
Chapter-1

Introduction

1.1 General introduction

Cancer has been a growing global threat for the past several years. Cancer is defined as the uncontrolled, abnormal proliferation of normal cells and leading to tumor formation. Its characteristics are entirely different from that of normal cells. Cancer cells are immature, and they won't undergo apoptosis. When the normal cells are damaged, they are identified and removed by our immune system, but cancer cells have the ability to confuse the immune system and remain and form the tumor. They have the capacity for metastasis. The tumours are malignant, that they can invade neighbouring tissues and form a tumor there. Even the size and shape of cancer cell varies from that of the normal cells.

Cancer is caused by the mutations accumulating by the internal and external factors, thus disrupting the molecular signalling. So, cancer is the result of a series of events, not a single one. And the sequence of events causing multiple genetic defects are generated by the exposure to dietary, environmental, infectious agents, and lifestyle factors (Herceg, 2007). Epidemiological evidence reveals that cancer can be reduced by the increased uptake of fruits and vegetables, avoidance of smoking, control of infections, avoidance of over sun exposure, increased physical activity and reduced alcohol consumption and red meat (Anand *et al.*, 2008).

Cancer statistics show that frequently observing cancer in India is lifestyle-dependent, with offending factors such as low socioeconomic status, alcohol usage and tobacco usage, multiple pregnancies and poor sexual hygiene (Desai, 2002). In India, among 1,00,000 populations, 80 are affected by cancer (population being 1.27 billion) (Ali *et al.*, 2011). Every year around 8,00,000 new cases and 5,50,000 deaths are reported in India. Lung cancer (19.45%), liver cancer (9.1%) and stomach cancer (8.8%) are the most common ones found in India (Ali *et al.*, 2011). In 2017, 9.6

million people lost their lives due to this chronic disease in the world, and it is observed that every sixth death in the world is caused by cancer and making it the second cause leading to death, the first cause being cardiovascular diseases (Wang *et al.*, 2016).

Death is not the only factor to be considered. Cancer also leads to depression, other mental illnesses and financial burden. The treatment is often costly and challenging for an average family to afford. The treatment usually involves surgery, chemotherapy and radiation therapy. Chemotherapy is the most practiced one, but its side effects and drug resistance seem to be the major limitations. So considering all these facts, many studies have been done, and many are ongoing to replace synthetic drugs with natural ones to reduce the side effects and financial burden. Chemoprevention is another remedy against cancer. It is the administration of natural, synthetic or biological agents that can delay or reduce the appearance of malignancy (Steward and Brown, 2013).

Carcinogenesis or tumorigenesis is a multistep process that involves the initiation, promotion and progression. Initiation is the irreversible or stable change occurring to a target cell spontaneously or due to exposure to carcinogens. Thus, mutation initiates at the cellular genome (Cox, 1994). Promotion is the subsequent changes leading to neoplastic transformation in the activated cell unless and until repeated and prolonged exposures stimulate it, thus upsetting the cellular balance (Upton, 1986). Progression is the last step in cancer development which involves further changes to the neoplasm resulting in cell detachment from the primary tumor and invading other tissues and organs, thus leading to metastatic growths (Devi, 2004). Different cancer genes like oncogenes and anti-oncogenes play an essential role in cancer development. Gain of function mutations happening in the oncogenes,

leading to the abnormal cell multiplication and loss of function mutations occurring in the anti-oncogenes leading to the inactivation of cell differentiation and apoptosis are the main factors leading to cancer (Khambete and Kumar, 2014).

The multifactorial mechanism produced by the inherited and environmental events mutates benign cells to malignant cells through a series of molecular changes (Hemminki and Mutanen, 2001). In this multistep process of cancer development, promotion is very much related to oxidative stress arising from exogenous and endogenous sources. Reactive oxygen species (ROS) and free radicals are constantly being produced in human beings under natural conditions. To counter them, the antioxidant system has evolved to expose the body against those free radicals during high oxidative stress. Many of the fruits and vegetables we consume are rich in antioxidants, which seems to reduce the chances of getting cancer. In addition to this, many medicinal plants rich in flavonoids and phenols possess antioxidant activity, which helps maintain the balance between antioxidants and oxidants, thus fighting oxidative stress (Scalbert *et al.*, 2005). Also, antioxidant supplements are provided to those under chemotherapy treatment to reduce the side effects (Conklin, 2000). Even in chemoprevention, the antioxidants play a significant role, and thus it becomes indispensable to fight cancer.

Inflammation stands as a causal agent in certain types of cancer (Birbach *et al.*, 2011). Inflammation is the immune system's natural response to harmful stimuli that can be activated by many non-infectious and infectious factors like irradiation, toxic compounds, damaged cells, pathogens etc. (Chen *et al.*, 2018). Chronic inflammation is said to be a predisposing factor that leads to carcinogenesis. In contrast, acute inflammation contributes to cancer regression. However, many epidemiologic studies support that the risk of cancer increases with chronic inflammatory diseases (Lu *et al.*,

2006). Chronic inflammation is dominated by plasma cells, lymphocytes and macrophages with different morphology (Balkwill and Mantovani, 2001). The inflammatory cells and macrophages generate numerous cytokines, growth factors and reactive nitrogen and oxygen species that can induce DNA damage. If these macrophages are persistently activated, they can trigger continuous tissue damage (Coussens and Werb, 2002). A microenvironment created by the above elements inhabits the persisting cell proliferation caused by the tissue damage, thus leads chronic inflammation to neoplasia (Lu *et al.*, 2006).

Herbal medicines having anticancer properties with additional benefits of providing antioxidants and anti-inflammatory activity have high priority in cancer drug development. Natural products have proven to be very useful in drug development and mechanistic investigations involving biological components that are important in cancer management. Taxol, camptothecin, bleomycin, phorbol esters etc., are just a few examples of natural products (Douros and Suffness, 2013).

In 1956, the National Cancer Institute began testing plant extracts for anticancer potential. Considering the microbial sources, the fungal group is of most significant interest. The medicinal benefits of macro fungi have been known and utilized for centuries. The use of many different types of fungus extracts exhibiting immunostimulatory, anti-inflammatory, and anticancer action in traditional medicine extends back to ancient China, Japan and other far Eastern nations (Hobbs, 1995).

Moreover, fungi have a considerable potential for producing a wide range of compounds that may be employed in a variety of applications. Fungal species are known to generate over 6,400 bioactive compounds. Because of their heavy metal tolerance and ability to absorb and bioaccumulate metals, fungi are frequently employed as reducing and stabilizing agents. Also, fungi can readily be cultured on a

large scale and generate nanoparticles with precise size and shape (Gade *et al.*, 2008). Fungi produce an enormous quantity of proteins and enzymes compared to other microorganisms, some of which could be utilized to synthesize nanoparticles quickly and sustainably (Guilger-Casagrande and Lima, 2019).

Due to the importance of natural product-derived cancer chemotherapeutic agents in chemotherapy, innovative, effective medicines with fewer side effects are a vital need. The present study explore the anticancer properties of a rare termite macro fungus called *Sclerotium stipitatum* Berk. *et. Curr.*. Moreover, the antitumor, antioxidant, and anti-inflammatory properties of ethanol extract of fungus *Sclerotium stipitatum* were investigated in depth. This thesis summarises the findings.

1.2 *Sclerotium stipitatum* Berk. *et Curr.*

Sclerotium stipitatum is a hypogean fungus usually associated with termite nests under the soil. It was first identified by Berkeley in 1862 (Berkeley, 1862). Currently *S. stipitatum* is suspected as a *Xylaria* species based on a molecular study conducted on it (Latha *et al.*, 2015). The shape of *S. stipitatum* varies in size and shape, and size is more or less equal to a lemon. However, when compared to other fungus, their habitat is quite different (Anto *et al.*, 2015).

In Kerala, among the tribal colonies, this fungus has got tremendous significance. They used to preserve this fungus because of its magical medicinal properties (Shortt, 1867). This fungus was used for various illnesses like stomach pain, jaundice, arthritis, cholera etc. (Balakrishnan and Anil, 2001). But due to its extreme habitat preference and rare occurrence, researchers haven't explored this fungus, and its properties are not scientifically proved. And it is not yet reported from anywhere outside the Malabar region of Kerala. Here we take the opportunity to go in detail to

find the best medicinal properties of this fungus and synthesize silver nanoparticles using the aqueous extract. The objectives of the studies are given below.

1.3 Objectives

The objectives of the present work are as follows

- To study the molecular characterization of *S. stipitatum*.
- To evaluate the preliminary chemical screening, GC-MS and LC-MS analysis.
- To determine the in-vitro cytotoxicity and in-vivo antitumor activity.
- To evaluate the in-vivo antioxidant and anti-inflammatory potential.
- Mycosynthesis of silver nanoparticles and its characterization.