Assessment of Biological Potential of Cinnamon-Coated Silica Nanoparticles in Combination with Doxorubicin

By

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A thesis submitted in partial fulfilment of requirements for the degree of

Master of Philosophy in Biochemistry/Molecular Biology

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بسم الله الرحمن الرحيم

"Read! In the Name of your Lord Who has created (all that exists). He has created man from a clot (a piece of thick coagulated blood). Read! And your Lord is the Most Generous. Who has taught the writing by the Pen? He has taught the man that which he knew not"

[Quran, 96: 1-5]

DECLARATION

I hereby declare that the work presented in the following thesis is my own effort and the thesis is my own composition and that, to the best of my knowledge, no part of this thesis has been previously presented elsewhere by anyone for any other degree. All references herein have been duly acknowledged.

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CERTIFICATE

This thesis, submitted by **Ms. Lubna Tabassum** to the Department of Biochemistry, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan, is accepted in its present form as satisfying the thesis requirement for the Degree of Master of Philosophy in Biochemistry/Molecular Biology.

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Dated: **October 18, 2023**

DEDICATION

Firstly, I am grateful to Allah Almighty for the guidance, strength, and power of mind, protection, skills and the healthy life He has given me.

This study is wholeheartedly dedicated to my beloved parents, who were my source of inspiration and strength when I thought of giving up and who continually provided their moral, spiritual, emotional and financial support.

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Acknowledgments

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I would like to pay heartfelt gratitude to my senior **Muhammad Hamid Siddique**, PhD Scholar, for his kindness, encouragement and support throughout. May Allah grant him with good health, blessed life and success in his future endeavors.

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Lubna Tabassum

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List of Abbreviations

MSNPs	Mesoporous silica-based nanoparticles
WNF	World Naturopathic Federation
XRD	X-ray diffraction analysis
FTIR	Fourier-transform infrared spectroscopy
UV	Ultraviolet
WHO	World Health Organization
CNS	Central Nervous System
DDS	Drug Delivery System
PEG	Poly-ethylene Glycol
IRB	International Review Board
TRP	Total Reduction Potential
TAC	Total Antioxidant Capacity
DPPH	2,2-diphenylpicrylhydrazyl
ROS	Reactive Oxygen Species
COX	Cyclo-Oxygenase Enzyme
SSRI	Selective Serotonin Reuptake Inhibitor
BDNF	Brain Derived Neurotrophic Factor

Abstract

Naturopathy offers a conventional treatment approach utilizing plant extracts either alone or in combination with therapeutic drugs. Significant progress has been made in the field of nanomedicine, bringing about a revolution in the global pharmaceutical industry. Nanoparticles, with their ability to deliver drug to specific target sites, have gained attention for increasing drug bioavailability and minimizing side effects. In the present study, we evaluated the biological potential of cinnamon-loaded Mesoporous Silica-based Nanoparticles (MSNPs) alone and in combination with the chemotherapeutic drug doxorubicin, carrying out both in vitro and in vivo assays. Nanomedicine formation was confirmed using different techniques like FTIR, UV/Vis and XRD. Toxic effects of nanomedicine alone and in combination were evaluated using Brine Shrimp cytotoxicity assay. Antioxidant potential of nanomedicine was evaluated using total antioxidant assay, total reducing power assay, while free radical scavenging potential was evaluated by DPPH assay. In vivo assays were also performed including anticoagulant, antidepressant and analgesic assays. Brine shrimp assay results demonstrated that cinnamon didn't show any significant cytotoxicity. The comparison with the controls of antioxidant assays illustrated that antioxidant capacity of nanomedicine was significantly enhanced when used in higher concentrations. Likewise, notable anticoagulant potential was observed during *in vivo* analysis which correlated directly with dosage concentration. Same pattern was observed when nanomedicine was administered to anti-depressant and analgesic models which indicate the potential of cinnamon and cinnamon-loaded MSNPs as valuable therapeutic agents for a wide range of diseases.

1. Introduction

1.1 Naturopathy

Naturopathy is a diverse medicinal spectrum that delineates the use of natural and preventive therapies. Naturopathic practitioners are highly skilled in primary care, focusing on prevention, identification, management, and treatment of various illnesses. The goal of naturopathy is not solely to treat diseases, but to restore overall wellness by considering the individual patient's needs rather than relying on common symptoms. Rooted in the concept of nature's healing power, naturopathic medicine takes a holistic approach, favoring non-invasive treatments and minimizing the use of surgery and conventional drugs. It recognizes the interplay between physical, emotional, and psychological well-being, aiming to restore complete health. By utilizing natural remedies, naturopathic medicine seeks to provide prompt healing without excessive reliance on symptom management. With its foundations dating back to Hippocrates, naturopathy acknowledges the significance of environmental factors that can disrupt the body's normal functioning and contribute to illness. The vitalistic philosophy of naturopathic medicine emphasizes the healing power of nature, utilizing resources such as soil, water and sunlight for effective disease management (Jagtenberg *et al*., 2006)

The World Health Organization (WHO) recognizes naturopathy as a globally effective and culturally accepted traditional medicine system that is affordable, curative, and potent in promoting health and well-being (Homberg *et al*., 2022).

World Naturopathic Federation (WNF) defines naturopathy as a distinct healthcare system which is rooted in longstanding traditional principles and practices, delivered by medically trained practitioners, and offers a wide range of natural treatment options to cater to the needs of patients. Naturopathy emphasizes natural therapies, preventive care, and the holistic treatment of individuals. It recognizes the body's innate healing abilities and aims to restore overall wellness by addressing physical, mental, and emotional aspects. Naturopathy is based on the belief in the healing power of nature and the utilization of natural resources for disease management and health promotion (Kohli & Kohli, 2014). A study conducted over the course of one year, confirmed that

a significant portion (approximately 6.2%) of the Australian population, who were experiencing severe diseases and related risk factors, sought primary care from naturopathic practitioners (Steel *et al*., 2020).

The naturopathic perspective highlights that air, fresh water, and sunlight are natural sources of therapeutic agents that are often deficient in urban areas. Given the challenges posed by the adverse effects of global climate, diminishing rainfall, and excessive use of antibiotics, individuals are seeking naturopathy as a treatment option that offers the potential for relief without unwanted side effects. The increasing prevalence of health issues globally has led to the recognition of clinical naturopathy as a distinct and vital component of promoting overall well-being. Its noteworthy advancements have demonstrated a positive and comforting impact on patients, as observed in the uplifting and relieving responses reported by (Wardle & Sarris, 2014).

Susan E. Cayleff presents four key points that summarize the essence of American naturopathic history as follows:

i. The holistic approach of naturopathy emerged as a response to the dominant medical practices of the early 19th century in the United States, aiming to challenge and resist the influence of conventional medicine.
ii. Naturopathy became a diverse and dynamic movement during the 20th century, characterized by intense debates and conflicting perspectives.
iii. It represents a collection of varied practices and remedies with the goal of providing accurate and effective treatment options.
iv. The promotion and contribution to family health by practitioners of naturopathy were predominantly undertaken by women (Cayleff, 2016).

Despite the limited supportive evidence regarding its efficacy, naturopathy is a remarkable approach that not only focuses on healing but also on preventing infections. Various naturopaths employ diverse methods of treatment, with herbalism and homeopathy being the most prevalent. While naturopaths may be drawn to conventional drugs due to the lack of substantial evidence, many individuals still seek a natural approach to address their health issues (Elder, 2013).

According to Fleming and Gutknecht (2010), naturopathy is often criticized for its holistic approach that focuses on treating the entire individual based on their symptoms. This approach has

gained reliance from oncologists and cardiologists due to its notable effectiveness in targeting various conditions (Cayleff, 2016). However, naturopaths express concerns about the adverse impact of symptom suppression on the natural healing process, particularly in relation to detoxification (Pizzno & Snider, 2015).

According to a survey conducted in the USA by an American naturopath, approximately 92% of natural healing responses are attributed to detoxification (Allen *et al*., 2011). Similarly, in Canada, detoxification is predominantly favored in the natural healing process for addressing various diseases (Temple, 2015). Moreover, naturopathy plays an active role in the treatment of cancer, as conventional medication struggles to effectively target the diverse nature of cancer cells (Ahmed *et al*., 2015).

According to Ahmed and co-workers, (2015), the holistic approach of naturopathy demonstrates better results in cancer patients. Taking into account the findings of a short study, naturopathy is primarily favored for addressing various illnesses through herbal remedies. Cinnamon, an ancient medicinal plant known for its significant antioxidant properties, is one such herbal treatment used in this approach.

1.2 Cinnamon

The Cinnamomum genus, which belongs to the Lauraceae family, is documented in Table 1.1 indicating its scientific classification. This genus consists of approximately 250 aromatic evergreen trees predominantly located in Asia (Chen *et al*., 2016). Among the species within this genus is *Cinnamomum zeylanicum*, a small evergreen plant commonly found in Sri Lanka and India. It is commonly known as cinnamon and serves various purposes such as enhancing the flavor of food and being utilized as an herbal remedy (Ranasinghe *et al*., 2013; Morgan *et al*., 2014)

Table 1.1: Scientific classification of Cinnamomumzeylanicum

Scientific Classification	
Kingdom	Plantae
Phylum	Magnoliophyta
Class	Magnoliopsida
Order	Laurels
Family	Lauraceas
Genus	Cinnamomum
Specie	Zeylanicum

1.2.1 Chemical Composition of Cinnamon

The chemical composition of cinnamon can vary depending on its geographical origin and processing conditions. Cinnamon bark has a nutritional composition that includes moisture (9.9%), carbohydrates (22.6%), protein (4.65%), fiber (20.3%), total ash (3.55%), calcium (1.6%), phosphorus (0.05%), iron (0.04%), sodium (0.01%), potassium (0.4%), vitamin B1 (0.14 mg/100 g), vitamin B2 (0.21 mg/100 g), vitamin C (39.8 mg/100 g), and niacin (1.9 mg/100 g). It also contains various chemical constituents, including cinnamaldehyde, gum, tannin, mannitol, coumarins, and essential oils. The root bark oil contains camphor as its main component, while cinnamon leaf oil is rich in eugenol. Cinnamon oleoresin, obtained through solvent extraction, is a concentrated liquid containing over 50% volatile oil (Thomas and Kuruvilla, 2012).

Cinnamon bark is recognized as a therapeutic option for treating diverse ailments due to its rich assortment of essential oils. It comprises approximately 45% to 65% cinnamaldehyde, as noted by Cheng (1983). Another significant component is eugenol, which ranges between 12% and 18% concentration. In addition to these primary components, there are also trace amounts of other essential oils such as 2'-benzoloxycinnamaldehyde, 2'-hydroxycinnamaldehyde, arabinoxylan, cinnzeylanin, and cinnzeylanol (Isogai, 1977; Gowda, 1987; Lee, 1999).

Cinnamon oleoresin, obtained through solvent extraction, is a concentrated liquid containing over 50% volatile oil. Furthermore, essential oils and compounds can be found in the root, stem, and

leaves of cinnamon, albeit in varying quantities, which hold importance for industrial applications (Ranasinghe *et al*., 2013).

1.2.2 The biological activity exhibited by cinnamon

Cinnamon demonstrates a range of biological activities, such as antibacterial, anti-inflammatory, antioxidant, anticancer, antifungal, and anti-diabetic effects (Joshi *et al*., 2009). These activities are primarily attributed to the presence of cinnamaldehyde and its cinnamon derivatives.

Antioxidant and anti-inflammatory effects of Cinnamon are attributed to its bioactive compounds like cinnamaldehyde. It can scavenge free radicals and reduce oxidative stress, thus protecting cells from damage. Cinnamon also displays anti-inflammatory effects by inhibiting pro-inflammatory molecules and pathways (Shen *et al*., 2012; Chen *et al*., 2016; Herdwiani *et al*., 2016).

1.2.3 Mode of Action of Cinnamon

Antioxidant and anti-inflammatory effects of Cinnamon are attributed to its bioactive compounds like cinnamaldehyde. It can scavenge free radicals and reduce oxidative stress, thus protecting cells from damage. Cinnamon also displays anti-inflammatory effects by inhibiting pro-inflammatory molecules and pathways (Shen *et al*., 2012; Chen *et al*., 2016; Herdwiani *et al*., 2016). The compound 2-cinnamaldehyde found in cinnamon target both topoisomerase I and II, which are enzymes involved in DNA replication and repair, as well as telomerase activity, which is crucial for cancer cell immortality. (Shen *et al*., 2012; Chen *et al*., 2016; Herdwiani *et al*., 2016).

1.2.4 Therapeutic Applications of Cinnamon

In addition to its culinary uses, cinnamon can be employed as a therapeutic agent for various ailments. In China, India, and Sri Lanka, cinnamon is utilized as a medicinal treatment for gastric, respiratory, and gynecological issues (Ranasinghe *et al*., 2013).

Cinnamon has been documented to have potential benefits in addressing neural disorders such as Parkinson’s and Alzheimer's disease (Rao and Gan, 2014). Furthermore, cinnamon plays a significant role in the treatment of type 2 diabetes mellitus (Gruenwald *et al*., 2010). Cinnamon is

also recognized for its ability to improve circulation of blood in the uterus (Minich and Msom, 2008). Moreover, it contributes to the prevention of excessive bleeding by improving coagulation time (Hossein *et al*., 2013; Rao and Gan, 2014). Its prominent use is often associated with dental toothpaste, where it aids in alleviating toothaches, combating oral microbes, eliminating bad breath, and addressing other dental issues.

In reference to the study done by Morgan and his colleagues in (2014), body weight, changes in blood parameters and histopathology, serve as indicators to assess the toxicity of a compound. Although Cinnamon exhibits minimal toxicity in most cases, its high doses can have an impact on liver and kidney function. However, cinnamon is generally considered non-toxic and non-carcinogenic. Various toxicity tests, such as the in vivo bone marrow micronucleus test and the in vitro cell micronucleus test, have shown negative results for cinnamon compounds (Yun *et al*., 2018). Dermatologists also note that cinnamon oil has limited toxicity in terms of skin allergies and rashes, although it may cause a sensation of oral burning. Despite its low toxicity, cinnamon can be utilized as a remedy for diverse ailments (Perry *et al*., 1990). Further research is needed to explore the molecular mechanisms underlying the effects of cinnamon.

1.3 Chemotherapy

Cancer, characterized by the uncontrollable proliferation of cells, remains a significant global cause of mortality. Approximately 3 million cancer cases were reported in Europe which led to a million cancer related deaths (Ferlay *et al*., 2007). In the United States, cancer-related deaths rank second only to those caused by cardiovascular disease. Despite significant advancements in cancer treatment over the past five decades, it remains a prominent health issue, prompting extensive endeavors in the quest for novel therapeutic strategies. While in ancient times, the sole approach to treating rapidly dividing cells in the body was through surgical removal, the landscape changed in 1940 with the development of various chemical agents. These agents played a pivotal role in directly or indirectly causing the demise of cancer cells by impeding their growth (Shewach & Kuchta, 2009)

One of many such treatments is chemotherapy which refers to the administration of cytotoxic chemicals, i.e. chemicals which possess the ability to kill cells, with the objective of eradicating or reducing the tumor in certain cases. This reduction in tumor size aims to alleviate tumor-associated symptoms and potentially extend life expectancy. Intravenous administration is the primary method for delivering cytotoxic drugs with cell killing properties, with the aim to, in some cases, eradicate the tumor or, at least, reduce the tumor burden and thereby reduce the tumor-related symptoms and perhaps prolong life.

An example of such cytotoxic chemicals is Doxorubicin which is extensively utilized as a chemotherapeutic agent in the treatment of leukemia.

1.3.1 Doxorubicin

Doxorubicin, is a chemotherapeutic medication used to treat abnormal cell growth or uncontrolled cell division in the body. Doxorubicin belongs to the class of anthracyclines. It is widely used in the treatment of various types of cancer, including leukemia, breast cancer, ovarian cancer, and other solid tumors. Doxorubicin was first isolated and identified in the early 1960s.

1.3.2 Structure of Doxorubicin

Doxorubicin has a complex chemical structure. It is an anthracycline antibiotic and belongs to the class of anthracyclines. The chemical formula of doxorubicin is C27H29NO11, and its systematic name is (8S,10S)-10-[(2R,4S,5S,6S)-4-amino-5-hydroxy-6-methyloxan-2-yl]oxy-6,8,11-trihydroxy-8-(2-hydroxyacetyl)-1-methoxy-7,8,9,10-tetrahydrotetracene-5,12-dione (Tacer *et al*., 2013) as shown in the figure 1.1. The structure of doxorubicin consists of a tetracyclic anthraquinone core with various functional groups attached, including a daunosamine sugar moiety (Arcamone *et al*., 1972). The specific arrangement of atoms and bonds in the molecule gives doxorubicin its therapeutic properties as an anticancer agent.

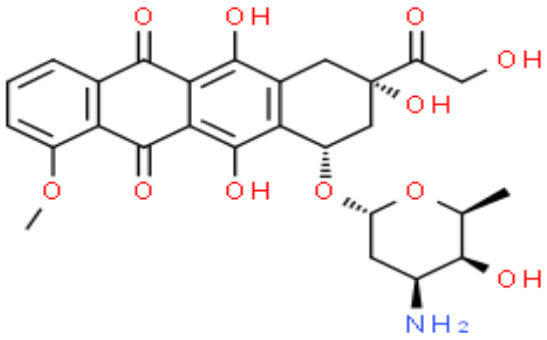

Figure 1.1: Molecular structure of doxorubicin (Zhang et al., 2018)

1.3.3 Mode of Action of Doxorubicin

The mode of action of doxorubicin involves multiple mechanisms that contribute to its anticancer effects. Doxorubicin primarily functions by intercalating with nuclear DNA as well as mitochondrial DNA (Ashley and Poulton, 2009) which means it inserts itself between the DNA base pairs, disrupting the DNA structure and inhibiting DNA replication and transcription. Additionally, doxorubicin inhibits the activity of topoisomerase II enzyme (Bodley *et al*., 1989) which is responsible for DNA unwinding during replication and transcription. This inhibition leads to the accumulation of DNA breaks and further prevents DNA repair. Moreover, doxorubicin generates reactive oxygen species (ROS) that cause oxidative damage to cells, leading to cell deaths (Iqbal *et al*., 2008). The combined effects of DNA intercalation, topoisomerase II inhibition, and ROS generation makes doxorubicin a potent anticancer drug. The mode of action of doxorubicin is shown in figure 1.2.

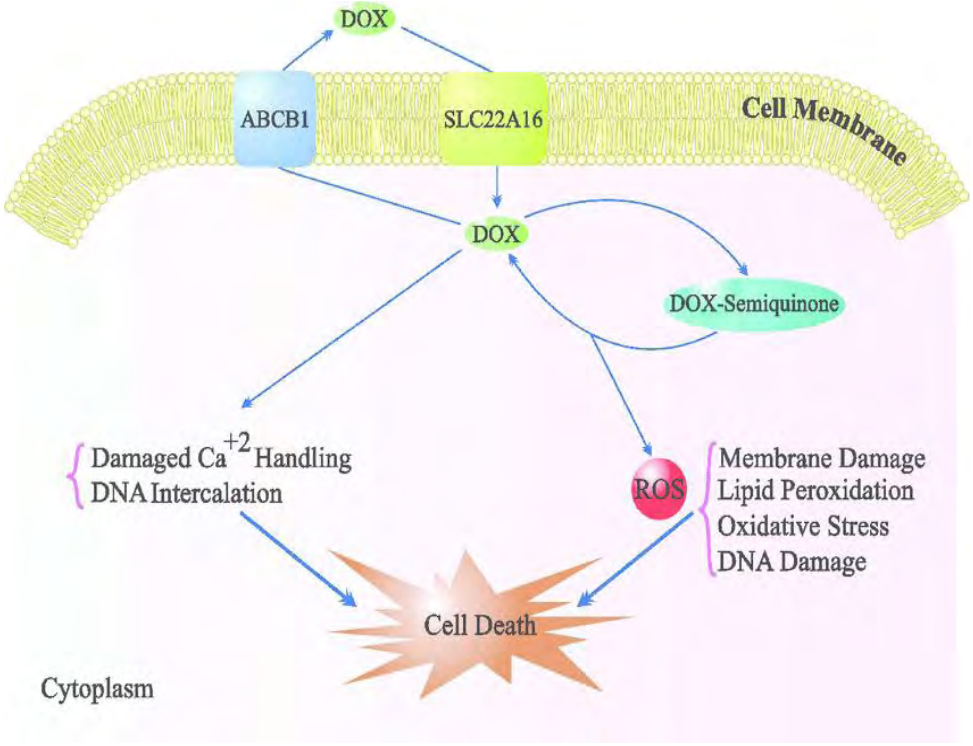

Figure 1.2: Mode of action of doxorubicin in living cells (Yang et al., 2014)

1.3.4 The adverse effects of doxorubicin

Although doxorubicin is a good anticancer drug, it also triggers apoptosis in normal cells and disrupts their normal functions, as illustrated in figure 1.3. The activity of anthracyclines is based on their ability to inhibit topoisomerase II, which occurs when they intercalate between DNA base pairs. This process generates hydroxyl free radicals that contribute to both the antitumor effects and the toxicity to healthy tissues (Lebrecht *et al*., 2005). Among the healthy tissues, myocardial tissue is particularly vulnerable to free radical damage (Young *et al*., 1981). The main dose-limiting side effects of anthracyclines are acute myelosuppression (bone marrow suppression) and cumulative dose-related cardiotoxicity (Chatterjee *et al*., 2010). Anthracycline-induced cardiomyopathy, which can lead to congestive heart failure, is often irreversible. Other toxicities associated with anthracyclines, such as stomatitis, nausea, and vomiting, hair loss, and "radiation recall" reactions, are generally reversible (Hortobagyi *et al*., 1997).

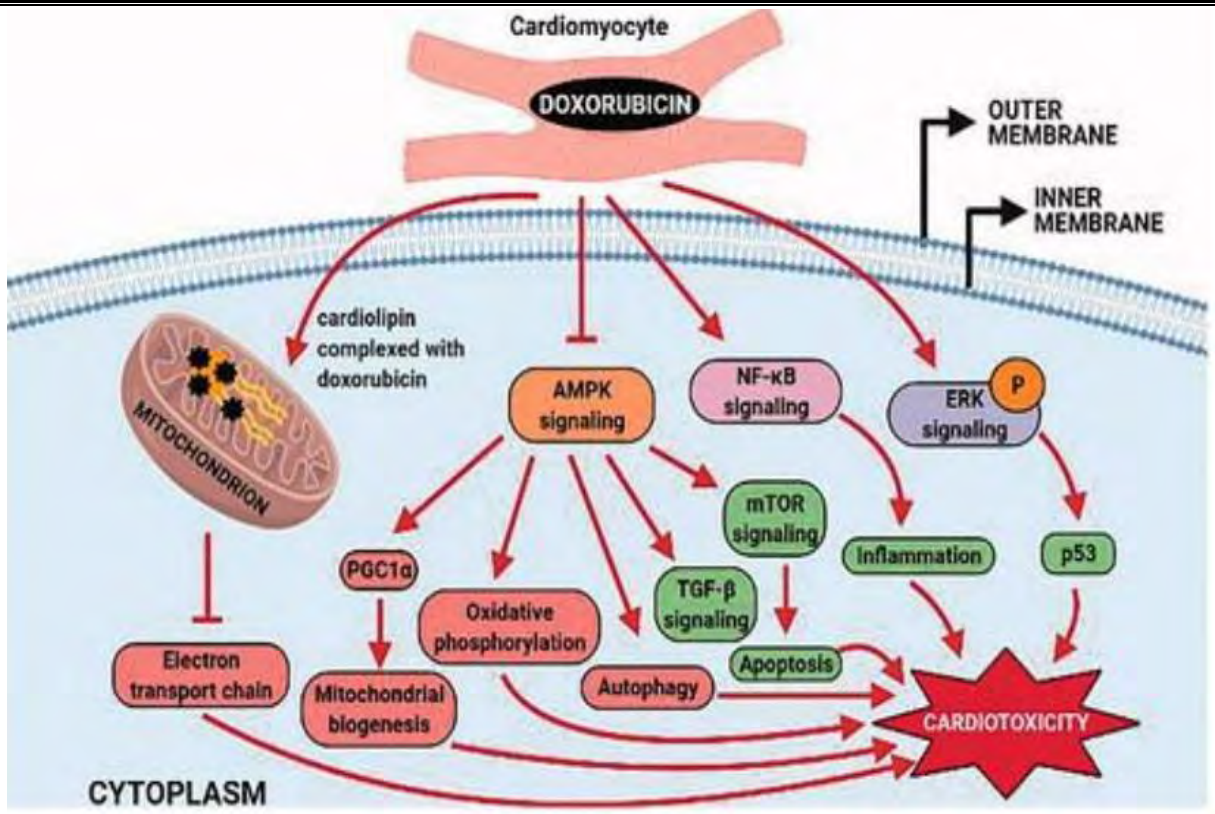

Figure 1.3: Toxic effect of doxorubicin in normal body cell (Shivakumar et al., 2012)

1.4 Nano medicine

Nano medicine is a field of medicine that utilizes nanotechnology for the prevention, diagnosis, and treatment of diseases. It involves the application of nanoscale materials and devices, having diameter ranging from 1 to 100 nanometers in size, to interact with biological systems at the molecular level (Sinha *et al*., 2006). The production of nanomedicines using nanotechnology is a rapidly growing field worldwide due to its wide range of applications (Singh *et al*., 2006). Nano medicines have a broad range of applications in the medical field, including the advancement of controlled drug delivery systems (DDS). These systems are designed to minimize undesirable side effects and improve the effectiveness of treatment by precisely targeting specific sites in the body (Wilczewska *et al*., 2012). The properties of nanoparticles can be adjusted by manipulating their size, structure, and surface area, providing advantages over larger particles such as microparticles. Nanoparticles offer a high surface-to-mass ratio, which can be beneficial in various applications (Thomas *et al*., 2005). By modifying the surface of nanoparticles with different functional groups,

their interactions with cellular and molecular components can be facilitated, leveraging their size similarity to biomolecules (Faraji & Wipf, 2009). In therapeutics, nanoparticles are utilized as carriers, allowing drugs to be covalently linked to their surfaces, resulting in effective therapeutic solutions (Mohanraj *et al*., 2006).

1.5 Types of Nanoparticles

There are various types of nanoparticles used in different fields. Nanoparticles can be either artificially produced or found naturally. Various types of nanoparticles, including carbon base nanotubes, metal nanoparticles, magnetic nanoparticles, inorganic nanoparticles, and polymeric nanoparticles, are synthesized, characterized, and utilized in diverse therapeutic systems (Sanvicens & Marco, 2008).

1.5.1. Inorganic Nanoparticles

Inorganic nanoparticles are nanoparticles composed of inorganic material. The structure of inorganic nanoparticle can vary depending on the specific material and synthesis method used. However, in general, inorganic nanoparticles often exhibit a core shell structure. The core of the nanoparticle refers to the central region composed of the inorganic material itself. It can be a single element (i.e. gold, silver) or a compound (i.e. metal oxides, semiconductor materials). The size and shape of the core can vary and are typically in nanometer range. The shell or surface coating surrounding the core is an essential component of inorganic nanoparticles. It serves multiple purposes, such as providing stability, controlling surface properties, enhancing biocompatibility, and enabling functionalization. The shell can be a layer of organic molecules, polymers or other inorganic materials. The overall structure of nanoparticles allow for unique properties, such as large surface-to-volume ratio, tunable optical and electronic properties and high reactivity. These features make inorganic nanoparticles suitable for a wide range of applications, including drug delivery, imaging, catalysis, and energy storage (Faraji & Wipf, 2009).

1.5.2 Magnetic Nanoparticles

Magnetic nanoparticles predominantly consist of an iron core encapsulated within a coating composed of polyethylene glycol (PEG). These nanoparticles have garnered significant interest in the field of controlled drug delivery systems and the mitigation of chemotherapy-related side

effects, owing to their exceptional magnetic properties such as magnetic excitation, thermally activated spin, and zero temperature spin (Kodama, 1999). The ability to enhance the therapeutic potential of drugs has attracted considerable attention towards magnetic nanoparticles, as they facilitate drug deposition at specific targets by leveraging the influence of a magnetic field (Mody *et al*., 2014). Figure 1.4 illustrates the various applications of magnetic nanoparticles, highlighting their versatility and wide range of uses.

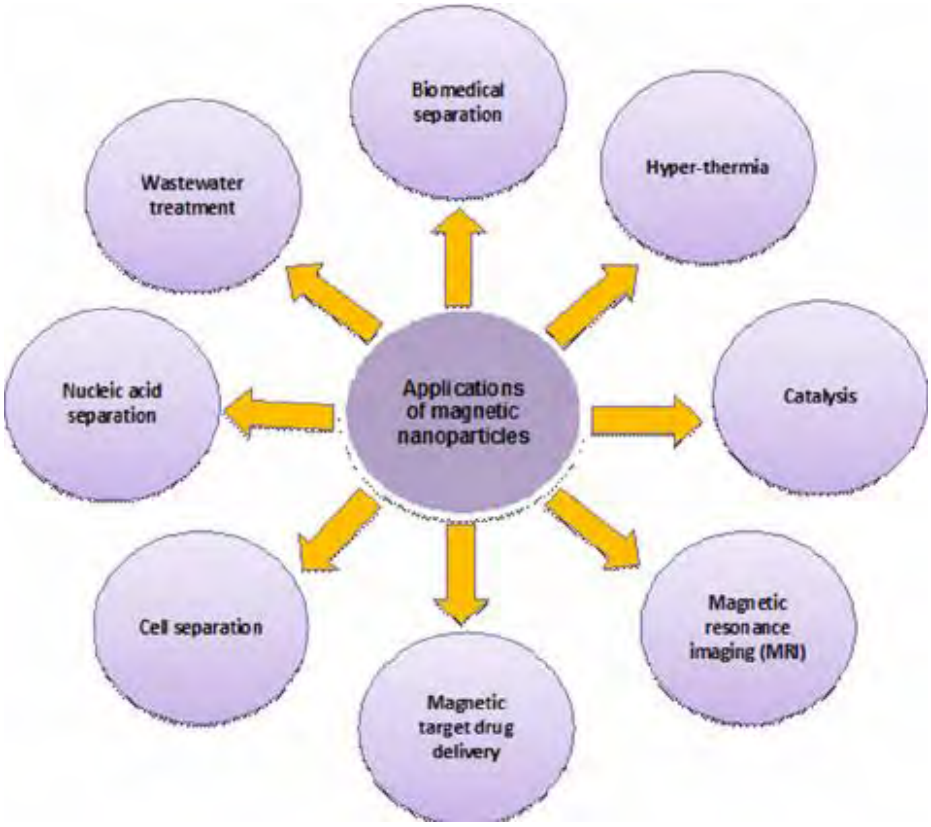

Figure 1.4: Application of magnetic nanoparticles in nanomedicines (Mornet et al., 2006)

1.5.3 Polymeric Nanoparticles

Polymeric nanoparticles are nanoscale particles composed of polymers. These nanoparticles are typically made from biocompatible and biodegradable polymers such as poly(lactic-co-glycolic acid) (PLGA), polyethylene glycol (PEG), and chitosan (Kumari *et al*., 2010). Polymeric nanoparticles play a crucial role in drug delivery, thanks to their versatility, making them well-

suited for addressing the complexities of diseases and drug toxicity (Begines *et al*., 2020).They can be used for drug delivery systems, gene therapy, imaging agents, and tissue engineering, among others. The polymeric coating of these nanoparticles provides stability, controlled release of drugs, and the ability to target specific sites in the body. (Masood, 2016). Figure 1.5 illustrates the process of synthesizing polymeric nanoparticles.

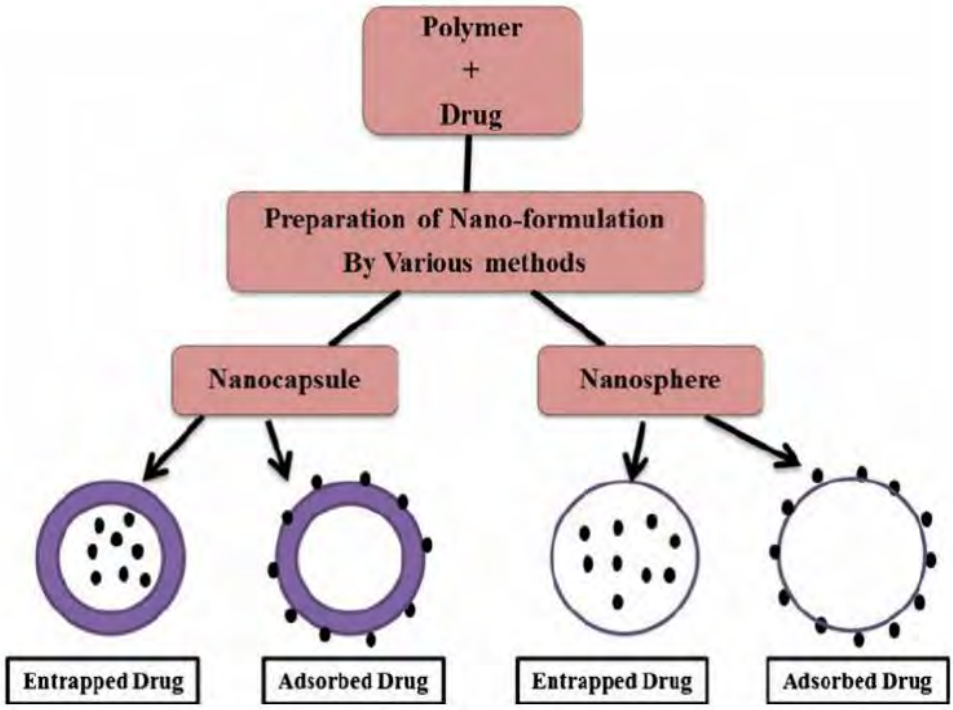

Figure 1.5: Polymeric nanoparticle synthesis and working mechanism (Vauthier & Bauchemal, 2009)

1.6 Characterization of nanoparticles

In order to characterize nanoparticles the following techniques are used

➢ Fourier transform infra-red spectroscopy

➢ X-ray diffraction analysis

➢ UV visible spectroscopy

1.6.1 Fourier Transform Infrared Spectroscopy (FTIR)

Fourier transform infrared spectroscopy (FTIR) is a technique used to analyze the chemical composition of a sample by measuring the interaction of infrared radiation with the sample's molecular bonds. FTIR spectroscopy is commonly employed for the characterization of nanoparticles' stabilization using infrared rays. The analysis typically covers a size range of 400 to 4000 cm-1, and spectra are recorded using an FTIR spectrophotometer. This technique allows for the identification of absorption bands at various spectral regions, which indicate the presence of functional groups and provide insights into drug loading onto nanoparticles (Sarmento *et al*., 2006), as depicted in figure 1.6.

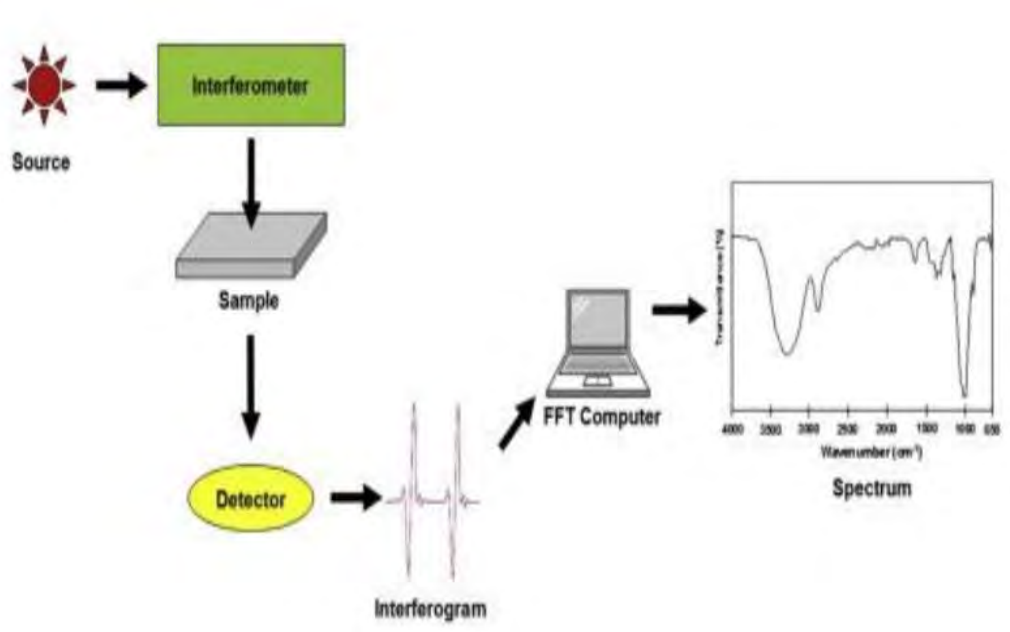

Figure 1.6: Working principle of FTIR (Sarmento et al., 2006)

1.6.2 X-ray Diffraction Analysis

X-ray powder diffraction (XRD) is a non-destructive analytical technique that is utilized for studying the crystal structure and properties of a material. It provides valuable insights into the structure of a crystal, including its geometry, lattice constants, and other parameters. XRD is

capable of identifying the type of material, determining particle size and orientation, assessing sample purity, and even detecting the presence of amorphous content (Hassellov *et al*., 2008).

It involves exposing a sample to X-ray radiation and analyzing the diffraction pattern of the X-rays that are scattered by the sample. The X-rays interact with the atoms in the sample, causing constructive interference and producing a distinct pattern of diffracted X-rays. By employing Bragg's equation (Tiwary *et al.,* 2013), the inter-particle spacing can be determined.

Bragg's equation $2d\sin\theta = n\lambda$,

Where "d" represents the inter-planar spacing, "n" is the order of reflection (a positive integer), and λ denotes the wavelength of the incident radiation, typically X-rays. This equation enables the measurement of inter-particle spacing, providing valuable information about the arrangement and distance between crystal planes. A schematic diagram illustrating the working principle of XRD is depicted in figure 1.7.

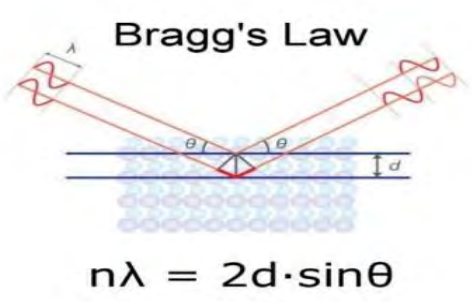

Figure 1.7: X-rays diffraction analysis of nanoparticles (Dorofeev et al., 2012)

1.6.3 UV Visible Spectroscopy

UV-visible spectroscopy is a technique used to analyze the absorption and transmission of light in the UV and visible regions of the electromagnetic spectrum. It measures the intensity of light absorbed or transmitted by a sample at different wavelengths. This method is crucial for confirming nanoparticle synthesis and characterizing their optical properties, such as size, shape, and composition, including the detection of surface plasmon resonance peaks (Ramamurthy *et al*., 2013). Additionally, UV-visible spectroscopy allows for the quantification of nanoparticle concentration and the tracking of optical property changes under different conditions modifications

(Haiss *et al*., 2007). The experimental setup of the UV/Vis spectrophotometer is depicted in figure 1.8.

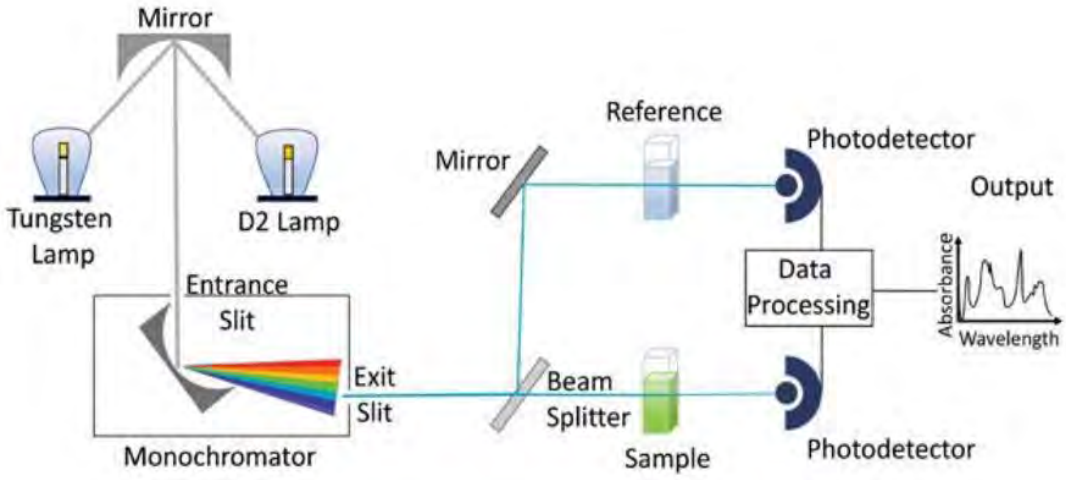

Figure 1.8: Experimental set up of UV visible spectroscopy (Ramamurthy et al., 2013)

1.7 Therapeutic Applications of Nano medicine

Despite the availability of various cancer treatment options, each approach is associated with distinct side effects and can harm both cancerous and healthy cells. Considering the significant side effects and high costs associated with current therapies, there is a global demand for affordable and more efficient medicines with fewer adverse effects on normal cells (Kawalec *et al*., 2015). Nanoparticles serve as promising tools for early cancer detection and targeted drug delivery systems, enabling precise targeting of cancer cells and minimizing the toxicities caused by conventional chemotherapeutic agents (Sinha *et al*., 2006).

1.8 Drug Delivery System

Creating a drug delivery system is important for several reasons. Some of them are listed below:

- To protect drugs from degradation by biological system
- To control the release of Nanodrug at desired location
- To improve the bio-availability of the drug

1.9 Nano medicine Specificity

The successful targeting of specific locations within the biological environment relies on two key components: the target and the carrier. The target refers to the specific body part or organ that requires treatment, while the carrier acts as a vehicle or transporter responsible for delivering the drug and enhancing its binding to the target. This can be achieved by modifying the carrier's molecular structure. There are various types of targeting strategies employed in nanomedicine to achieve this objective. They are listed below:

- Active targeting
- Targeted drug delivery
- Passive targeting
- Stimuli response drug delivery

1.9.1 Active Targeting

Active targeting in nanomedicine is characterized by the presence of specific receptors exclusively found on tumor cells, rather than normal cells. As a result, drug-loaded nanoparticles can selectively and efficiently target cancerous cells while sparing normal cells.

1.9.2 Passive Targeting

Passive targeting in nanomedicine takes advantage of the distinguishing characteristics of cancerous cells, such as the presence of porous surfaces resulting from endothelial tissue lining. These porous surfaces facilitate the targeted delivery of drug-loaded nanoparticles specifically to cancer cells, distinguishing them from normal cells (Haley & Frankel, 2008).

1.9.3 Targeted Drug Delivery

A controlled drug delivery system (DDS) offers a potential solution compared to traditional drug forms (Wilczewska *et al*., 2012) which has low efficiency, non-specific targeting, uncontrolled release, and uneven distribution of the drug. Implementing a targeted drug delivery system can improve efficiency, enabling precise targeting, and providing higher drug concentrations at specific target sites (Allen, 2004). By considering factors such as biocompatibility, biodegradability, effective drug encapsulation, and appropriate drug dosage, we can enhance the

efficiency and achieve specific targeting of drugs to the desired/targeted pathological site (Allen, 2004).

1.9.4 Stimuli Responsive Drug Delivery

Stimuli-responsive drug delivery refers to a type of drug delivery system that can release drugs in response to specific external stimuli or internal physiological conditions. These stimuli can include changes in temperature, pH, light exposure, magnetic fields, or the presence of specific molecules or enzymes (Lammers *et al*., 2012).The purpose of stimuli-responsive drug delivery is to provide controlled and targeted drug release, enhancing the therapeutic efficacy while minimizing side effects. The drug delivery system can be designed to respond to the specific stimulus present at the target site, allowing for on-demand drug release and personalized treatment approaches.

1.10 Combination Therapy

In recent times, there has been a significant increase in the severity of diseases. While conventional treatments exist for various ailments, chemotherapy has become less effective due to side effects and drug resistance. To address the shortcomings of chemotherapy and improve its efficacy while minimizing side effects, new therapeutic approaches are needed. A new line of treatment called combination therapy is extensively used nowadays which refers to the use of multiple treatments or drugs together to improve the effectiveness of a treatment regimen. One such kind of combination therapy is the use of medicinal plants in combination with conventional chemotherapeutic drugs, such as doxorubicin. Medicinal plants are gaining significance due to their ability to offer effective treatment with minimal side effects. Cinnamon, known for its antioxidant properties, has been utilized in combination with doxorubicin as a therapeutic approach to mitigate the side effects associated with chemotherapy.

1.11 Aim

The aim of this study is to evaluate the biological potential of cinnamon-coated silica nanoparticles in combination with doxorubicin for enhanced therapeutic efficacy.

1.12 Objectives

The objectives of this study were:

- To synthesize and characterize cinnamon-coated silica nanoparticles.
- To improve the biocompatibility of the drug to reduce the side effects associated with chemotherapy.
- To analyze cytotoxicity of nanomedicine in vitro.
- To assess the antioxidant properties of nanomedicine.
- To investigate the potential of the nanomedicine in a rat model for its analgesic, anticoagulant, and antidepressant effects.
- To check the efficacy of cinnamon-coated silica nanoparticles in combination with doxorubicin.

2. Materials and Methods

Various biological assays were conducted in Molecular Cancer Therapeutics Laboratory, Biochemistry Department, Quaid i Azam University, Islamabad, to analyze the active biological potential of Cinnamon in combination with synthesized mesoporous silica nanoparticles. These assays, both in vivo and in vitro, were performed following standardized procedures and optimized protocols. Multiple tools, materials, and techniques were employed to achieve the desired results. The study received approval from the Institutional Review Board (IRB) of Quaid i Azam University, Islamabad.

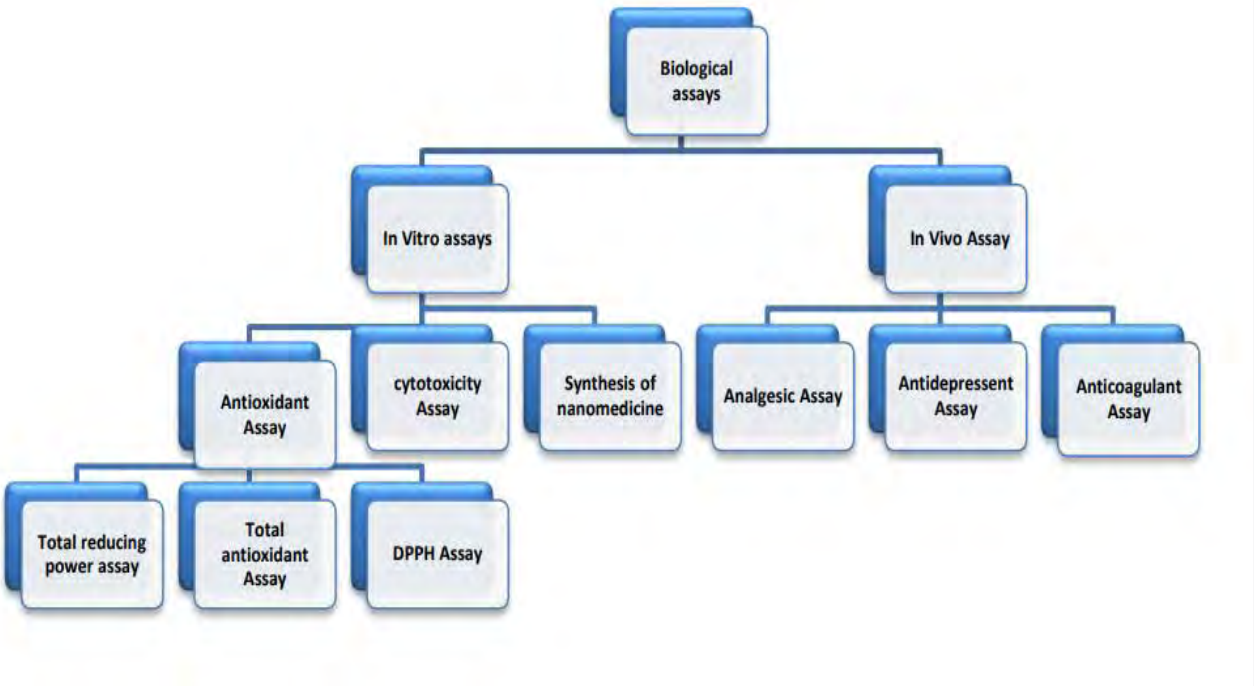

Figure 2.1: Experimental Design

2.1. In vitro Assays

2.1.1. Synthesis of Mesoporous Silica nanoparticles (MSNPs)

Chemical method was employed in this study to manufacture mesoporous silica nanoparticles, which are widely utilized in targeted drug delivery and biomedical applications. These nanoparticles were used as carriers and loaded with drugs to create a targeted drug delivery system.

The chemicals used in the development, coating, and loading of the mesoporous silica nanoparticles are listed in Table 2.1.

Table 2. 1: Reagents utilized in the synthesis and loading of MSNPs

S.No	Chemical Reagent	Reagent Formula	Function
1.	CTAB (Cetyl trimethyl ammonium bromide)	C19H42BrN	Surfactant
3.	DMSO (dimethyl sulfoxide)	C2H6OS	Solvent
4.	Ammonia solution (29%)	NH4OH	Used to maintain PH
5.	Polyethylene Glycol (PEG)	C2nH4n+2On+1	Polymer compound for coating of nanoparticles.
5.	Tetra methyl Ortho silicate	SiC8H20O4	Source of silica

2.1.1.1. Synthesis Protocol

The pH adjustment of deionized water was achieved by gradually adding NH4OH, followed by heating the solution on a hot plate at 50°C. As the solution heated, Cetyl trimethyl ammonium bromide (CTAB) was introduced while stirring, and a pH of 10.3 was maintained throughout the process. Subsequently, the solution was allowed to cool to room temperature. The addition of tetra methyl ortho silicate to the solution led to the observation of turbidity and precipitation after a brief 2-minute incubation. This introduction of tetra methyl ortho silicate induced a notable change, resulting in the solution's turbidity and the formation of precipitates. In order to isolate the precipitates, a filtration step was carried out, followed by the drying of the separated solids in an oven at 98°C. The solution obtained post-calcination underwent an incubation at 500°C for a duration of 5 hours as part of the experimental process. After obtaining dried form of MSNPs loading cinnamon was carried out following the protocol given by Balaure et al., 2017,

Sonication of a mixture containing 200mg of MSNPs with 200ul of CEO and 2ml of chloroform was carried out for 20 minutes. The solvent was evaporated completely to get CEO-MSNPs.

2.1.1.2 Characterization of Mesoporous Silica-Based Nanoparticles

The confirmation of MSNP synthesis was achieved through characterization using the following techniques:

a. X-ray Diffraction Spectroscopy
b. Fourier Transformed Infrared Spectroscopy (FTIR)
c. UV/Vis Spectroscopy

a) X-ray Diffraction Spectroscopy

X-ray diffraction (XRD) data was obtained using an X-ray spectrophotometer, where the absorbance at a wavelength of 15 angstroms was measured. The XRD analysis was conducted using a voltage of 40 kV and a current of 30 milliamperes. The obtained XRD data provides information about the size and nature of the compound.

b) Fourier Transformed Infrared Spectroscopy (FTIR)

FTIR spectroscopy was conducted on a 400 µl sample, using an FTIR spectroscope with a resolution of 4 cm-1 and a scan range of 400 to 4000 cm-1. The FTIR analysis was performed at the Department of Biochemistry, Quaid-i-Azam University, Islamabad.

c) UV/Vis Spectroscopy

UV/Vis spectroscopy was conducted at the Department of Biochemistry, Quaid-i-Azam University, Islamabad. The spectrum was obtained using a spectrophotometer at room temperature, covering the range of 250 to 800 nm.

2.1.2. Brine Shrimp Assay (Cytotoxicity Assay)

To determine the optimal drug dosage for the in vivo assay, a cytotoxicity assay was conducted (Meyers *et al*., 1982). The assay involved testing the percentage of mortality in cultured Brine shrimp larvae exposed to the drug. The necessary materials for the Brine shrimp assay are listed in Table 2.2.

Table 2.2: Particulars needed for Brine Shrimp Assays

S.No.	Material	Concentration	Company Name
1.	Brine shrimp eggs	As per requirement	Ocean star international Inc.
2.	Sea salt	17g	-
3.	Distilled water	500ml	-

a) Steps Involved In Brine Shrimp Assay

In order to prepare sea water for Brine shrimp egg hatching, 17g of sea salt was measured and incorporated into 500ml of distilled water. This saline solution, along with the Brine shrimp eggs, was subjected to approximately 30 minutes of heating on a hot plate. Subsequent to this heating, the Brine shrimp eggs were carefully placed under incubation conditions for a duration of 24 hours. After this period, the hatching of Brine shrimp eggs within the sea water was observed using fluorescent light. Dilutions of different concentrations were prepared in triplicate to determine the optimal drug dosage. In a 96-well micro titer plate, 10 Brine shrimp eggs were added using a magnifying glass. Isopropanol and saline water was used as positive and negative controls respectively and were run in triplicate alongside the sample. After incubating the Brine shrimps for 24 hours, the number of alive and dead shrimps was recorded to calculate the percentage mortality using the following formula:

$$\text{Percentage mortality} = \frac{(\%\ \text{killed in treated} - \%\ \text{killed in control})}{(100 - \%\ \text{killed in control})} \times 100$$

b) Dilutions

To analyze the cytotoxicity of the drug, various dilutions were prepared at different concentrations.

Table 2.3: Dilutions for Brine Shrimp Assay

S.No	Drug	Conc.1 (ul/ml)	Conc.2 (ul/ml)	Conc.3 (ul/ml)	Conc.4 (ul/ml)
1.	Cinnamon Essential Oil(CEO)	4	2	1	0.5
2.	MSNPs	2	1	0.5	0.25
3.	CEO+MSNPs	4+2	2+1	1+0.5	0.5+0.25
4.	Doxorubicin	1	0.5	0.25	0.125
5.	CEO coated MSNPs (CcMSNPs) + Doxorubicin	4+1	2+0.5	1+0.25	0.5+0.125

2.1.3 Assessment of Antioxidant Activity

To evaluate the ability of the compound to scavenge free radicals, various antioxidant assays were conducted (Halliwell, 1990). Antioxidant activity refers to the capacity of a compound to protect another compound from oxidation by undergoing oxidation itself. In order to characterize cinnamon as an antioxidant, three different in vitro assays were performed following an optimized protocol.

2.1.3.1 1,1 Diphenyl picryl hydrazyl (DPPH) assay

The scavenging percentage assay using DPPH was conducted to determine the free radical scavenging activity of the compound, as proposed by Blios. The method reported by Moein et al. (2008) was followed to measure the free radical scavenging activity of the desired compound under optimized conditions. The specific requirements for performing the scavenging percentage assays are provided in Table 2.4.

Table 2.4: Reagents Required for DPPH Assay

S.No	Reagent	Concentration
1.	Ascorbic Acid	1mg/ml
2.	DPPH	4.8mg of DPPH dissolved in 50ml of Methanol

a) Steps Involved In DPPH Assay

Triplicate wells of a 96-well microtiter plate were filled with 20µl of different dilutions of the compound. DPPH reagent was added to each well containing the compound, and the plate was incubated at 37oC for 30 minutes. Ascorbic acid at a concentration of 1mg/ml was used as the positive control, while distilled water served as the negative control. The absorbance of the samples was measured using a spectrometer at a wavelength of 517nm.

b) Dilutions

Table 2.5: Dilutions of Sample used in DPPH Assay

S.No	Drug	Conc.1 (ul/ml)	Conc.2 (ul/ml)	Conc.3 (ul/ml)	Conc.4 (ul/ml)
1.	Ascorbic Acid	1	0.5	0.25	
2.	Cinnamon Essential Oil(CEO)	4	2	1	0.5
3.	MSNPs	2	1	0.5	0.25
4.	Cinnamon Coated MSNPs(CcMSNPs)	4+2	2+1	1+0.5	0.5+0.25
5.	Doxorubicin	1	0.5	0.25	0.125
6.	Cinnamon Coated MSNPs + Doxorubicin	4+1	2+0.5	1+0.25	0.5+0.125

2.1.3.2 Total Reducing Power (TRP) Assay

The assessment of the drug's antioxidant potential was conducted by following protocol given by Moein *et al*., (2017), and TRP (Total Reducing Power) values were obtained. The reagents employed in the TRP assay are listed in Table 2.6.

Table 2.6: Reagents Required for TRP assay

S.No	Reagents	Concentration	Preparation
1.	Trichloro acetic Acid	10%	100g of C2HCL3O2 IN 1000ml of distilled water
2.	Phosphate Buffer	1%	by dissolving 1.42g of Na2HPO4 and 1g of NaH2PO4 IN 50ml of distilled water
3.	Ferric Chloride	0.1%	By adding 10g ferric chloride in 1000ml of distilled water
4.	Potassium Ferricyanide	1%	By adding 100g of potassium ferricyanide in 1000ml of distilled water

a) Steps Involved in TRP Assay

The experimental procedure involved a series of systematic steps. Firstly, 150µl of the test samples, each at different dilutions, were carefully introduced into individual Eppendorf tubes. Following this, an equivalent volume of potassium ferricyanide and phosphate buffer (500µl) was added to each respective Eppendorf tube. Subsequently, the Eppendorf tubes were subjected to an incubation period at a controlled temperature of 50°C for a duration of 20 minutes. After the incubation phase, the addition of Trichloroacetic acid (500µl) to each Eppendorf tube was undertaken. This was followed by employing a centrifuge to spin the solution at 3000 rpm for 10 minutes. This centrifugation process facilitated the separation of different components within the solution. Upon the completion of centrifugation, approximately 500µl of the upper layer, referred to as the supernatant, was transferred to fresh Eppendorf tubes. Further processing involved adding 500µl of distilled water to the Eppendorf tubes and subsequently introducing 100µl of a solution containing 0.1% ferric chloride. To evaluate the reducing power of the samples, the absorbance of each dilution was measured at a wavelength of 630nm using spectrophotometer. Ascorbic acid was employed as positive control while water served as negative control in this assay.

b) Dilutions

The total reducing power of the drug was determined using dilutions of different samples. The prepared dilutions are provided in Table 2.7.

Table 2.7: Dilutions of sample used in TRP Assay

S.No	Reagent	Conc.1 (ul/ml)	Conc.2 (ul/ml)	Conc.3 (ul/ml)	Conc.4 (ul/ml)
1.	Ascorbic Acid	1	0.5	0.25	
2.	Cinnamon Essential Oil(CEO)	4	2	1	0.5
3.	MSNPs	2	1	0.5	0.25
4.	Cinnamon Coated MSNPs(CcMSNPs)	4+2	2+1	1+0.5	0.5+0.25
5.	Doxorubicin	1	0.5	0.25	0.125
6.	Cinnamon Coated MSNPs + Doxorubicin	4+1	2+0.5	1+0.25	0.5+0.125

2.1.3.3 Total Antioxidant Capacity (TAC) Assay

The phosphomolybdate method, proposed by Phatak et al. (2014), was employed to estimate the total antioxidant capacity (TAC) value. This assay quantitatively determines the antioxidant capacity of cinnamon by utilizing various spectrophotometric techniques. The reagents utilized in the phosphomolybdate method for determining TAC values are presented in Table 2.8.

Table 2.8: Reagents Required for TAC Assay

S.No	Reagents	Concentration
1.	H2SO4	1.63ml
2.	NaH2PO4	1.679g
3.	(NH4)2MoO4	0.247g

a) Steps Involved In TAC Assay

Firstly, the TAC reagent was prepared by accurately weighing the specified amounts of Diammonium molybdate, sodium dihydrogen phosphate, and sulfuric acid, which were 0.247g, 1.679g, and 1.63ml, respectively, according to the protocol. Next, 20µl of each sample dilution was added to triplicate wells of a 96-well micro titer plate. Subsequently, 180µl of the TAC reagent was added to each well containing the sample, followed by incubation at 95°C for 90 minutes. The micro titer plates loaded with the sample and TAC reagent were then allowed to cool to room temperature after the incubation period. Ascorbic acid was used as the positive control, while water served as the negative control. Once cooled, the absorbance of each well was measured at 630nm using a spectrophotometer. The TAC values were calculated using the provided formula:

$$\textbf{TAC value} = \frac{\mathbf{100}}{\mathbf{2.651}} \boldsymbol{X\ absorbance\ value}$$

a) Dilutions

In order to determine TAC value following dilutions were prepared enlisted in table 2.9.

Table 2.9: Dilutions of sample used in TAC Assay

S.No	Reagents	Conc.1 (ul/ml)	Conc.2 (ul/ml)	Conc.3 (ul/ml)	Conc.4 (ul/ml)
1.	Ascorbic Acid	1	0.5	0.25	
2.	Cinnamon Essential Oil(CEO)	4	2	1	0.5
3.	MSNPs	2	1	0.5	0.25
4.	Cinnamon Coated MSNPs(CcMSNPs)	4+2	2+1	1+0.5	0.5+0.25
5.	Doxorubicin	1	0.5	0.25	0.125
6.	Cinnamon Coated MSNPs + Doxorubicin	4+1	2+0.5	1+0.25	0.5+0.125

2.2 In Vivo Assays

After confirming the promising antioxidant activity exhibited by the compound through various in vitro assays, further investigations were conducted to assess its active biopotency in an animal model. These investigations included anticoagulant assay, antidepressant assay, and analgesic assay. The Sprague Dawley rat was chosen as the animal model for these in vivo assays. The study strictly adhered to ethical guidelines and obtained approval from the Bioethics Committee. The rats were procured from the primate facility of the biological sciences faculty at Quaid-i-Azam University, Islamabad. Careful attention was given to the treatment and nutrition of the rats, ensuring they were provided with a healthy and balanced diet. Additionally, the rats were housed in aluminum cages during the experimental period.

Experimental Groups

In order to evaluate the activity of cinnamon-coated MSNPs in animal rats, the rats were divided into different groups and received intraperitoneal administration of the test compound. The groups were as follows:

i. Group –I Negative control (saline)
ii. Group- II Positive control (a different drug for different assays)
iii. Group- III 1mg/ml of doxorubicin
iv. Group- IV 4mg/ml of cinnamon
v. Group-V 0.5mg/ml of MSNPs
vi. Group-VI Cinnamon + MSNPs
vii. Group-VII Cinnamon coated MSNPs + doxorubicin

2.2.1 Antidepressant Assay

Antidepressant assay is a type of experimental test or study conducted to evaluate the potential antidepressant activity of a substance or drug. The tail suspension method, as described by Steru *et al*. (1985), was employed to evaluate the antidepressant activity of the drug. This method involves suspending rats in the air, as it is believed that depression symptoms and pain perception share a common neurochemical pathway. To assess the antidepressant activity of cinnamon and its combination, the tail suspension method was utilized, with fluoxetine serving as a positive control.

a) Procedure

The experiment involved the selection of six distinct groups of rats, with each group comprising three rats. To establish a baseline for comparison, an initial reading was acquired approximately 2 hours prior to the commencement of the experiment by suspending rats in the air. The rats were then administered with the test compounds, where fluoxetine is used as the positive control and saline is used as the negative control. This was followed by an incubation period of one hour to allow for the effects of the compounds to take place. After the completion of the incubation period, the rats' tails were gently tied with a string and suspended in the air for a duration of six minutes. The suspension was carefully performed to ensure the rats' safety and comfort.

The entire duration of the suspension, from the moment the rats are suspended to the point when they cease to move was recorded using video equipment. This allows for accurate assessment and analysis of the rats' behavior during the experiment.

2.2.2 Analgesic Assay

An analgesic assay is a method used to evaluate the pain-relieving properties or analgesic activity of a substance. The analgesic activity of various test compounds was assessed using the hot plate assay, following the protocol proposed by Eddy and Leimbech in 1953. In this assay, a heated plate was employed, and the response of rats to the thermal stimulus, indicated by paw withdrawal, was used to evaluate the analgesic efficacy of the test compounds.

a) Procedure

The administration of the test compound, positive control, and negative control was carried out via intra-peritoneal injection. Diclofenac and saline water was taken as positive and negative control respectively. The hot plate was adjusted such that to maintain temperature of 55 degree Celsius. Individually, each rat received the drug and was then placed onto the hot plate. Using a stopwatch, the time interval was carefully recorded from the moment the rat was positioned on the hot plate until it engaged in its first paw lick as a response to the heat. This process was systematically repeated, and additional readings were taken to ensure the accuracy of the data collected.

2.2.3 Anti-coagulant Assay

Anticoagulant assay is a method used to evaluate the ability of a substance or drug to prevent blood from clotting. It measures the effectiveness of the substance in inhibiting the coagulation cascade and formation of blood clots. Anticoagulants are substances or drugs that prevent or inhibit blood from clotting. They are commonly used in medical treatments to prevent the formation of blood clots, which can lead to serious conditions such as deep vein thrombosis, pulmonary embolism, and stroke. Anticoagulants play a crucial role in the treatment of thromboembolic disorders, as they serve as important mediators (Hirsh *et al*., 2005). Anticoagulant assay was performed to in order to check the anticoagulation properties of cinnamon and combination drug.

a) Procedure

The administration of samples to rats were carried out via intraperitoneal injection. To assess the effects of the drug, blood samples were gathered from the tails of each group of rats using sterilized scissors, with the tails being cleaned beforehand using spirit to maintain hygiene. A clean glass slide was used to observe the blood samples, with the help of a toothpick. The toothpick was used to continuously move the blood in a circular motion on the slide until a fibrin thread appeared. The

coagulation time was recorded using a stopwatch as soon as the fibrin thread appeared on the slide. The assay was repeated to obtain additional readings, and the results were analyzed and interpreted.

2.3 Statistical Analysis

After conducting the tests, the results were interpreted as mean values accompanied by their respective standard deviations. A significance level of $p<0.05$ was considered to indicate statistical significance. To statistically analyze the results obtained from the various in vivo assays and in vitro assays, the software GraphPad Prism was employed, utilizing the One-way Analysis of Variance (ANOVA) which is a test of significance used to compare means across multiple groups and determine if there are significant differences between them. Additionally, for group comparisons, the Tukey's post hoc test was applied

3. Results

3.1 Characterization of MSNPs

Following results were obtained from different characterization techniques which confirmed formulation of mesoporous silica nano-particles.

3.1.1 X-ray Diffraction

Figure 3.1 illustrates the significant diffraction peaks observed at 29° during X-ray diffraction of mesoporous silica-based nanoparticles, confirming their amorphous nature and size. The X-ray diffraction was performed at a voltage of 30KV and 30mA.

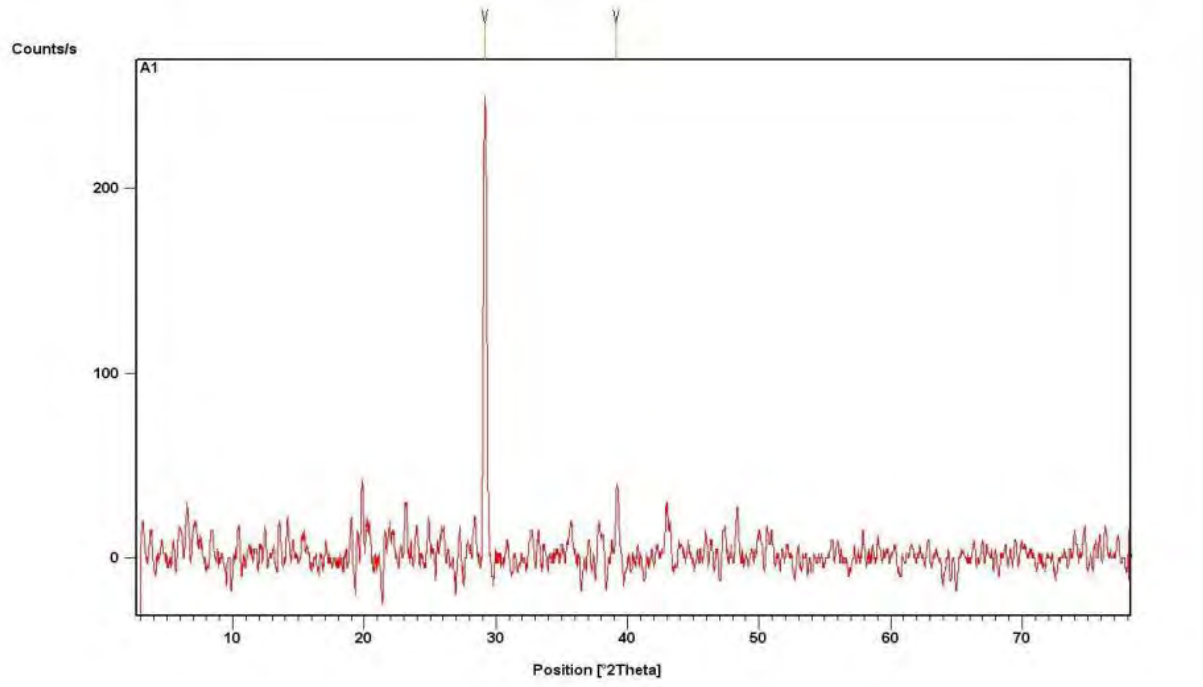

Figure 3.1: X-ray Diffraction Analysis

3.1.2 UV/Vis Spectroscopy

A liquid cuvette was used for optical absorbance spectra in a wavelength range of 250 to 800 nm. UV-Vis spectroscopy of Cinnamon loaded MSNPs is depicted in figure 3.2.

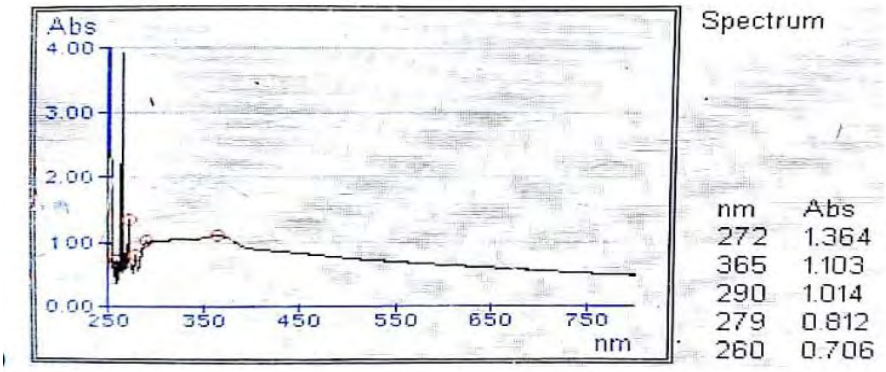

Figure 3.2: Absorbance spectra of MSNPs

3.1.3 Fourier Transformed Infrared Spectroscopy

FTIR analysis was conducted to identify the presence of functional groups. The absorbance spectra obtained from FTIR revealed distinct functional groups. It showed characteristic peaks in the range of 1800-600cm-1. 1679cm-1 exhibiting aldehyde carbonyl, 1573cm-1 showing aromatic functional group, 1450cm-1 showing alcohol functional group and 1248cm-1 representing aromatic esters and the stretching vibrations of phenolic groups. The peak at 973 cm − 1 is assigned to the C-H bending vibration absorption while the peak at 685 cm − 1 shows the vibration absorption of alkenes.

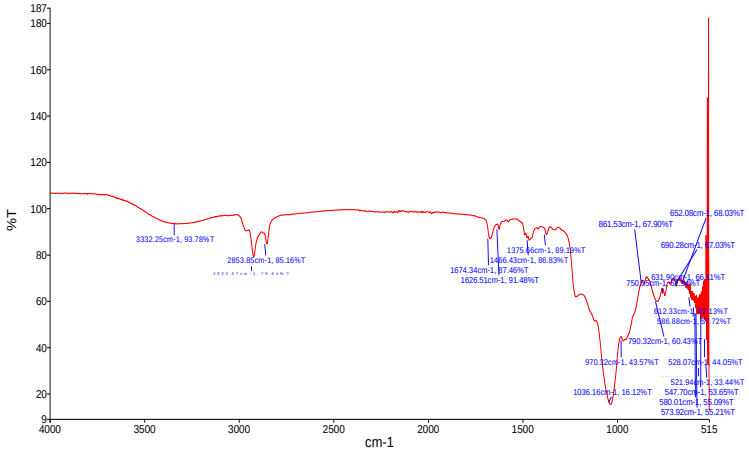

Figure 3.3: FTIR Analysis of Cinnamon-coated MSNPs

3.1.4 Brine Shrimp Assay

Brine shrimp cytotoxicity assay was performed in order to determine mortality percentage of different dilutions of our sample alone and in combination with chemotherapeutic drug i.e. doxorubicin. For this assay isopropanol was taken as positive control which showed 100 percent mortality rate and saline water was taken as negative control whose mortality rate is zero. Mortality rate of different dilutions of samples showed is in the range of 0% to 100%. Combination of doxorubicin with CcMSNPs significantly decreased the mortality of doxorubicin. This lethality potential gradually decreases with decrease in concentration. Similarly, mortality rate of cinnamon coated MSNPs showed a gradual decrease with decrease in concentration.

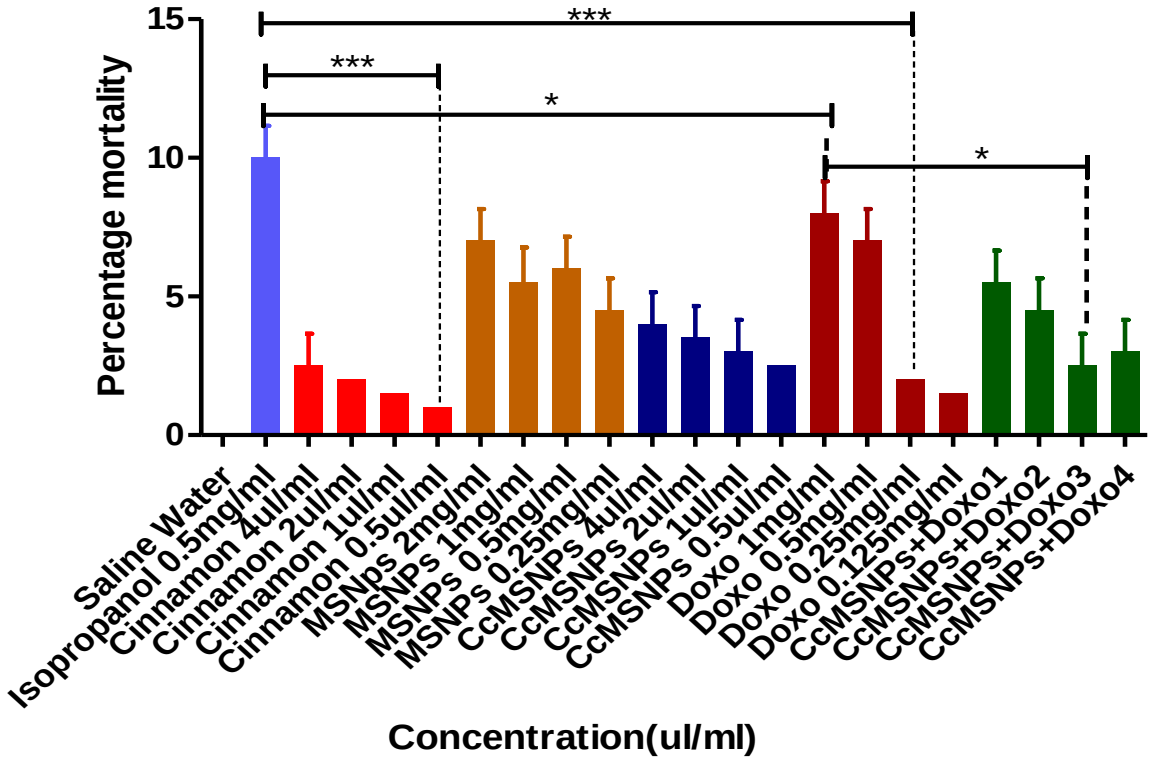

Figure 3.4: Assessing percentage mortality and cytotoxicity of Cinnamon Coated MSNPs and their combination with Doxorubicin.

3.1.5 Total Antioxidant Assay (TAC)

In order to determine total antioxidant capacity, TAC assay was carried out. Ascorbic acid (1mg/ml) was taken as positive control which showed highest antioxidant activity. Further different dilutions of sample, alone, and in combination were taken. Highest concentration of cinnamon (4mg/ml) showed maximum absorbance which gradually decreases with decrease in concentration. Similarly, antioxidant capacity of Nano-medicine also significantly decreases with decrease in concentration. Further, One-way Analysis of Variance (ANOVA) showed that highest concentration of Cinnamon coated MSNPs in combination with chemotherapeutic drug showed maximum TAC capacity as compare to chemotherapeutic drug alone.

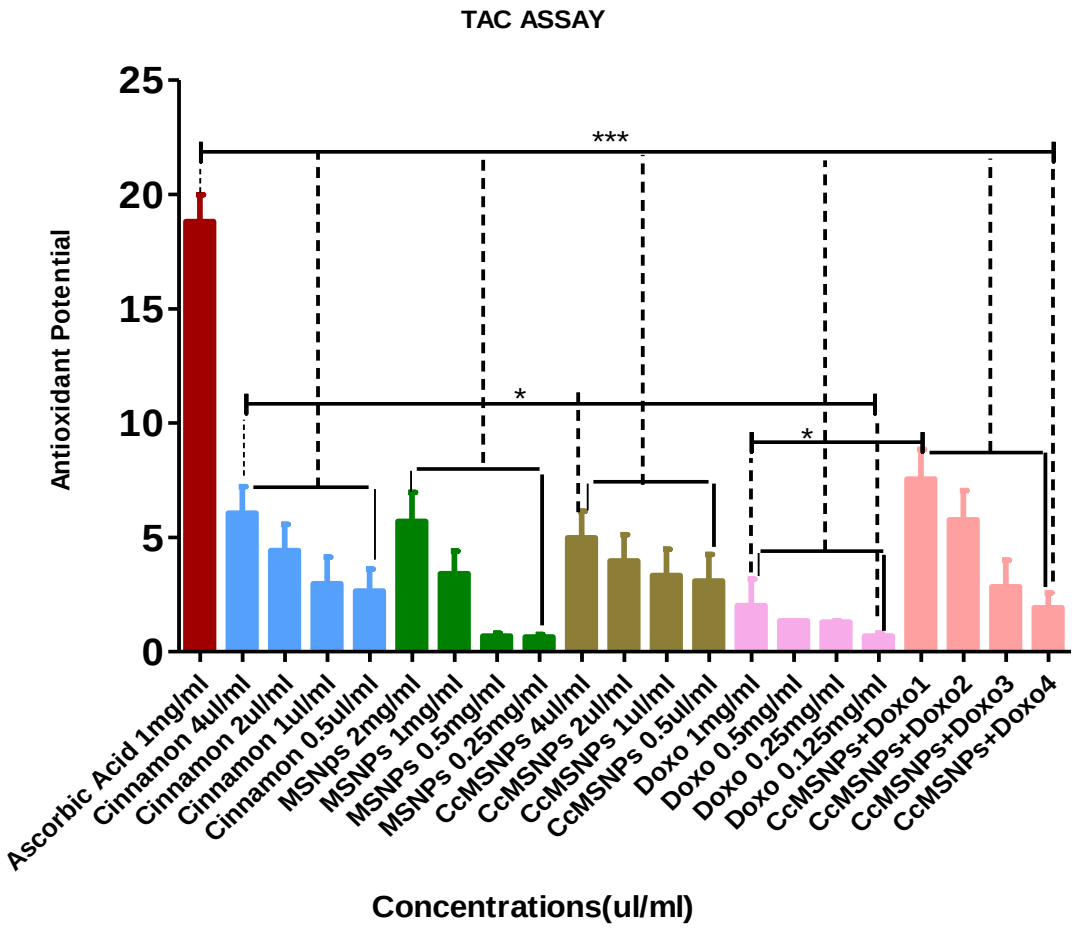

Figure 3.5: Evaluation of antioxidant potential of different groups of Cinnamon Coated MSNPs and their combinations

3.1.6 Total Reducing Power (TRP) Assay

Total reducing power assay was carried out to find out the reducing potential of different dilutions of sample, alone, and in combination with doxorubicin. Ascorbic acid was taken as positive control which exhibited highest reduction potential. Cinnamon in its highest concentration showed maximum reduction potential after positive control. Decrease in cinnamon concentration showed a gradual decrease in reducing power. Loading cinnamon onto MSNPs decreases its reduction potential but it showed a significant increase when combined with the chemotherapeutic drug doxorubicin.

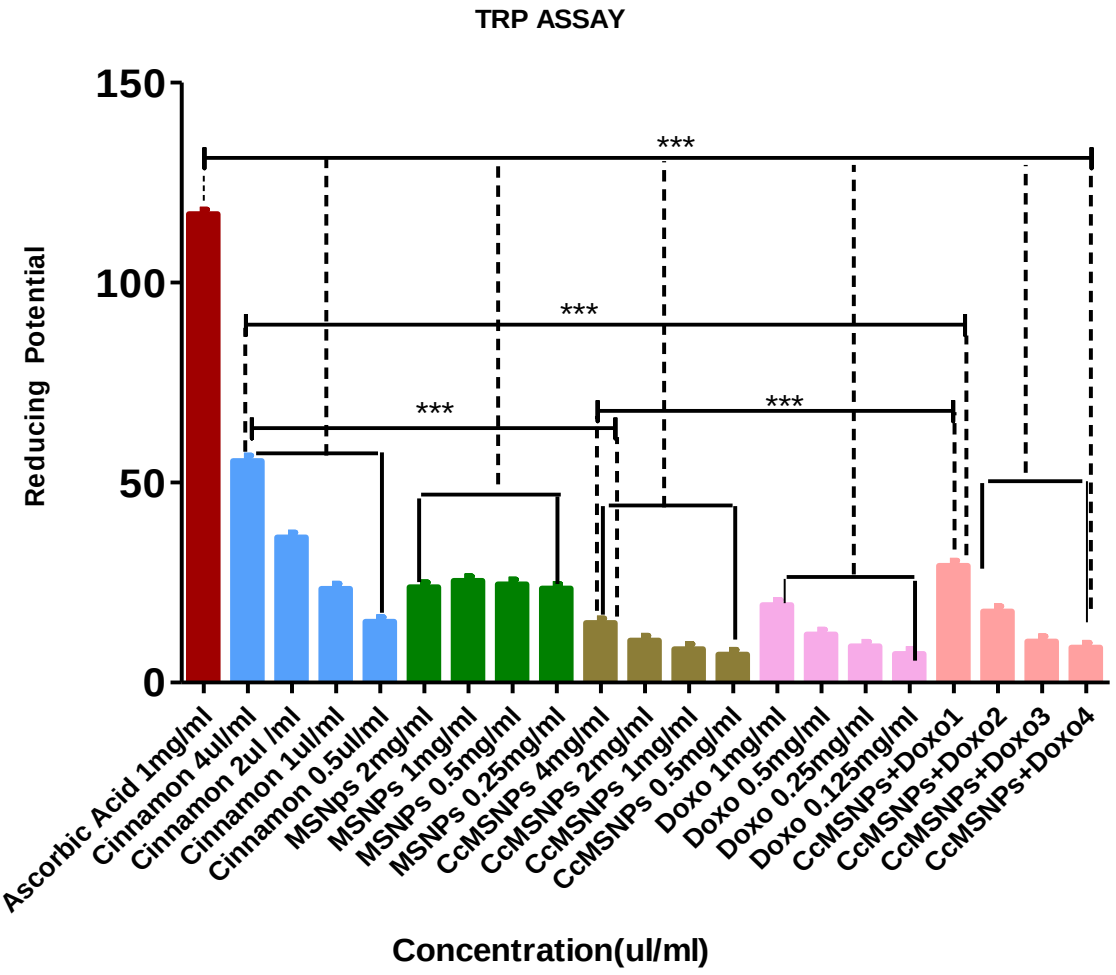

Figure 3.6: Evaluation of Reduction Potential of Cinnamon Coated MSNPs and their combination.

3.1.7 Free radical Scavenging Assay (DPPH)

DPPH assay was carried out to evaluate the free radical scavenging activity of different dilutions of cinnamon, alone, and in combination with doxorubicin. Ascorbic acid was used as positive control which showed highest free radical scavenging activity. Cinnamon in its highest concentration showed maximum scavenging activity after positive control. Loading cinnamon onto MSNPs reduced its scavenging activity while combining it with doxorubicin reduced it scavenging activity even further at significant level. The significance was found out using One-way Analysis of Variance (ANOVA).

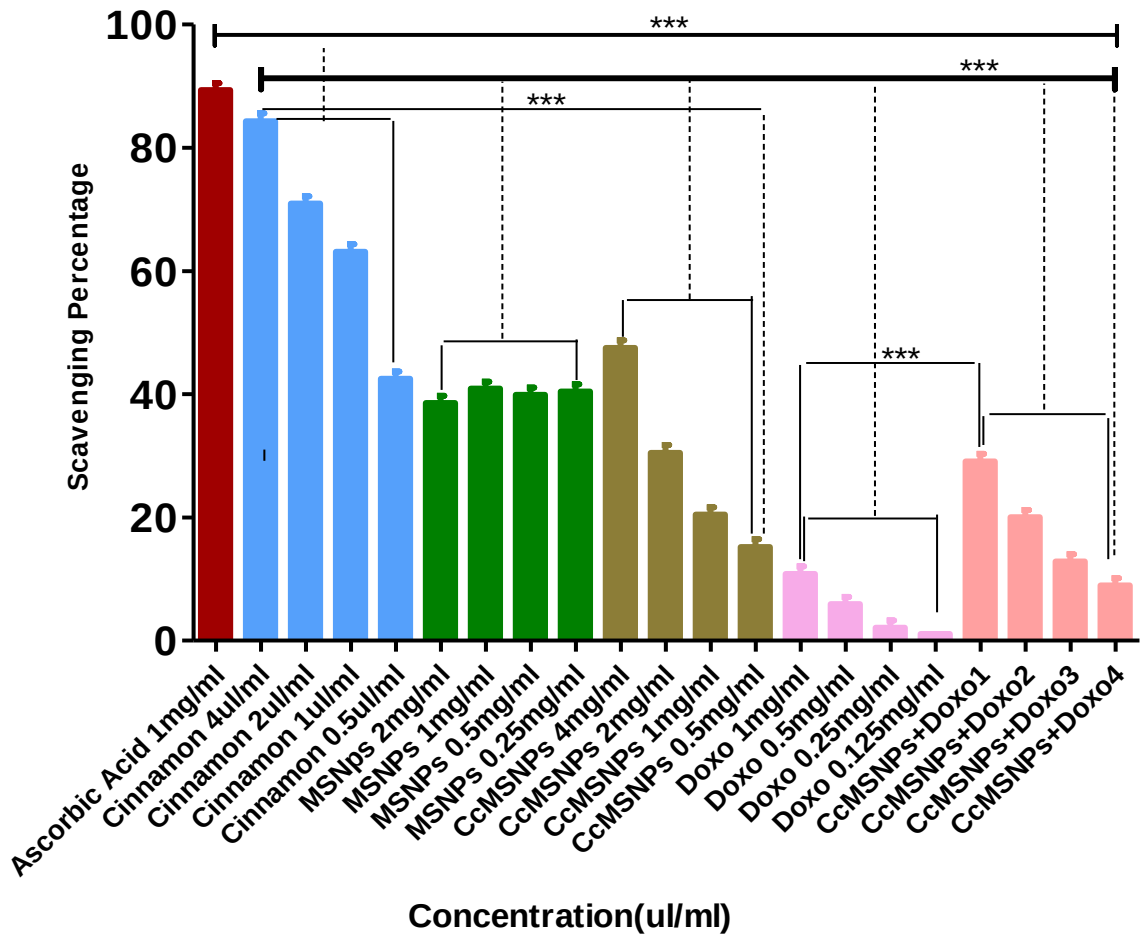

Figure 3.7: Illustration of the scavenging activity of Cinnamon coated MSNPs and their combinations

3.2 In Vivo Assays

Anticoagulant, analgesic (hot plate assay), and anti-depressant assay was carried out to evaluate the anticoagulant, analgesic and antidepressant activity of cinnamon coated MSNPs and their different combinations.

3.2.1 Anti-depressant Assay

Antidepressant assay was carried out, using tail suspension method, to evaluate the antidepressant activity of Nano medicine and their different combinations. Fluoxetine was used as a positive control which showed maximum activity. Cinnamon showed significant antidepressant activity. Even though cinnamon coated MSNPs showed less antidepressant activity in combination but when used alone its antidepressant activity was the highest.

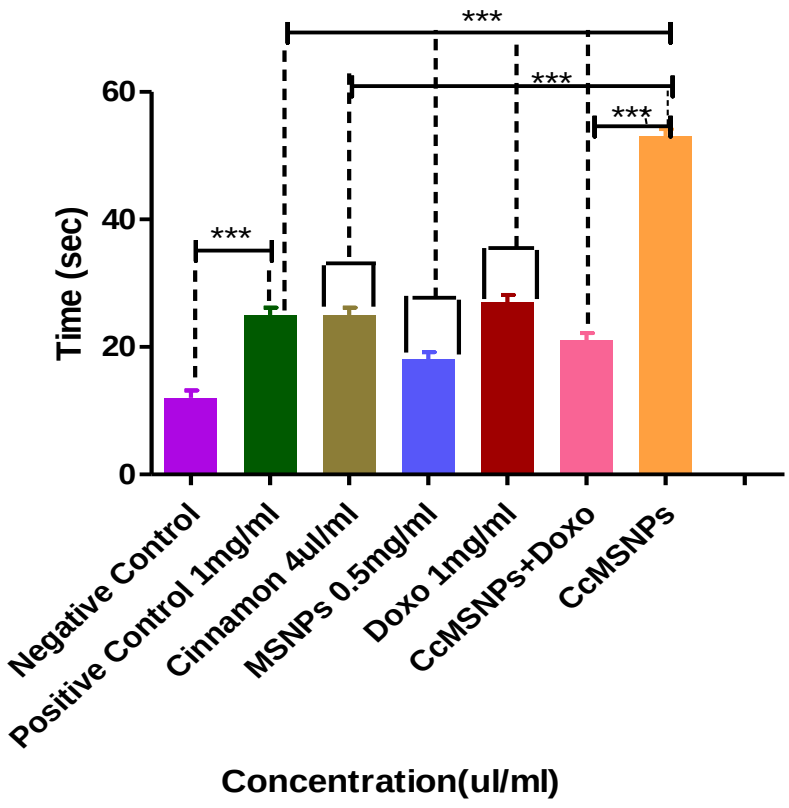

Figure 3.8: Depiction of the anti-depression activity of cinnamon coated MSNPs and their different combinations

3.2.2 Anticoagulant Assay

Anticoagulant potential of cinnamon, cinnamon coated MSNPs and their combination with doxorubicin was evaluated against positive control aspirin, by performing anticoagulant assay. Anticoagulant activity was observed by measuring the time taken for blood to clot after dosing. Aspirin (positive control) showed highest anticoagulant activity. Cinnamon exhibited highest anticoagulant activity after aspirin. Doxorubicin alone and cinnamon coated MSNPs alone showed lesser anticoagulant activity but their combination showed an increase in anticoagulant activity.

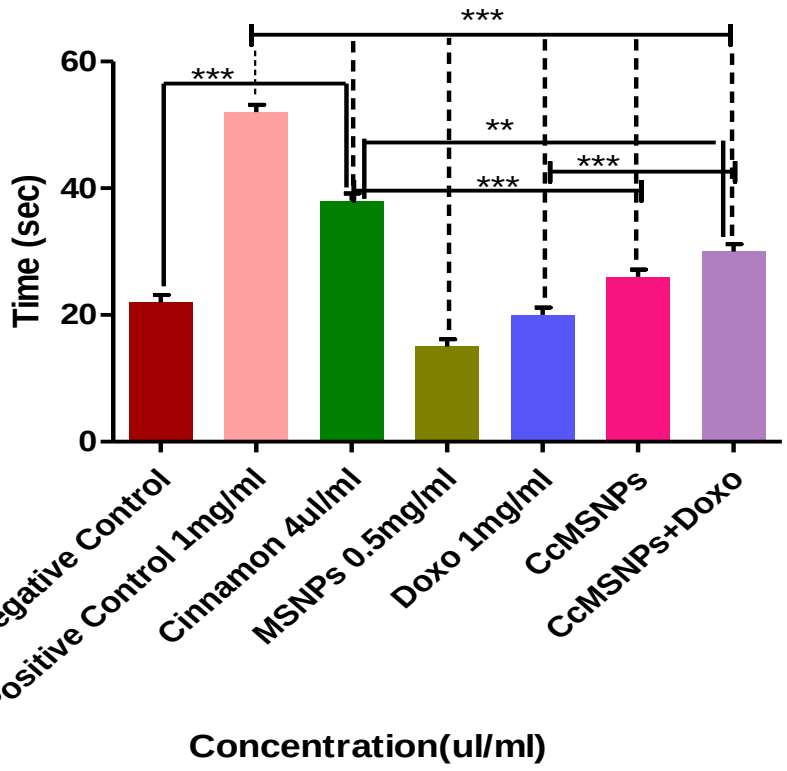

Figure 3.9: Illustration of the Anticoagulant activity of Cinnamon Coated MSNPs and their different combinations.

3.2.3 Analgesic Assay

In order to evaluate analgesic activity of different combinations and dilutions of samples, analgesic assay was carried out. Rats were given different doses and then analgesic activity was measured by Hot plate method while recording the time taken by rats to lick their paw for first time. Recorded values were plotted on graph and then One-way Analysis of Variance was carried out which showed that positive control (diclofenac potassium) exhibited maximum analgesic activity. It also showed that doxo had lower activity but combining it with CcMSNPs enhanced its analgesic potential. Also, cinnamon loaded onto MSNPs showed a significant increase in analgesic potential as compare to cinnamon alone.

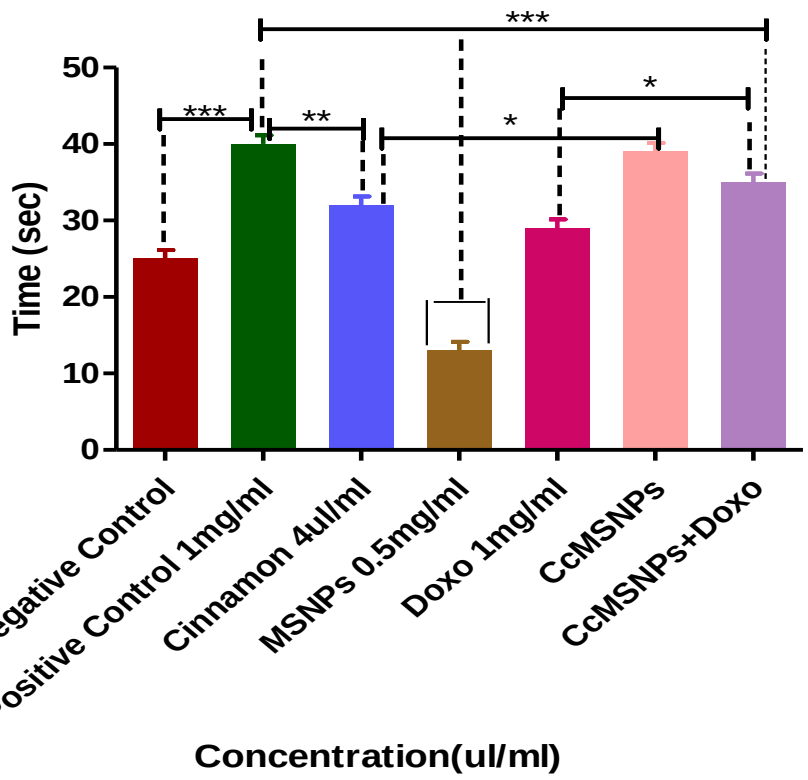

Figure 3.10: Depiction of Analgesic activity of Cinnamon coated MSNPs and their different combinations.

4. Discussion

Cinnamaldehyde is the primary substance that corresponds to the therapeutic effects of cinnamon. Cinnamon show anticancer activity by interfering with both topoisomerase I and II as well as telomerase activity. These enzymes are involved in DNA repair and replication which is crucial for cancer cell immortality (Shen *et al*., 2012; Chen *et al*., 2016; Herdwiani *et al*., 2016). The purpose of this study was to assess the effectiveness of nanomedicine in combination with chemotherapy (DOXO) through various in vivo and in vitro tests. The results demonstrated that the combined therapy exhibited significant therapeutic potential due to its ability to deliver drugs directly to the targeted site and enhance bioavailability. Following drug delivery, the mesoporous silica nanoparticles (MSNPs) are excreted from the body in a soluble form, known as Ortho silicic acid (Slowing et al., 2008).

The XRD and FTIR analysis of nanoparticles showed that the nanoparticles synthesized were of 18.18nm in size which is in accordance with previous studies, which state that efficient size for nanoparticles to act as a drug vehicle, must be less than 50nm (Zhang *et al*., 2008).

Brine shrimp assay was used to determine cytotoxic effects of cinnamon essential oil, doxorubicin, cinnammon coated silica nanoparticles and their combination at various concentrations. Cinnamon didn't show any significant cytotoxicity at any concentration (Maridass, 2008). Doxorubicin showed maximum cytotoxicity when used in highest concentration of 1mg/ml which gradually decreases with decrease in concentration. Doxorubicin works by interfering DNA through iron complex formation (Keizer et al., 1996). When doxorubicin was used in combination with nanomedicine, its cytotoxicity was reduced which may have been reduced by cinnamon (Wang et al., 2004). Cytotoxicity of nanomedicine in combination with doxorubicin decreases gradually with decrease in concentration. Excessive Reactive Oxygen Species (ROS) leads to oxidative stress which in turn causes many diseases, including cancer. Oxidative stress can be reduced by taking antioxidants which can reduce these oxidative species by various means. Cinnamon is one such known antioxidant.

The antioxidant potential of cinnamon and nanomedicine was evaluated by carrying out Total antioxidant capacity, Total reducing power and 2,2-Diphenyl-1-picrylhydrazyl assays taking

Ascorbic acid as a positive control in all these assays, which is an excellent antioxidant. Ascorbic acid neutralize free radicals by either donating electrons to free radicals, or by directly scavenging reactive oxygen species. Ascorbic acid also adds to antioxidant activity through enhancing activity of other antioxidant enzymes in the body Njus *et al* (2020). Cinnamon showed maximum antioxidant activity at maximum concentration of 4ul/ml. The antioxidant potential of cinnamon decreases gradually with decrease in concentration (Ashfaq, M. H., Siddique, A., & Shahid, S. (2021). The same trend was followed by doxorubicin. Doxorubicin act as a pro-oxidant increasing ROS production by interacting with antioxidant enzymes (Jabłońska-Trypuć *et al*., 2018). Nanomedicine in combination with doxorubicin showed a significant increase in antioxidant potential. Antioxidant ability of cinnamon and nanomedicine was also checked through (TRP) total reducing power assay, which actually assesses the ability of a substance to donate electrons to free radicals and reduce them.

To check anticoagulant effect, anticoagulant assay was performed. Aspirin was used as a positive control in anticoagulant assay. Aspirin demonstrates excellent potential against pyrexia and other infections by inhibiting actions of COXI and COXII (Vane and Botting (2003). Cinnamon exhibited maximum anticoagulant activity by inhibiting platelet function (Mehrpouri *et al*., 2020). Doxorubicin exhibited low anticoagulant activity which is in accordance with prior studies as doxorubicin doesn't show any significant anticoagulant activity (Kim et al., 2011). Anticoagulant assay results showed an increase in anticoagulant activity of doxorubicin when combined with nanomedicine potentially by inhibiting platelet function, which may have been aided by the antioxidant potential of nanomedicine.

Antidepressant activity of test sample was evaluated by using tail suspension method. Fluoxetine was taken as a positive control which showed maximum antidepressant activity, exhibiting 100% potential. Fluoxetine act as an antidepressant, primarily affecting levels of serotonin in the brain by acting as a selective serotonin reuptake inhibitor (SSRI) (Faingold & Randall, 2011). Cinnamon exhibited maximum antidepressant activity as it has a positive impact on Brain Derived Neurotrophic Factor (BDNF) (Aryanezhad et al., 2021). Results showed that antidepressant activity of cinnamon when loaded onto Mesoporous silica-based Nanoparticles enhanced notably, potentially by inhibiting the reuptake of serotonin through the plasma membrane and increasing

the concentration of dopamine and also by increasing the bioavailability and targeted drug delivery of drug (Faingold & Randall, 2011).

Analgesic activity of test compound was assessed using hot plate assay to check the analgesic efficacy of nanomedicine. Diclofenac was used as a positive control in this assay which exhibited maximum analgesic activity. Diclofenac works by inhibiting the enzymes cyclooxygenase-1 and cyclooxygenase-2 (COX-1, COX-2) which in turn inhibit prostaglandin production, thereby decreasing inflammation and its associated symptoms, thus performing its action Gan (2010). Cinnamon also showed significant analgesic activity which is in accordance with previous studies (Esmaeili et al., 2020) Moreover, assay results showed that cinnamon when loaded onto Mesoporous silica-based nanoparticles demonstrated enhanced analgesic activity by inhibiting the enzymes cyclooxygenase-1 and cyclooxygenase-2 (COX-1, COX-2) Bariguian Revel *et al*., (2020). This increase in analgesic activity could be attributed to high bioavailability and targeted drug delivery of nanoparticles. Doxorubicin alone showed low analgesic activity but when combined with CcMSNPs (Cinnamon-coated Mesoporous Silica Nanoparticles), its analgesic activity was increased notably. This suggest a synergistic effect between doxorubicin and CcMSNPs (Cinnamon-coated Mesoporous Silica Nanoparticles), leading to an increased analgesic effect that excel the individual effect of doxorubicin.

5. Conclusion

This study investigated the biological potential of cinnamon loaded onto Mesoporous Silica Nanoparticles (MSNPs) alone and in combination with Doxorubicin. The results demonstrated that Cinnamon exhibited no cytotoxic effect and displayed maximum antioxidant potential. When combined with MSNPs, the antioxidant capacity increased significantly. Additionally, the combination of cinnamon coated MSNPs and Doxorubicin showed enhanced antioxidant activity. Furthermore, cinnamon displayed high anticoagulant, antidepressant, and analgesic activities when used alone, while the combination with MSNPs showed varying effects. These findings highlight the potential of cinnamon and cinnamon-loaded MSNPs as valuable agents in cancer treatment and require further exploration in future studies.

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