2

REVIEW OF LITERATURE

Cancer is a severe health problem that continues to be a leading cause of death worldwide. Increasing knowledge of the molecular mechanisms underlying cancer progression has led to the development of a vast number of anticancer drugs. However, the use of chemically synthesized drugs has not significantly improved the overall survival rate over the past few decades. As a result, new strategies and novel chemoprevention agents are needed to complement current cancer therapies to improve efficiency. Naturally occurring compounds from plants known as phytochemicals serve as vital resources for novel drugs and are also sources for cancer therapy. A number of these phytochemicals are naturally occurring biologically active compounds with significant antitumor potential. The development of effective and side-effects free phytochemical based anticancer therapy begins with the testing of natural extracts (from dry/wet plant material) for potential anticancer biological activity (Choudhari *et al.*,2020).

The review of literature relevant to the study entitled "Drug Release Analysis and Anticancer Potential of *Tabebuia pallida* Silver Nanoparticles Loaded Liposomes against Molt-3 Cells - *in vitro"* is discussed under the following headings.

2.1 Leukaemia

2.1.1 Types of Leukaemia

2.1.2 *Treatment for Leukaemia*

2.2 Free radicals

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2.4.1 Reactive Oxygen Species

2.4.1.1 Hydroxyl radical (HO•)

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2.1 Leukaemia

Leukaemia is a cancerous disease in which blood cells display abnormal proliferation and invade other tissues. It is one of the biggest health issues globally. Almost half a million new leukaemia cases were diagnosed in 2018 (Bray *et al.,* 2018). Blood cancers affect the production and function of blood cells. They are the most common cancer types in children from birth to 14 years of age and account for around 3% of all cancers diagnosed in developed countries. Blood cancer survival rate in adults is about 50%. Although survival in children is higher and improving, blood cancer is still the major cause of cancer death in paediatric patients. There are three major types of blood cancer. Leukaemias are caused by the rapid production of abnormal white blood cells. Lymphomas are a type of blood cancer comprising abnormal lymphocytes, a type of white blood cells that fight infections. These cells multiply and collect in lymph nodes and other tissues and impair the lymphatic system's functionality to remove unnecessary fluids from the body and fight infections. Finally, myeloma is a cancer of the plasma cells, which produce disease- and infection-fighting antibodies (Chulián *et al.,* 2020)

2.1.1 Types of Leukaemia

Leukemia is a production of abnormal leukocytes either as a primary or secondary process. Based on the rapidity of proliferation, they can be classified as acute or chronic, and myeloid or lymphoid based on the originator cell (Fig. 1). Predominant subtypes are Acute Myeloid Leukemia (AML) and Chronic Myeloid Leukemia (CML), involving the myeloid chain; and Acute Lymphoblastic Leukemia (ALL), and Chronic Lymphocytic Leukemia (CLL) involving the lymphoid chain. Other less common variants such as mature B-cell and T-cell leukemias, NK cell-related leukemias, to name a few, arise from mature WBC cells (Chennamadhavuni *et al.,* 2021)

The four major subtypes of leukemia are

- Acute lymphoblastic leukemia (ALL) is a heterogeneous haematological malignancy that occurs mainly in children (median age at diagnosis $\approx$ 15 years); however, ALL in adults accounts for $\approx$ 20% of all leukaemia cases (National Comprehensive Cancer Network, 2019). ALL is typically characterized by the proliferation of large number of immature lymphoid cells in the bone marrow, peripheral blood and other organs. With an improved

understanding of the pathogenesis of ALL and the recent advances in targeted therapies, the rates of complete remission and overall survival in patients with ALL have improved significantly, primarily in the paediatric population. Indeed, 5-year overall survival in children with ALL is 86–89%, with the rate declining with increased age (overall survival in adults ≈ 41%) (Heo et al., 2019).

- **Acute myeloid leukemia AML** is a genetically heterogeneous malignancy characterized by proliferation and accumulation of myeloid blast cells in the bone marrow, peripheral blood, and lymphoid tissue. It is an aggressive haematological cancer associated with signifcant humanistic impact (Joshi *et al.,* 2019).
- **Chronic lymphocytic leukemia (CLL),** the most frequent type of leukaemia in adults, and is a lymphoproliferative disorder that is characterized by the expansion of monoclonal, mature $CD5^{+}CD23^{+}$ B cells in the peripheral blood, secondary lymphoid tissues and bone marrow. CLL is an incurable disease with a heterogeneous clinical course, for which the treatment decision still relies on conventional parameters such as clinical stage and lymphocyte doubling time (Bosch and Dalla, 2019)
- **Chronic myeloid leukemia (CML)** is a clonal disorder of the hematopoietic stem cell compartment defined and driven by the *BCR-ABL1* gene rearrangement and the tyrosine kinase it encodes. Clinically, it is accompanied by an expansion of mostly myelo-erythroid progenitors that maintain the ability to differentiate terminally into neutrophils (Krishnan *et al.,* 2021).

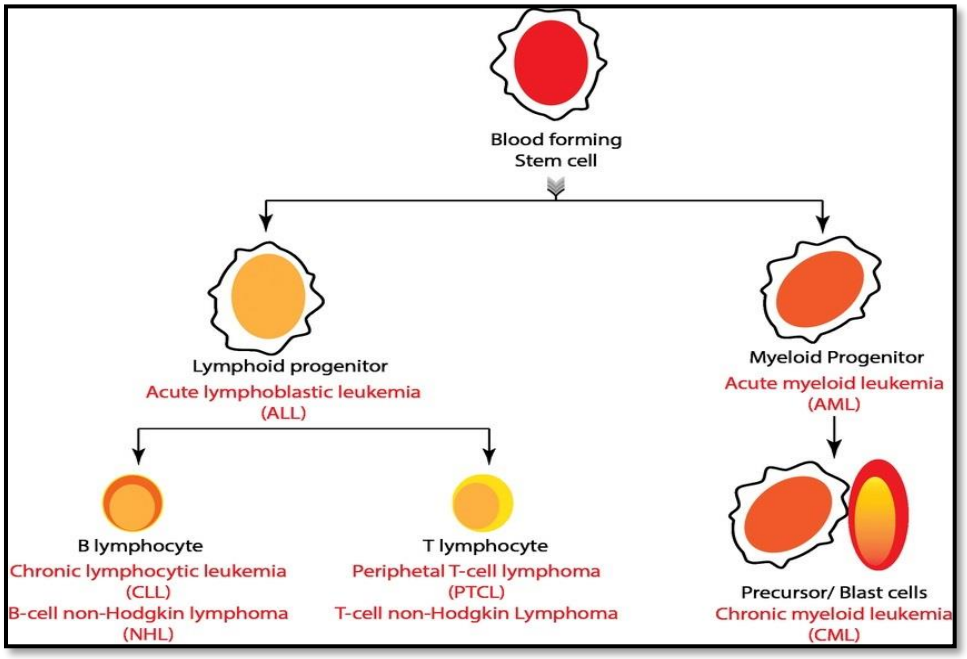

Fig. 1 Different types of Leukaemia based on its origin (Nighat et al., 2020)

2.1.2 Treatment for Leukaemia

The mainstays of leukemia treatment for adults have been chemotherapy, radiation therapy, and stem cell transplantation. Over the last two decades, targeted therapies have also become part of the standard of care for some types of leukemia. Different types of leukemia require different combinations of therapies. Although much progress has been made against some types of leukemia, others still have relatively poor rates of survival. And, as the population ages, there is a greater need for treatment regimens that are less toxic. Standard leukemia treatments for children have been chemotherapy, radiation therapy, and stem-cell transplant. Despite great improvements in survival for children with some types of leukemia, some children do not respond to standard treatments or experience a relapse of their disease. Others live with the side effects of chemotherapy and radiation therapy for the rest of their lives, highlighting the need for less toxic treatments. Now researchers are focusing on targeted drugs and immunotherapies for the treatment of leukemia in children. Newer chemotherapy drugs are also being tested. So an anticancer drug with a fewer side effects, which targets only the cancer cells to avoid side effects is the need of the hour (*National Cancer Institute, 2021*)

2.2 Free radicals

A free radical is a molecule containing an unpaired electron in its external molecular orbital; typically, radicals are reactive and short-lived intermediates (Rafael, 2018). Free radicals are small diffusible molecules that are highly reactive because of the unpaired electron. Free radicals were initially thought to be oxygen centered radicals called reactive oxygen species (ROS) but also include a subgroup of reactive nitrogen species (RNS) and are all a product of normal cellular metabolism. ROS and RNS have been established to play a double role as beneficial and harmful specie based on their beneficial and deleterious effect on biological systems. The beneficial roles occur at low to moderate concentrations while the deleterious effects occur at high concentrations where the ROS/RNS production surpasses the antioxidant ability to balance it (Obeagu, 2018).

2.3 Source of free radicals

Free radicals can be formed from both exogenous and endogenous substances. Exogenous sources include environmental triggers, such as exposure to cigarette smoke, UV radiation, heavy metal ions (Iron, copper, cadmium, nickel, arsenic, and lead), ozone, allergens, drugs or toxins, pollutants, pesticides, or insecticides (Antunes *et al.,* 2018, Mahajan *et al.,* 2018 and Oke *et al.,* 2019). The main endogenous sites of cellular redox-reactive species generation-including ROS and reactive nitrogen species (RNS) comprise mitochondrial electron transport chain (ETC), endoplasmic reticulum (ER), peroxisomes, membrane-bound NADPH oxidase (NOX) isoforms 1–5, dual oxidases (Duox) 1 and 2 complexes, and nitric oxide synthases isoforms 1–5 (NOS1–3). The complexes I and III of mitochondrial ETC produces superoxide anion (Sharifi *et al.,* 2020). Some life style habbits like smoking, alcohol consumption, adequate or inappropriate diet, exercise; training or untrained condition also contributes to the generation of free radicals (Antonioni *et al.,* 2019 and Wu *et al.,* 2019). The diverse source of free radicals is given in Fig.2

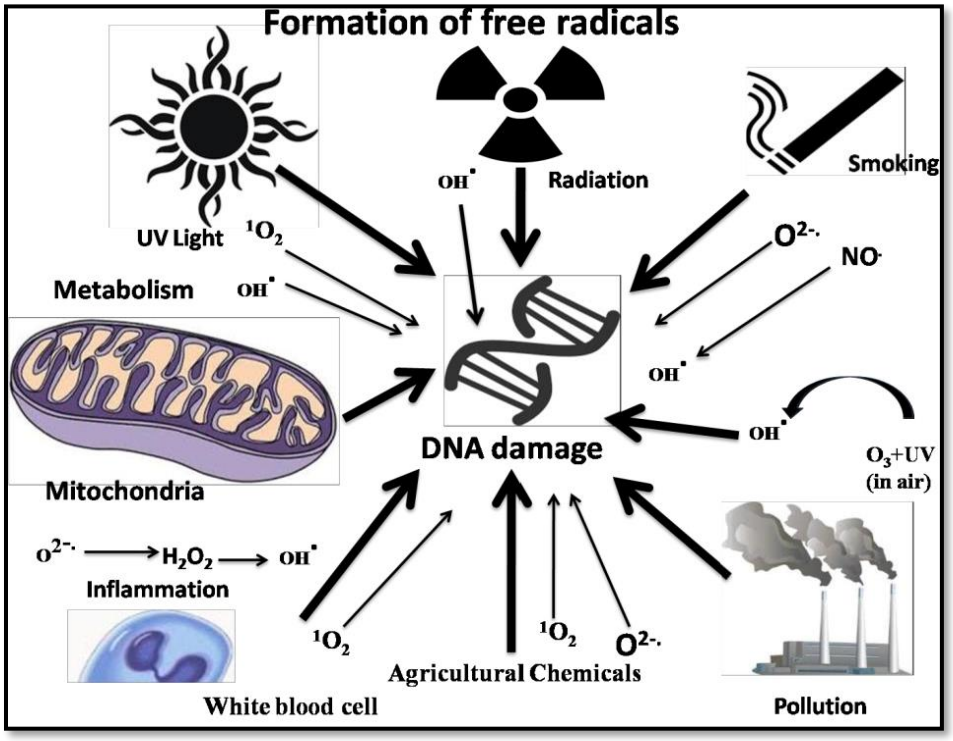

Fig. 2: Diverse source of free radicals (Pathak *et al.*, 2017)

2.4 Types of free radicals:

In the various fields of biology and medicine, free radicals are more generally known as ROS or RNS. The most important ROS are the hydroxyl radical (HO•), the superoxide radical anion ($O_2^{\bullet-}$) and peroxyl radicals (ROO•) as well as non-radical species such as hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$) (Losada *et al.*, 2017). The major reactive nitrogen species include nitric oxide ($^{\bullet}NO$) and peroxynitrite ($O=NOO^-$) (Ishimoto *et al.*, 2018). The ROS and RNS are known to play dual roles as species that may be either deleterious or beneficial in living systems. The beneficial effects of ROS/RNS tend to occur at low to moderate concentrations and involve their participation in various physiological roles and in numerous cellular signaling pathways. The harmful effects of free radicals occur in biological systems when there is an overproduction of ROS and/or RNS, on the one hand, and a deficiency of antioxidant enzymes or low molecular weight antioxidants on the other. A sustained and delicate balance between the beneficial and harmful effects of ROS/RNS is an important aspect of healthy organisms, and is achieved by a collection of mechanisms that are described as 'redox regulation'. Oxidative/nitrosative stress

results from an imbalance between the formation of ROS/RNS and the impaired ability of an organism to detoxify these reactive intermediates or to repair the damage caused by reactive species. Because alterations in their metabolism and signaling from healthy cells, cancer cells exhibit an increased formation of ROS/RNS which is counterbalanced by enhanced antioxidant defense mechanisms (Poprac *et al.,* 2017).

2.4.1 Reactive Oxygen Species

ROS play an important role in tumorigenesis and affect multiple biological processes such as cell proliferation, genomic instability, inflammation, resistance to apoptosis and metabolic reprogramming. Increased levels of ROS are observed in a number of cancer cell lines. In a tumor cell, ROS are primarily generated by the mitochondria. ROS levels are often elevated in cancer, however, high levels of ROS can have deleterious effects therefore, and cells have evolved mechanisms in order to maintain a proper balance of ROS (Weinberg *et al.,* 2019)

2.4.1.1 Hydroxyl radical (HO•)

Hydroxyl radicals are highly reactive species that attack most of the organic molecules. They are highly oxidizing in nature which is attributed to their oxidation potential. In addition, owing to their nonselective nature, many susceptible organic molecules can easily be removed or degraded using hydroxyl radical (e.g., acids, alcohols, aldehyde, aromatics, amines, ethers and ketones). This radical degrades organic compounds which makes it harmful if produced in excess and in close proximity to cells (Lyngsie *et al.,* 2018). Owing to their short life span (10^{-9} s), the hydroxyl radicals are produced in situ by either oxidation of water or hydroxide ions and also the hydroxyl radical is formed during the Haber–Weiss reaction, by the Fenton reaction (Chen, 2019) or by decomposition of peroxynitrite (Fujiwara *et al.,* 2019).

2.4.1.2 Superoxide radical anion ($O_2^{\bullet-}$)

Superoxide, which is an anion radical, is produced by the one-electron reduction of molecular oxygen. Superoxide $^{\bullet}O_2^-$ is detrimental and is mainly produced as a by-product of mitochondrial respiration (especially in Complexes I and III, in the electron transport chain ETC) (Sousa *et al.,* 2018), where a small percentage of the electrons in the ETC chain escape from it, as well as by several other enzymes, which catalyse the electron transfers directly to molecular

oxygen under strongly reducing conditions, as occurs in the mitochondrial matrix. It is also generated in the immune system to eliminate invading micro-organisms. In phagocytes, the enzyme NADPH oxidase produces $^{\bullet}O_2^-$ in large quantities for use in the oxygen-dependent destruction mechanisms of invading pathogens (Valenta *et al.,* 2020).

2.4.1.3 Peroxyl radicals (ROO•)

Peroxyl radicals (ROO·) are formed by a direct reaction of oxygen with alkyl radicals (R·). Decomposition of alkyl peroxides (ROOH) also results in peroxyl (ROO·) and alkoxyl (RO·) radicals. Irradiation of UV light or the presence of transition metal ions can cause hemolysis of peroxides to produce peroxyl and alkoxyl radicals. Peroxyl and alkoxyl radicals are good oxidizing agents (Santos *et al.,* 2019). Peroxy radical (ROO^-) is an important radical during the lipid oxidation process (Zhang *et al.,* 2022)

2.4.1.4 Hydrogen peroxide (H_2O_2) radical

H_2O_2 is a strong oxidant, having a reduction potential of 1.76 V at pH 7.0, 25°C. (Sanghai and Tranmer, 2022). Hydrogen peroxide (H_2O_2) is generated in numerous biological processes. It transmits cellular signals, contributes to oxidative folding of exported proteins, and, in excess, can be damaging to cells and tissues. Although a strong oxidant, high activation energy barriers make it unreactive with most biological molecules. Its main reactions are with transition metal centers, selenoproteins and selected thiol proteins, with glutathione peroxidases (GPxs) and peroxiredoxins (Prxs) being major targets (*Christine, 2018*).

2.4.1.5 Singlet oxygen ($^1O_2$)

Singlet oxygen ($^1O_2$) is a higher energy state molecular oxygen species. It is one of the most active intermediates involved in chemical and biochemical reactions. Singlet oxygen is not a free radical but it can be formed during some free radical reactions and can trigger the formation of free radicals. It can be formed by macrophages during phagocytosis. Singlet oxygen has approximately equivalent properties as $O_2^{\bullet-}$ with the best affinity to Trp, His, Tyr, and Cys residues of proteins. It has been demonstrated now that $^1O_2$ can react with many kinds of biological molecules such as DNA, proteins and lipids (Edge *et al.,* 2021)

2.4.2 Reactive Nitrogen Species

Apart from these ROS, nitrogen species also play a significant role in causing oxidative damage and tissue dysfunction and act as molecular signals. Thus, nitrogen-containing species, which are at present known as Reactive Nitrogen Species (RNS), including nitric oxide (NO·), that is comparatively unreactive, and its derivative namely peroxynitrite ($ONOO^-$), is a prevailing oxidant, able to cause damage to various biological molecules (Kapoor et al., 2019). Reactive oxygen and nitrogen species are essential for normal physiological processes and play important roles in cell signaling, immunity, and tissue homeostasis. However, excess radical species are implicated in the development and augmented pathogenesis of various diseases (Ferreira et al., 2018). In general, RNS have a very short life, in the order of micro- or nanoseconds, and readily react with numerous cellular components including lipids, nucleic acids, and proteins. This process, which occurs via a free-radical chain reaction, causes damage and forms harmful secondary products such as lipid peroxides and other lipid adducts.

2.4.2.1 Nitric oxide (NO·)

Nitric oxide (NO) is a small molecule that contains one unpaired electron on the antibonding, hence a radical. It is soluble in aqueous and lipid media, a property that enables it to readily diffuse through cytoplasm and plasma membranes (Habib and Ali, 2011). It has a half-life of only a few seconds in aqueous environment and a greater stability in environment with lower oxygen concentration. NO is generated in biological tissues through a tightly regulated process by specific nitric oxide synthases (Obeagu, 2018).

2.4.2.2 Peroxy nitrite **($O=NOO^-$)**

In inflammed tissues, the reaction of nitric oxide and superoxide leads to the formation of an extremely reactive peroxynitrite (ONOO−), which is a well known oxidizing and nitrating agent that exhibits high reactivity at physiological pH. The peroxynitrite formed can attack a wide range of biomolecules via direct oxidative reactions or indirect radical-mediated mechanisms thus triggering cellular responses leading to cell signaling, oxidative injury, committing cells to necrosis or apoptosis. Cellular DNA is an important target for ONOO− attack, and can react with deoxyribose, nucleobases or induces single strand breaks (Rizwan *et al.*, 2019). The toxicity of

NO• increases when it reacts with superoxide to form peroxynitrite, ONOO– (a very strong oxidant), at a diffusion-limited reaction rate. Peroxynitrite may react with aromatic amino acid residues (e.g., tyrosine) to form nitrotyrosine that can lead to enzyme inactivation. Protonation of peroxynitrite occurs at pKa 6.8 to yield peroxynitrous acid that decomposes spontaneously to yield hydroxyl free radical and • NO2 (Sharma *et al.*, 2018).

2.5 Oxidative stress mediated diseases

It is well comprehended that oxidative stress plays a significant role in degenerative senescence. ROS have been found to be involved in the pathogenesis of various cellular processes, and is also associated with numerous diseases like cardiovascular, cancer, myocardial infarction, muscular degeneration, neurodegenerative, hypertension, stroke, and respiratory diseases, as depicted in the Fig. 3. The rise in ROS concentration in cells have also been associated with ageing, however, it cannot be considered as the only determining factor responsible for ageing. Moreover, in age-related diseases, the elevated concentration of ROS has been involved in the impairment of mitochondria and cellular oxidative damage (Islam, 2017).

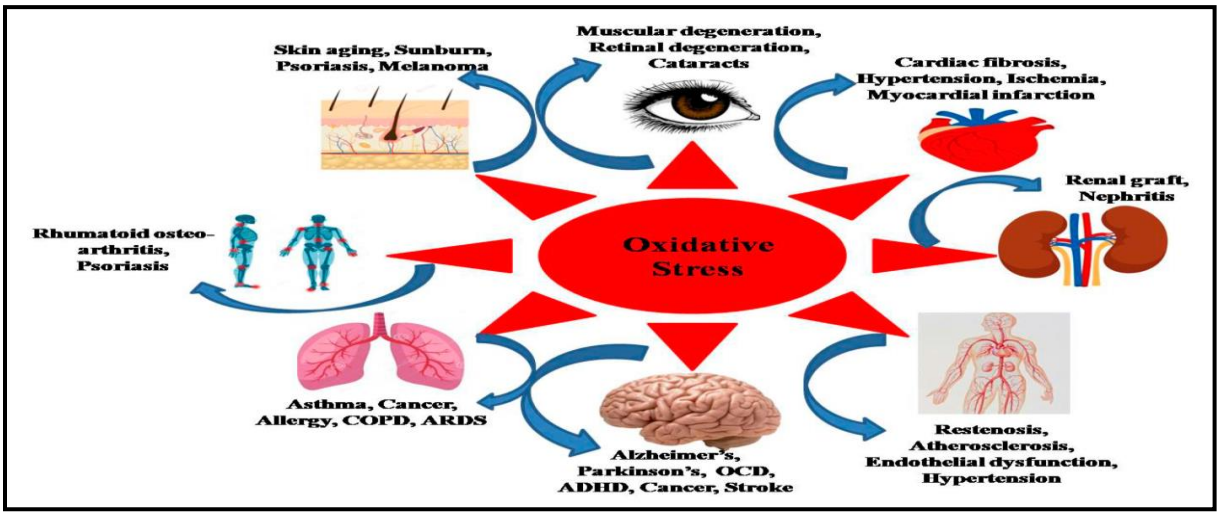

Fig. 3: Oxidative stress mediated diseases (Kumar *et al.*, 2020)

2.6 Role of ROS in cancer

ROS are maintained in a dynamic balance by a series of reduction-oxidation (redox) reactions in biological systems and act as signaling molecules to drive cellular regulatory pathways (*Zhang et al.,2016*). Excessive oxidative stress derived from ROS accumulation deregulates the

antioxidative defense system, which is closely associated with various diseases (*Scialò et al.,2017 and El-Kenawi and Ruffell, 2017*), especially cancers (*Moloney and Cotter, 2018*). A physiological concentration of ROS that maintained in equilibrium is necessary for normal cell survival. Ectopic ROS accumulation promotes cell proliferation and consequently induces malignant transformation of normal cells by initiating pathological conversion of physiological signaling networks. Excessive ROS levels lead to cell death by damaging cellular components, including proteins, lipid bilayers, and chromosomes (Wang et al., 2021). *Elevated ROS levels, accompanied with down-regulation of cellular antioxidant enzyme systems, result in malignant transformation via different molecular targets, such as NF-κB and nuclear factor (erythroid-derived 2)-like-2 factor (Nrf2). Signaling cascades regulated by these key factors generate an inflammatory environment leading to the suppression of apoptotic cell death, tumor proliferation, angiogenesis, and metastasis; which cumulatively augment initiation, development, and progression of malignant neoplasms.* Therefore, both scavenging abnormally elevated ROS to prevent early neoplasia and facilitating ROS production to specifically kill cancer cells are promising anticancer therapeutic strategies, in spite of their contradictoriness and complexity *(Aggarwal et al., 2019).*

2.7 Role of RNS in cancer

It is estimated that infectious diseases and chronic inflammation account for approximately 25% of cancer-causing factor. Under chronic inflammation, reactive nitrogen species are produced from not only inflammatory cells but also epithelial cells. RNS cause DNA damage in organs under inflammation, leading to cancer development. Chronic inflammation causes various types of damage to nucleic acids, proteins, and lipids via RNS generation, resulting in tissue damage. The tissue injury may activate progenitor/stem cells for tissue regeneration. Stem cells are damaged by RNS from inflammation, and the resulted mutations can accumulate, which could generate cancer stem cells (Murata, 2018). RNS also activate transcriptional factors (NF-KB, STAT-3) and bring about cellular proliferation, genomic instability, angiogenesis, resistance to apoptosis, invasion, and metastasis. The presence of inflammatory mediators in the tumour microenvironment inhibits or promotes inflammation-induced cancer, depending on various stages of immune surveillance of the tumor i.e. by immunoediting, immunoprocessing, and immunoevasion. Myeloid derived suppressor cells are immature myeloid progenitor cells. They are the major immune-suppressor

cells in the tumour inflammatory microenvironment that activate transcriptional factor NF-KB, STAT-3 to bring about tumour progression (Shrihari, 2017).

2.8 Antioxidants

Antioxidants are structurally diverse group of small organic molecules and large enzymes that comprise complex systems of overlapping activities working synergistically to enhance cellular defense and to combat oxidative stress resulting from various reactive oxygen species and reactive nitrogen species. The term antioxidants may refer to either industrial chemicals that may be added to products to combat oxidation or to natural products that are found in foods, plants and tissue (Moussa et al., 2019).

Chronic and degenerative diseases are caused not only by a combination of genetic and environmental factors, but also by disruptions in prooxidant/antioxidant homeostasis. When homeostasis is maintained, ROS are deactivated by endogenous and exogenous antioxidants. The intensification of free radical processes induces strong and prolonged oxidative stress and leads to permanent changes in the structure of DNA, proteins and lipids. These processes damage cellular structures and genes, causing metabolic disorders and neoplastic transformation (George and Abrahamse, 2020). The human body is not completely defenceless against these threats. Enzymatic and non-enzymatic defence systems protect the body against the toxic and mutagenic effects of ROS, RNS and xenobiotics (Pizzino *et al.*, 2017). Their activity can be enhanced by incorporating biologically active foods rich in antioxidants (hydrophilic and lipophilic), vitamins and minerals into the human diet (Corrochano *et al.,* 2019 and Khan *et al.*, 2019). Dietary proteins strengthen the immune system and decrease the risk of carcinogenesis (Vidal *et al.,* 2019).

The antioxidants are classified into two major categories they are enzymic antioxidants and non enzymic antioxidants. The major enzymic antioxidants include superoxide dismutase, catalase, and glutathione peroxidase. The non-enzymic antioxidants include vitamin C, E, and β-carotene or natural antioxidants such as flavonoids, tannins, coumarins, phenolics, and terpenoids (Aziz et al., 2019).

2.9 Role of medicinal plants in cancer therapy

Medicinal plants have been used from the beginning of human civilization, which is mostly evident from the ancient script and traditional herbal medicine recipe. Human civilization is using plant as a source of food, shelter, and medicine for almost same time (Mohammad, 2020). Cancer is a severe health problem that continues to be a leading cause of death worldwide. Increasing

knowledge of the molecular mechanisms underlying cancer progression has led to the development of a vast number of anticancer drugs. However, the use of chemically synthesized drugs has not significantly improved the overall survival rate over the past few decades. As a result, new strategies and novel chemoprevention agents are needed to complement current cancer therapies to improve efficiency (Choudhari et al., 2020).

Researchers mentioned that herbal-based medicines are found to be one of the best choices for treating and/or preventing incidence of cancer. This is mainly because of the varieties of active substances that plants contained which work against many types of cancers in several mechanisms. These compounds can be extracted and can be used alone or in combination with other anticancer treatments. In comparison with synthetic drugs, these natural compounds are found to be naturally available, cheaper, and easy to administer orally and have low or minimal side effects, and they are found to be rich of various biologically active chemotypes (Hassan, 2019). Overtime researchers detected that plants found to be enriched with natural compounds called secondary metabolites and these metabolites characterized by several biochemical methods make them effective antitumor agents. These compounds can be classified into "three main groups which are: terpenoids, phenolics and the extremely diverse alkaloids (Seca and Pinto, 2018). Till date, several plant-based compounds have been reported for their anticancer activity, and among them, a good number of compounds is clinically successful as well.

2.10 Nanotechnology

Nanobiotechnology is a novel concept and area of nanotechnology that has attracted worldwide interest. Nanoparticles are particles between 1 and 100 nanometres in size and are made up of carbon, metal, metal oxides or organic matter (El Shafey, 2020, Rzayev et al., 2021, Ramalingam et al., 2021 and Murthy et al., 2021). Chemical conditions, reaction circumstances e.g., temperature and pH which can change the structural attributes of nanoparticles such as size and shape. Nanotechnology is the utilisation of nanoparticles that have a very small size and a much larger surface area than its bulk form (Arasu et al., 2019, Roy, 2021, Savunthari et al., 2021 and Kaur and Roy, 2021). Nanomaterials have a variety of properties, including chemical, optical, and thermal capabilities (Al-Dhabi and Valan, 2018). Several bulk materials possess different properties when studied at the nanoscale. One known reason for this phenomenon is because of their higher aspect ratio. For different nanoparticles, this can result in a variety of characteristics. As a response, nanomaterials have considered as potential alternative for use in a

variety of biological applications (Valsalam et al., 2019a; Valsalam et al., 2019b; Abd et al., 2019). Due to their biocompatibility, anti-inflammatory and antimicrobial action, effective drug delivery, bioactivity, bioavailability, tumor targeting, and biological absorption, NPs are frequently utilized in biological, medical and environmental applications (Magdalane et al., 2018; Al-Dhabi et al., 2019; Salem and Fouda, 2021 and Khalith et al., 2021).

2.10.1 Types of nanomaterials:

Nanoparticles can be organized into four material-based categories; they are as follows:

- **Carbon-based nanomaterials:** Generally, these Nanomaterials contain carbon, and are found in morphologies such as hollow tubes, ellipsoids or spheres. Fullerenes (C60), carbon nanotubes (CNTs), carbon nanofibers, carbon black, graphene (Gr), and carbon onions (Kumar and Kumbhat, 2016).
- **Inorganic-based nanomaterials**: These nanomaterials include metal and metal oxide nanoparticles. They can be synthesized using various metals such as Au or Ag NPs, metal oxides such as TiO_2 and ZnO NPs, and semiconductors such as silicon and ceramics.
- **Organic-based nanomaterials:** These include nanomaterials made mostly from organic matter. The utilization of noncovalent (weak) interactions for the self-assembly and design of molecules helps to transform the organic nanomaterials into desired structures such as dendrimers, micelles, liposomes and polymer nanoparticles.
- **Composite-based nanomaterials:** Composite nanomaterials are multiphase nanoparticles and nanostructured materials with one phase on the nanoscale dimension that can either combine nanoparticles with other nanoparticles or nanoparticles combined with larger or with bulk-type materials (e.g. hybrid nanofibers) or more complicated structures, such as metal-organic frameworks. The composites may be any combinations of carbon-based, metal-based, or organic-based nanomaterials with any form of metal, ceramic, or polymer bulk materials (Jeevanandam *et al.,* 2018).

2.10.2 Silver nanoparticles

Certain materials, due to their exemplary medicinal properties, have been part of the medicinal domain since time immemorial. Silver (Ag), due to its extraordinary range of bactericidal properties and therapeutic abilities, has been a part of medical treatment and management of various diseases since ancient times. In nanomedicine, silver nanoparticles are extremely important due to their attractive physicochemical properties and biological

functionality, including their high antimicrobial efficiency and relatively non-toxic, wide spectrum of bactericidal properties (Durán *et al.,* 2016), anticancer properties and other therapeutic abilities, their unique ability to form diverse nanostructures and their relatively low manufacturing cost (Sohn *et al.,* 2015). Silver nanoparticles are intensively explored nanostructures ranging between 1 and 100 nm, primarily used for unconventional and enhanced biomedical applications in such areas as drug delivery, wound dressings, tissue scaffolding and protective coating applications *(Almatroudi, 2020).*

2.10.3 Methods of silver nanoparticles synthesis

Many approaches and methods have evolved for the effective synthesis of silver nanoparticles, including physical, chemical (Chemical reduction method, Electrochemical method, Pyrolysis and Irradiation-assisted chemical method) and biological techniques. While physical and chemical methods are commercially more cost-effective, the biological methods are relatively less harsh on the environment (Hulkoti and Taranath, 2017). The size, shape, structure, physical, chemical and biological properties of nanoparticles depend on the synthetic method. Several synthetic methods have been reported by the researchers. Mainly, the three most important approaches have been given. The chemical reduction method is the most common and widely used method which involves the reduction of Ag^{+} species to Ag° using reducing agents like $NaBH_4$, $LiAlH_4$. Physical methods (Laser, Arc-discharge, Ball-milling and Vapour condensation) usually involve high-energy consumption during synthesis. In biological methods, AgNPs have been synthesized by using fungi, plants and bacteria and do not employ any toxic reducing agents (Arif and Uddin, 2021). The schematic representation of various methods for synthesis of silver nanoparticles is given in Fig. 4.

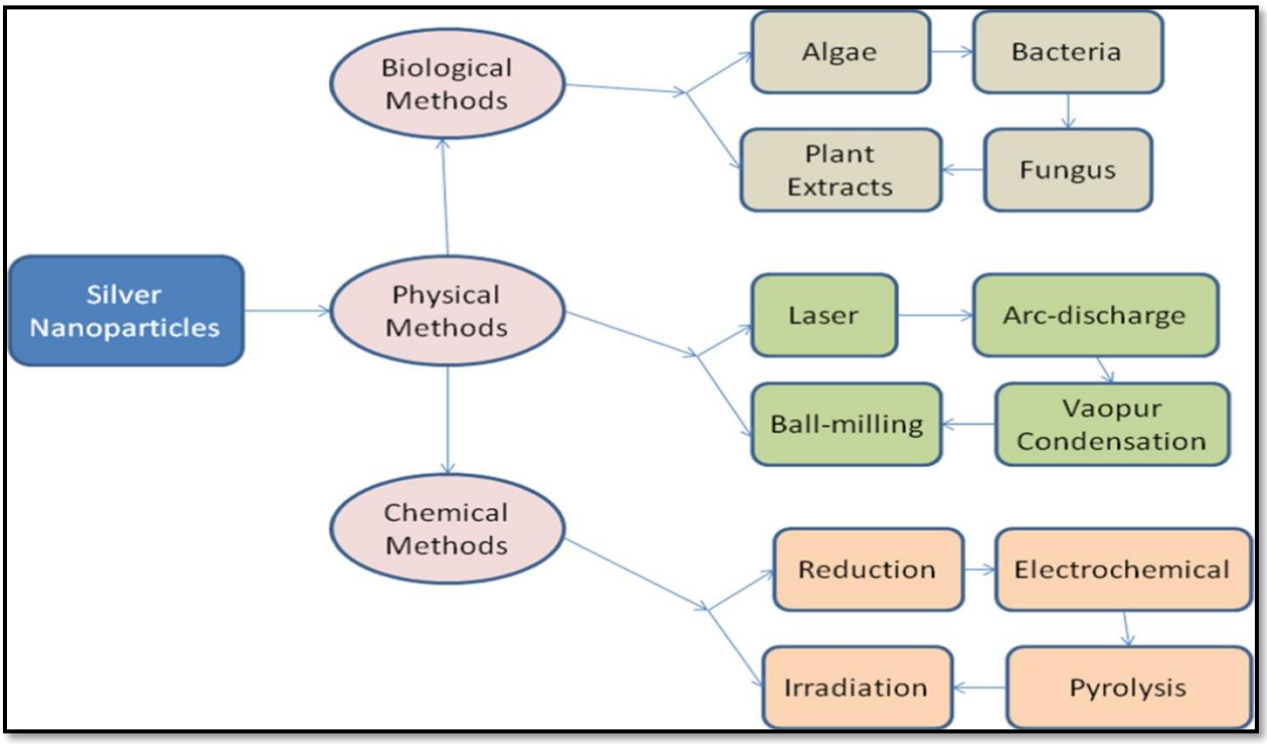

Fig. 4: The schematic diagram for the various methods of synthesis of silver nanoparticles (Arif and Uddin, 2021)

2.10.4 Green synthesis of silver nanoparticles using plant extracts

Green synthesis is the biological method of synthesizing nanoparticles. Green synthesis of AgNPs is the most accepted method as it provides various advantages over conventional techniques (chemical and physical methods). The technique is eco-friendly, easy, no sophisticated instruments and chemicals are required. No toxic chemicals are involved as reducing agents and stabilizing agents are derived from plants (Nadaroglu et al., 2017). Plants provide free reducing, stabilizing, and capping agent and also cost of microorganism and culture media is reduced, ultimately reducing the overall cost of the formulation (Mousavi et al., 2018). This method is a good alternative to conventional methods of nanoparticles synthesis. The product formed using this method is more stable with the desired shape and size (Thakur and Mohan 2019; Bedlovicova and Salayova, 2017).

Naturally occurring phytoconstituents consist of numerous primary and secondary metabolites such as proteins, amino acid, vitamins, nucleic acids and alkaloids, terpenoids, flavonoids, saponins and phenols (Silva et al., 2019). These primary and secondary metabolites in

plant extract act as reducing agents for silver ions by getting oxidized and coats the newly developed particles. In the presence of oxygen, such as in silver nitrate (AgNO3), these metabolites lose their electron and become oxidized via common cellular procedures, thus act as reducing agents (Sanjay, 2019; Ghosh, 2019). The steps involved in the green synthesis of silver nanoparticles are elucidated in Fig. 5.

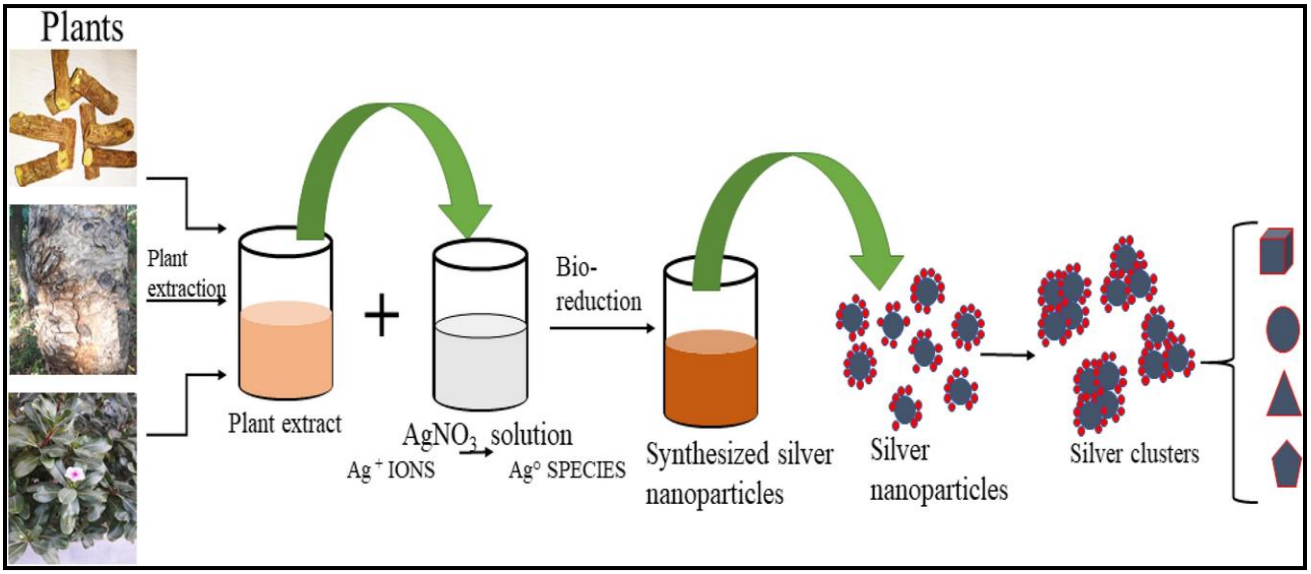

Fig. 5: Synthesis of AgNPs through Green synthesis method (Jain et al., 2021)

2.10.5 Characterization of silver nanoparticles

The expansion of nanotechnology in various research areas has led to the need to use analytical techniques for the analysis and characterization of nanoparticles. Several characterization techniques are available, including microscopic, separation and spectroscopic techniques. Nanoparticles are usually characterized in the literature by their size distribution, morphology, surface properties, stability and interactions (López and Mizaikoff, 2016). The main characterization techniques of nanoparticles in general and AgNP in particular are given in the table 1.

Table: 1 Principal techniques for evaluation of the physicochemical characteristics of nanoparticles

S.NO	TECHNIQUES	PHYSICOCHEMICAL CHARACTERISTICS ANALYZED
1	X-ray photoelectron spectroscopy (XPS)	Elemental and chemical composition at the surface
2	Zeta potential	Stability referring to surface charge
3	Infrared spectroscopy (MS)	Structure and conformation of bioconjugates, functional group analysis.
4	Scanning Electron Microscopy (SEM)	Size and size distribution, shape, aggregation.
5	Transmission Electron Microscopy (TEM)	Size and size distribution, shape heterogeneity, aggregation.
6	Dynamic Light Scattering (DLS)	Hydrodynamic size distribution
7	Near-Field Scanning Optical Microscopy (NSOM)	Size and shape of nanomaterials
8	Nuclear Magnetic Resonance (NMR)	Structure, composition, purity.
9	Mass Spectrometry (MS)	Molecular weight, composition, structure.
10	Atomic Force Microscopy (AFM)	Size and size distribution, shape, structure, aggregation.

2.10.6 Therapeutic applications of silver nanoparticles

Although AgNPs are used in a variety of applications including thin films, surface coatings, batteries, cosmetics, textile industry, food industry, energy harvesting and conductors, medical applications have attracted most attention due to increasing life threatening diseases worldwide and multidrug resistance challenges (Shanmuganathan *et al.,* 2019). Upon reaching nanoscale, silver particles have different physicochemical properties and create exceptional biological activities. This distinctiveness of silver nanoparticles widens their application in antibacterial, anti-fungal, anti-viral, anti-inflammatory, anti-angiogenic and anti-cancer therapy. Recent studies have indicated that AgNPs do not harm humans and kill viruses, bacteria and other eukaryotic

microorganisms without any adverse effects in diluted concentrations (Sukriye and Cigdem, 2019). The AgNPs plays a crucial role contributing to the development of novel antimicrobial agents, biomaterial and medical device coatings, drug delivery formulations, detection and diagnosis platforms, tissue restoration and regeneration materials and performance-enhanced therapeutic alternatives. In addition, AgNPs can be incorporated as additives into membrane, bone cement, denture base, tooth implant, fractured bone, catheters and hydrogel to prevent or reduce formation of biofilm or any medical pathogens as well as improve and fasten the recovery of bone growth, wound and gums recovery (Yang *et al.,* 2021). Various biomedical applications of silver nanoparticles are described in Fig. 6.

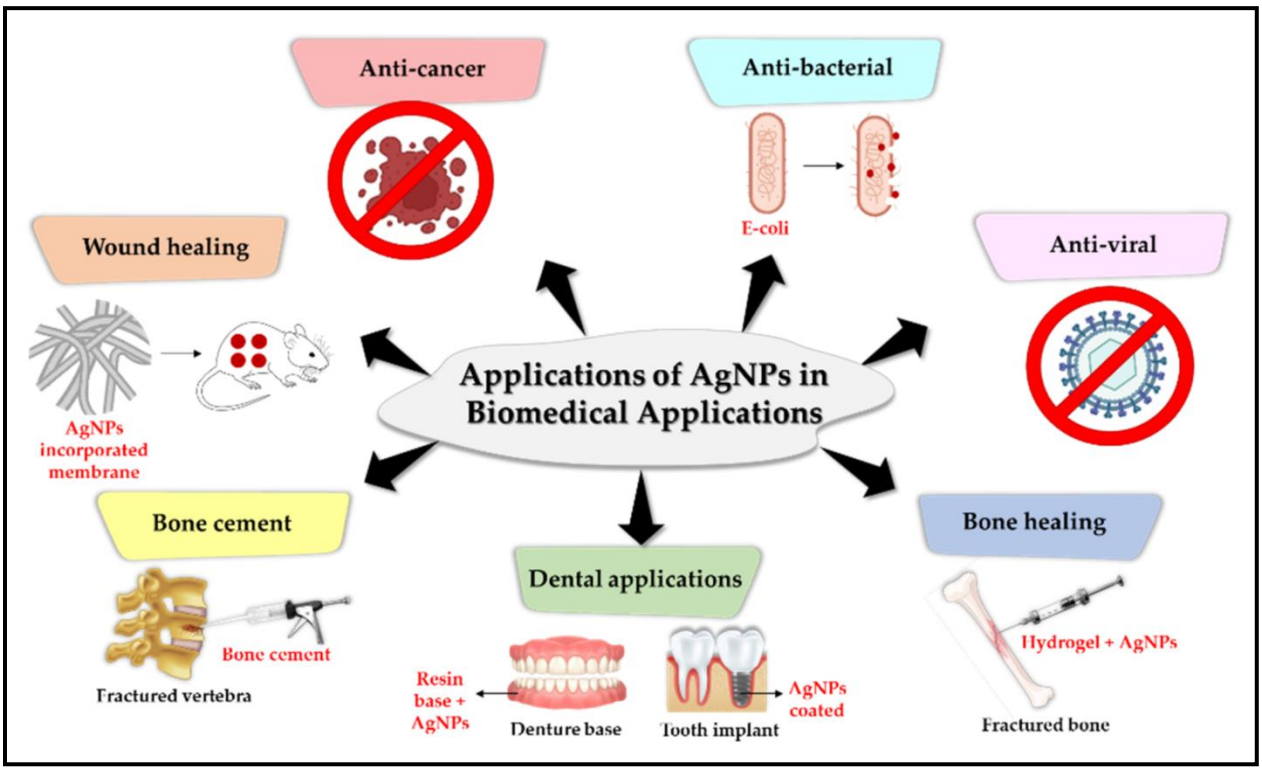

***Fig. 6: Biomedical applications of silver nanoparticles (*Naganthran *et al.*, 2022)**

2.10.7 Role of silver nanoparticles in cancer therapy

For effective cancer therapy, it is essential to develop or engineer a drug or gene delivery system that has an excellent ability to target tumor cells sparing the normal healthy cells. It enhances therapeutic efficacy, thereby shielding normal cells from the effect of cytotoxicity. It can

be achieved by the well-organized delivery of NPs into the tumor microenvironment (TME), indirectly targeting cancer cells (Gavas et al., 2021)

The therapeutic potential of nanoparticulated silver relies on its unique mode to induce cell death in mammalian cells. Regardless of their physical and chemical properties, such as heterogeneity in size, shape, and capping material, their way of action to induce cancer cell death is rather dogmatic. Following their uptake, mostly by endocytosis-related mechanisms, AgNPs are collected in endosomes, of which the organelles are then directed to lysosomal fusion. The lysosomal acidic environment leads to an increased release of silver ions from the AgNPs, of which the reactive ions then unbalance cellular homeostasis and based on the biological feature of the targeted cell, which leads to apoptotic cell death (Cameron *et al.*, 2018). This type of action is traditionally referred to as the "Trojan-horse"-type mechanism and implies that the cytotoxic feature of AgNPs emerges only following their uptake by the cells (Hsiao *et al.*, 2015).

It is worth noting that the toxicity of AgNPs is not a direct effect of free Ag^+ but is caused by the oxidative stress of Ag nanoparticles. It was found that AgNPs can induce oxidative stress by producing ROS in cells, leading to cytotoxicity, apoptosis, and necrosis of cancer cells, which is a new step in the treatment of tumors. The mechanism of noble metal nanoparticles promoting tumor cell apoptosis by cytotoxicity is shown in Fig. 7 (*Rui et al., 2022*).

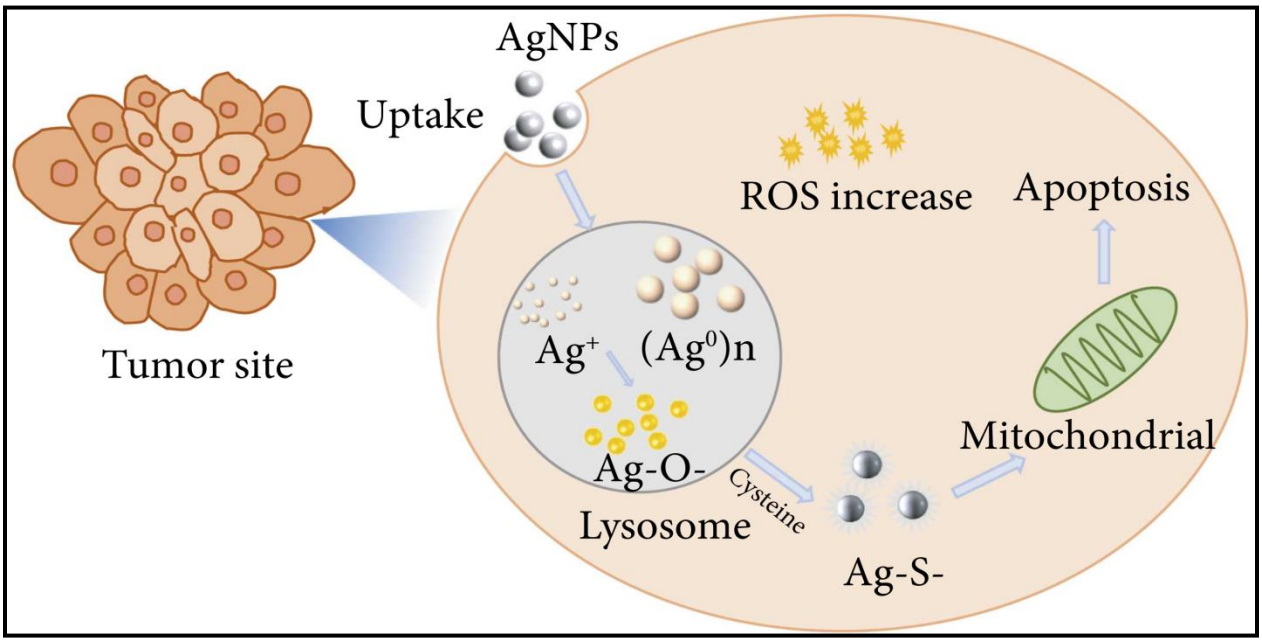

Fig. 7: Mechanism of silver nanoparticles promoting apoptosis of tumor cells by cytotoxicity *(*Rui et al., 2022*)*

2.11 Liposomes

Liposomes are spherical, closed structures composed of phospholipids in the colloidal size range of 5–200 nm and contains one or more concentric/non-concentric membranes, of around 4 mm thickness. The liposomes consist of amphiphilic phospholipids with hydrophilic head and hydrophobic tail, which aids in unique characteristics such as self-sealing of liposomes in aqueous media. Shortly, liposomes in a size range of 5–200 nm have been noticed for encapsulation of hydrophilic or hydrophobic drugs into aqueous phase/bilayer membrane, which made a pavement of liposomes for drug delivery applications. In recent past, much research has been focused on the delivery of antibiotics (Marchianoa et al., 2020 and Cha et al., 2019), genes (Chunyan et al., 2020 and Rodrigues et al., 2020), antifungal (Bezerra et al., 2020 and Oliveira et al. 2020), anti-inflammatory (Zhang et al., 2020 and Zhang and Michniak, 2020) and anti-cancer drugs (Bai et al., 2020 and Li et al., 2020) and also used in many pharmaceutical, biological and medical fields. A significant advantage of systemic liposomes as drug formulations is high biocompatibility, low immunogenicity, biodegradability, increased efficiency, prolonged drug half-life, targeted delivery, lowered systemic toxicity and protection of sensitive molecules, with enhanced pharmacokinetics. The utmost advantage of systemic liposomes incorporation and release of two different materials with different solubility's simultaneously (Aghdam et al., 2019).

2.11.1 Types of liposomes

Liposomes classified into unilamellar, multilamellar, oligolamellar, and multi vesicular vesicles based on the number of phospholipid bilayers, as shown in Fig. 8. The desirable size of liposomes for drug delivery applications ranges from 50 to 200 nm. Liposome size is the major factor for efficient delivery of drugs into the body. The size of liposomes shows significant effect on the pharmacokinetics of liposomes and drugs encapsulated into the liposomes. The size of liposomes less than 200 nm shows increased circulation and residence time of liposomes in the blood, enhanced in vivo drug release from liposomes and significant accumulation into the tumour cells (Andra et al., 2022).

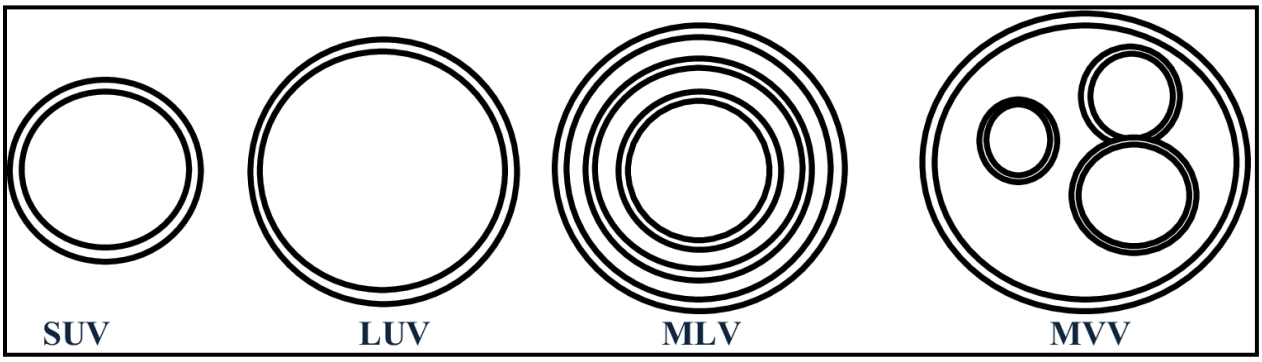

Fig. 8: Classification of liposomes (Andra et al., 2022).

2.11.2 Methods of liposome preparation

Liposome preparation can be done by conventional methods such as Bangham method [thin film hydration], ether/ethanol injection method, reverse phase evaporation method, detergent depletion method, heating method, microfluidic channel method, membrane extrusion method, homogenization and sonication method. Novel methods for liposomal-based drug delivery involve freeze drying, dual asymmetric centrifugation [DAC] and supercritical fluid [SCF] methods since a decade. Depo-foam liposome technique, lysolipid thermally sensitive liposome technique, non-PEGylated liposome technique and stealth liposome techniques are the innovative techniques used for the delivery of drugs in the recent past (Bulbake et al., 2017).

2.11.3 Thin Film Hydration Method [Bangham Method]

The Bangham method is the first commonly used and simple method for liposome preparation. This method utilizes an organic solvent (dichloromethane, chloroform, ethanol and chloroform–methanol mixture) to dissolve lipids; further the organic solvent can be removed by evaporation under vacuum at a temperature of 45–60ºC to form a thin lipid film. Subsequently, the thin lipid film gets hydrated in aqueous media by continuous agitation up to 2 h at a temperature of 60–70ºC where it swells to produce round closed liposomes (Zhang, 2017). Finally, sonication is performed to reduce the size of the liposomes to nanoscale. The schematic representation of Bangham method is represented in Fig. 9.

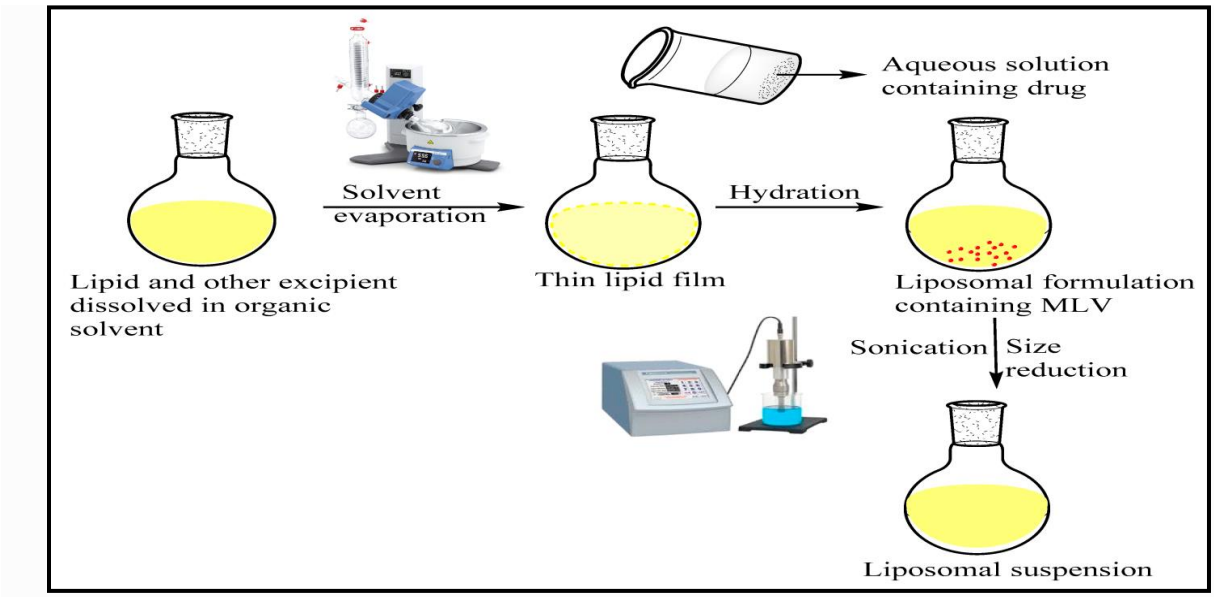

Fig. 9 The schematic representation of Bangham method for the preparation of liposomes (Kesharwani *et al.*, 2021)

2.11.4 Drug-Loading Methods

The drug loading into the liposomes can be either passive or active. The passive drug-loading method involves encapsulating the drug agent during the preparation of liposomes. The drug can be encapsulated within the inner aqueous space or embedded in the bilayer of liposomes by means of covalent, ionic, electrostatic, noncovalent, or steric interactions between drug molecules and lipids. The main disadvantage of this approach is the low encapsulating efficiency, and thus leading to an additional step of free drug removal. The active drug loading approach, also called remote drug loading, involves loading the drug agent after empty liposomes are produced. The transmembrane gradient of pH or ion concentration is the driving force to promote the drug diffuse across the membrane into the inner core of liposomes. The drug-entrapment process takes around 5 min to 30 min, and a high loading efficiency (above 90%) can be reached (Liu *et al.*, 2022).

2.11.5 Administration Route of Liposomal Drugs

Like many different drugs, NP-based liposomal medicines can be administered from a wide variety of routes. In other words, oral consumption and distinct injection methods such as intravenous (I.V.) administration and various local injections are among the common

administration routes of liposomal drugs *(Daeihamed et al.,2017).* The usage of nanoparticles, including liposomes, for drug delivery via oral administration has been highlighted as an effective strategy since the nanoparticles increase the bioavailability of medicines, improve their interaction with cells, and prevent any modifications in the molecular structure of the drug due to enzymes and gastric juices in the gastrointestinal tract. Moreover, they have the ability not only to enhance the release of remedial molecules into the mucosal and epidermal layer but also to protect drugs from unwanted changes during the first pass effect. Intravenous injection is used as the primary administration route for many liposomal drugs approved by the FDA or other authorities. On the other hand, subcutaneous (S.C.), intradermal (I.D.), intraperitoneal (I.P.), and intramuscular (I.M.), classified under the title of the local injection, are also utilized for administration of liposomal drugs *(Rommasi and Esfandiari, 2021).*

2.11.6 Drug delivery of liposomes to cancer cells

Numerous strategies have been developed for the purpose of producing liposomes that selectively target tumour cells and deliver anticancer agents to tumour sites. The surface functionalization of liposomes facilitates the active and passive targeting and release of drugs into the tumor site *(*Riaz *et al.*, 2018*).*

Passive targeting

Passive targeting is a strategy that depends solely on the pathophysiological characteristics of tumour tissues for drug targeting. Liposomal drug formulations translocate freely across the endothelium of capillaries into the interstitial fluid due to leaky tumour vessels. The pores that lie between the endothelial cells of tumour microvasculature vary considerably in size. Gap sizes between endothelial cells that line normal capillaries range between 5 and 10 nm, whereas the gap sizes between endothelial cells of tumour capillaries range between 100 and 780 nm which enables liposomes to engage in passive targeting. Consequently, ideal targeting can be obtained if liposomes are prepared with a size range appropriate for extravasation into tumour tissues and not normal tissues. The phenomenon that enables effective liposomal accumulation in tumours is termed as the EPR effect (Alavi and Hamidi, 2019).

Due to the EPR effect, blood capillaries in cancerous tissues have greater permeability and a limited fluid return to the lymphatic circulation. Therefore, liposomes up to 400 nm in size and their encapsulated drugs can preferentially accumulate within the microenvironment of solid tumours owing to the inadequate lymphatic drainage of extravasated molecules. The consequent accumulation of liposomes in solid tumours provides improvements in drug delivery as there are higher local drug concentrations available. The EPR effect may be optimized by preparing liposomes with particle sizes ranging between 40 and 200 nm as they have exhibited greater extravasation (Maeda *et al.,* 2013).

Active targetting

In order to further minimize off-target side effects, different strategies have been employed to design actively targeted liposomes. Active targeting involves directly targeting drug payloads at the target site. Preparing actively targeted liposomes generally involves the conjugation of targeting ligands onto the surface of liposomes such as peptides, monoclonal antibodies, and aptamers. Ligands may be attached to liposomes in a multitude of ways, including direct attachment to lipids or attachment at the terminal end of Poly Ethylene Glycol chains. Ligand-lipid-PEG conjugated micelles may be incorporated into preformed liposomes via the postinsertion technique. One other commonly utilized approach is ligand incorporation into the step of liposome formulation (Latifa *et al.,* 2021).

2.12 Candidate plant- Tabebuia pallida

Tabebuia is a genus of flowering plants in the family Bignoniaceae. *Tabebuia pallida* is commonly known as trumpet trees, Cuban Pink Trumpet Tree, Pink Tabebuia, Pink Trumpet Tree, White Cedar. It is native to the Caribbean islands. It is a Semi-deciduous tree, about 10-15 metres tall, with a grey-brown bark. It shows simple or 3-leaflets. Leaves are elliptic in shape and are about 16×6 cm with glossy dark green colour. Flowers are funnel-shaped and long about 8 cm, of white or pale pink colour. The fruits are 12-18 cm long capsules with a diameter of about 1 cm, containing many winged seeds. It easily reproduces by seeds. It is cultivated as showy flowering street tree (Mahbubur *et al.,* 2016).

- Kingdom : Plantae
- Subkingdom : Viridiplantae

- Division : Tracheophyta
- Subdivision : Spermatophytina
- Class : Magnoliopsida
- Family : Bignoniaceae
- Genus : Tabebuia
- Species : *Tabebuia pallida*

Plate-1: Tabebuia pallida

Tabebuia pallida found to possess antibacterial, cytotoxic, antioxidant, anti- hypotensive, free radical scavenging properties. It was used in the treatment of various ailments in folk medicine (Mahbubur et al., 2019). But a very few or negligible literature are available to prove the medicinal properties of *Tabebuia pallida* scientifically. So an attempt was made to validate the anticancer potential of *Tabebuia pallida*.

The present study was designed into four phases and the methodology adopted for each phase is discussed in the next chapter.