The present study investigates the anti-cancer and multi drug resistance (MDR) reversal potential of hydroalcoholic Allium cepa, Allium sativum, Boerhavia diffusa and Eclipta alba extracts through in vitro as well as in vivo experiments. Plant Extracts were able to induce apoptosis in both cell lines and cancer induced animals, promising result was given by EAE and ASE. RT-PCR analysis revealed that the mRNA expression of NFkB was markedly decreased in vitro and in vivo upon treated with the PE, preferably by EAE. Our data suggest the presence of bioactive compounds in PE, capable of augmenting liver carcinoma cells by lowering the level of MMP, ROS, induction of apoptosis, altered NFkB signaling and reversing MDR but among all extracts EAE shown the superlative result. These results suggest that EAE is a novel anti-cancer and potent MDR reversal agent and may be a potential adjunctive agent for tumor chemotherapy.

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Dr. Sriram Seshadri did Master's in Endocrinology and Ph. D. in Male Reproductive Physiology. He has over 8 years of Teaching experience and 12 years experience in Research. Currently, he is working at Institute of Science, Nirma University as Assistant Professor. He has guided 2 Ph. D. students and more than M. Sc. 30 students.

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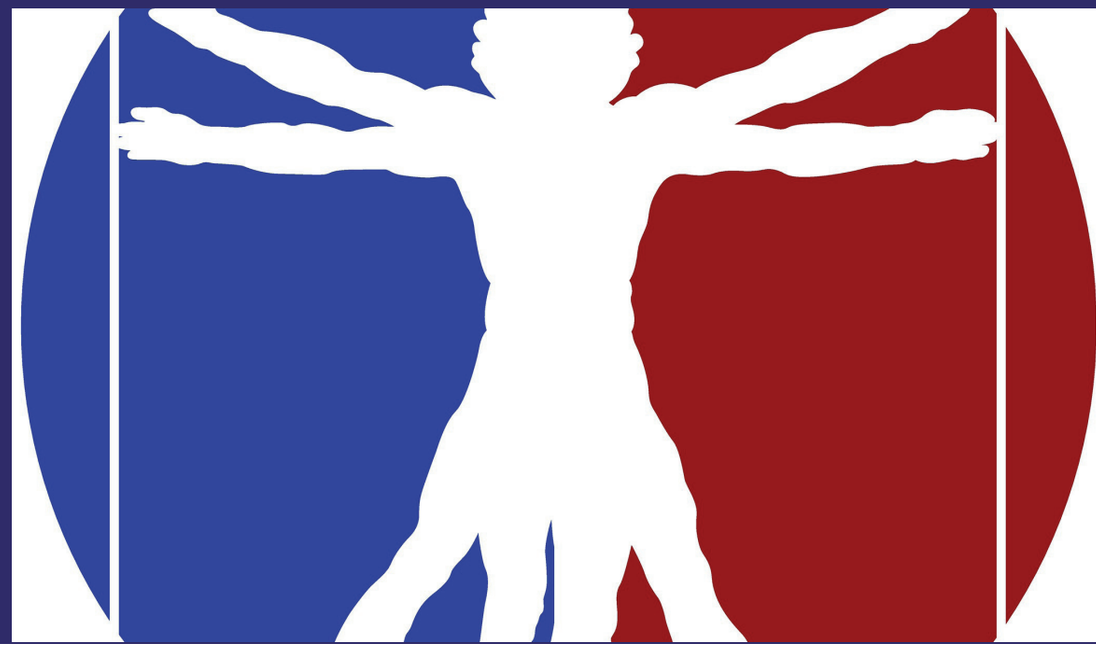

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Harshita Chaudhary

Plant extracts as Anti-Cancer agents

Anti cancer & MDR reversal plant extracts

Sriram Seshadri, H. Chaudhary

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Chapter 1: Introduction

1.1. Cancer

Despite multiple approaches to therapy and prevention, cancer remains a major cause of death worldwide. Cancer is a complex, multi-step process characterized by misregulated signal transduction and altered metabolism. Cancer cells divide faster than normal cells and their growth rates have been reported to correlate with increased metabolic flux during cell transformation (Mizrachy-Schwartz1 et al., 2007). Only 5–10% of all cancer cases can be attributed to genetic defects, whereas the remaining 90–95% has their roots in the environment and lifestyle. The lifestyle factors include cigarette smoking, diet (fried foods, red meat), alcohol, sun exposure, environmental pollutants, infections, stress, obesity, physical inactivity and xenobiotics.

1.2. Liver Cancer

Liver cancer is among the seven leading causes of cancer deaths worldwide. Prognosis is usually poor, and no effective chemotherapeutic treatment is presently available (Hussain et al., 2001). One explanation may be that the major function of the liver is to process and detoxify numerous structurally diverse compounds (Vessey, 1996). Being the metabolic "engine-room of the body", liver has a pivotal role in regulation of physiological processes. It is involved in several vital functions such as metabolism, secretion and storage. The bile secreted by the liver has, among other things, an important role in digestion. Further, detoxification of a variety of drugs and xenobiotics occurs in the liver itself. Almost all the drugs, foods and water constituents are metabolized and detoxified in the liver, and as such it is often exposed to maladies resulting in a number of clinical syndrome and diseases. Because of variations in liver dysfunctions and difficulties encountered in reaching to a proper diagnosis, a physician is rarely able to provide specific treatment. At the most, supportive and symptomatic treatments are given but the

multiplicity of deranged functions renders the treatment still more complicated.

Modern (allopathic) drugs exhibit severe toxicity, thus there is a definite need to search alternate drugs having maximum therapeutic value with no or least toxicity. Liver diseases are among the most serious disorders. They may be classified as acute or chronic hepatitis (inflammatory liver diseases), hepatisis (non-inflammatory diseases) and cirrhosis (degenerative disorder resulting in fibrosis of the liver). The liver diseases are mainly caused by toxic chemicals (certain antibiotics, chemotherapeutics, peroxidised oil, aflatoxin, di-ethyl nitrosomine, carbon tetrachloride, acetaminophen, chlorinated hydrocarbons, etc.), excess consumption of alcohol, infections and autoimmune disorders (Kumar et al., 2011).

1.3. Understanding cancer treatment options and terminology

Part of the difficulty in exploring treatment options is the confusing terminology used in medical literature. Terms used to describe different categories of treatment include *investigational, proven, standard/conventional*, *complementary and alternative methods* (Cassileth, 1998). Understanding these terms will help to evaluate the different treatment options to be considered.

Investigational treatments are those undergoing a carefully structured, closely monitored process to determine if therapies are effective treatments for cancer. Before a drug can be used regularly to treat patients, it is first studied and tested in laboratory test tubes and then in animals. If discovered to be a promising treatment, further studies in humans are done. A cancer treatment clinical trial involves a research study that evaluates a new treatment or combination of treatments in humans. Evaluating a new drug or therapy is a long process that involves distinct phases, each with a different goal. As a result of this lengthy and careful process, *investigational* treatments may become *proven* treatments. In summary*, proven* treatments

are those which have gone through the aforementioned phases of clinical research and, as a result, can be incorporated as part of the standard therapy for a specific type of cancer (Gordon, 2000).

Standard, or *conventional*, treatments are those that have found to have benefit for a specific population of patients and are typically taught as part of the curriculum for medical students or other health care professionals. For some types of cancer, standard therapy may cure disease or significantly prolong survival. In other cases, standard therapy may have a less profound impact on the likelihood of remission. Presently, surgery, radiation therapy and chemotherapy are the most common *conventional* treatments for cancer in this country (Druss et al., 1999). There are also standard, conventional treatments to manage side effects or symptoms associated with cancer and cancer treatments. *Standard* treatments are culturally defined, however, which means what is considered standard in one culture may be different from another. Very few studies have looked at differences in cancer survival between developed nations. When differences are seen, it is difficult to know whether those differences are due to stage at diagnosis, conventional treatments used, complementary / alternative treatments used, or other patient variables. Currently, there are no known differences in treatment outcome between advanced nations (Richardson et al., 2001)

Complementary and *alternative* medicine (CAM) includes a broad range of healing approaches, therapies and philosophies. CAM therapies are used with the intent to reduce stress, improve well-being, prevent illness, avoid or minimize side effects and symptoms, and/or to control or cure disease. *Complementary* approaches may also be considered to *complement* the body's natural abilities to heal. *Alternative* therapy is the term often used to refer to treatments that are used in place of conventional treatments (Ernst et al., 1998). The term *alternative* is also used to indicate treatments outside of conventional medicine used with the intent to treat the disease, as opposed to promoting wellness or managing symptoms.

Despite of several chemotherapeutic, cytotoxic and immunomodulating agents are available in western medicine to treat cancer. Besides being enormously expensive, these drugs are associated with serious side effects and morbidity. A number of undesired side effects sometimes occur during and after chemotherapy also. The resistance of tumors to multiple chemotherapeutic drugs has been recognized as a major reason for the failure of cancer therapy (Gottesman and Pastan, 1993).The phenomenon of tumor drug resistance became a hotspot of cancer research after the emergence of a novel type of resistance discovered by, when it was shown that a glycoprotein of 170 kDa called P-glycoprotein, correlated with the degree of drug resistance in several Chinese hamster ovary cell lines (Juliano and Ling, 1976). The phenomenon called multidrug resistance subsequently appeared as a major impediment to the curative treatment of a variety of malignancies. Reversal of drug resistance offers the hope of increasing the efficacy of conventional chemotherapy.

1.4. Multi Drug Resistance and Cancer

Cancer multidrug resistance is defined as the cross-resistance or insensitivity of cancer cells to the cytostatic or cytotoxic actions of various anticancer drugs which are structurally or functionally unrelated and have different molecular targets. Pharmacokinetic studies dealing with the absorption, distribution, metabolism and clearance of administered drug have been useful in elucidating the levels of drugs in cancer cells and it seems that there are two factors that are primarily responsible for multidrug resistance: i) Individual specificity with regard to variations in absorption, metabolism and delivery of drugs to target tissues. This factor is influenced by individual's genetic pattern which generates various cellular responses that obstruct the drug from reaching to threshold levels inside the cells required for its pharmacological action. ii) Tumor specificity in terms of origin, vasculature and tissue function. Tumors located in parts of the body where the drug is not accessible or tumors with compromised vasculature often show resistance to chemotherapy. The former specificity is linked to

acquired resistance where the altered genetics of the cancer cells exhibit mechanisms that lead to MDR and the latter specificity is responsible for the inherent or natural resistance conferred to certain types of tumors which do not respond to standard chemotherapy drugs from the beginning (Gottesman, 2002).

Tumors derived from tissues, the physiological role of which requires high expression of transporter proteins exhibit intrinsic multidrug resistance to cytostatic agents even before chemotherapy is initiated. The MDR in tumors derived from other tissues appears phenotypically upon induction of genes coding for transporter proteins by a cytostatic agent resulting into acquired MDR during the course of the treatment. Multiple drug resistance (MDR) in cancer is often associated with overexpression of the mdr-1 gene, which encodes a P-glycoprotein (P-gp). P-glycoprotein is considered to be of prognostic relevance in different tumor types. Past researches of about 35 years have thrown up various hypothesis related to the mechanisms of MDR development and also the modulators tailored to address this problem (Mohd, 2008)

1.5. CAM, CANCER and MDR

"Complementary and alternative" therapies are actually a vast collection of disparate, unrelated regimens and products, ranging from adjunctive modalities that effectively enhance quality of life and promising antitumor herbal remedies now under investigation, to bogus therapies that claim to cure cancer and that harm not only directly, but also indirectly by encouraging patients to avoid or postpone effective cancer care (Cassileth, 1999).

CAM has become a much more accepted option for individuals facing cancer and other chronic diseases in recent years. Most commonly, people use CAM to improve their overall health, wellness and to manage specific symptoms associated with their disease or its treatment. Now a day, people use CAM in an attempt to directly treat the cancer. There are different categories of CAM

which were used to treat cancer. Anticancer complementary and alternative medicines (CAMs) are being used worldwide. Cancer is a complex disease and CAM must be an integrated part of the research studies of basic tumor biology and drug design. To overcome the menace of cancer and its drug resistance, there is need to assess a variety of natural sources and products, unravel pathways underlying that can affect various molecular targets and can potentially be used for the prevention as well as treatment of cancer and reversal of multi drug resistance (MDR) alone or along with existing technologies (Eisenberg et al., 1993).

Herbal remedies typically are part of traditional and folk healing methods with long histories of use. Herbal medicine remains one of the common forms of therapy available to much of the world‘s population. Since before the days of Aristotle and Hippocrates people have been using medicinal plants for treatment of their ailments. According to the World Health Organization, about three quarters of the world‘s population currently uses herbs and other forms of traditional medicine to treat diseases (Vecchia and Tavani, 1998; Wattenberg, 1998)

Indian medicinal plants are the essence of Ayurveda and Ayurvedic treatments. When used judicially and clocking with the basic principles they produce miraculous effects. Their role cannot be confined to mere curation of disease but they also used being of human body. Hence, Ayurvedic drugs are rightly called the “Elixirs of Life”. Ayurvedic Herbs played important role in the treatment, from ancient time to this most modern time. Indian Medicinal Plants/ Herbs show good result in disease cure (Mukhtar and Ahmad, 1999*).*

Ayurveda is the medical/Health care system which uses this as treatment base with theoretical principles. There is need of some more to be done to find out the pharmacological action of it. Even in USA, use of plants and phytomedicine has increased dramatically in the last two decades. However, only few medicinal plants have attracted the interest of scientists to

investigate the remedy for neoplasm (tumor or cancer). Hence, an attempt has been made to evaluate some medicinal plants used for the prevention and treatment of cancer (Rock, 1998).

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