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EFFECT OF MEDICINAL PLANTS ON EHRLICH ASCITES CARCINOMA CELLS (A REVIEW)

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ABSTRACT

Cancer is the uncontrolled growth of cells in the body. Drugs have been developed to treat cancer. These drugs act to control the growth of cells. Medicinal plants have been sources of effective drugs used in cancer treatment over the past few decades. Testing medicinal plants for their cancer chemotherapeutic functions involve the use of models of cancer cells. Ehrlich ascites carcinoma cells are a common transplantable tumor that has been employed in several studies to mimic mammary tumors in animals. The tumor is able to survive and grow in animals. Several studies have shown the universality of the use of these tumor cells in testing for medicinal plants with anti-cancer properties.

Keywords: Ehrlich, cancer, plants, carcinoma, ascites

INTRODUCTION

Cancer is a group of disease characterized by an abnormal growth of cells in vertebrates that can lead to death. These cancer cells have the ability to invade and destroy normal cells (Madhuri and Pandey, 2009). Cancer is not just one disease, but a generic term used to describe a group of more than two hundred diseases sharing common characteristics. Cancers are characterized by their unregulated and unlimited growth as well as the spread of cells to other regions of the body (Corner, 2001; Yarbro, Frogge and Goodman, 2005). Surgical removal of the original tumour is not always a successful treatment in malignant disease, due to microscopic spread. Malignant tumours are often irregular in shape, with ill-defined margins (Wolfe, 1986; Walter, 1977). Chemotherapy is recognized as a major treatment option for the control of advanced stages of these malignancies. There is currently a large and ever-expanding global population that prefers the use of natural products in treating and preventing medical complications (Gautam *et al.*, 2007; Jassim and Naji, 2003). The worldwide upsurge in the use of herbal preparations and active ingredients isolated from medicinal plants have provided the pharmaceutical industry with one of its most important sources of lead compounds, as up to 40% of modern drugs are derived from natural sources, using either the natural substance or a synthesized version. Medicinal plants possess immunomodulatory and antioxidant properties, leading to anticancer activities. Plants contain several phytochemicals, which possess strong antioxidant activities. The antioxidants may

prevent and cure cancer and other diseases by protecting the cells from damage caused by 'free radicals' – the highly reactive oxygen compounds.

Some of these substances are believed to have potential as cancer chemopreventive or therapeutic agents (Pezutto, 1997; Christou *et al.*, 2001; Mukherjee *et al.*, 2001). Furthermore, over 100 new products are in clinical development, particularly as anti-cancer agents and anti-infectives (Gautam *et al.*, 2007; Harvey, 2008; Jassim and Naji, 2003).

Most of these substances exert their chemotherapeutic activity by blocking the cell cycle progression and triggering apoptotic cell death. Therefore, the induction of apoptosis in tumor cells has become an indicator of the tumor-treating ability of naturally derived bioactive substances (Smets, 1994; Paschka *et al.*, 1998). The increase of cancer incidence occurs at a faster rate of 48% in developed countries and 52% in developing countries (Parkin *et al.,* 1993). There is no satisfactory treatment for cancer, and the search for new drugs continues (Elangovan *et al.*, 1994; Kanna and Kannabiran, 2010).

Because of the high death rate associated with cancer and the serious side effects of chemotherapy and radiation therapy, many cancer patients seek alternative and/or complementary treatment methods (Madhuri and Pandey, 2009).

Medicinal plants are used in various countries in the treatment and prevention of cancer. New anti-cancer drug development from medicinal plants has been shown to be a source of cheap and accessible agents that can decipher between normal cells and tumour cells (Cragg and Newman, 2005). Over the years, researchers have focused on the anticancer activity of plants (Saluja *et al.*, 2010; Muthuraman *et al.*, 2008; Sowemimo *et al.*, 2007). More than 50% of all modern drugs in clinical use are of natural products, many of which have the ability to control cancer cells (Rosangkima and Prasad, 2004). Medicinal plants have been known to be good sources of effective anticancer drugs (Cragg and Newman 2005) such as taxol, vincristine and camptothecin. Despite the development of new drugs, cancer is the largest cause of mortality globally and claims over 6 million lives every year (Abdullaev *et al.,* 2000). Hence, the need to search for new drugs that could prolong the life span of patients. Researchers have recently focused on the use of Ehrlich ascites carcinoma cells in the investigation of plants reported to cure cancer locally (Ozaslan *et al.*, 2010; Gupta *et al.*, 2004; Bromberg *et al.*, 2010). Ehrlich ascites carcinoma cells are described as transplantable tumours used in the testing of anticancer drugs in mice (Ozaslan *et al.*, 2011; Lawal *et al*., 2012). Ehrlich ascites carcinoma (EAC), an animal model of mammary tumor, is a rapidly growing undifferentiated malignancy with very aggressive behaviour (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 and Evans 1964; Nusse 1981; Bhattacharyya *et al.*, 2004; Bredov *et al.*, 2004; Izhevatkin 2004; Luksiene *et al.*, 2006; Prabhakar *et al.*, 2006; Dongre *et al.*, 2007). It is known that Ehrlich ascites tumor cells do not have H-2 histocompatibility antigens (Chen and Watson, 1970), which is the major reason, for their fast proliferation in almost any mouse host (Patt and Straube, 1956). The kinetics of intact EAC cell proliferation in vivo has been reported in detail (Izhevatkin, 2004; Lawal *et al.*, 2012; Ozaslan *et al.*, 2016; Lawal *et al.*, 2019). The EAC cell implantation induces a semblance of a local inflammatory reaction, which results in a progressive ascitic fluid formation in the abdominal cavity of murine (Nascimento *et al.*, 2006, Lawal et al., 2012). The ascitic fluid is essential for tumor growth,

since it constitutes a direct nutritional source for tumor cells (Gupta *et al.*, 2004). This ascites tumor was obtained from the Ehrlich mouse carcinoma, which originated as a tumor of the mammary gland. Intraperitoneal injection of the tumor emulsion produces ascites (Loewenthalh and Jahn, 1932).

It is now well recognized that apoptosis is a mode of cell death used by multi-cellular organisms to eradicate cells in diverse physiological and pathological settings (). Recent evidence also shows that suppression of apoptosis by tumor promoting agents in pre-neoplastic cells is an important mechanism in tumor promotion (Shibata *et al.*, 1996).

Many plant-derived products have been reported to exhibit potent antitumour activity against several rodent and human cancer cell lines (Lin et al., 1996). Several natural compounds have been reported to induce cell death in Ehrlich tumor cells *in vivo* and *in vitro* (Lawal et al., 2016).

These plants are used against various types of tumours/cancers such as sarcoma, lymphoma, carcinoma and leukaemia. Many of these medicinal plants have been found effective in experimental and clinical cases of cancers. Literature abounds in Nigeria on medicinal plants included in important anticancer decoctions against tumours such as *Securidaca longepedunculata*, *Nymphaea lotus and Tetrapleura tetraptera* with varying activities on Ehrlich ascites carcinoma cells.

Ehrlich Ascites Carcinoma

Ehrlich ascites tumor cells is an undifferentiated tumour that originated spontaneously as a carcinoma of the mammary gland of a stock mouse (Stewart et al., 1959).

Ehrlich ascites carcinoma (EAC), an animal model of mammary tumor, is a rapidly growing undifferentiated malignancy with very aggressive behaviour (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 and Evans 1964; Nusse 1981; Bhattacharyya *et al.*, 2004; Bredov *et al.*, 2004; Izhevatkin 2004; Luksiene *et al.*, 2006; Prabhakar *et al.*, 2006; Dongre *et al.*, 2007). It has been reported that Ehrlich ascites tumor cells lack H-2 histocompatibility antigens (Chen and Watson, 1970), which apparently is the reason for their rapid proliferation in almost any mouse host (Patt and Straube, 1956). The kinetics of intact EAC cell proliferation in vivo has been studied in detail (Izhevatkin, 2004). The EAC cell implantation induces per se a local inflammatory reaction, which results in a progressive ascitic fluid formation (Nascimento *et al.*, 2006). The ascitic fluid is essential for tumor growth, since it constitutes a direct nutritional source for tumor cells (Gupta *et al.*, 2004).

This ascites tumor was obtained from the Ehrlich mouse carcinoma, which originated as a tumor of the mammary gland. Intraperitoneal injection of the tumor emulsion produces ascites (Loewenthalh and Jahn, 1932). Solid tumors are obtained by subcutaneous injection of fresh ascitic fluid containing cancer cells, and ascites tumors are obtained by intraperitoneal injection into mice. Upon intraperitoneal injection of fresh ascetic fluid containing about 1 million cancer cells, mice regularly develop large amounts of milky ascites (5-20 ml.) in 7-14 days and die in 2-6 weeks. The tumor generally has 100 per cent takes and regresses in about 5 per cent. The milky fluid contains about 5 to 100 million cancer cells/ml usually about 25

million cancer cells, and about 5-10 per cent of normal cells. Cancer cells in ascitic form are much larger than those in solid form. Hemorrhagic exudates were occasionally observed in 12-14-day-old ascites tumors. Inoculation of the ascitic fluid containing a fairly large number of erythrocytes had no inhibitory effect on the normal course of ascites formation. The frequency of appearance of hemorrhagic ascites increased with time, so that at 21 days as many as 50 per cent of animals showed hemorrhagic ascites. At this time, the fluid had a tendency to clot (Sugiura, 1953).

It is well known that the microenvironment of the cells of in vivo ascites tumors is generally hypoxic. EAC cells were grown in the peritoneal cavity of mice in the low end of normal physiologic oxygen tension between 2.6 and 5.2% (Lin and D'Rosario, 2003). These cells are considered to be only transiently oxygenated when fluctuating into the vicinity of blood vessels of the peritoneum. The frequency of such transient oxygenations diminishes as the tumor volume increases, thereby causing an increasing proportion of quiescent cells registering in earlier investigations (Probst *et al.*, 1988).

Formation of ascites and pleural effusion is a common problem for patients with advanced stage of cancer (Verheul *et al.*, 2000). These fluid accumulations cause severe symptoms such as abdominal distention, shortness of breath and fatigue.

***Securidaca longepedunculata* and Ehrlich ascites carcinoma cells**

Lawal et al. (2012) first reported the in vivo and in vitro anti-tumour activity of *Securidaca longepedunculata*, a savannah shrub used locally in Nigeria for numerous medicinal functions, against Ehrlich Ascites Tumour cells. The study was able to ascertain the traditional claim on the cytotoxicity of medicinal plants as shown by the IC_{50}. The study was also able to go further to show that angiogenesis could be involved in the tumour–inhibitory activity of some of the plants as shown the Ascitic fluid volume /weight and bodyweight changes. The administration of some of the extracts caused a restoration of hematological parameters as depicted by the PCV. The study was also able to detect the relative pro-apoptotic ability of the plants as revealed by the DNA fragmentation assay results. The study revealed some of the safe doses in which the plant shows effective anti-tumour potential as revealed by the doses and viability tests/tumour count. The study also revealed the life prolongation ability of the plants as shown by the Mean Survival time and the Increased life span.

Ehrlich Ascites carcinoma cells and Nigerian medicinal plants

A study by Lawal *et al.* (2019) compared the cytotoxicities of several medicinal plants viz *Securidaca longepedunculata*, *Tetrapleura tetraptera*, *Morinda lucida*, *Spondias mombin* and *Nymphaea lotus* on Ehrlich ascites carcinoma cells. The cytotoxicities of some of these plants have been reported in literature. However, this work was the first to report comparison of the cytotoxicities of these plants on Ehrlich ascites carcinoma (EAC) cells. Aqueous extracts of *Securidaca longepedunculata*, *Tetrapleura tetraptera*, *Morinda lucida*, *Spondias mombin* and ethanol extracts of *Tetrapleura tetraptera*, *Spondias mombin*, *Nymphaea lotus* were prepared. The different extracts were used to test for cytotoxicity on Ehrlich ascites carcinoma cells using the Trypan blue dye exclusion principle. All extracts caused dose-dependent increase in mortality of EAC cells. IC_{50} values of the extract range from 11.48 for Aqueous extract of

Tetrapleura tetraptera to 2691µg/ml for ethanol extract of *Nymphaea lotus*. Aqueous extract of Tetrapleura tetraptera was observed to be the most cytotoxic with an IC_{50} of 11.48 µg/ml compared to 5-fluorouracil with 2.88 µg/ml.

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