its co-products (FPC)	Pulp & Peel of fig fruit	FPC of peel contained higher antioxidant activity than of pulp FPC.
Food packaging	Chitosan film based on Fig	Fig leaves	Chitosan based on fig leaves extract used for protection and increasing shelf life of food.
Baking	Cookies	Powder of Fig fruit	Fig powder containing cookies have more nutrients than market product.
Dairy	Milk desserts sugar free	Dried fig and CMC	CMC and fig powder enhanced dessert properties.
Oil	Canola Oil	Pulp and peel extract	Oxidative stability as they have good phenolic content.
Confectionery	Toffee	Fruit powder of Fig	Toffee products were assessed for their physio-chemical parameters.

2.9.1 Nanoparticles Synthesized with *Ficus carica* Extract

Fig is a typical fruit and is component of diets that promote health. Silver NPs were biosynthesized from 1mM AgNO3 solution with extract of dried fruit of fig. Fig fruit extract acted as reducing and encapsulating agent to reduce toxicity on NPs. AgNPs by using fruit extract of *Ficus carica* results showed no deleterious or toxic effects of the AgNPs on animal body (i.e. liver, heart, brain, and kidney). This confirms that the protective potential of different metabolites present in fig fruit reduced the toxicity and enhanced the biocompatibility of silver nanoparticles [59]. Nickel oxide nanoparticles have been prepared using *Ficus carica* leaf extract. 0.1 M nickel oxide solution was used to prepare NiO2 NPs where Ficus carica leaf extract was reducing agent. The study found that NPs were essential for various biological assays mainly bacterial and fungal strains [60].

2.9.2 Therapeutic Efficacy of Plant Extracts

It is necessary to mention that plant extracts can be considered as one of the extensive and promising fields studied at the present moment; the potential of the undertaken developments is aimed at changing the world of healthcare for the better, offering people safe and efficient treatments for numerous sicknesses. Their therapeutic value is attributed to a vast array of bioactive compounds they produce, such as: their therapeutic value is attributed to a vast array of bioactive compounds they produce, such as: These antioxidants are proven to have anti-inflammatory and anti-cancer effects; these are phenolic compounds [61]. These are alkaloids that are used to treat pains and infections since they have antibacteria [62]. Terpenoids as agents possess anti-inflammatory, anti-tumor, and immunomodulatory effect. Essential oils; have many uses including; antimicrobial and antifungal [63]. Finally there is Therapeutic potential of plant extracts against a wide spectrum of diseases Here are some of what it looks like: The curcumin which equals to Curcuma longa displays the curcumin having powerful effect on decreasing inflammation and preventing cell damage; thus, the curcumin may be recognized as a contender against diseases for instance arthritis and Alzheimer's disease [64]. Resveratrol found in grapes and red wine, resveratrol demonstrates cardio protective effects and potential benefits against neurodegenerative diseases [65]. Ginger (Zingiber officinale), Gingerol the bioactive from ginger is an antiemetic and has anti-inflammatory properties, beneficial for nausea and pains. Camellia sinensis leaves have a very rich polyphenol content especially epigallocatechin gallate (EGCG), green tea has anti-cancer and cardiovascular health effects [66].

Chapter 3

Methodology

3.1 Preparation of *Ficus carica* L. Extract

Fresh *Ficus carica L.* leaves were washed with tap water and then washed with distilled water twice. Weighed 50g leaves and fine chopped, added into 500ml beaker. Added 250ml distilled water in the beaker containing fine chopped leaves. Placed the beaker in water bath at 55-60°C for 45 minutes. Stir the mixture time to time after every 10 minutes. After 45 minutes, the mixture was left to attain room temperature. Filter the mixture with filter paper. The extract can be stored at 4°C until utilized for Sodium metasilicate solution reduction [67].

3.2 Synthesis of Silica Nanoparticles (SiNPs)

1g of Sodium metasilicate salt was added in 20ml distilled water to make precursor salt solution of 0.4M concentration. Sodium metasilicate (Na2SiO3) solution was added dropwise into 100ml freshly prepared *Ficus carica L.* extract with constant stirring. pH of the solution was maintained at 8-9 by adding 1M HCl dropwise in it. Keep the reaction mixture at 60-70°C for 1 hour for reduction process to occur. Centrifuge the reaction mixture at 4000rpm for 10-15 minutes. Removed the supernatant and pallet was kept in oven for drying at 80°C for 2 hours [67].

3.3 Characterization of Silica Nanoparticles

Characterization helps us to analyze the composition and structure of nanoparticles. This analysis also helps us evaluate the effectiveness of the method used to obtain the material. There are two main types of characterization techniques: qualitative and quantitative [68]. Fourier Transform Infrared (FTIR) Spectroscopy is used to find the functional groups that are present in the nanoparticles. Also it is used to determine the molecules and their functional group composition [69]. Ultraviolet-Visible (UV-Vis) Spectroscopy (UV-Vis spectrophotometry) is utilized for the qualitative and quantitative analysis of the chemical elements within the nanoparticles. X-Ray Diffraction (XRD) analysis is conducted to determine the crystalline nature of the nanoparticles. Size of nanoparticles is determined by using Debye-Scherer equation i.e.

$$D = 0.94\frac{\lambda}{B\cos\Theta} \tag{3.1}$$

Scanning Electron Microscopy (SEM) imaging is used to visualize the morphological property and size of the individual silica NPs [69].

3.4 Animal Selection

Twelve adult male rats (*Sprague Dawley*) weighing 140-160gm of six to seven weeks old were used for the experiment. The rats were kept in animal house with specific pathogen free (SPF) environment, with a controlled atmosphere. Temperature was maintained at 22 $\pm$ 2 in Celsius scale, with humidity of 55% $\pm$ 10%, and 12 hours of light and dark cycle [70].

3.5 Dosage Optimization

Dose of silica nanoparticles was optimized on the base of the tolerated dose of silica NPs that is 100mg/kg induced for 1-3 months orally [71]. The optimized

high dose i.e., 22.5mg of SiNPs and low dose i.e., 7.5mg was administered orally on daily basis for 21 days. Whereas the plant extract dose was optimized at the rate of 200mg/kg as reported earlier [72].

TABLE 3.1: Doses of SiNPs and Plant Extract

Dose Type	**Dose Amount**	**Reference**
Low Dose of SiNPs	7.5mg/150g	[71]
High Dose of SiNPs	22.5mg/150g	[71]
Plant Extract Dose	30mg/150g	[72]

3.6 Experimental Plan

This experiment utilized a four-group design, each having 3 rats, in a controlled animal facility for a period of 21 days.

Group 1: This group acted as a control, animals were provided with normal diet and purified water for 21 days.

Group 2: This group was given a plant extract at a standard dose with purified water intake and diet.

Group 3: This group acted as the low-dose treatment group. Animals in this group were given a low dose of SiNPs along with a normal diet and purified water.

Group 4: This group was given a high dose of SiNPs with normal water and diet[70].

3.7 Morphological Assay

Body weight of all rats in each group was measured every two days with the help of electronic weight measuring machine from treatment day to end day of experiment [73].

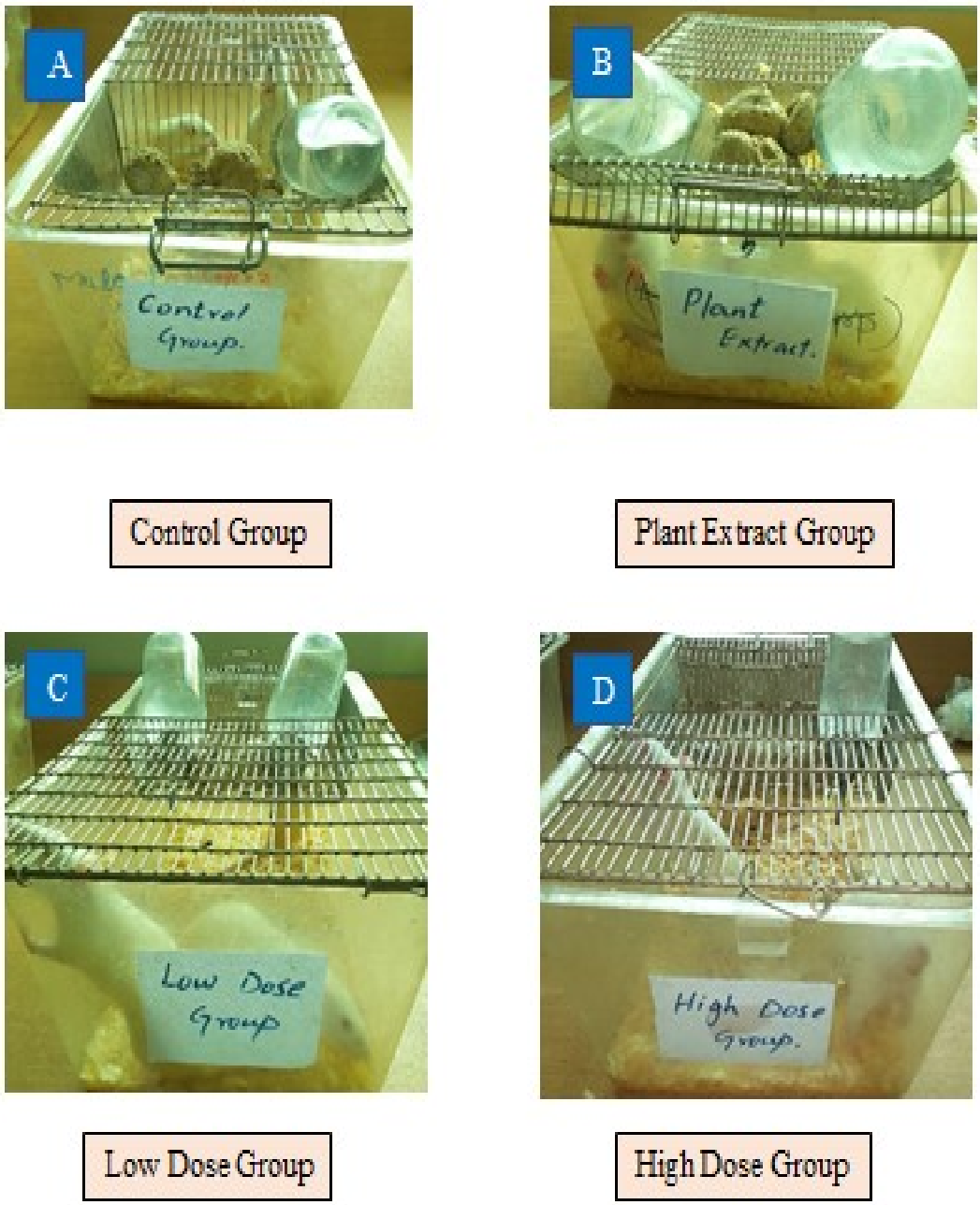

FIGURE 3.1: Animals Grouping

3.8 Animal Dissection

After 21 days of dosing, rats were dissected to get blood samples for biochemical and histological assays to check the toxicity of SiNPs. Rats were anesthetized with chloroform and then euthanized for blood collection and tissue isolation for testing. After removal, the liver tissue samples was placed in solution called neutral-buffered formaldehyde (NBF) to keep them safe for further examination [74].

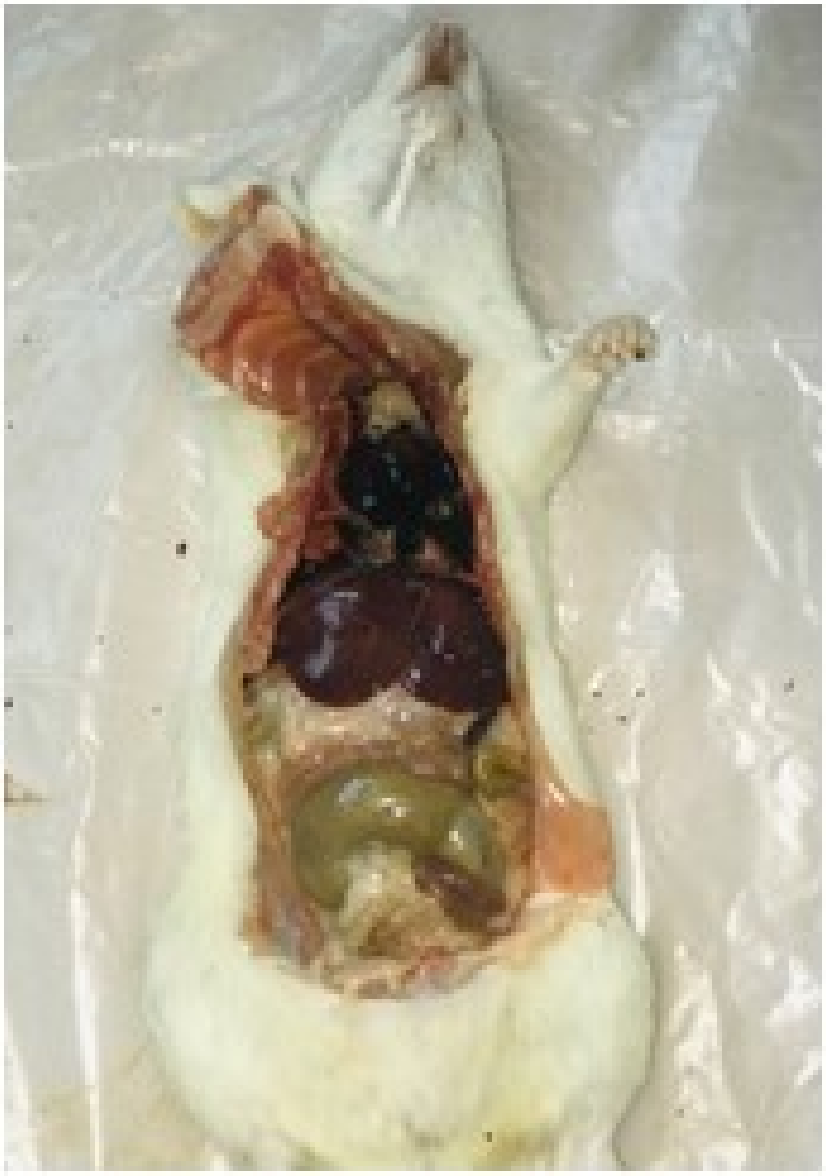

FIGURE 3.2: Animal Dissection

3.9 Hematological Analysis

The blood samples underwent a complete blood count (CBC) using an automated hematology analyzer (model 2800 Hematology Auto-Analyzer). This analysis assessed various parameters, including the number of white blood cells (WBC), red blood cells (RBC), hemoglobin concentration (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) [75].

3.10 Biochemical Assays

Blood was drawn from the rats to assess several biochemical parameters. This analysis included liver function tests (LFTs), renal function tests (RFTs), C reactive proteins (CRP) and erythrocyte sedimentation rate (ESR). Rats were administered anesthesia. Blood was collected via cardiac puncture and stored in chilled centrifuge tubes at -20°C [73].

FIGURE 3.3: Blood samples collection for tests.

3.11 Histopathological Analysis

Liver tissues were collected and fixed in 10% formalin, paraffin-embedded, sectioned, and stained with hematoxylin and eosin (H&E) for subsequent histological examination using standard techniques [76].

3.12 Statistical Analysis

The data was subjected to one-way analysis of variance (ANOVA) to assess the difference between groups as mean standard deviation [75].

FIGURE 3.4: Liver samples collection for Histo analysis.

Chapter 4

Results and Discussion

4.1 Plant Extract Preparation and Synthesis of Nanoparticles

Plant extract was prepared by heating fine chopped *Ficus carica L.* leaves in 100ml of distilled water and filtered (Fig:4.1A). After filtration, 0.4M salt solution in 50ml of plant extract was heated at 80oC for 3 hours. A color change results in the indication of reduction process and NPs synthesis (Fig:4.1B) [67].

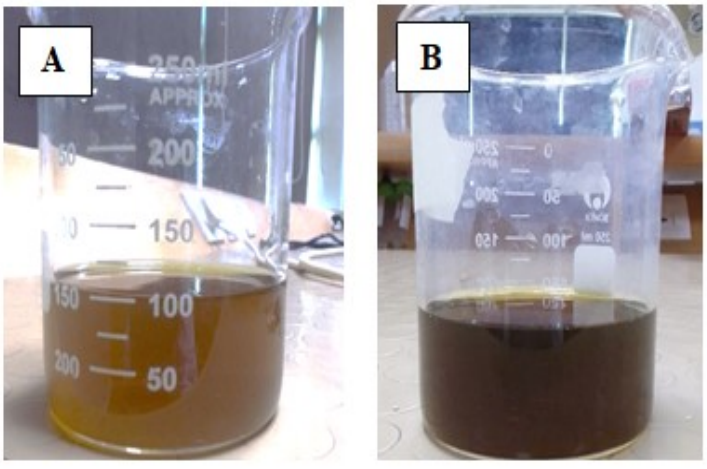

FIGURE 4.1: (A) Plant extract of *Ficus carica L.* leaves. (B) Plant extract mixed with sodium metasilicate salt solution and color change indication of synthesis of NPs.

4.2 Characterizations of Nanoparticles

For confirmation of synthesis, crystallinity, size and morphology of nanoparticle, characterization is important to validate them as they nano scaled in size and cannot be seen with naked eye. Characterizations of SiNPs were performed by using UV-Vis spectrophotometry, FTIR, XRD and SEM.

4.2.1 UV-Vis Spectrophotometry

UV-Vis, a widely used technique, was performed to assess the optical properties of synthesizes silica NPs. Range of wavelength used from 200 to 1200nm. Synthesized SiNPs showed maximum absorbance peak in visible region that is at 304nm that was the indication point of synthesis of SiNPs. There has been reported the maximum absorption band of Silica NPs at 310nm wavelength [77]. Moreover, there is also been reported the maximum absorbance of Silica NPs at 297nm wavelength that proven the synthesis of SiO2 NPs [78]. Figure 4.2 shows the UV-Vis absorption spectrum, a graph of optical absorbance versus wavelength.

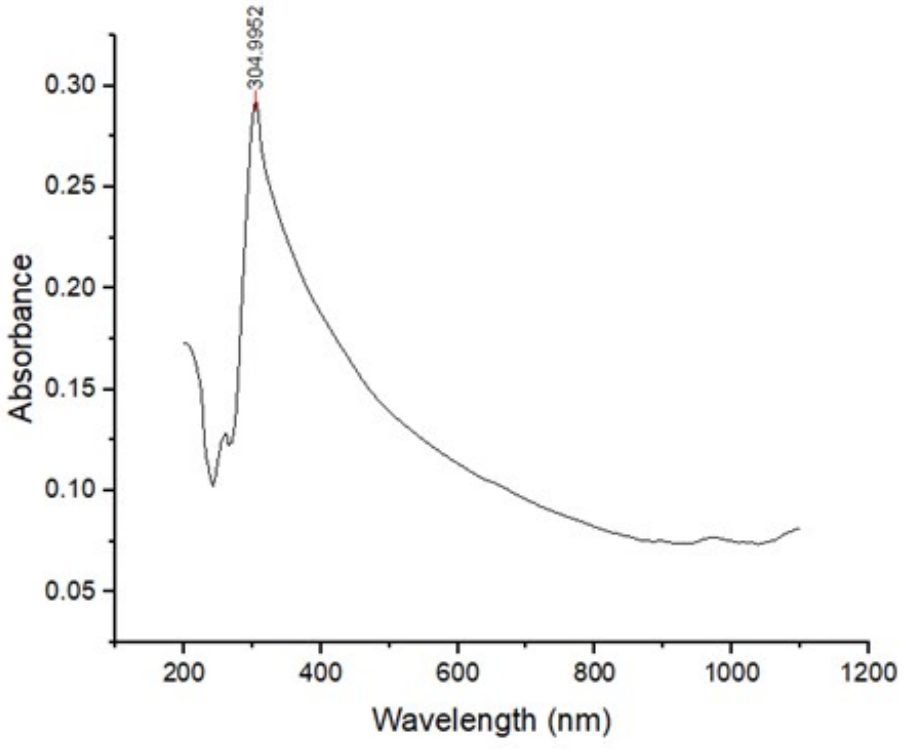

FIGURE 4.2: UV-Vis absorption spectrum of SiNPs

4.2.2 FTIR Analysis

FTIR (Thermofisher Nicolet Summit Pro) analysis of both plant extract and SiNPs was done to identify the functional groups present in them. Results obtained from FTIR analysis were analyzed by Origin software. Confirmation of SiNPs was done by FTIR data (Fig: 4.3).

Multiple peaks at 2342.9cm-1, 1530.2cm-1, 1059cm-1 and 458cm-1 were observed. Peak formed at 458cm-1 is due to symmetric stretching of vibration of Si-O-Si i.e. siloxane group that are actually silica nanoparticles. Peak that is detected at 1059cm-1 is related to C-O stretching of primary alcohol.

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Peak at 2385.804cm-1 and 2279.646cm-1 represents NH compounds relating amino acids. Peak at 1132.743cm-1 indicates the CH3 bond that is towards organic phosphorous compound [81]. Peak at 3922.77cm-1 represents the presence of amine salts. Peaks at 1911.5 and 1692.03 represents C=O stretch i.e. conjugated aldehyde. Peak analyzed at 1529.203 represents C-H bond stretching that is alkane. Peak at 729cm-1 represents the presence of C-H weak stretch of Benzenoid. Peak of 587 is showing the presence of C-I stretch that is towards Halo compounds and peak at 488.495 represents the C-OH bond that is phenol [82].

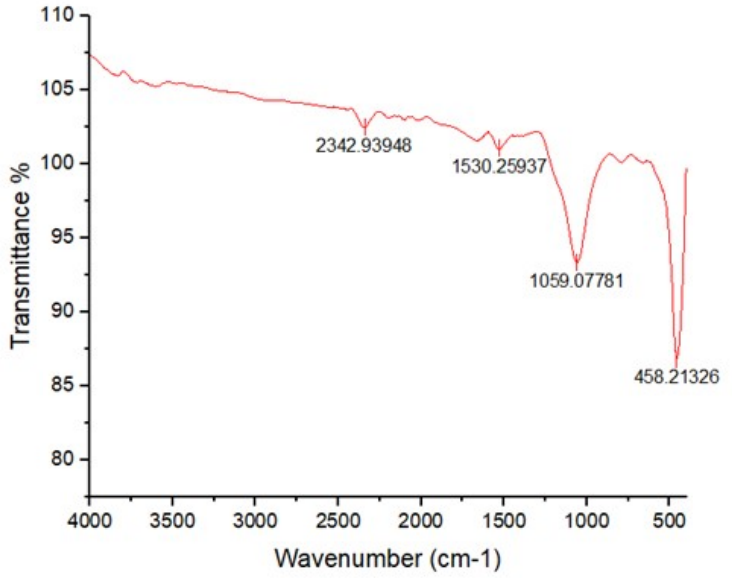


FIGURE 4.3: FTIR spectrum of SiNPs

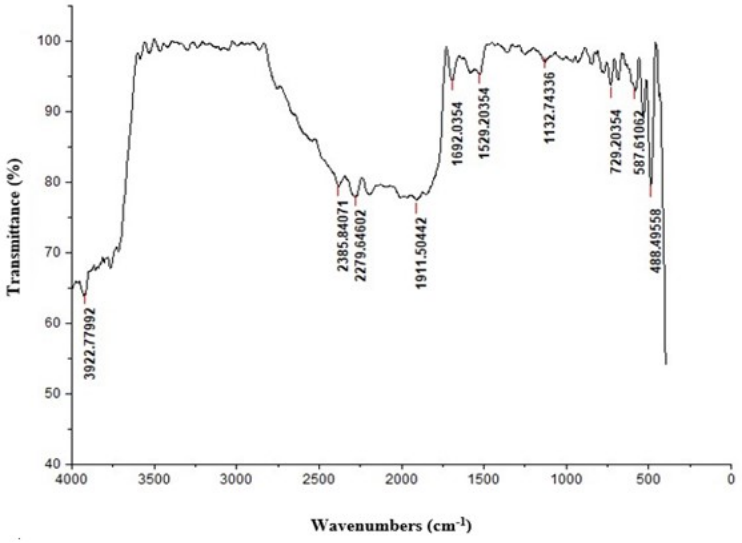
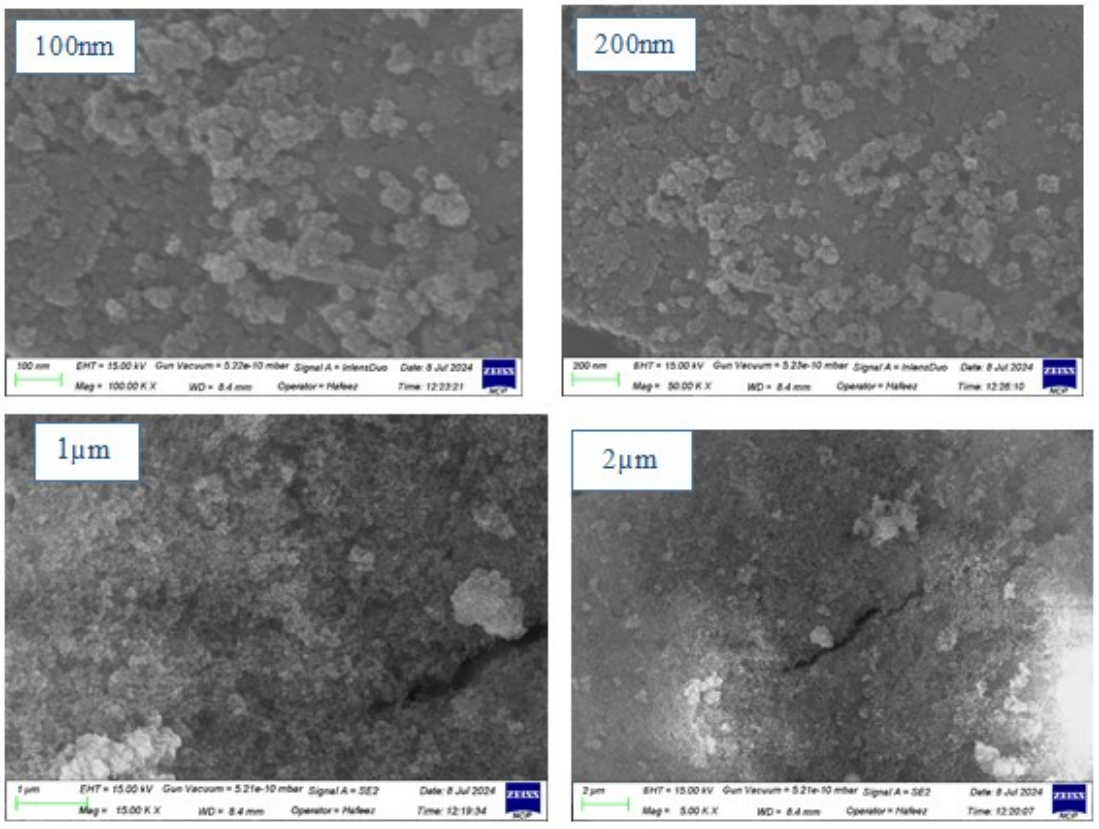


FIGURE 4.4: FTIR spectrum of Ficus carica L. leaf extract

4.2.3 SEM-EDX

SEM (Scanning Electron Microscopy) was done to identify the morphology and size of the synthesized nanoparticles and EDX (Energy Dispersive X-Ray) (MIRA 3, TESCAN) was also performed to confirm the elemental composition of Silica NPs synthesized by Ficus carica L. leaf extract. According to SEM, the size of nanoparticles is 19nm-33nm. Image formed by SEM is shown in Figure 4.5 which

demonstrate the homogenous formation of SiNPs in amorphous form at different resolutions. Somehow same results are reported about the agglomerated form of SiNPs homogenously distributed [77].



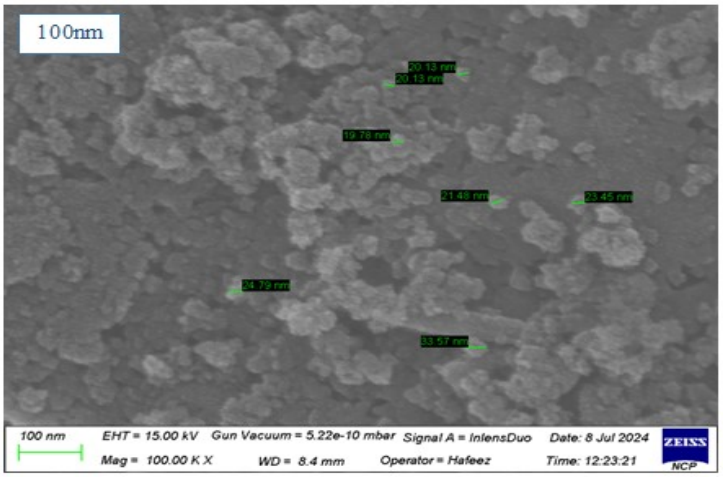


FIGURE 4.5: SEM Images of SiNPs under different resolutions.

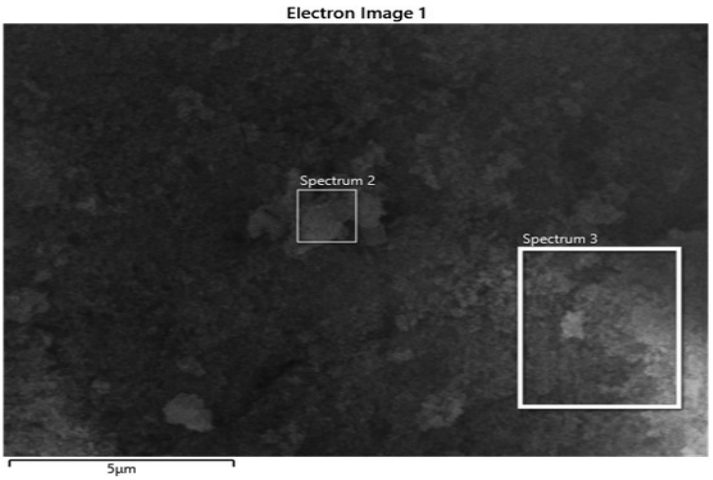


FIGURE 4.6: SEM of Silica Nanoparticles at 5micron

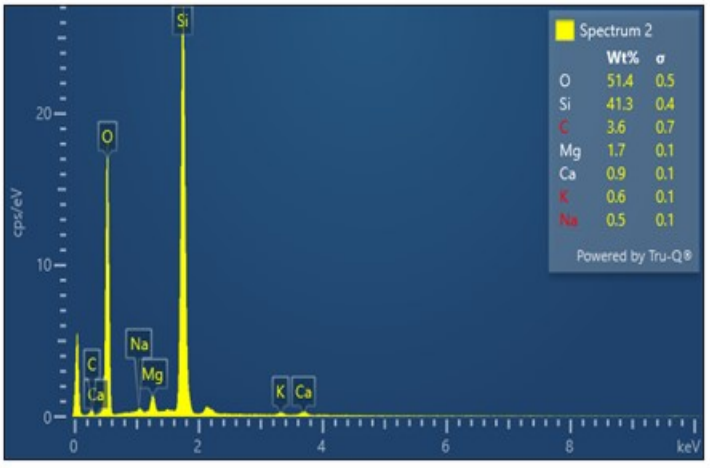


FIGURE 4.7: EDX of Spectrum 2 of SiNPs.

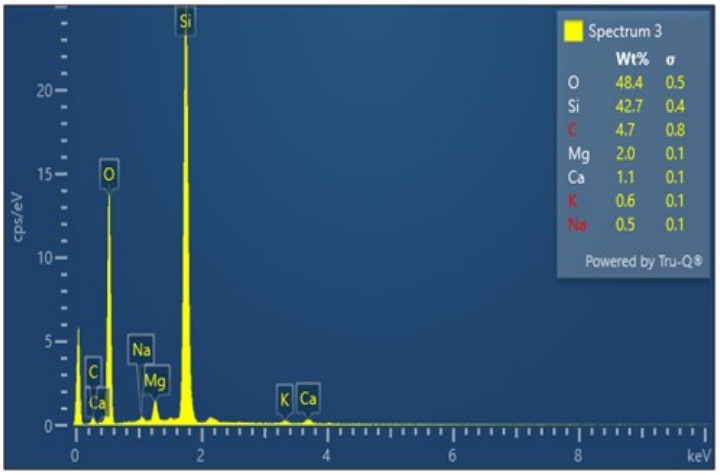


FIGURE 4.8: EDX of Spectra 3 of SiNPs

Figure 4.6 demonstrate the elemental composition of NPs. Results obtained were analyzed by Origin. EDX spectra are presented in the form of peaks corresponding to Si and O fIG:(4.7)(4.8). All these peaks contain Si and O elements at highest. This shows that 90-95% pure Si and O elements are present.

Trace elements like Calcium (Ca), Sodium (Na), Magnesium (Mg), Potassium (K), Chlorine (Cl) and Carbon (C) are also present. As the synthesis of NPs was Ficus carica leaf extract based, so these elements must be adhered from this extract to SiNPs. Yadav and Fulekar [83] reported 94 to 97% pure SiNPs with highest peaks of Si, O and C.

4.2.4 XRD

XRD is best technique to analyze the crystalline or amorphous nature of the nanoparticles. Dry powder NPs were used to perform XRD analysis. Peak was obtained at $2\Theta = 22.21$ which confirms the amorphous nature of the Si NPs (Fig:4.9). These results are following the observation reported [77][84] that if XRD pattern obtained at peak of 20-25, nature of silica nanoparticles will be amorphous.

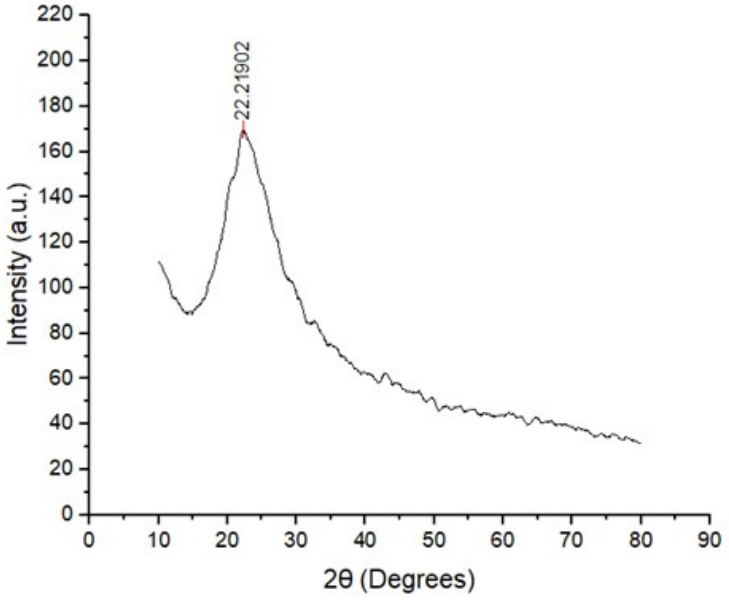


FIGURE 4.9: XRD pattern of Silica nanoparticles

4.3 Body Weight

The body weight of animal models play vital role in determining the overall health of the body. During 21 days of experiment, the weight of the control group gradually increased.

Whereas the weight of plant extract group decreases in first week and then remained consistent. On the other hand, the weight of low dose and high dose group increased gradually which might be due to the use of nanoparticles that causes the water retention due to the accumulation of globular proteins in the blood plasma.

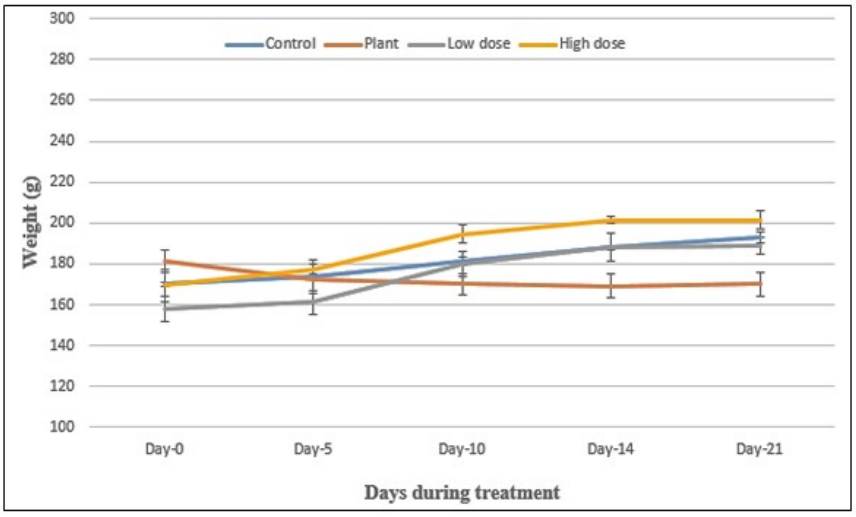


FIGURE 4.10: Graph representing weight of all groups during the 21 days experiment.

In control group, weight increases from 170 to 193g during 21 days. Weight of plant extract group decreased gradually from 181 to 170g. Weight of low dose and high dose group animals increased from 158 to 188g and 169 to 201g respectively. This weight increase was also observed and reported [85] while inducing AgNPs in rats. They reported 5g increase in body weight. High dose group animals also showed a significant weight increase during treatment days. The results obtained are consistent with the results previously reported by Noori M. Luaibi [86] with increase in body weight during AgNPs induction in rats. Table: 1 demonstrate the comparison of weight of all groups' animals.

TABLE 4.1: Mean±SD (n=3) Weight of Plant extract, Low dose and High dose groups compared with the Control group by one-way ANOVA.

Group	Days				
	0	5	10	14	21
Control	170 ± 1.15ab	174 ± 1.15a	181.33 ± 1.76ab	188 ± 1.15a	193 ± 2.65a
Plant Ext.	181.33 ± 5.81b	172.67 ± 6.96a	196.67 ± 5.21b	203.33 ± 5.81a	200 ± 6.11a
Low Dose	158 ± 6.43a	161.33 ± 5.81a	180 ± 6.00a	188 ± 6.93a	188.67 ± 4.06a
High Dose	169.33 ± 7.69ab	177.33 ± 4.67a	194.66 ± 4.66ab	201.33 ± 1.76a	201.33 ± 4.37a
P Value	0.12	0.22	0.07	0.083	0.23

4.4 Hematological Assays

Hematological parameters such as white blood cell count (WBC), red blood cell count (RBC), hemoglobin (Hb) concentration, hematocrit (HCT), platelet count etc. are sensitive indicators of changes in the body due to toxic effects of any material. After 21 days of experiment, hematological tests were done to evaluate the toxicity of SiNPs on animal models that are as follows:

4.4.1 Hemoglobin (HB)

Hemoglobin, a heme and globin containing protein present in blood of almost all vertebrates and it develops in the bone marrow. Its prime function is to carry and transport oxygen to body tissues as well as carry carbon dioxide to remove it from body. Hemoglobin indices were measured for all four groups' animals. Results are given in Figure: 4.11.

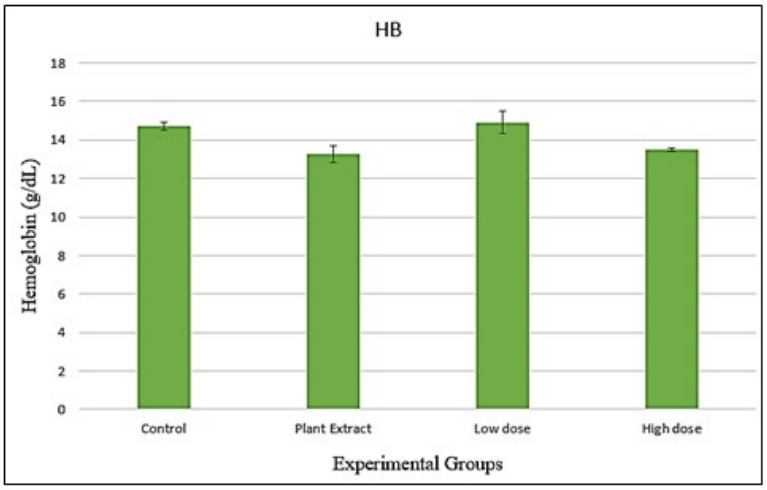


FIGURE 4.11: HB (g/dL) of all experimental groups

There is minimum differences between the HB indices of all groups. As compared to control group, HB in plant extract group and high dose group increased while in low dose group decreased.

The values are almost in correspondence to the results that are reported [85] upon testing AgNPs who observed that p>0.3 in not significant value which showed their AgNPs do not seems like affecting these parameters at tested doses. Mean values for HB are given in Table: 4.2. p>o.o5 mean that data obtained is insignificant and minimum to no toxicity on this blood parameter occurred.

This data supports the concepts of T. N. Almanaa [87] who reported that a decline of Hb due to SiNPs cause acute toxicity in rats. Mean values of HB for all groups are given in Table: 4.2.

TABLE 4.2: Mean±SD values for HB of all groups compared by one-way ANOVA.

Parameter	**Control**	**Plant Ext.**		**Low dose**		**High dose**		**P Value**
HB	14.7 ± 0.2ab	13.25 0.45a	±	14.91 0.59b	±	13.5 0.10ab	±	0.08

4.4.2 RBCs

Red Blood Cells (RBCs) that are also termed as Erythrocytes are important elements in blood and body as they contain HB proteins and carry oxygen and carbon dioxide towards and away from body tissues respectively. Fig.4.12 demonstrating the differences between RBCs values of all groups. There is a slight difference between values of RBCs in all groups which is seeming like SiNPs induce less to no toxicity on RBCs of animal model [87]. RBC count in low dose slightly increased as compared to control but showed no significant differences as p value is given in Table: 4.3.

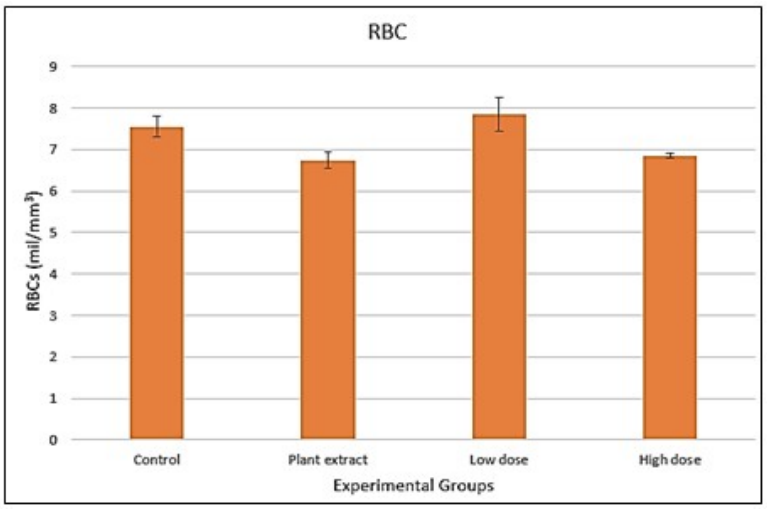


FIGURE 4.12: RBCs (mil/mm3) of all Groups

TABLE 4.3: Mean±SD values for RBCs of all groups compared by one-way ANOVA.

Parameter	**Control**	**Plant Ext.**	**Low dose**	**High dose**	**P Value**
RBCs	7.55 ± 0.24a	6.74 ± 0.19b	7.85 ± 0.41a	6.85 ± 0.005b	0.091

4.4.3 WBCs

White Blood Cells (WBCs) also termed as leukocytes that help body against infections. They flow through our blood and reach the target area. White blood cells defend the body by consuming foreign substances and cellular waste, and by eliminating harmful pathogens. WBCs values for all groups are shown as graph in Fig.4.10 which demonstrates a slight increase in WBCs count. There is reported that increase in WBCs can be reason for inflammation or damage in body [88]. So, the values presented in Fig: 4.13 demonstrate a very slight increase of WBCs in plant and low dose group while WBCs count decreased in high dose group. This slight change in WBCs is in accordance with the work that is already reported who suggested no affect of SiNPs on this blood parameters by tested dose [87]. Table: 4.4 demonstrating the mean values with standard deviation ($p>0.05$) which corresponds to the work reported by N. Almanaa [87].

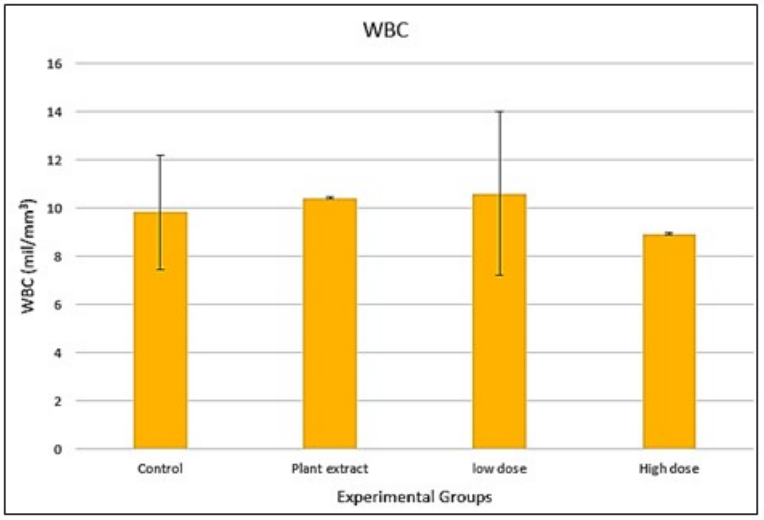


FIGURE 4.13: Graph showing WBCs (mil/mm3) in all groups.

TABLE 4.4: Mean±SD values for WBCs of all groups compared by one-way ANOVA.

Parameter	**Control**	**Plant Ext.**		**Low dose**		**High dose**		**P Value**
WBCs	9.83 ± 2.36a	10.42 0.06a	±	10.60 3.40a	±	8.92 0.05a	±	0.935

4.4.4 HCT

Hematocrit (Ht or HCT) is the %age of Red Blood Cells (RBC) in the blood. Figure 4.14 is the graphical representation of the HCT level in all experimental groups. As shown, HCT in plant group and high dose group decreased as compared to control group and in low dose given group, HCT level is equal to the control group.

Mean values of HCT level in all experimental groups in Table: 4.5 with p values significant (p>0.05) which depicts the similar results reported by Ozdan Akram Ghareeb (2021) [89] that upon administering AgNPs, HCT level decreased in rats which shows some alteration in blood osmotic level resulting in increased the plasma level.

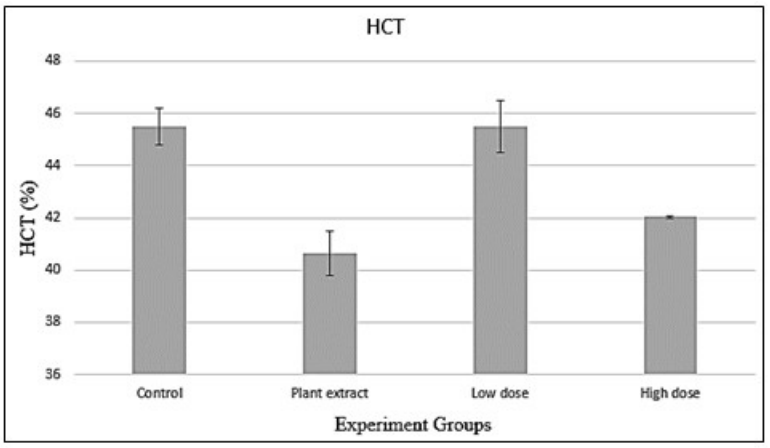


FIGURE 4.14: HCT % of all experimental groups

TABLE 4.5: Mean±SD values for HCT of all groups compared by one-way ANOVA.

Parameter	**Control**	**Plant Ext.**		**Low dose**	**High dose**		**P Value**
HCT	45.5 ± 0.7b	40.65 0.85a	±	45.5 ± 1.0b	42.05 0.05a	±	0.021

4.4.5 MCV

MCV stands for Mean Corpuscular Volume. It is a measure of the average volume or size of red blood cells (RBCs) in a blood sample. The MCV value is an important parameter in a complete blood count (CBC) and is useful in diagnosing different types of anemia.

In Figure 4.15 all groups MCV values are demonstrated by graphs which shows MCV level decreased in low dose group and increased in high dose group while in plant extract group, this value is almost equal to the control group. In contrast, T. N. Almanaa [87] Silica NPs administered at multiple doses induce metabolic, hepatic and inflammatory disorders with the increased value of MCV in both low and high dose animals. The reduced level of MCV might be due to decreased level of erythrocyte formation [90].

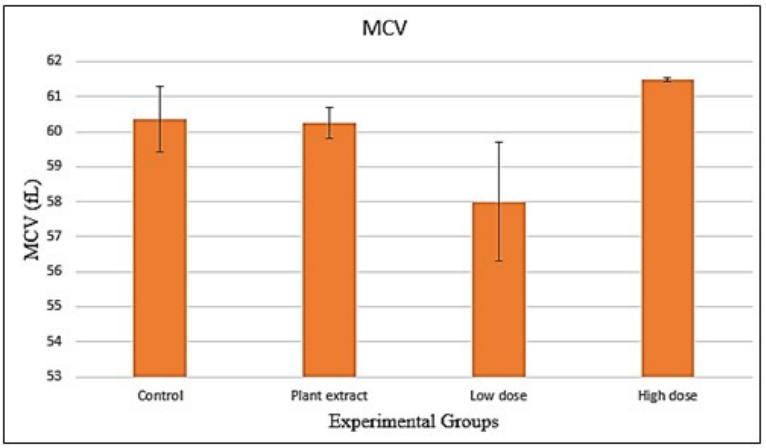


FIGURE 4.15: MCV (%) values of all groups.

Mean values of MCV of all experimental groups are given in table 4.6 with p>o.o5 which indicates the insignificant value of data for MCV.

TABLE 4.6: Mean±SD values of MCV for all experimental groups compared by one-way ANOVA.

Parameter	**Control**	**Plant Ext.**		**Low dose**	**High dose**		**P Value**
MCV	60.35 ± 0.95a	60.25 0.45a	±	58 ± 1.7a	61.5 0.05a	±	0.243

4.4.6 Platelet Count

Platelets, also termed as Thrombocytes, found in blood. They are formed in bone marrow and are involved in blood clotting process (hemostasis). As shown in Figure 4.16, platelets of plant extract group and high dose group decreased as compared to control and low dose group increased than control. Values obtained are in correspondence with R. Rehman [85] who reported decreased platelet count in high dose and increased platelet count in low dose that depicts the tested doses not affecting the animal.

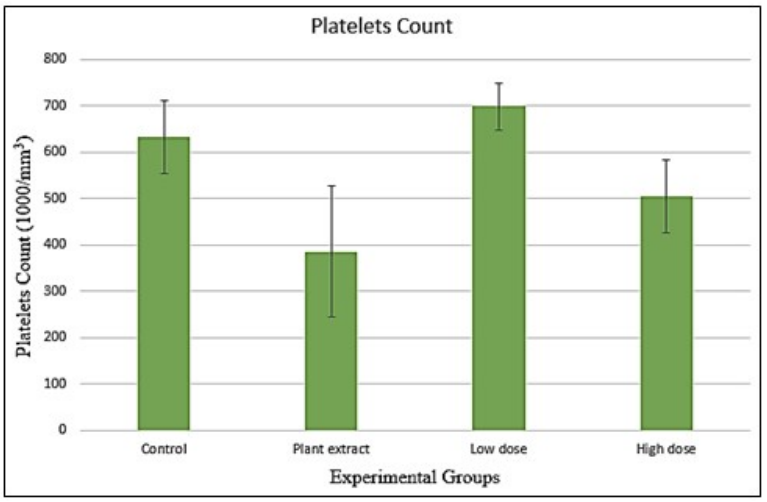


FIGURE 4.16: Graphical representation of Platelets Count

Table 4.7 showing all mean values of experimental groups with p>0.05 which is insignificant data supporting work of R. Rehman [85] with less to no toxic effect of NPs on model organisms.

TABLE 4.7: Mean±SD values of Platelets count for all groups compared by one-way ANOVA.

Parameter	Control	Plant Ext.	Low dose		High dose		P Value
Platelets	633 ± 78a	386 ± 142a	698.5 49.5a	±	504.5 77.5a	±	0.22

4.5 Inflammatory Markers

Inflammatory markers are biological measurements that are used to predict the inflammation in body. These markers are present in blood, urine or other body fluids. Bio inflammatory markers were used to assess any inflammation in animal body.

4.5.1 ESR

ESR stands for erythrocyte sedimentation rate. It's a simple blood test used to assess the presence of inflammation in the body. The test measures how quickly

red blood cells settle at the bottom of a test tube over a period of one hour. Normally, red blood cells settle relatively slowly. Figure 4.17 represents the graphical representation of ESR values of all groups. It is shown that ESR rate or plant extract and high dose are comparable to control group and in low dose group, ESR value is elevated. Low value of ESR depicts no inflammation in body while elevated level of ESR is sign of inflammation [91].

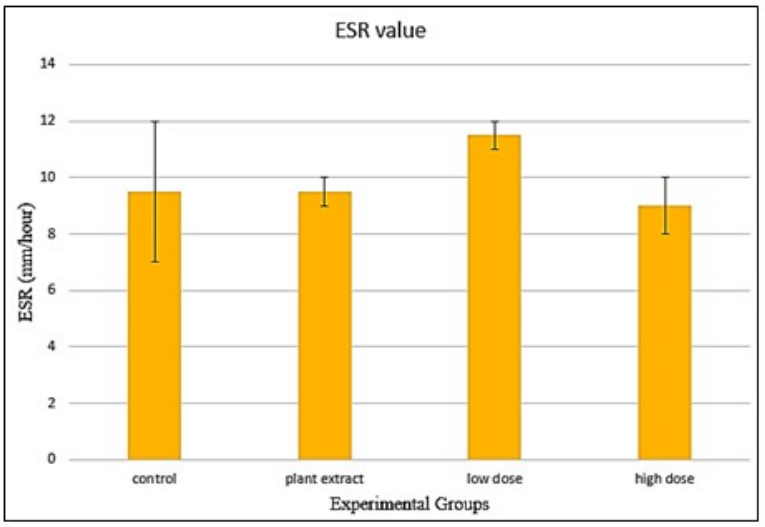


FIGURE 4.17: Graphical representation of ESR value for all groups.

Mean values of ESR of all groups are given in Table: 4.8 with p>0.05 that is insignificant showing less inflammation on average.

TABLE 4.8: Mean±SD values of ESR for all groups compared by one-way ANOVA.

Parameter	**Control**	**Plant Ext.**	**Low dose**	**High dose**	**P Value**
ESR	9.5 ± 2.50a	9.5 ± 0.50a	11.5 ± 0.50a	9 ± 1.0a	0.631

4.5.2 CRP

C-reactive protein (CRP) is a substance produced by the liver in response to inflammation. Its primary role is as a marker of inflammation in the body. When there is inflammation present, CRP levels in the blood increase rapidly, typically

within hours. This makes CRP a useful biomarker in clinical practice for detecting and monitoring inflammatory conditions. Figure 4.18 demonstrating the values of CRP in all groups. A minor increase in CRP level of plant extract group and high dose of SiNPs group can be seen. Where in low dose of SiNPs group, CRP is equal to the control group.

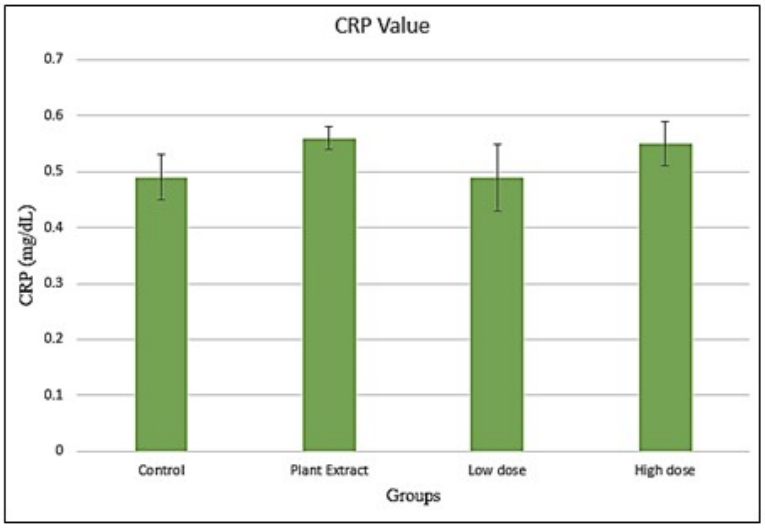


FIGURE 4.18: CRP of all groups

S. T. Rafik [92] reported that increased CRP level is due to inflammation in body. Following their report, a slight inflammation is seen in plant extract group and high dose of SiNPs but not of extreme.

TABLE 4.9: Mean±SD values of CRP for all groups compared by one-way ANOVA.

Parameter	**Control**	**Plant Ext.**	**Low dose**	**High dose**	**P Value**
CRP	0.49 ± 0.04a	0.56 ± 0.02a	0.49 ± 0.06a	0.55 ± 0.04a	0.534

4.6 Biochemical Tests

Biochemical tests refer to a set of laboratory procedures used to identify and characterize various substances in biological samples such as blood, urine, cerebrospinal fluid, and others.

4.6.1 RFTS

Renal function tests are a group of tests that are commonly performed to assess how well the kidneys are functioning. These tests are essential in diagnosing and monitoring various kidney conditions, as well as evaluating overall health. RFTs are as followed:

4.6.1.1 Creatinine

Creatinine is essentially a metabolite of creatine phosphate, a compound that acts as a source of energy in muscle. Creatinine is present in the bloodstream (serum creatinine) and is filtered out of the body at a constant rate by the kidneys (glomerulus and the proximal tubule). As shown in Figure 4.19, Creatinine level in all treated groups is decreased as compared to control group. According to Monir Doudi [93] the level of creatinine in blood reflects how well the kidneys are filtering waste products. This filtration process happens in tiny structures called glomeruli. Higher-than-normal creatinine levels can signal reduced kidney function or renal damage.

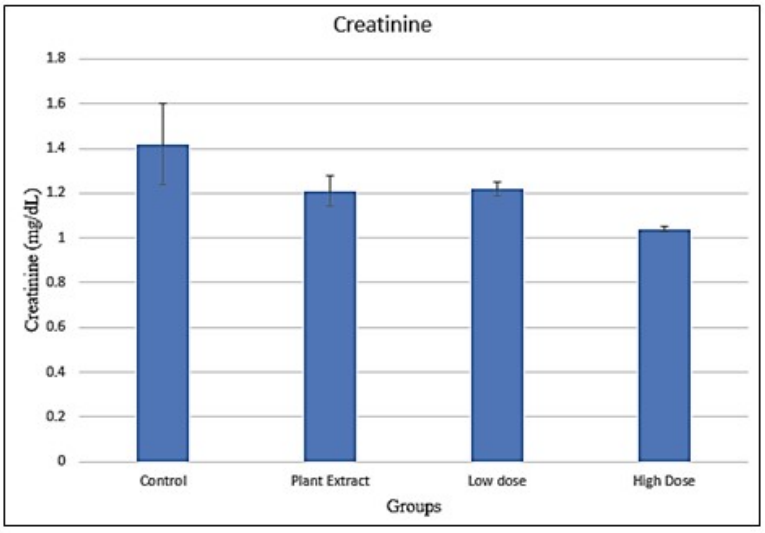


FIGURE 4.19: Creatinine (mg/dL) value of all groups.

From Monir Doudi [93] report, it can be concluded that treated groups do not show any damage to renal function. Mean values with standard deviation are given in Table:4.10.

TABLE 4.10: Mean±SD values for Creatinine of all groups compared by one-way ANOVA.

Parameter	Control	Plant Ext.	Low dose	High dose	P Value
Creatinine	1.42 ± 0.18a	1.21 ± 0.07a	1.22 ± 0.03a	1.04 ± 0.01a	0.184

4.6.1.2 BUN

BUN stands for Blood Urea Nitrogen. It is a common laboratory test used to assess kidney function and overall health. BUN measures the amount of nitrogen in blood that comes from urea. From figure: 4.20, it is shown that BUN level is elevated in all three treated groups but up to slight extent.

Elevated level of BUN cause renal function damage in such a way that it do not filter urea properly [85]. Low dose group increased creatinine more as compared to high dose that have less increased level. Same results are reported where low dose of AgNPs induced more creatinine as compared to high dose AgNPs [85].

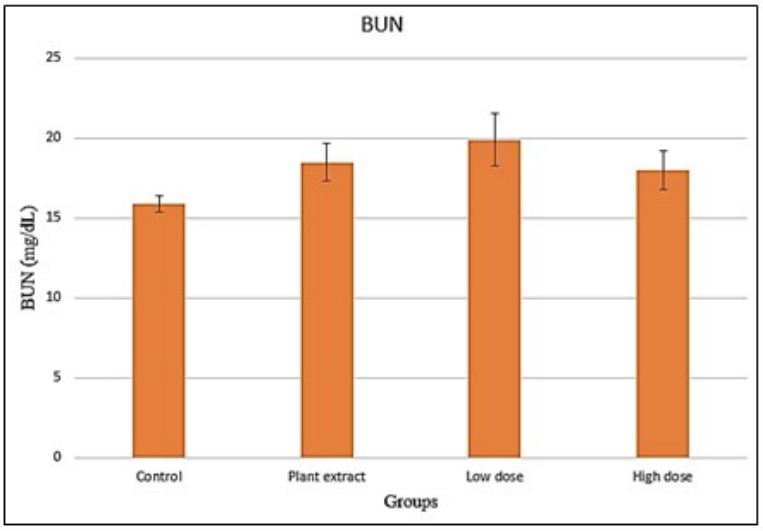


FIGURE 4.20: BUN (mg/dL) value of all groups

Mean values of BUN of all groups are shown in table: 4.11 with p value

TABLE 4.11: Mean±SD values for BUN of all groups compared by one-way ANOVA.

Parameter	Control	Plant Ext.	Low dose	High dose	P Value
BUN	15.9 ± 0.50a	18.5 ± 1.15a	19.9 ± 1.65a	18 ± 1.2a	0.276

4.6.1.3 Urea

Urea, is the key element that is measured in RFTs, that is used to test the health and function of kidneys. The concentration of urea in the blood increases when the kidneys are not functioning properly, leading to reduced excretion of urea. It is the marker for the Nitrogen metabolism in the body. In figure: 4.21, urea level is slightly elevated in treated groups that depicts a slight change in kidney function. This elevated level can be due to renal function disorder.

It is the marker for the Nitrogen metabolism in the body. In figure: 4.21, urea level is slightly elevated in treated groups that depicts a slight change in kidney function. This elevated level can be due to renal function disorder. Similar results have been reported the increased level of urea in animal models while inducing SiNPs in them and reported a renal damage [87]. Table: 4.11 shows the Mean and standard deviation values of Urea of all groups.

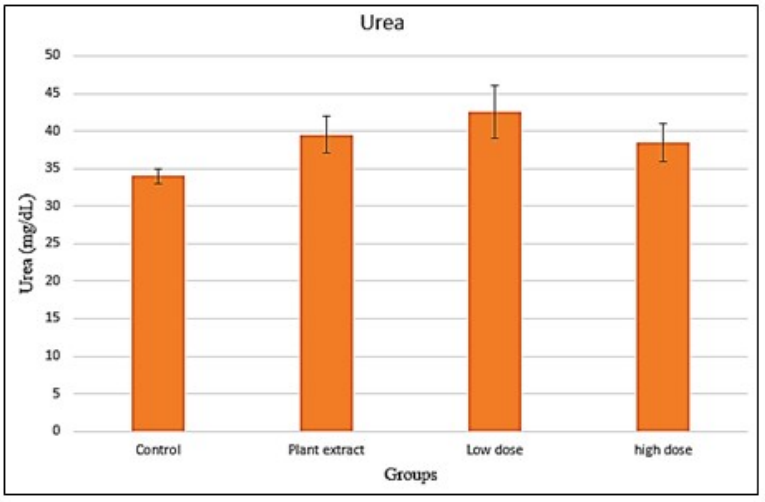


FIGURE 4.21: Urea (mg/dL) value of all groups

TABLE 4.12: Mean±SD values for Urea of all groups compared by one-way ANOVA.

Parameter	Control	Plant Ext.	Low dose	High dose	P Value
Urea	34 ± 1.00a	39.5 ± 2.5a	42.5 ± 3.5a	38.5 ± 2.5a	0.267

4.6.2 LFTs

Liver function tests (LFTs), also known as liver enzyme tests or liver panel, are a group of blood tests used to assess the health and function of the liver. They typically include several different tests that measure various enzymes, proteins, and substances produced or excreted by the liver.

4.6.2.1 ALT

Alanine aminotransferase (ALT), is practically most recognized enzymes in the liver. It is the first step (stage) in examining liver functioning. An increased level in ALT shows the liver damage because these enzymes are present in the cells of liver and when liver gets damage, cells throw out the enzyme in blood stream that elevated their level in blood [93].

In figure 4.22, it is shown that ALT level in plant group is same as control but in low dose and high dose of SiNPs, ALT level is reduced than control which shows minimum to no damage to liver by NPs. Mean values with standard deviation are shown in Table: 4.11.

TABLE 4.13: Mean±SD values for ALT of all groups compared by one-way ANOVA.

Parameter	Control	Plant Ext.	Low dose	High dose	P Value
ALT	78.5 ± 178a	76 ± 121a	59 ± 2.0a	34 ± 15a	0.44

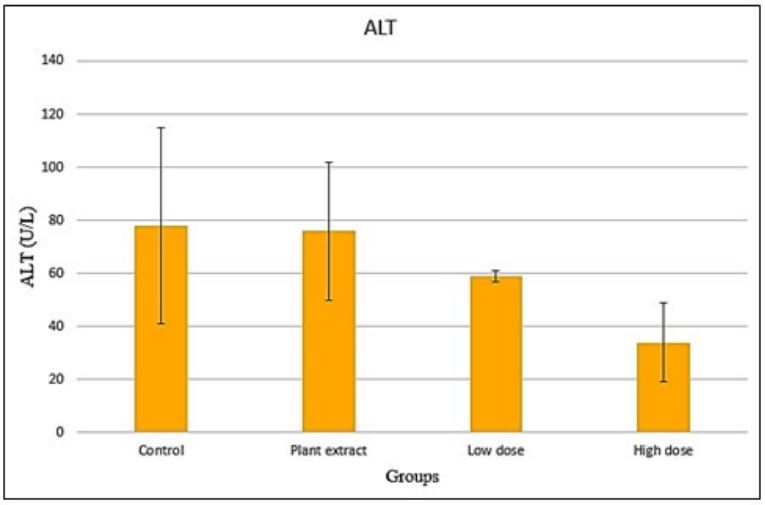


FIGURE 4.22: ALT (U/L) value of all groups

4.6.2.2 AST

AST, or Aspartate aminotransferase, is an enzyme found in your liver, but also in small amounts in your heart, kidneys, muscles, and brain. AST is the first element that is measured to examine the liver functioning. Increase in AST is the indication of the liver damage [93]. In figure: 4.23, it is shown that AST level in plant is slightly increased while in SiNPs doses of high and low, the level of AST is decreased as compared to control group which demonstrate that no liver damage occurred in case of SiNPs. This study contradicts the results of T. N. Almanaa [87], who observed an increase in the level of AST in blood serum of rats upon administration of Silica NPs.

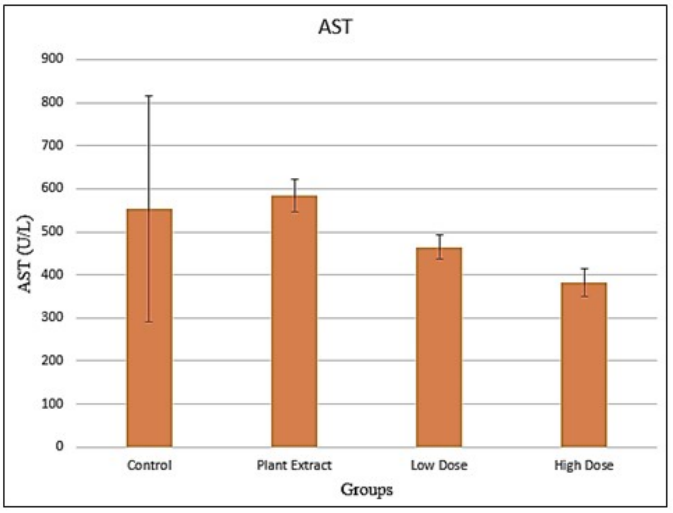


FIGURE 4.23: AST (U/L) value of all groups

Mean values with standard deviation are given in table below with p>0.05.

TABLE 4.14: Mean±SD values for AST of all groups compared by one-way ANOVA.

Parameter	Control	Plant Ext.	Low dose	High dose	P Value
AST	554 ± 262a	584 ± 39a	464.5 ± 27.5a	382.5 ± 31.5a	0.67

4.6.2.3 Bilirubin Total

Bilirubin is a yellowish pigment that forms when red blood cells break down. The liver helps remove bilirubin from blood. Bilirubin total is a part of a liver function test that measures the amount of bilirubin in blood. After biochemical testing of blood, it is seen that Bilirubin total is same in all four groups. There is no difference between these values.

This study is contradictory to the results reported, decrease in value of Bilirubin in all groups treated with SiNPs and they reported no toxicity of NPs in animal [94]. Hence, no effect of SiNPs observed on bilirubin level of blood serum. Mean values of bilirubin are given in Table: 4.12.

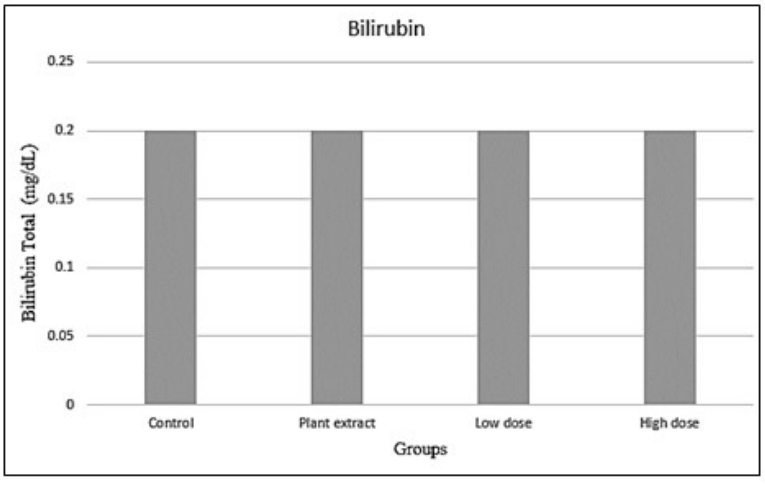


FIGURE 4.24: Bilirubin Total (U/L) value of all groups

TABLE 4.15: Mean±SD values for Bilirubin of all groups compared by one-way ANOVA.

Parameter	Control	Plant Ext.	Low dose	High dose	P Value
Bilirubin	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	—

4.6.2.4 Albumin

Albumin is a protein produced by the liver that plays a vital role in various bodily functions. It helps maintain fluid balance in the body, transports hormones and other substances throughout the bloodstream, and contributes to wound healing. In biochemical testing, albumin level in plant extract group is decreased while in high and low doses of SiNPs, albumin level is increased that depicts a slight change in liver function.

These results contradict the results reported in (2017) [94], who observed decrease in the albumin level of mice upon administering SiNPs and report no toxicity. Figure 4.25 represents the graphical representation of albumin levels in all groups and Table: 4.13 represents the Mean and SD values of albumin of all experimental groups.

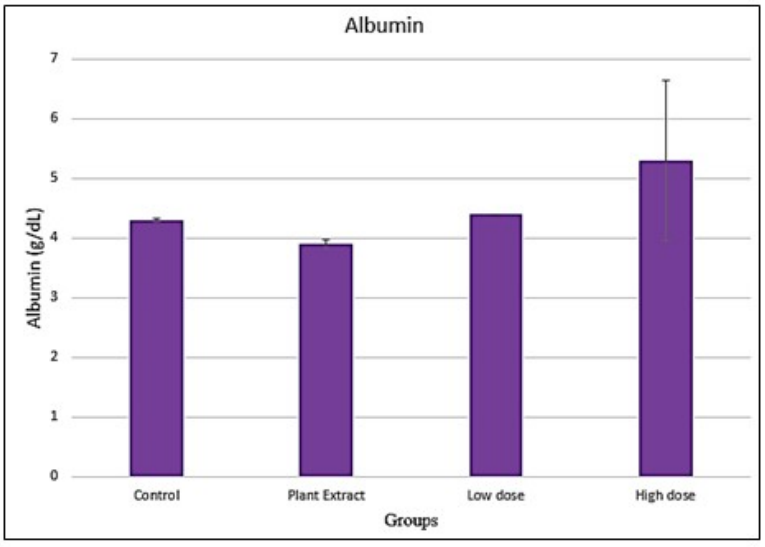


FIGURE 4.25: Albumin (g/dL) value of all groups

TABLE 4.16: Mean±SD values for Albumin of all groups compared by one-way ANOVA.

Parameter	Control	Plant Ext.	Low dose	High dose	P Value
Albumin	4.3 ± 0.025a	3.9 ± 0.08a	4.4 ± 0.00a	5.3 ± 1.34a	0.595

4.7 Histopathological Analysis of Liver Tissues

Histochemical analysis of liver tissue carried in order to assess the toxicity on liver tissues.

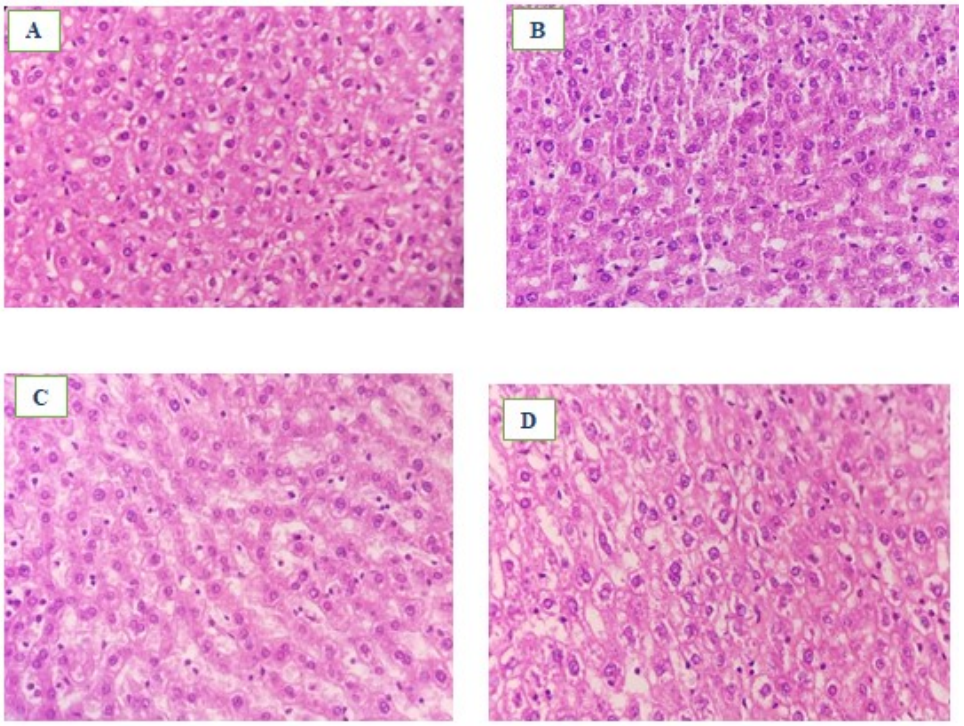


FIGURE 4.26: Histo analysis of Liver Tissue of (A) Control, (B) Plant Extract group, (C) Low dose (SiNPs) group and (D) High dose (SiNPs) groups.

Histological analysis of liver tissue of control group (Fig:4.26A) shows normal histological structures. No cell damage or inflammation in liver tissues and cells are in normal size [94]. Histomicrographs of liver tissue from rats of plant extract group (Fig:4.26B) shows a normal liver tissue with a minor change in cellular structure as compared to control one. Micrographs of liver tissue from rat of low dose of SiNPs group (4.26C) shown a very slight change in morphology of liver cells where nuclei of each cell reduced their size and slight necrosis is seen while

21 days of administration of SiNPs. Micrographs of liver tissue of rats from high dose group are shown in (Fig:4.26D). There can be seen a start of necrosis in liver tissues and nuclei of cells gets expanded which demonstrate that upon 21 days of induction of SiNPs, hepatocytes tend to necrosis at very earlier stage.

Chapter 5

Conclusion and Future perspective

5.1 Conclusion

Several studies have been made on synthesis and use of SiNPs for variety of functions and to overcome the negative effects of SiNPs induced in animal bodies. Main aim of this study was to synthesize silica nanoparticles in a way to reduce its toxicity up to maximum. Several different protocols were applied for green synthesis of SiNPs with *Ficus carica L.* leaf extract. One of them with little optimizations get successful in synthesizing the SiNPs. Characterization techniques confirmed their synthesis and nano range size (19-33nm). After synthesizing and assessing its toxicity on animal model, study depicts that green synthesized SiNPs have a slight effect on overall parameters of blood, kidney and liver. A slightly less in vivo toxicity is seen. These findings underscore the feasibility of utilizing natural extracts for eco-friendly nanoparticle synthesis, highlighting the importance of further research to explore their efficacy in various therapeutic and diagnostic roles.

5.2 Future Work

Upon further optimizations of their charge, surface area, size etc, SiNPs can be created in a way to have no toxicity factor in them. Then they can be best used for drug delivery system, textile industry, food additives etc. Creative ideas, new learnings, efforts and work on this tiny world where nano sized NPs are being used, can be revolutiozed in number of fields. Nanoparticles can be utilized in environmental cleanup. Their high surface area and adsorption capacity make them effective in removing pollutants and contaminants from the environment. In electronics, silica nanoparticles are being explored for their potential in improving the performance of devices like transistors and solar cells.

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