CHAPTER I

INTRODUCTION

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Cancer is known as a group of disease complications which are commonly characterized by the uncontrolled growth and proliferation of cells, resulting in death (American Cancer Society, 2014). It is indeed a pandemic burden affecting people of all ages, races, incomes, and statuses; principally by commencing a state of sustaining proliferative signaling, avoiding the growth suppressors, protecting the defective cell death, accelerating the replicative immortality, inducing angiogenesis, and finally by activating invasion and metastasis (Torre *et al.,* 2015; Bette and Mandic 2014; Herbein and Kumar 2014; Hanahan and Weinberg 2011; Jemal *et al.,* 2011). However, cancer has been commonly noticed to develop in older people; and interestingly 78% of all cancer diagnoses are being conducted in people 55 years of age or older (American Cancer Society 2014). Cancer is usually triggered by tobacco usage, exposure to infectious microorganisms, consumption of unhealthy diet, and by the inherited genetic alterations, hormones, and immune suppressive conditions (Bhagirath *et al.,* 2015; Wu *et al.,* 2015; Leiserson *et al.,* 2013, Vandin *et al.,* 2012). Cancer initiation has long been known to require only a single exposure to a carcinogen with the concomitant effect on DNA damage. Though a single genetic change may suffice the onset of a malignant tumor, most of the researches pointed to a multistep process of chronological alterations in many oncogenes, tumor–suppressor genes, or microRNA genes (Caja and Vannucci 2015, Miyagi *et al.,* 2015, Zarogoulidis *et al.,* 2015; White *et al.,* 2014, Weinstein *et al.,* 1988). Cancer promotion involves multiple exposures to the carcinogens that do not trigger the DNA damage directly; rather this stage involves the conversion of benign to malignant tumors which in turn continue to progress in their degree of malignancy and heterogeneity (Torgovnick and Schumacher 2015).

Treatment of cancer is till date ambiguous; however, decline of cancer progression may be set by the site specific surgery, irradiation, chemotherapy, hormone therapy, immune therapy, and finally by the drugs that specifically interfere with cancer cell growth (i.e., targeted therapy) (Punglia *et al.,* 2010).

Cannabinoids are naturally occurring compounds found in the *Cannabis sativa* plant and are classified into three categories based on their origin: plant-derived cannabinoids such as Δ9-tetrahydrocannabinol (Δ9-THC), endocannabinoids and synthetic molecules that mimic the structure of either plant or mammalian cannabinoids. More than 120 phytocannabinoid entities

are found in cannabis plant extracts, with the main clinical interest being cannabidiol (CBD) and tetrahydrocannabinol (THC) (Bogdanović *et al.,* 2017; De Petrocellis *et al.,* 2013; Ramos *et al.,* 2012; Press *et al.,* 2020). In recent years, cannabinoids have gained more interest in treating side effects associated with chemotherapy, such as nausea. There is also growing interest in the anti- cancer potential of *Cannabis sativa* secondary metabolites. However, there is little data to support their use or effectiveness in cancer treatment. Research has demonstrated that cannabinoids possess some anti-cancer properties since the identification of cannabinoid receptor type 1 (CB1) and cannabinoid receptor type 2 (CB2) (Scarlett *et al.,* 2018; Pisanti *et al.,* 2017). Recent studies had demonstrated that there anticancerous and preventive activity on albino mice. CBD has been found to elicit anti-proliferative and apoptotic effects in breast cancer cell lines, MDA-MB 321 and MDA-MB 436 (Shrivastava *et al.,* 2011; Ramer R *et al.,* 2013). Several studies have acknowledged cannabidiol possesses anti-cancer properties against several cancers, but none has reported their effect with co-treatment with other cancer therapies (Lukhele *et al.,* 2014). Despite the lack of a described and understood molecular mechanism of action, CBD is still the most effective cannabinoid for use in developing anti-cancer drugs (Braun IM *et al.,* 2020). Researchers have resorted to animal tumor models as they are a reliable predictor in preclinical cancer research, given that they can produce reliable tumor growth. Although many studies have examined the impact of cannabis on prostate cancer in cell culture experiments, very few papers have established if these effects occur in in vivo animal models. Few publications have determined whether these effects occur in in vivo animal models, regardless of the fact that several studies have explored the efficacy of cannabinoids on prostate cancer in cell culture studies (Marzo, 2018). Numerous studies have been done on CBD concerning various cancers, including prostate and lung cancer (Singh *et al.,* 2021). Some of these studies discovered that the extracts of cannabis enhanced with CBD effectively reduced the growth of tumors in androgen receptor- positive LNCaP xenografts but amplified tumor growth in androgen receptor-negative DU-145 xenografts. Correspondingly, to determine the effects of CBD in vivo, the xenograft mouse models (Shen *et al.,* 2017). The migration/invasion and viability of the HNSCC cells have been drastically reduced in a dose- and time-dependent manner (Go *et al.,* 2020; Ladin *et al.,* 2016). Although these studies show anticancerous potential of EAC induced cancer in albino mice. The chemical compositions of cannabis extracts called phytocannabinoid. Main phytocannabinoids are-Δ9–tetrahydrocannabinol

(THC), cannabinol (CBN), cannabichromene (CBC), cannabidiolic acid (CBDA), cannabigerol (CBG). This compounds shows anticancerous properties. So this is designed to observe doses depended and time duration depended activity of cannabis extract.

Figure 1: *Cannabis sativa* plant (Source-Internet)

Cannabinoids have been used in cancer therapy to reduce the side effects include nausea, alleviate pain, and lack of appetite. However, pre-clinical studies have shown anti- cancer properties of cannabinoids for many cancers in mammalian cells and biological models (Dariš *et al.,* 2018). Cannabinoids have been attracting a great deal of interest as a source of anti-cancer drugs since the early 1970s because of their ability to regulate cell growth, invasion, and cell death (Guzmán *et al.,* 2001, Dariš *et al.,* 2018). Velasco *et al.,* (2012) also has been reported that cannabinoids inhibits the tumour metastasis and induce growth arrest (Blázquez *et al.,* 2008). Tumour ability of cannabinoids was first reported by Munson *et al.,* (1975) where cannabinoids were found to be involved in the inhibition of tumours and prolonged life of mice bearing Lewis lung adenocarcinoma. The involvement of cannabinoid receptors in antitumor genic effect of cannabinoids was described by Galve (2000), subsequently many independent studies have reported anti-tumour activity in vitro on different cancer cell lines including lung, glioma, pancreas, lymphoma, prostate, uterus and breast carcinoma cells (Alexander *et al.,* 2009). Cannabinoids have to be shown to be involved in inhibiting tumour cells by altering the cell signalling receptors further inducing apoptosis or inhibiting angiogenesis of tumour cell.

Cannabis has been used both medically and recreationally. The possibility that cannabis could be effective in the treatment of cancer has attracted a lot of attention.

Ehrlich ascites tumor cells are an undifferentiated tumor that spontaneously developed from a stock mouse's mammary cancer (Stewart *et al.,* 1959). Ehrlich ascites carcinoma (EAC) is one of the most prevalent experimental cancers and is very important for modeling. Ehrlich and (Apolant *et al.,* 1905) employed it as an experimental tumor by transplanting tumor tissues subcutaneously from mouse to mice after it first manifested as a spontaneous breast cancer in a female mouse (Aktaş *et al.,* 1996; Taşkin *et al.,* 2002).

Ehrlich ascites carcinoma (EAC), an animal model of mammary tumor, is a rapidly growing undifferentiated malignancy with very aggressive behavior (Luksiene *et al.*, 2006), which is able to grow in almost all strains of mice (Dongre *et al.,* 2007) and is frequently employed in cancer research (Kim *et al.,* 1964; Bhattacharyya *et al.,* 2004; Bredov *et al.,* 2004; Izhevatkin, *et al.,* 2004; Luksiene *et al.,* 2006; Prabhakar *et al.*, 2006; Dongre *et al.,* 2007). Ehrlich ascites tumor cells have been shown to lack H-2 histocompatibility antigens, which is thought to be the cause of their fast proliferation in virtually any mouse host (Chen *et al.,* 1970; Patt *et al.,* 1956).

In-depth research has been done on the kinetics of intact EAC cell growth in vivo (Izhevatkin, *et al.,* 2004). The EAC cell implantation causes a local inflammatory response that leads to the gradual development of ascitic fluid (Nascimento *et al.,* 2006). Since it serves as a direct source of sustenance for tumor cells, ascitic fluid is crucial for the formation of tumors (Gupta et al., 2004). The Ehrlich mouse carcinoma, which began as a mammary gland tumor, was used to create this ascites tumor. Ascites is produced by intraperitoneal injection of the tumor emulsion (Loewenthalh and Jahn *et al.,* 1932).5-10% of normal cells and one million malignant cells are combined. Ascitic cancer cells are significantly larger than solid cancer cells. Ascites tumors aged 12–14 days occasionally exhibited hemorrhagic exudates. The normal course of ascites production was unaffected by the injection of ascitic fluid that contained a sizable amount of EAC is either employed as ascites or a solid form; if ascites fluid contains the tumor cell that injects i.p., the ascites form is obtained (Okay, 1998; Zeybek, 1996). Due to its undifferentiated nature and quick rate of proliferation, EAC resembles human malignancies that are the most susceptible to treatment. Some researchers claimed that various plant extracts were beneficial against EAC because of the similarity (Ozaslan *et al.*, 2007, 2009a, 2009b; Cragg and

Newmann, 2004). For EAC induced tumor in albino mice, cannabis extracts had used as a treatment and prevention purpose.

Cannabis sativa active ingredients cannabinoids, sometimes known as marijuana or hashish, have long been used in traditional medicine to treat a variety of ailments, including pain, depression, amenorrhea, inflammation, and lack of appetite (Kovalchuk *et al.,* 2020). There is currently a resurgence in interest in comprehending how cannabinoids exert their medicinal benefits and in their for use in additional applications. Cannabinoid ligands may be useful therapeutics for the management of pain, inflammation, Parkinson's disease, multiple sclerosis, and the wasting and emesis brought on by chemotherapy for cancer and AIDS, according to ongoing research (Porter, 2001). The use of cannabis in cancer treatment has recently attracted new interest. Animal models have shown that cannabinoids have antitumoral effects on a variety of tumour xenografts, including thyroid epithelioma (Bifulco, 2001), glioma (Galve, 2000), and lung adenocarcinoma (Munson *et al.,* 1975). The activation of either CB1 (Melck 2002) or CB2 receptors, or both (McKallip, 2001), can mediate the anticancer effects of plant, synthetic, and endocannabinoids.

It became evident that the majority of the effects of marijuana in the brain and peripheral tissues were caused by activation of these two G-protein-coupled cannabinoid receptors after the discovery of the two specific molecular targets for THC, CB1 and CB2 (Pertwee, 1997). The immunomodulatory effects of cannabis are well documented. Nowadays, there are many well-known cannabis cultivars, and each one has a unique composition of different compounds. Many studies have demonstrated the effects of single cannabinoids, such as THC and CBD, on inflammation and cancer cell growth (Kovalchuk *et al.,* 2020). Other components of the plant (such as minor cannabinoids, terpenes, terpenoids, flavonoids, and others) may act synergistically with cannabinoids and can be useful from a therapeutic point of view. The modulating effect of these compounds is known as “an entourage effect;” such modulation is typically positive which means that the medicinal effect of the whole plant extract is more significant than the effect of isolated compounds (Russo *et al.,* 2019). Like with any other drug, the effects significantly depend on the concentration. In the future, with more research being done, we might gain more insight into the potential immunostimulatory effect of individual cannabinoids or cannabis extracts. This knowledge can help medical professionals to integrate cannabis extracts into cancer targeted therapy, potentially as adjunct therapy. The special

extracts with strong anti-neoplastic activities should be identified that are not cytotoxic to normal cells and can sensitize cancer cells to further treatment without reducing the immune responses. Then, these extracts can be combined with immunotherapy, and such combination may have a synergistic action. The results of the retrospective analysis performed with patients with melanoma, renal carcinoma and non-small cell lung cancer when cannabis was used in combination with an immunotherapeutic agent Nivolumab showed a decrease in response rate (RR) but no changes in progression-free survival (PFS) and overall survival (OS). More studies are needed to investigate the possible interactions between cannabinoids and immunotherapy drugs (Taha *et al.*, 2019). In such case, in vivo research would be beneficial to study cannabis as a neoadjuvant therapy before starting biologics. This should be followed by studies testing whether there is an enhancement in the adaptive immune responses mediated by T cells. By directing cytotoxicity to cancer cells, cannabis extracts might increase the release of tumor antigens followed by the enhanced antigen presentation. Also, changes in the tumor microenvironment such as the MDSC and Treg infiltration should be evaluated. The second approach could focus on the possibility of reversing the tumor-induced immunosuppression by using extracts. By finding extracts that can influence this particular pathway, one can combine them with immunotherapy to show a synergistic action. In addition, the assessment of macrophage polarization can be done by studying cytokines. Cannabis extracts that polarize macrophages to the M1 type and do not possess the anti-inflammatory properties can further be combined with immunotherapy.

In the present study cannabis extracts had used for the treatment and prevention purpose of induced Ehrlich Ascites Carcinoma (EAC) cells in albino mice for the following objectives:-

1.1 Objectives of this research

- **To observe the effects of cannabis extract on albino mice.**
- **To evaluate the responses of cannabis extract for the treatment of EAC cells induced cancer.**
- **To determine the preventive efficacy of cannabis extract on EAC cells induced cancer.**