Evaluation of anticancer activity of ethanol extract of *Pterocarpus marsupium* ROXB. bark against Ehrlich Ascites Carcinoma (EAC) bearing Swiss Albino mice

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IN

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Submitted by

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OCTOBER – 2016

Certificates

EVALUATION CERTIFICATE

This is to certify that the work embodied in this dissertation entitled **"Evaluation of anticancer activity of ethanol extract of *Pterocarpus marsupium* ROXB. bark against Ehrlich Ascites Carcinoma (EAC) bearing Swiss Albino mice",** submitted to "The Tamil Nadu Dr. M.G.R. Medical University", Chennai, in partial fulfillment to the requirement for the award of Degree of **Master of Pharmacy** in **Pharmacology**, is a bonafide work carried out by **Mr. SUFIYAN.N [Reg.No.261425231]**, during the academic year 2015-2016, under my guidance and direct supervision in the Department of Pharmacology, J.K.K. Nattraja College of Pharmacy, Kumarapalayam.

Internal Examiner **External Examiner**

CERTIFICATE

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DECLARATION

I hereby declare that the dissertation entitled **"Evaluation of anticancer activity of ethanol extract of *Pterocarpus marsupium* ROXB. bark against Ehrlich Ascites Carcinoma (EAC) bearing Swiss Albino mice",** has been carried out under the guidance and supervision of **Dr. R.SHANMUGA SUNDARAM, M.Pharm., Ph.D.,** Vice Principal, Department of Pharmacology, J.K.K. Nattraja College of Pharmacy, Kumarapalayam, in partial fulfillment of the requirements for the award of degree of **Master of Pharmacy** in **Pharmacology** during the academic year 2015-2016.

I further declare that, this work is original and this dissertation has not been submitted previously for the award of any other degree, diploma associate ship and fellowship or any other similar title.

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Date: **Reg.No.261425231**

Dedicated to
Almighty,
My beloved family,
Teachers and
Friends.

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1. INTRODUCTION

Herbal medicine is the oldest form of healthcare known to mankind. They have been used by all cultures since time immemorial (Bhowmick et al., 2010). Medicinal Plants are of great importance in the case of an individual with respect to his health and to the community as well. The medicinal values of the plants are based on the presence of certain chemical substances, which produce a definite physiological action in the human body.

Plants are Inevitable to man for his life. Plants have always been common source of medicament either in the form of traditional preparations or pure active principles. All phyta of plants yield official and unofficial products of medicinal importance. The history of herbal medicine is as old as human civilization. India is virtually herbarium of the whole world. Even though the biological active compounds of some herbal drugs or theirextracts are known, they are prescribed widely because of their effectiveness, minimum side effects in clinical experiences and relatively low costs. Alkaloids, tannins, flavonoids, and phenolic compounds are the most important bioactive phytoconsitutents (Okwu., 1999 and 2001). Medicinal Plant based drugs are advantageous because, they are simple, effective, and exhibit broad-spectrum activity. Thus we see that the importance of herbs in the management of human ailments cannot be overemphasized. It makes us clear that the plant kingdom has got a plentiful of active ingredients, which is very much valuable in the management of a huge number of diseases which are intractable. But these complimentary components give the plants as a whole a safety and efficiency much superior to that of its isolated and pure active components (Shariff., 2001). As per Ayurvedic, Indian medicinal plants are rich source of substances that have several therapeutic properties. About 75-85 % of the world's population, plant derived products still play an essential role in primary healthcare mainly in the developing countries (Dobhal et al.,2013).Indeed ,well into the twentieth century , much of the pharmacopoeia of scientific medicine was derived from the herbal care of the native people.

According to the World Health Organisation, over 80% of the world’s population or 4.3 billion people rely upon such traditional plant based systems of medicine to provide them with primary health care (Attiso., 1983).

Medicinal plants are used to treat illness and diseases for thousands of years. They have gained economic importance because of their application in pharmaceutical, cosmetic, perfumery and food industries. The interest in herbal systems of medicine is growing day-by-day because nature can cure many diseases. Herbs are staging a comeback and herbal 'renaissance' is happening all over the globe. The herbal products today symbolise safety in contrast to the synthetics that are regarded as unsafe to human and environment. Although herbs had been priced for their medicinal, flavouring and aromatic qualities for centuries, the synthetic products of the modern age surpassed their importance, for a while. However, the blind dependence on synthetics is over and people are returning to the naturals with hope of safety and security.

Over three-quarters of the world population relies mainly on plants and plant extracts for health care. More than 30% of the entire plant species, at one time or other was used for medicinal purposes. It is estimated that world market for plant derived drugs may account for about Rs.2, 00,000 crores. Presently, Indian contribution is less than Rs.2000 crores. Indian export of raw drugs has steadily grown at 26% to Rs.165 crores in 1994-'95 from Rs.130 crores in 1991-'92. The annual production of medicinal and aromatic plant's raw material is worth about Rs.200 crores. This is likely to touch US $5 trillion by the year 2050.

It has been estimated that in developed countries such as United States, plant drugs constitute as much as 25% of the total drugs, while in fast developing countries such as China and India, the contribution is as much as 80%. Thus, the economic importance of medicinal plants is much more to countries such as India than to rest of the world. These countries provide two third of the plants used in modern system of medicine and the health care system of rural population depend on indigenous systems of medicine.

Green plants synthesise and preserve a variety of biochemical products, many of which are extractable and used as chemical feed stocks or as raw material for various scientific investigations. Many secondary metabolites of plant are commercially important and find use in a number of pharmaceutical compounds. However, a sustained supply of the source material often becomes difficult due to the factors like

environmental changes, cultural practices, diverse geographical distribution, labour cost, and selection of the superior plant stock and over exploitation by pharmaceutical industry. Plants, especially used in Ayurveda can provide biologically active molecules and lead structures for the development of modified derivatives with enhanced activity and reduced toxicity. The small fraction of flowering plants that have so far been investigated have yielded about 120 therapeutic agents of known structure from about 90 species of plants (Joy et al.,).

Some of the useful plant drugs include vinblastine, vincristine, taxol, podophyllotoxin, camptothecin, digitoxigenin, gitoxigenin, digoxigenin, tubocurarine, morphine, codeine, atropine, pilocarpine, capscicine, allicin, curcumin, artemesinin and ephedrine among others. In some cases, the crude extract of medicinal plants may be used as medicaments. On the other hand, the isolation and identification of the active principles and elucidation of the mechanism of action of a drug is of paramount importance. Hence, works in both mixture of traditional medicine and single active compounds are very important. Where the active molecule cannot be synthesised economically, the product must be obtained from the cultivation of plant material (Mukherjee & Mukhhetjee, 2005).

Major pharmaceutical companies are currently conducting extensive research on plant materials gathered from the rain forests and other places for their potential medicinal value. Interest in herbal medicines has continued to grow. This is shown in several ways, for example, by increased retail sales of herbal medicinal products in Europe and the USA as well as the greater awareness among the public and healthcare professionals about natural health products and complementary therapies. Industrially produced new herbal products, mainly based on single-herb extracts standardized for its specific active ingredient, continue to be developed.

In India knowledge of medicinal plant is very old, and medicinal properties of plants are described in 3500-1500 B.C, from which Ayurveda developed. In Ayurveda the ancient well known treatise are Charak samhita dealing mostly with plants in sashrat samhita in which surgery is also maintained. In Egypt the people were familiar with medicinal properties of plants and animals. Greek scientists contributed much to the knowledge of natural history. Hippocraties (460-370 B.C) is referred to as father of medicine and is remembered for his famous writings on kingdom, which is considered

authoritative even in the 20th century. The substances from the plants were isolated, the structure was elucidated and the pharmacologically active constituents were studied. In 1934-1960 simultaneous applications of disciplines developed like organic chemistry, biochemistry, biosynthesis, pharmacology and modern methods and techniques of medicinal chemistry including paper, thin layer chromatography, gas chromatography and spectrophotometry.

Plants have provided the lead molecules for a large number of diseases. During the past 40 years numerous novel compounds have been isolated from plant sources and many of these substances have been demonstrated to possess interesting biological activities. India is the treasure house of herbs and more than 9000 different herbs with varying medicinal properties are present. Ayurveda an ancient system of Indian medicine has recommended a number of drugs from indigenous plant and animal sources for the treatment of several diseases and disorders. More than 13,000 plants have been studied during the last 5 years (Trease and Evans; Shah & Qadry 1996; Varrote Tyler-iynn et al., 1996).

1.1. Herbal Medicines

Herbal Medicine sometimes referred to as Herbalism or Botanical Medicine, is the use of herbs for their therapeutic or medicinal value. An herb is a plant or plant part valued for its medicinal, aromatic or savory qualities. Herb plants contain a variety of chemical substances that has therapeutic value.

Herbal Medicine is the oldest form of healthcare known to mankind. Herbs had been used by all cultures throughout history. It was an integral part of the development of modern civilization. Primitive man observed and appreciated the great diversity of plants available to him. The plants provided food, clothing, shelter and medicine. Much of the medicinal use of plants seems to have been developed through observations of wild animals and by trial and error. As time went on, each tribe added the medicinal power of herbs in their area to its knowledge base. They methodically collected information on herbs and developed well–defined herbal pharmacopoeias. Indeed, well into the 20th century much of the pharmacopoeia of scientific medicine was derived from the herbal lore of native peoples. Many drugs commonly used today are of herbal origin. Indeed, about 25 % of the prescription drugs dispensed in the United States contain at least one active ingredient derived from plant material. Some

are made from plant extracts; others are synthesized to mimic a natural plant compound.

Major pharmaceutical companies are currently conducting extensive research on plant materials gathered from the rain forests and other places for their potential medicinal value (WHO, 1993).

1.2. Synthetic Drug: Dominance

Pharmaceutical research took a major leap when alongside natural plants chemistry, pharmacologists, microbiologists and biochemists began to unravel the chemistry of natural products in human beings, animals, plants and microorganisms. Advances in synthetic organic chemistry led to the identification of many chemical molecules that offered more opportunities to develop novel compounds. Many new drugs emerged by this route, particularly those now being used to treat infections, infestations, cancers, ulcers, heart and blood pressure conditions. Many drugs were developed through random screening of thousands of chemicals synthesized as dye-stuffs and the like; many others resulted from serendipity arising from sharp eyed observations of physicians and scientists. Example of such drugs includes sulphonamides, isoniazid, antipsychotics, antihistamines and penicillin. Emergence of the modern pharmaceutical industry is an outcome of all these different activities that developed potent single molecules with highly active for a wide variety of ailments. The drugs produced in many cases improved on nature, a new range of local anesthetics from cocaine avoided its dangerous effects on blood pressure, and chloroquine is much less toxic than quinine. These successes and many more like them resulted in reduced interest in natural products drug discovery and many major drug companies almost neglected such divisions. Work on developing new drugs for the treatment of the world's major diseases such as malaria, trypanosomiasis, filariasis, tuberculosis, schistosomiasis, leshmaniasis and amoebiasis came almost to a standstill. In addition, although botanical medications continued to be produced in every country, the clinical efficacy of these was usually not evaluated and the composition of these complex mixtures was only crudely analyzed. Thus herbal medicines became the domain of 'old wives tales' and quack medicine, exploitation of the sick, the desperate and the gullible. Sadly, herbal medicines continued to reflect poor quality control both for materials and clinical efficacy (Patwardhan et al.,)

1.3. Development of Phytomedicines for various diseases

Medicinal plants play a key role in the human health care. About 80% of the world population relies on the use of traditional medicine, which is predominantly based on plant materials (WHO, 1993). The traditional medicine refers to a broad range of ancient natural health care practices including folk tribal practices as well as Ayurveda, Siddha, Amchi and Unani. These medical practices originated from time immemorial and developed gradually, to a large extent, by relying or based on practical experiences without significant references to modern scientific principles. These practices incorporated ancient beliefs and were passed on from one generation to another by oral tradition and guarded literature. Although herbal medicines are effective in the treatment of various ailments very often these drugs are unscientifically exploited and improperly used. Therefore, these plant drugs deserve detailed studies in the light of modern science**.**

1.4. Phytotherapeutic Approach of Drug Development

In phytotherapeutic approach, the emphasis is on the development of a new drug whose extraction and fractionation have emanated on the basis of therapeutic activity. The standard fraction of an active extract or mixture of fractions may prove better therapeutically, less toxic and inexpensive compared to pure isolated compound drugs. However, crude plant preparations require modern standards of safety and efficacy. Modern bioassay methods and physiochemical profile do provide ways and means of developing quality control as well as determining the expiry date of crude preparations or fractions. Standardized herbal preparations may serve as inexpensive and useful drugs to the masses.

Herbal drugs have gained importance in recent years because of their efficacy and cost effectiveness. These drugs are invariably single plant extracts or fractions thereof or mixtures of fractions of extracts from different plants which have been carefully standardized for their safety and efficacy (Suck Dev 1997).

1.5. Traditional Wisdom

Lag phase for botanical medicine is now rapidly changing for a number of reasons. Problems with drug resistant microorganisms, side effects of modern drugs, and emerging diseases where no medicines are available, have stimulated renewed interest in plants as a significant source of new medicines. Pharmaceutical scientists are

experiencing difficulty in identifying new lead structures, templates and scaffolds in the finite world of chemical diversity. A number of synthetic drugs have adverse and unacceptable side effects. There have been impressive successes with botanical medicines, most notably quinghaosu, artemisinin from Chinese medicine. Considerable research on Pharmacognosy, Chemistry, Pharmacology and Clinical therapeutics has been carried out on Ayurvedic medicinal plants. Numerous molecules have come out of Ayurvedic experimental base, examples include Rawolfia alkaloids for hypertension, psoriases in vitiligo, hypolipidemic agents, mucana pruriens for Parkinson's disease, piperidines as bioavailability enhancers, baccosides in mental retention, pierosides in hepatic protection, phyllanthins as antivirals, curcumine in inflammation and many other steroidal lactones and glycosides as immunomodulators. A whole range of chronic and difficult to treat diseases such as cancers, cardiovascular diseases, diabetes, rheumatism and AIDS all require new effective drugs. Most developing countries have relied and will continue to rely on traditional natural medicines due to the deterrence of high costs of modern allopathic medicines (Patwardhan et al.,)

Current estimation indicates that about 80% of people in developing countries still rely on traditional medicine based largely on various species of plants and animals for their primary healthcare. Four out of ten Americans used alternative medicine therapies in 1997. Total visits to alternative medicine practitioners increased by almost 50 from 1990 and exceeded the visits to all US primary care physicians (Suck Dev 1997).

1.6. Cancer

Cancer is a general term applied to a series of malignant diseases which may affects many different parts of the body. These diseases are characterized by rapid and uncontrolled formation of abnormal cells which may mass together to form a growth or tumour, or proliferate throughout the body. Initiating abnormal growth at other sites, if the process is not arrested, it may progress until it causes the death of the organism. Cancer is commonly encountered in all higher animals, and plants also develop growth that resembles cancer. Next to heart disease, cancer is a major killer of mankind. Cancer is basically a disease of cells characterized by the loss of normal cellular growth, maturation and multiplication, and thus homeostasis is disturbed.

Carcinogens

Carcinogens, the agents that cause cancer, have been classified into three broad groups' *viz* physical, chemical and biological.

Physical agents

Ultraviolet and ionizing radiations are mutagenic and carcinogenic, which may damage DNA in several ways. Ultraviolet radiation catalyzes the formation of covalent pyrimidine dimer, thus distorting the A-T, C-G bases pairing sequence in DNA strand. Ionizing radiation (X-rays, G-rays) can break the backbone of DNA molecule either by altering the base sequences structurally or by deletion of the bases from the backbone. Apart from direct effects on DNA, X-rays and G-rays cause free radicals to form in the tissues which can lead to oxidative DNA damage.

Chemical agents

It is estimated that 80% of human cancers are due to environmental factors, principally chemicals. A variety of compounds *viz*., polycyclic aromatic hydrocarbons, nitrosamines, alkylating agents and other inorganic and naturally occurring compounds are carcinogenic. Generally all carcinogens are electrophiles which attack nucleophilic groups in the DNA and RNA and proteins and thus damage the cell.

Many of the alkylating agents can act directly on the target molecules (direct carcinogens) but there are other compounds, which cannot act directly and require prior metabolism to become carcinogens (procarcinogens). Thus chemical carcinogens can be classified as

- Initiating agent – which is capable of initiating cells only.
- Promoting agent – capable of causing the expression of initiated cell clones.
- Progressor agent – which can convert initiated cell or a cell in the stage of promotion to a potentially malignant cell.
- A complete carcinogen has all the properties of initiating, promoting and progressor agents.

Another potential source of exogenous transforming mutations are certain biochemical processes which generate significant quantities of reactive oxygen species (ROS) and free radicals that are estimated to cause alterations in the DNA. Therefore, processes inherent in the cell and not necessarily dependent on exposure to exogenous agents may cause carcinogenesis. But it must be emphasized that environmental carcinogens significantly increase the risk of cancer and carcinogens do accelerate the process of carcinogenesis. The interaction of an "ultimate" carcinogen (i.e. the active electrophile) with DNA targets determines the formation and permanent fixation of transforming lesions.

Biological agents

The oncogenic viruses are well known and form a very diverse group of carcinogenic agents. They include members of all major families of DNA viruses that infect vertebrates except very small Parvovirus's and the very large Poxviruses. On the other hand, only one family of RNA viruses, the retroviruses, can cause tumors. The tumor viruses vary in complexity of their genomes, in the types of neoplasm they induce and in their requirement for co-factors in tumor genesis. RNA tumor viruses are characterized by the presence of RNA-dependant DNA polymerase (reverse transcriptase). Once the virus core enters the cell, the reverse transcriptase transcribes the single stranded RNA viral genome into double stranded DNA copies. The original viral RNA becomes degraded and the viral DNA copy (provirus) is then inserted (integrated) by a covalent linkage into the host cell DNA.

Endogenous DNA damage from normal oxidation is enormous. The steady state of oxidative damage in DNA is about one million oxidative lesions per rat cell. This high background suggests that increasing the cell division rate must be a factor in converting lesions to mutations and thus cancer. Raising the level of either DNA lesions or cell division will increase the probability of cancer. Just as DNA repair protects against lesions, guards the cell cycle and protects against cell division if the lesion level gets too high. If the lesion level becomes still higher, can initiate programmed cell death (apoptosis).

1.7. Main Features of Cancer

- Excessive cell growth, usually in the form of tumor.
- Invasiveness, i. e. the ability to grow into surrounding tissue.

- Undifferentiated cells or tissue.
- The ability to metastasize or spread to new sites and establish new growth;
- A type of acquired heredity in which the progeny of cancer cells also retain cancerous property.
- A shift of cellular metabolism towards increase in production of macromolecules from nucleosides and amino acids, with an increased catabolism of carbohydrates for cellular energy. Such behavior of cancer cells lead to illness in the host as a result of

❖ Pressure effect due to local tumor growth;
❖ Destruction of the organ involved by the primary growth;
❖ Systemic effect as a result of new growth.

1.8. Causes of Cancer

Many factors are implicated in the causation of cancer. These factors are listed as

- Exposure to the carcinogenic hydrocarbons or to excessive radiation.
- Hereditary factors: A "cancer family syndrome" has been described by the Lynch *et al.,* The hereditary factors involved in the causation of cancer are chromosomal abnormality, enzymes, immune defence system, hormonal imbalance etc.
- Cultural factors: cultural factor play a dominant role by causing about 70 % of all cancers. The important amongst are diet, smoking, drinking and sexual habits.
- Occupational factors: These factors are ionizing radiation, chemicals and other substances for example- coal tar, mustard gas, chromium, hematite, nickel and asbestos can trigger lung cancer in employees working in chemical, insulation and gas factories.
- Viruses: Though it is known that viruses cause cancer in animals, their role in human cancer has not been proved.

1.9. Cell Cycle

Cellular multiplication involves passage of cell through a cell cycle. The various phases of cell cycle are characterized as:

- The interval following cell division to the point where DNA synthesis starts, known as the presynthetic phase G_1.

- After mitosis some of the daughter cells pass into a resting phase or non proliferative phase G_0 and do not re-enter the cell cycle phase G_1 immediately. They may enter the G_1 phase later.
- DNA synthesis phase (S).
- The premitotic or postsynthetic (G2) phase fallows. In this phase RNA and protein synthesis takes place.
- Mitotic phase (M) follows.

1.10. Criteria for Antineoplastic Drug (Anticancer drug)

The term 'anticancer drug' is emotive and can build up false hopes among cancer sufferers. Investigator in the United States National Cancer Institute (NCI) program have used the term cytotoxic, antitumour and anticancer to describe the activity of the compound isolated according to the following definitions. A cytotoxic agent is toxic to tumour cell *in- vitro* and if this toxicity transfers through the tumour cell *in vivo*, the agent is said to have antitumour activity. The term anticancer is reserved for material, which are toxic to tumour cell in clinical trials.

1.11. An antineoplastic drug should be:

(1) Cytotoxic: To inhibit the cancer cell metabolism, particularly synthesis of protein and nucleic acid, in order to prevent cell growth, differentiation, vascularization of the new growth etc.

(2) Mitostatic: To disrupt the process of cell division, to prevent the uncontrolled number of cycles of cell division and growth, to retard the proliferation of the cancerous tissue.

(3) Nontoxic: The drug should be nontoxic to the rest of the body of the patient; it should not cause any side effect such as renal or hepatic dysfunction, neurotoxicity, hypersensitivity etc.

(4) Target oriented: It should be site-selective targeting in action to the cancerous region and not cause the same effect (or at least not cause them to the same degree as on cancer) on the other part of patient's body.

The drug should be effective in small and few doses, should not be expensive, should have longer half-life, freely available on the market, etc., These criteria for an anticancer drug are a tall order. Hardly there is any drug, synthetic or natural, that meets with all these qualification and so the choice is dictated by the maximum

compliance of the criteria and the philosophy of the '*Lesser evil'*. With the presence of a large number of different types of cancer, each a kind of a syndrome, no single drug can be expected to be effective against more that are, at the best a few of related cancers (Barar 2003; Singh & Lippman 1998).

1.12. Plants in the Treatment of Cancer

In the face of failure to find synthetic drugs against cancer, thousands species of plants have been screened since a long time, for antineoplastic activity, in the hope of discovering effective natural products. Compounds have been isolated from hundreds of species and their activities in suppressing tumours induced in laboratory animals have been evaluated. Such work is still going on in several laboratories throughout the world. The Natural Product Drug Development Program of the U.S. National Cancer Institute has identified about 3,000 species of plants and animals as useful in dealing with one or the other aspect of cancer management. Basing on *in vitro* data, a large number of species have been identified to be of promise and taken to clinical trials. However, products of hardly a handful of plant species, such as the Vinca alkaloids, taxol, camptothecin, podophyllotoxin, etc., have passed through the rigorous tests to be officially used against certain types of cancer and are now available in the market.

1.13. Plants in the management of cancer

In addition to the handling of the cancer proper, a host of synthetic or plant based drugs are used in the biomedical system in the management of cancer, which is distinct from treatment aimed at a cure. The better hope of usefulness of plants lies in the areas of detection, prevention, management of symptoms inherent in the disease or incidental to the treatment, post-cure management such as the recovery of the body to full and normal functioning, prevention of remission, and management of symptoms of incurable cancers, to keep the patients in the maximum possible comfort.

1.14. Plants in the prevention of cancer

There is a prevailing hope that 'someday people should be able to avoid cancer or delay its onset by taking specially formulated pills or foods. Chemoprevention is the attempt to use natural and synthetic compounds to intervene in the early pre-cancerous stages of carcinogenesis, before the invasive disease begins, as prevention of cancer is immensely better than its uncertain cure.

Food has been identified as one of the most promising sources of chemopreventive agents. These include vitamins A (and its analogues), C and E, which are obtained by us only from plants. Some plant products without any recognised nutritional value such as indoles, isothiocyanates, dithiolthiones and organosulphur compounds have been shown to be chemopreventive. Dithiolthiones and organosulphur compounds are abundant in broccoli, cauliflower and cabbage. Genistein from soyabean, and epigallocatechin, the bulk of solid material in brewed tea have also been found to be chemopreventive, as well as turmeric, ginger and saffron.

Among the plant-based chemopreventives, β-carotenes, the precursors of vitamin A, are rated high. In addition to carrots, they are present in a large number of plants, particularly abundant in the leafy vegetables. These food plants also provide the dietary fibre that is believed to prevent colon cancer. It is not yet very clear how the chemopreventive agents function. Some are believed to prevent the mutations that can lead to cancer, some halt the process of excessive proliferation of altered cells, and some hasten apoptosis (death of cells) of altered cells, while some function as antioxidants and scavenge the free radicals that may trigger cancer (VandeCreek et al., 1999).

1.15. Cancer Chemotherapy

The chemotherapy of neoplastic disease has become increasingly important in recent years. An indication of this importance is establishment of a medical specialty in oncology in which the physician practices various protocol of adjuvant therapy. Most cancer patient now receives some form of chemotherapy, even though it is merely palliative in many cases. The relatively high toxicity of most anticancer drugs has fostered the development of supplementary drugs that may alleviate these toxic effects or stimulate the regrowth of depleted normal cells. There is a cogent reason why cancer is more difficult to cure than bacterial infections. One is that there are qualitative differences between human and bacterial cells. For example bacterial cell have distinctive cell walls, and their ribosome differ from those of human cells. In contrast, the differences between normal and neoplastic human cells are mostly quantitative. Another difference is that immune mechanism and other host defences are very important in killing bacteria and other foreign cells, whereas they play a lesser role in killing cancer cells.

1.16. INDIAN SCENARIO

The global context sketched above suggest several tremendous opportunities for India, a country with unrivalled terms of diversity of medicinal systems and practices, in addition to being a major storehouse of biological diversity, with 2 of the 4 mega diversity areas of the world located within its borders. In addition several concerns arise in relation to the current consequences of participation in the market, with regard to the sustainable and equitability of prevailing practices in the sector.

To add to all these aspects, the market in India has been shown to be highly inefficient and imperfect. The need of the hour then is to replant India's participation in the expanding global market. Such an overview could form the basis of a renewed development of India's medicinal plant sector, and a strategic exploitation of other comparative advantage in the global market on a sustainable and equitable basis.

1.17. Drug designing for cancer

In designing specific regimens for clinical use, a number of factors must be taken into account. Drugs are generally more effective in combination and may be synergistic through biochemical interactions. These interactions are useful designing new regimens. It is more effective to use drugs that do not share common mechanisms of resistance and that do not overlap in their major toxicities. Drugs should be used as close as possible to their maximum individual dose and finally, drugs should be used as close as possible to discourage tumor growth and maximize dose intensity (the dose gives per unit time, a key parameter in the success of chemotherapy). Based on experimental tumor models, it is necessary to eradicate all tumor cells. The fraction of cells killed with each treatment cycle is constant, with regrowth between cycles. Thus, it is desirable to achieve maximal cell kill with each cycle, using the highest drug dose possible, and to repeat dose as frequently as tolerated. Since the tumor cell population in patients with visible disease exceeds 1gm, or 10^9 cells, and since each cycle of therapy kills less than 99% of the cells, it is necessary to repeat treatments in multiple cycles to kill all the tumor cells.

The activity of many of the drugs currently used in cancer chemotherapy can probably be ascribed to inhibition of nucleic acid synthesis, but mechanism of action differs widely. Some compounds are mitotic inhibitors for example colchicine, podophyllotoxin, vincrystine and maytansine, and they act by binding to the protein

tubulin in the mitotic spindle, preventing polymerization and assemble into microtubules, and after cell division, the microtubules are transformed back to tubulin.

Although podophyllotoxin is a tubulin binder, it is intriguing that the semisynthetic anticancer drugs etoposide and teniposide derived from it have a different mode of action. These drugs inhibit DNA synthesis and replication via the enzyme topoisomerase II. Camptothecin derivatives, topotecan and irinotecan, exert their cytotoxic action through inhibition of topoisomerase I system. Topoisomerase are fundamental enzyme complex involved in DNA replication by their ability to break and reseal the DNA strands.

With the identification of an increasing number of molecular targets associated with particular cancers, high throughput screening of compounds against a range of such targets now forms the basis of anticancer drug discovery. Examples are the cyclin dependant kinases, which, together with their cyclin parameters, play a key role in the regulation of cell cycle progression, and inhibition of their activity delays or arrest progression at specific stages of cell cycle. These are over 2000 kinases so for identified for genomic studies and all have a common site, the position where the ATP, that is, the source of phosphate that is donated, is bound. The moderately antitumor flavonoids, quercetin, is an early example of natural product compound class that ultimately led to CDK inhibitors. This flavonoids resembles an ATP mimic where the planar bicyclic chromone ring system is an isostere of adenine. Quercetin exerts its antitumor effect through blocking cell cycle progression at the G0/G1 interface, consistent with Cyclin dependant kinase inhibition.

Taxol is a naturally occurring highly derivatised diterpene belonging to taxane group of compounds present in genus *Taxus* under family Taxaceae. A derivative of taxol-taxofere has been reported to have better bioavailability and pharmacological properties. The bio target of taxol is microtubule responsible for formation of mitotic spindle necessary for cell division which causes detrimental effects leading to blockage of cell cycle (Wilson and Gisvold 2004).

2. Plant Profile

(Wealth of India, 2003 [Anon]; Devasagayam 2007; Wealth of India, CSIR, 1969; Indian Medicinl Plants [Kitikar & Basu] 1975, 1987, 1999; The Flora of Orissa; Saxsena & Brahmam, 1994; Sharma et al., 2005; Sharma 2003; Swain & Das 2007; Nadkarni 1976; Kapoor, 1989).

Botanical Name: *Pterocarpus marsupium* Roxb.
Botanical Source: The plant consists of the bark, leaves, heartwood of *Pterocarpus marsupium P. marsupium*

Order: Fabales

Family: Leguminosaa (Fabaceae)

Subfamily: Faboideae

Tribe: Dalbergieae

Genus: *Pterocarpus*

Species: *marsupium*

Authority: Roxb.

Vernacular names: (Sharma 2003; Swain & Das 2007)

1. Sanskrit: Pitasala, Bijaka, Murga
2. Hindi: Bijasal
3. English: Malabarkino; Indian Kino Tree
4. Bengali: Pitsal
5. Nepalese: Bijasar
6. Sinhalese: Gammalu
7. German: Malabarkino
8. Kannada: Honne
8. French: Pterocarp
9. Unani: Dammul-akhajan
10. Arabian: Dammul Akhwayn
11. Persian: Khoon-e-siyaun-shan
12. Tamil: Vengai
13. Telugu: Yegi
14. Malayalam: Venga

P. marsupium, also known as Malabar kino, (Gamble, 1935) Indian kino tree or vijayasar, is a medium to large, deciduous tree that can grow up to 30 metres tall. It is

native to India, Nepal, and Sri Lanka, where it occurs in parts of the Western Ghats in the Karnataka-Kerala region and also in the forests of Central India. Parts of the Indian kino (heartwood, leaves, and flowers) have long been believed to have medicinal properties in Ayurveda (The Flora of Orissa, Saxsena & Brahmam, 1994). In Karnataka the plant is known as honne or kempu honne. The Kannada people in India make a wooden tumbler from the heartwood of this herb tree (Saldanha, Flora of Karnataka, 1984).

Bark of *Pterocarpus marsupium marsupium*

Leaves of *Pterocarpus*

2.1 Habitat

A moderate to large deciduous tree about 90ft or more high, commonly found in hilly region of central and peninsular India (Andhra Pradesh, Bihar, Gujarat, Kerala, Madhya Pradesh, Maharashtra, Karnataka, Orissa, Tamilnadu, Uttar Pradesh); found at 3000 ft in Gujarat, Madhya Pradesh and Himalayan & sub Himalayan tracts-Nepal (Kapoor, 1989) and Sri Lanka. It grows on a variety of formation provided the drainage is good. It prefers a soil with a fair proportion of sand though it is often found on red loam with a certain amount of clay. The normal rainfall in its natural

habitat ranges from 75 to 200cm but it attains its largest size in parts of Mysore and Kerala, where the rainfall is even higher. It is a moderate light demander and the young seedlings are frost-tender (Wealth of India, CSIR, 1969).

Parts used: Bark, Leaves, Kino (gum)

2.2 Pharmacognostical Characteristics

Morphology

It is a moderate-sized to large deciduous tree. bark grey, longitudinally fissured and scaly. The older trees exude a blood red gum-resin.

Description

Leaves: compound; with 5 to 7 leaflets, 3 to 5 in long, oblong or elliptical with wavy margin or rounded or obtuse or retuse ends, glaucous beneath, secondary nerves close and parallel, over 12 cm each side.

Flowers: yellow, , up to 1.5 cm long, corolla papilionaceous, exserted beyond calyx, Stamen 10, split in 2 bundles , yellow, in very large, dense bunches.

Fruits: 2 to 5 cm long, roundish, winged, with one seed. Legume indehiscent, orbicular, compressed, broadly hardened winged around margin, usually single seeded, seeds subreniform, hilum small.

The Heartwood: is golden to yellowish brown with dark streaks staining yellow when damp and turning darker on exposure, strong and tough.

2.3 Microscopic Characteristics

The wood consists of vessels, tracheids, fibre tracheids and wood parenchyma all the elements being lignified and filled with tannin. Vessels are medium sized drum shaped, scattered, leading to semiring-porous conditions, tyloses present. Tracheids are long, abundant, thick walled, with tapering ends and simple pits on the side walls. Xylem parenchyma is small, thick walled with blunt ends; rectangular simple pitted surrounding the vessel. A few crystal fibers are observed in tangential section of the wood. Tree bark yields a reddish gum known as Kino gum, which becomes brittle on hardening and is very astringent. Sclerenchyma diffused pores Red marks are resin canals 8 Stem hairs overlapping metaxylem and protoxylem.

2.4 Chemical Constituents

Researches in the past have established the genus *Pterocarpus* to be the rich sources of polyphenolic compounds. All active principles of *P. marsupium* are thermostable.

The primary chemical components of P. marsupium are pterosupin, pterostilbene, isoliquiritigenin, liquiritigenin, epicatechin, kinotannic acid, kinoin, kino-red beta-eudesmol,
marsupol, carpusin and marsupinol.

The plant contains pterostilbene 4-5%, alkaloids 0.4%, tannins 5%, protein, pentosan, pterosupin, pseudobaptigenin, liquiritigenin, isoliquiritigenin, garbanzol, 5-de-oxykaempferol, P-hydroxybenzaldehyde, beudesmol, erythrodirol-3-monoacetate, l-epicatechin, marsupol, carpusin, propterol, propterol B, marsupinol, irisolidone-7- O-A-L-rhamnopyranoside, have been obtained mainly from the heartwood and root.
The gum kino from the bark provides non-glucosidal tannins - kinotannic acid, kinonin (C28H24O12), kino-red (C28H22O11), pyrocatechin, pyrocatechin acid & small quantities of resin, pectin and gallic acid.

Aqueous extract of the heartwood of *Pterocarpus marsupium* contains 5 new flavonoids C-glucosides namely 6-hydroxyl-2-(4-hydroxybenzyl)-benzo-furan-7-C-â-D-glucopyr anoside, 3-(á -methoxy-4-hydro xybenzylidene) -6-hydroxybenzo-2(3H)-furanone-7-C-â- D-glucopyranoside, 2-glucopyranoside, 8-(C-â-D-glucopyranosyl)- 7,3,4-trihydroxyflavone and 1,2-bis (2,4-dihydroxy, 3-C-glucopyranosyl) – ethanedione and two known compounds C-â-D-glucopyranosyl-2,6-dihydroxyl benzene and sesquiterpene were isolated Ether extract of the roots of Pterocarpus marsupium consists of a new flavonol glycoside 6-hydroxy-3,5,7,4-te tramethoxyflavone 6-O-rhamnopyranoside, 8-hydroxy-4'-m ethoxyisoflavone-7-O-glucopyranoside.

A benzofuranone derivative 2,4'6-trihydroxy-4-methoxy benzofuran-3(2H)-one designated carpus in, 1,3-bis(4-hydroxyphenyl) propan-2-ol designated propterol, 1-(2,4-di hydroxyphenyl)-3-(4-hydroxyphenyl) propan-2-ol designated propterol, 6-hydroxy-7-O- methyl-3-(3-hydroxy-4-O-methyl benzyl) chroman-4-one. Ethyl acetate extract of root contains benzofuranone, marsupin, dihydrochalcone, pterosupin, stilbene, pterostilbene, aliquiritigenin, isoliquiritigenin.

Methanolic extract of heart wood c ontains an isoflavone 7-O-á-L-rhamno-pyranosyloxy-4'-methoxy-5-hydroxy-isoflavone. Three new isoflavone glycosides viz retusin 7-glucoside, irisolidone 7-rhamnoside and 5,7-dihydroxy-6-methoxy

isoflavone 7-rhamnoside have been isolated from the heartwood of Pterocarpus marsupium. 2,6-dihydroxy-2-(P-hydroxybenzyl)-3(2H)-benzofuran-7-C-â-D-glucopyranoside (Maurya et al., 2004; Gairola et a., 2010; Yogesh et al., 2010; Tiwari & Khare 2015).

2.5 Ethnomedicinal Uses (Tiwari et al., 2015)

Useful parts of the herb are heartwood, leaves, flowers, gum. The genus is widely distributed on the Earth and the astringent drug from this genus is known as "Kino". The phloem of stem contains red astringent fluid present in secretory cell, which exudes after given incision. Kino is odourless but has astringent taste and sticks in the teeth, colouring the saliva red in colour. As astringent it is used in diarrhoea, dysentery etc.

Bruised leaves are applied on fractures, leprosy, leucoderma, skin diseases, sores and boils, Constipation, depurative, rectalgia, opthamology, hemorrhages and Rheumatoid arthritis. Marsupin and Pterostilbene significantly lower the blood glucose levels useful in NIDDM. Bark is used as diuretic in Gabon and fresh leaves are used as food in Nizeria. Also is used in the form of powder or decoction in diarrhoea, and decoction is very useful for diabetic patients.

Stem in the treatment of neurological problems.

Leaves are used in GIT disorders, wood, stem bark, seed and flours are used in African traditional medicine, especially in the Cameroonian pharmacopoeia, for treating various diseases including hypertension, diabetes, intestinal parasitizes, renal and cutaneous diseases. The leaf paste is used as an ointment to treat skin diseases, sores and boils.

Wood: The heartwood is used as an ointment to astringent, bitter, acrid, cooling, anti-inflammatory, union promoter, depurative, urinary astringent, haemostatic, asthelmintic, constipating, anodyne alterant and rejuvenation. It is also useful in elephantiasis, inflammations, fractures bruises, leprosy, skin disease, leucoderma, erysipelas urethrorrhoea, diabetes, rectalgia, rectitis, opthalmopathy, diarrhea, dysentery, cough, asthma, bronchitis and greyness of hair.

Flower: The flower is used as appetizing and febrifuge and also taken to treat anorexia and fever.

Gum-resin: The gum is taken to treat bitter, styptic, vulnerary, antipyretic, anthelmintic and liver tonic. It is useful in spasmodic gastralgia, boils, gleet, urethrorrhoea, odontalgia, diarrhea, psoriasis, wound and ulcers, helminthasis, fevers, hepatopathy and ophthalmia.

Some facts: *P. marsupium* is a plant drug belonging to a group called '*Rasayana*' in Ayurvedic system of medicine. These '*Rasayana*' drugs are immunomodulators and relieve stress in the body. In India, Kannada peoples are used to make a wooden tumbler from the heartwood. Water is left overnight in the wooden tumbler and is consumed in the next morning to cure diabetes. Kol tribes in Odisha pound a paste mixture of the bark of *P. marsupium* with the bark of *Mangifera indica, Shorea robusta & Spondias pinnata* to treat some dysentery illness. The gum resin of this plant is the only herbal product ever found that regenerate beta cells that produce insulin in pancreas.

2.6 Biological activity

Although a large number of compounds have been isolated from various parts of *P. marsupium*, few of them have been studied for biological activity as shown in Table 1. The structure of some of these bioactive compounds has been presented in Figure 1. The bark contains l-epicatechin and a reddish brown colouring matter. The bark is occasionally employed for dyeing. The heartwood yields liquiritigenin, isoliquirtigenin, a neutral unidentified component, alkaloid and resin. The wood also contains a yellow colouring matter and an essential oil and a semi-drying fixed oil.

The tree yields a gum-Kino which exudes when an incision is made through the bark up to the cambium. It is odourless and bitter with astringent taste and colours saliva pink when masticated. Kino contains a non-glucosidal tannin kinotannic acid, kinoin and Kino-red, small quantities of catechol, protocatechuic acid, resin, pectin and gallic acid. The therapeutic value of Kino is due to Kino is due to kinotannic acid. Kino is powerfully astringent and was formerly used widely in the treatment of diarrhea and dysentery. It is locally applied in leucorrhoea and in passive haemorrhages. It is also used for toothache. The bark is used as an astringent and in toothache. The flowers are said to be used in fever. The bruised leaves are considered useful as an external application for boils, sores and skin diseases. The aqueous

infusion of the wood is said to be of use in diabetes and water stored in vessels made of the wood is reputed to have antidiabetic qualities (Anon, Wealth of India, 2003).

2.7 Medicinal use of various parts of *P.marsupium*

Various parts of the *P. marsupium* tree have been used as traditional ayurvedic medicine in India from time immemorial. The medicinal utilities have been described, especially for leaf, fruit and bark. The bark is used for the treatment of stomachache, cholera, dysentery, urinary

complaints, tongue diseases and toothache. The gum exude 'kino', derived from this tree, is used as an astringent (Singh et al., 1965). The gum is bitter with a bad taste. However, it is antipyretic, anthelmintic and tonic to liver, useful in all diseases of body and styptic vulnerant and good for griping and biliousness, opthalmiya, boils and urinary discharges. The flowers are bitter, improve the appetite and cause flatulence (Indian Medicinal Plants 1999). *P. marsupium* has a long history of use in India as a treatment for diabetes. It is a drug that is believed to have some unique features such as beta cell protective and regenerative properties apart from blood glucose reduction (WHO 1980; Chakravarthy et al., 1981). Some of the medicinal attributes of various parts of *P. marsupium* have been summarized (Yogesh et al., 2010) in table 2.

3. Literature Review

3.1. Anti-diabetic and antioxidant activity

P. marsupium demonstrates unique pharmacological properties, which include beta cell protective and regenerative properties as well as blood glucose lowering activity. The animal studies conducted have used various species including rats, dogs, and rabbits with induced diabetes and subsequent treatment with various extracts of *P. marsupium*. In all of these studies, *P. marsupium* was found to reverse the damage to the beta cells and actually repopulate the islets, causing a nearly complete restoration of normal insulin secretion (Chakravarthy et al., 1982; Manickam et al., 1997; Ahmad et al., 1991; Pandey & Sharma 1976; Shah 1967; Chakravarthy et al., 1982).

In one study it was shown that aqueous extract of *P. marsupium* modulates the inflammatory cytokine TNF-alpha in type 2diabetic rats and this has an indirect effect on PPAR-Gamma expression. By decreasing TNF-α, drug can upregulate the PPAR-Gamma and in turn the glucose metabolism (Halagappa et al., 2010).

The bark of *P. marsupium* is traditionally used in the Indian Ayurvedic system of medicine as an anti-diabetic drug. The compound that is responsible for antidiabetic activity is (-) epicatechin, a member of the catechin group of compounds belonging to the class of flavonoids (Zaid et al., 2002).

It has been shown that *P. marsupium* works by the regeneration of the beta cells and increase proinsulin biosynthesis. Marsupin and Pterostilbene significantly lowered the blood glucose level of hyperglycemic rats, and the effect was comparable to that of 1,1-dimethyl biguanide (metformin) (Manickam et al., 1997).

Overnight water stored in water tumblers made out of the heartwood of *P. marsupium* is used as a traditional therapy for patients of Diabetes mellitus especially in the state of Madhya Pradesh (Maheswari et al., 1980).

Isolated compounds from *P. marsupium* have been shown to enhance the conversion of Pro-insulin to insulin and stimulate cAMP content in the islets of Langerhans (Ahmad et al., 1991).

It is proposed that the flavonoid fraction of *P. marsupium* bark effectively reverses the alloxan induced changes in the blood sugar level and the beta cell population in the pancreas (Chakravarthy et al., 1980).

P. marsupium methanol extract has been found to cause normalization of serum protein and albumin levels, possibly through the increase in insulin mediated amino acid uptake, enhancement of protein synthesis and inhibition of protein degradation (Dice et al., 1978).

Administration of the bark extract to diabetic rats restored the levels of serum electrolytes, glycolytic enzymes and hepatic cytochrome p-450 dependent enzyme systems by inhibiting the formation of liver and kidney lipid peroxides (Gayathri and Kannabiran et al., 2010).

3.2. Cardiotonic activity

Cardiotonic activity was reported of the aqueous extract of heartwood of *P. marsupium*. This plant species contains 5,7,2-4 tetrahydroxy isoflavone 6-6 glucoside which are potent antioxidants and are believed to prevent cardiovascular diseases. The cardiotonic effect of the aqueous extract of heartwood of *P. marsupium* was studied by using the isolated frog heart perfusion technique. Calcium free Ringer solution was used as vehicle for administration of aqueous extract of *P.marsupium* as a test extract and digoxin as a standard (Mohire et al., 2007). Liquiritigenin and Pterosupin, the flavonoid constituents of *P. marsupium* are effective against reducing serum cholesterol levels, LDL cholesterol, and atherogenic index. Pterosupin being additionally effective in lowering serum triglycerides (Jahromi and Ray.,1993**).**

3.3. Hepatoprotective activity

Methanol extract of the stem barks of *P. marsupium* possesses significant hepatoprotective activity (Mankani et al., 2005).

3.4. Antioxidant activity

The whole aqueous extract of the stem bark of *P. marsupium* showed high anti-oxidant activity and protects the mitochondria against oxidative damage (Mohammadi et al., 2009).

Heartwood extracts of *P. marsupium* promotes wound healing in both normal and diabetic animals by topical application of the extracts (Singhal et al., 2013).

Ethanol extracts of the heartwood of *P. marsupium* is found to be useful in preventing allergic conditions and diseases such as asthma owing to its ability to decrease the increased eosinophilic , leucocytic count, prevention of mast cell degranulation (Suralkar et al., 2012).

Antidiarrhoeal activity of Ethanol heartwood extract of *Pterocarpus marsupium* was also studied (Dilpesh et al., 2011).

3.5. Antibacterial activity

Hexane, ethyl acetate and methanol extracts were tested against four selected Gram positive and Gram negative bacteria- *S. aureu*, *K. pneuminiae*, and *P. aeruginosa* (Sapha 1956; Gayathri and Kannibaran ., 2010). *In vitro*, it inhibits *Pseudomonas aeruginosa*, *Streptococcus pyrogens* and *Staphylococcus aureus*. Ethyl and methanol extracts were more sensitive to the bacteria than extracts made out of hexane. Both the extracts exhibited concentration dependent variation in their anti-bacterial activity. Similar observations have been reported where it has been showed that ethanol extracts of *P. marsupium* exhibited significant anti-ulcer and antioxidant properties in rats (Nair et al., 2005; Patil & Gaikwad, 2011).

3.6. Anti-inflammatory activity

P. marsupium has also shown strong potential for its antiinflammatory activity. In this study, an extract of *P. marsupium* containing pterostilbene has been evaluated for its PGE2- inhibitory activity in LPS-stimulated PBMC. In addition, the COX-1/2 selective inhibitory activity of *P. marsupium* extract was investigated (Hougee et al., 2005; Salunkhe et al., 2005).

3.7. Central Nervous System

The methanol extracts of *P. marsupium* has potent nootropic activity (Chauhan and Chaudhary, 2012).

3.8. Other Studies

Anti-cataract activity of *P. marsupium* in diabetes was observed (Vats et al., 2004).

Lukewarm aqueous suspension of 2g gum with jaggery is given early in the morning for a week to treat asthma (Patil et al., 2008).

Bark is useful in vitiated condition of kapha, pitta, elephantiasis, erysipelas, urethrorrhea, rectalgia, opthalmopathy, hemorrhages, dysentry, cough, and grayness of hair (Patil and Gaikwad., 2011).

20g of the stem bark boiled with 1 litre of water till 200ml along with 7 black pepper dried seeds of *Piper nigrum*) taken orally cures spermaturia, spermatorrhea, leucorrhoea, amenorrhoea, dysmennorhoea, menorrhagia and impotency (Behera and Mishra ., 2005).

Table 1. Primary chemical components from *Pterocarpus marsupium*

Neem compound	Source Biological	Reference
Liquiritigenin bark	Antidiabetic, Antihyperlipidemic effect	Jahromi & Ray 1993
Isoliquiritigenin bark	Antidiabetic	Jahromi & Ray 1993
Pterosupin	Antihyperlipidemic effect	Jahromi & Ray 1993
Epicatechin bark	Antidiabetic, Anthelmentic properties	
Pterostilbene bark	blood glucose levels, Anti-oxidant and anti tumor effects	Grover et al., 2005
Marsupinol bark	Antihyperlipidemic effect	Jahromi & Ray 1993

Table 2. Some medicinal uses of *Pterocarpus marsupium* as mentioned in Ayurveda

Parts	Medicinal use
Leaf	External application for boils, sores and skin diseases, stomach pain
Bark	Astringent, toothache
Flower	Fever
Gum-Kino	Diarrhea, dysentery, leucorrhoea, passive haemorrhages

3.9. Clinical studies and plausible medicinal applications

Although studies have been carried out on various biological activities of *P. marsupium* extracts and some of the isolated compounds in several animal models, a few reports are available on clinical studies with the extracts or the compounds and their medicinal applications (Anon, Wealth of India, 2003). *Pterocarpus marsupium* (PMS, *Leguminnaceae* family), commonly known as Bija, that has been

recommended as early as 1000 BC, by Sushruta for the treatment of diabetes. Various reports indicate the hypoglycemic activity of PMS both in experimental and clinical studies (Pandey & Sharma 1976; Remsberg et al., 2008; Manlio et al., 2005).

4. Aim and objective of the study

Since pre-historic days attempts are being made to find out suitable drugs from natural sources for treatment of different diseases. The rational approaches, experience of folk medicine provide a valuable approach in the search for the development of new and useful therapeutic agents. Gradually keeping in pace with the scientific interpretations of the drug actions, the causes of the diseases, and the development in the field of chemistry and technology, intensive efforts are being directed towards the design and synthesis of new drugs.

More recently a study by the World Health Organization (WHO) has shown that about 80% of the World's population still relies on traditional medicine. This is of interest to a natural product chemist for many reasons. There is the possibility that the herb used in the traditional medicine is harmful to the patient, in which case the treatment may do more harm the good. Conversely, there is the possibility that the herbs used are not effective at all. That may not be of concern for minor ailments, but in more serious cases an ineffective treatment could result in the death of the patient. Hopefully, however, the herbs used are effective. If that is the case then investigation of that remedy could be of benefit to the remaining 20% of the World's population.

Natural products still play a very important role in the medicine of the remaining 20% of the world's population. Between 1983 and 1994, 41% of new approved drugs have natural products as their source. This percentage becomes even higher when one examines anti-infective and anticancer compounds. For both classes, the percentage of drugs with natural products as their source increases to over 60%.

Because natural products are most important in the areas of anti-infective, antioxidants and anticancer agents, some of the important contributions to these drug classes are worth closer inspection. In particular the anticancer drugs will be examined as that is the area of our research.

Cancer is a multifactorial, multifaceted and multimechanistic disease requiring a multidimensional approach for its treatment, control and prevention. Cancer involves fundamental biological processes concerning disorganised cell replication, cell death and disorganization of organ structure. The annual estimates of cancer for the year

2001 in India is 0.98 million and the annual mortality in 2000 is 0.7 million. The incidence of cancer is on the rise, with multiple risk factors that involve interplay between genetic and environmental components.

The aim of the research is to find out new anticancer drugs from indigenous plants which are potent and nontoxic agents. These plants are traditional medicinal plants. Their chemical characterization, mode of action and toxicity studies are yet to be established. Present project deals with phytochemical and pharmacological evaluation of *of ethanol extract of Pterocarpus marsupium Roxb.* bark with special reference to *in-vitro* antioxidant studies (DPPH, ABTS, Nitric oxide and hydrogen peroxide) and *in vitro* cytotoxicity (MTTS) studies. Normally herbal products are free from side effects/adverse effect and they are low cost medicines, which will be beneficial for the people of this country. Keeping this in view, we have selected two plants from the Kolli hills, Namakkal District of Tamil Nadu which are used by the tribes for the treatment of different types of diseases. The main objective of this work is to develop potent anticancer agent having no minimum side effects from indigenous plants for the therapeutic management.

5. Plan of work

Phase I:
5.1. Phytochemical studies

- Collection and authentication of plants.
- Extraction of plant material by using various solvent systems.
- Preliminary phytochemical study for the identification of plant secondary constituents.

Phase II:
5.2. Pharmacological studies

- Evaluation of anticancer activity of ethanol extract of *Pterocarpus marsupium* ROXB. bark (EEPM) against Ehrlich Ascites Carcinoma (EAC) bearing Swiss albino mice.
- Evaluation of antioxidant enzymes and its parameters with special reference to cancer cell lines.

Phase III:
5.3. Statistical analysis

- The data's were presented as mean ± SEM and were subjected to statistical analysis by Dunnett's test followed by one way ANOVA. P-value less than 0.05 were considered statistically significant.

6. MATERIALS AND METHODS

6.1. Phytochemical Studies

Collection and Authentication of the Plant

The bark of *P. marsupium* were collected in the month of October 2015, from Kolli Hills, Namakkal district of Tamil Nadu state, India. The collected plants were authenticated by the Dr. G. Murthy, Botanical survey of India, Coimbatore, Tamil Nadu and the voucher specimen has been preserved in our laboratory for future reference (**BSI/SC/5/PG/15-16/OCT).**

6.1.1 Extraction of *Pterocarpus marsupium* bark

The barks of the tree were dried under shade with occasional shifting and made into coarse powder with a mechanical grinder. The powder material were first passed through sieve No.40 and then defatted with petroleum ether. The defatted powder materials thus obtained were further extracted by methanol for 72 hours in a Soxhlet extraction apparatus. The solvent was removed under reduced pressure and a semisolid mass was vacuum dried so yields a solid residue. After complete drying, the extract material was weighed and the extractive value in percentage was calculated with reference to the air dried sample. Thus an ethanol extract of *Pterocarpus marsupium* bark (EEPM) was obtained.

6.1.2. Preliminary phytochemical studies on ethanol extract of EEPM bark

The ethanol extracts were subjected to qualitative tests for the detection of various plant constituents like carbohydrates, glycosides, proteins and amino acids, fixed oils and fats, gums and mucilages, alkaloids, phytosterols, flavanoids, tannins and phenolic compounds, saponins, triterpinoids etc.

6.2 Pharmacological Studies

6.2.1. *In-Vivo* anticancer activity of *Pterocarpus marsupium* bark

Evaluation of anticancer activity of *Pterocarpus marsupium* against Ehrlich Ascites Carcinoma (EAC) bearing Swiss albino mice (Dacie & Lewis 1958; Wintrobe, 1961; D'Armour et al., 1965; Mazumder et al., 1997; Khanam et al., 1997; Gupta et al., 2000; Kakali et al., 2001; Ray et al., 2002; Rajkapoor et al., 2004; Gupta et al., 2004a; Gupta et al., 2004b; Sivakumar et al., 2005; Ojha et al., 2006; Ohkawa et al., 1979).

The present chapter deals with ethanol extract of *Pterocarpus marsupium* bark were evaluated for anticancer activity against Ehrlich Ascites Carcinoma (EAC) cells bearing Swiss albino mice.

6.2.1a Preparation of extract drug and mode of administration

For the present anticancer study we used two concentration of ethanol extract of *Pterocarpus marsupium* in the dose of 100mg/kg and 200 mg/kg. It was prepared in suspension form by dissolving the methanol extract of required quantity in sterile physiological saline containing 1% carboxyl methyl cellulose (CMC). This aqueous suspension of EEPM were administered intraperitonially in the dose of 100mg/kg and 200mg/kg throughout the experiment

6.2.1b Chemical and reagent

Vincristin (Oncocristin AQ, Sun Pharmaceutical India Ltd., Mumbai), Tryphan blue (Otta Kemi for Microscopy, Mumbai). 1-Chloro-2,4-dinitrobenzene (CDNB), Bovine serum albumin (Sigma chemical, St. Louis, MO, USA), Thiobarbituric acid, Nitro blue tetrazolium chloride (NBT) (Loba Chemie, Bombay, India), 5, 5'-dithiobis-(2-nitrobenzoic acid) (DTNB) (Sisco research laboratory, Bombay, India). Reduced glutathione, Ascorbic acid, 2,4- dinitrophenylhydrazine, ferric chloride (DNPH), 2,2-dipyridyl-*p*-phenylenediamine hydrochloride were obtained from S.D. Fine Chemicals, Mumbai, India. All the chemicals used in the present study are of analytical grade.

6.2.1c Animals

Studies were carried out by using adult Swiss albino mice weighing 20 $\pm$ 2 gm. They were obtained from Perundurai Medical College, Perundurai, Tamilnadu. The mice were grouped and housed in polypropylene cage containing paddy husk (procured locally) as bedding throughout the experiment and maintained under standard laboratory condition (Temp 25 $\pm$ 2^{o}C) and light (14 and 10 hr of light and dark, respectively). The animals had free access to the food and water the mice were acclimatized to laboratory condition for 10 days before commencement of the experiment. The study was approved by the Institutional Animal Ethical Committee of J.K.K. Nattraja College of Pharmacy, Komarapalayam.

6.2.1d Cancer cell line

EAC cells were obtained from Amala Cancer Research Center, Thrissur, Kerala, India. They were maintained by weekly intraperitoneal inoculation of 2 $x10^6$ cells / mouse

6.2.1e Treatment schedule

The Swiss albino mice were divided into 14 groups of each 10 animals (n=10) and given food and water *ad libitum*. All the groups were injected with EAC (10^6 cells/mice) intraperitoneally except normal groups. This was taken as day 0. On the first day, normal saline (0.9 % w/v, NaCl 5 ml/kg/day/mice) was administered to group-1. EAC control mice received only vehicle (propylene glycol 5 ml/kg/day/mice: group-2). The ethanol extracts of *Pterocarpus marsupium*, at the dose of 100 and 200 mg/kg/day/mice, were subsequently administered to animals in groups 3 and 4, respectively. Vincristine (0.8 mg/kg/mice), used as standard drug was injected to group 6, for 14 days intraperitoneally. On 15^{th} day, after the last dose and 18hr fasting five mice from each group were sacrificed for the study of anti-tumor activity, haematological parameter, and antioxidant enzymes estimation and rest of the animal of each group were kept to check the mean survival time (MST) and increase in the lifespan (%ILS) of the tumor scaring mice.

6.2.1f Tumor growth response

Anticancer effect of EEPM were assessed by observation of change with respect to body weight, ascetics tumor volume, packed cell volume, viable and non-viable tumor cell count, Mean survival time (MST) and percentage increase in life span (% ILS).

6.2.1g Tumor cell volume and packed cell volume

The mice were dissected for collecting ascetic fluid from peritoneal cavity. The transplantable tumor was carefully collected with the help of 5 ml sterile syringe and measured the tumor volume and the ascetic fluid was with draw in a graduated glass centrifuge tube and packed cell volume was determined by centrifuging at 1,000 g for 5 min.

6.2.1h Viable and non-viable cell count

For viable and non-viable cell counting the ascetic cell were stained by the tryphan blue (0.4 % in normal saline), dye exclusion test and count was determined in a

Neubauer counting chamber. The cells that did not take up the dye were viable and those that took the stain were non-viable.

6.2.1i Mean survival and Percentage increased in life span

The effect of EEPM on tumor growth was observed by MST and % ILS. MST of each group containing 5 mice were monitored by recording the mortality daily for 6 weeks and % ILS was calculated by using following equation.

MST= (Day of first death + day of last death)/2.

$$\% \text{ LLS} = \left(\frac{\text{Mean survival time of treated group}}{\text{Mean survival time of control group}} \times 100 \right)$$

6.3. Haematological Studies

For the total count of red blood cells and white blood cells, the blood was drawn in to RBC and WBC pipette respectively, then diluted and counted in a Neubauer counting chamber (Wintrob et al., 1961). The Hemoglobin concentration was determined by using Sahli's hemoglobin meter (D'Amour et al., 1965). Deferential count of Leukocytes was done on freshly drawn blood film using Leishman's stain. The RBC, WBC, Hb %, and differential count were estimated from the normal, EAC (control) standard and EEPM-treated groups (Wintrobe et al., 1961).

6.4. Biochemical Assay

After the collection of blood samples, the mice were sacrificed and their liver was excised, rinsed in ice cold normal saline followed by cold 0.15 M Tris-Hcl (pH 7.4), blotted dry and weighed. A 10% w/v homogenate was prepared in 0.15 M Tris-Hcl buffer and a portion was utilized for the estimation of lipid peroxidation and a second portion of the same after precipitating proteins with TCA was used for the estimation of glutathione. The rest of the homogenate was centrifuged at 1500 rpm for 15 minutes at 4^0C. The supernatant thus obtained was used for the estimation of superoxide dismutase, catalase and protein (D'Amour et al., 1965).

6.4a Estimation of Superoxide dismutase (SOD)

SOD activity of the brain tissue was analyzed using the method described by Kakkar et al. (1984). Assay mixture contained 0.1 ml of sample, 1.2 ml of sodium pyrophosphate buffer (pH 8.3, 0.052 M), 0.1 ml phenazine methosulphate (186 μM),

0.3 ml of 300 μM nitroblue tetrazolium, 0.2 ml NADH (750 μM). Reaction was started by addition of NADH. After incubation at 30^0 C for 90 s, the reaction was stopped by the addition of 0.1 ml glacial acetic acid. Reaction mixture was stirred vigorously with 4.0 ml of n-butanol. Mixture was allowed to stand for 10 minute, centrifuged and butanol layer was separated. Color intensity of the chromogen in the butanol layer was measured at 560 nm spectrophotometrically (LKB, Ultrospec II) and specfic activity of SOD was expressed as units/mg protein.

6.4b Estimation of Catalase (CAT)

Catalase activity was measured by the method of Aebi (1974). 0.1 ml of supernatant was added to cuvette containing 1.9 ml of 50 mM phosphate buffer (pH 77.0). Reaction was started by the addition of 1.0 ml of freshly prepared 30 mM H_2O_2. The rate of decomposition of H_2O_2 was measured spectrophotometrically from changes in absorbance at 240 nm. Activity of catalase was expressed as units/mg protein.

6.4c Lipid peroxidation (LPO)

The tissues were then homogenized in 0.1M buffer (pH 7.4) with a Teflon-glass homogenizer. Lipid peroxidation in this homogenate was determined by measuring the amounts of malondialdehyde (MDA) produced primarily, according to the method of Ohkawa et al. (1979). To 0.2 ml of tissue homogenate, 0.2 ml of 8.1% Sodium dodecyl sulphate (SDS), 1.5 ml of 20% acetic acid and 1.5 ml of 8% TBA were added. The volume of the mixture was made upto 4 ml with distilled water and then heated at 95^0 C on a water bath for 60 minute using glass balls as condenser. After incubation the tubes were cooled to room temperature and final volume was made to 5 ml in each tube. 5.0 ml of butanol:pyridine (15:1) mixture was added and the contents were vortexed thoroughly for 2 minute. After centrifugation at 3000 rpm for 10 min, the upper organic layer was taken and its OD read at 532 nm against an appropriate blank without the sample. The levels of lipid peroxides were expressed as n moles of thiobarbituric acid reactive substances (TBARS)/mg protein using an extinction coefficient of $1.56 \times 10^5 M^{-1} cm^{-1}$ (Dacie & Lewis 1958).

6.4d Estimation of reduced glutathione (GSH)

To measure the reduced glutathione (GSH) level, the tissue homogenate (in 0.1 M phosphate buffer pH 7.4) was taken. The procedure was followed initially as described by Ellman 1959. The homogenate was added with equal volume of 20% tetrachloroacetic acid containing 1 mM EDTA to precipitate the tissue proteins. The

mixture was allowed to stand for 5 minute prior to centrifugation for 10 minute at 2000 rpm. The supernatant (200µl) was then transferred to a new set of test tubes and added 1.8 ml of the Ellman's reagent (5,5'-dithio bis-2-nitrobenzoic acid) (0.1mM) was prepared in 0.3M phosphate buffer with 1% of sodium citrate solution). Then all the test tubes make upto the volume of 2ml. After completion of the total reaction, solutions were measured at 412 nm against blank. Absorbance values were compared with a standard curve generated from standard curve from known GSH (Ohkawa et al., 1979).

6.4e Estimation of protein content

The prepared 10% w/v liver homogenate in phosphate buffer solution (pH 7.4) was used for the estimation of protein content by using Alen, H., (1995) method. The prepared homogenate were centrifuged at 1500 g for 15 min at 4^{o}C. The Absorbance and Transmittance were measured in spectrophotometer at 540 nm. The protein content was calculated (Lowry et al., 1951). The amount of protein was expressed in gm of protein in 100 ml.

6.5. Statistical Analysis

The experimental results were expressed as the mean ± S.E.M. Data were assessed by the method of analysis of ANOVA, followed by Dunnet's t-test; p value of < 0.05 was considered as statistically significant.

7. RESULTS

7.1. Phytochemical studies

7.1.1. Extraction and preliminary phytochemical studies on ethanol extracts of *P. marsupium* bark (EEPM)

The dried powder materials of barks of *P. marsupium* were extracted with methanol in a Soxhlet apparatus. The yield was 7.06 % w/w. The tested ethanol extracts of *P. marsupium* showed the presence of phytoconstituents such as carbohydrates, sterols, glycosides, phytosterols and steroids, proteins & amino acids, flavonoids and triterpenoids. The results are shown in table no.4 and 5.

Table No.3 Data showing the extractive value of ethanol extract of *Pterocarpus marsupium*

Plant Name	Parts used	Method used	Solvent Used	%w/w yield Using
Pterocarpus marsupium	bark	Continuous hot percolation process	Ethanol (95 % v/v)	7.06

Table No. 4 Data showing the preliminary phytochemical screening of the methanol extracts of the *Pterocarpus marsupium*

Sl. No	Phytoconstituents	*P. marsupium*
1.	Carbohydrates	+
2.	Glycosides	+
3.	Alkaloids	-
4.	Phytosterol and steroids	+
5.	Flavonoids	+
6.	Proteins&Amino Acids.	+
7.	Tannins	-
8.	Resins	-
9.	Gums and mucilages	-
10.	Triterpenoids	+

+ Present; -Absent

7.2. Pharmacological studies

7.2.1.In-vivo anticancer activity of ethanol extracts of Pterocarpus marsupium bark against Ehrlich Ascites Carcinoma (EAC) bearing Swiss albino mice.

The investigations on the ethanol extracts of *Pterocarpus marsupium* (EEPM) bark revealed significant anticancer activity in EAC bearing mice. The effects of EEPM at the dose of 100 mg/kg and 200 mg/kg on various parameters are presented below:

7.2.1a Effect of mean survival time and tumour growth

The effect of EEPM at the dose of 100 mg/kg and 200 mg/kg on the mean survival time and % increase in life span of EAC bearing mice is shown in table no.19. In the EAC control group, the mean survival time was 22.21±0.25 days and it increased to 26.32±1.20, 24.26+0.33 (100 mg/kg) and 30.65±0.66, (200 mg/kg) days following EEPM treatment. The mean survival time in the standard drug group (vincristine 0.8 mg/kg), was found to be 31.25+40.90 days.

Treatment with EEPM at the doses of 100 and 200 mg/kg significantly ($p<0.01$) reduced the tumor volume, packed cell volume, and viable cell count in a dose dependent manner as compared to that of EAC control groups. Furthermore, nonviable tumor cell counts at different doses of EEPM were increased when compared with the EAC control group us shown in table no.18.

Table No.5 Effect of EEPM on survival time on EAC bearing mice.

S. No.	Experimental groups	Mean survival time (MST) days	% increase in life span
1	Normal control (normal saline 5 ml/kg b.w.)	-	-
2	EAC control	19.15±1.52	-
3	EAC + EEPM (100 mg /kg)	26.32±1.20	7.58
4	EAC + EEPM (200 mg /kg)	30.65±1.66	15.85
5	EAC + Vincristine (0.8 mg / kg) (standard)	31.25±2.38	31.74

All the values are expressed as mean ±SEM, n =8 in each group. Values are significantly different from standard (Vincristine). *P < 0.05; **P < 0.01; ***P < 0.001.

Fig No.1. Effect of EEPM on survival time on EAC bearing mice.

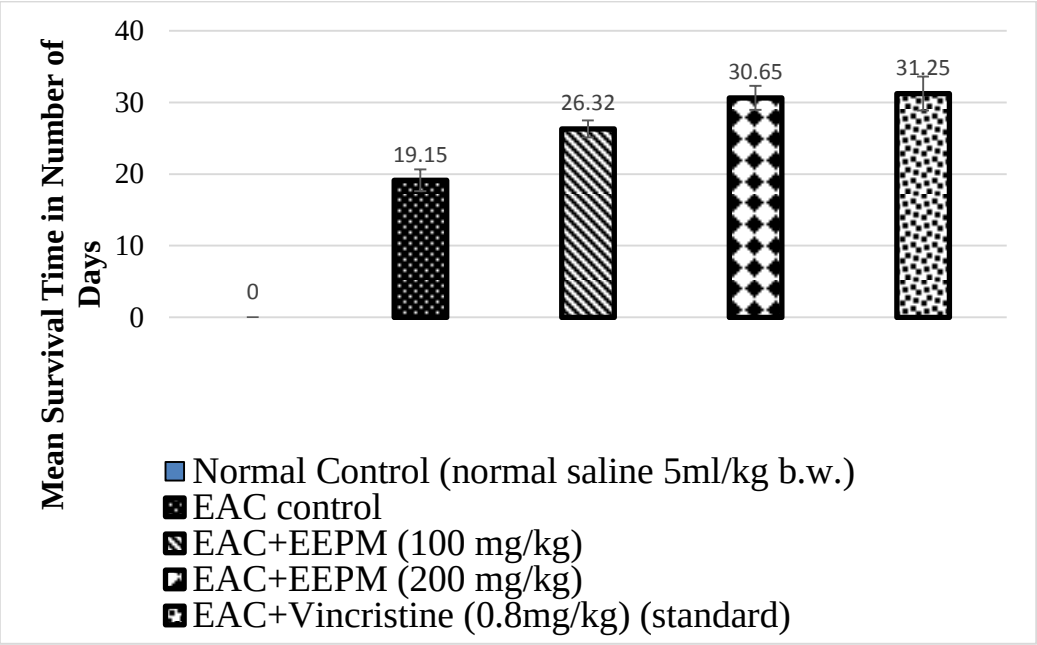
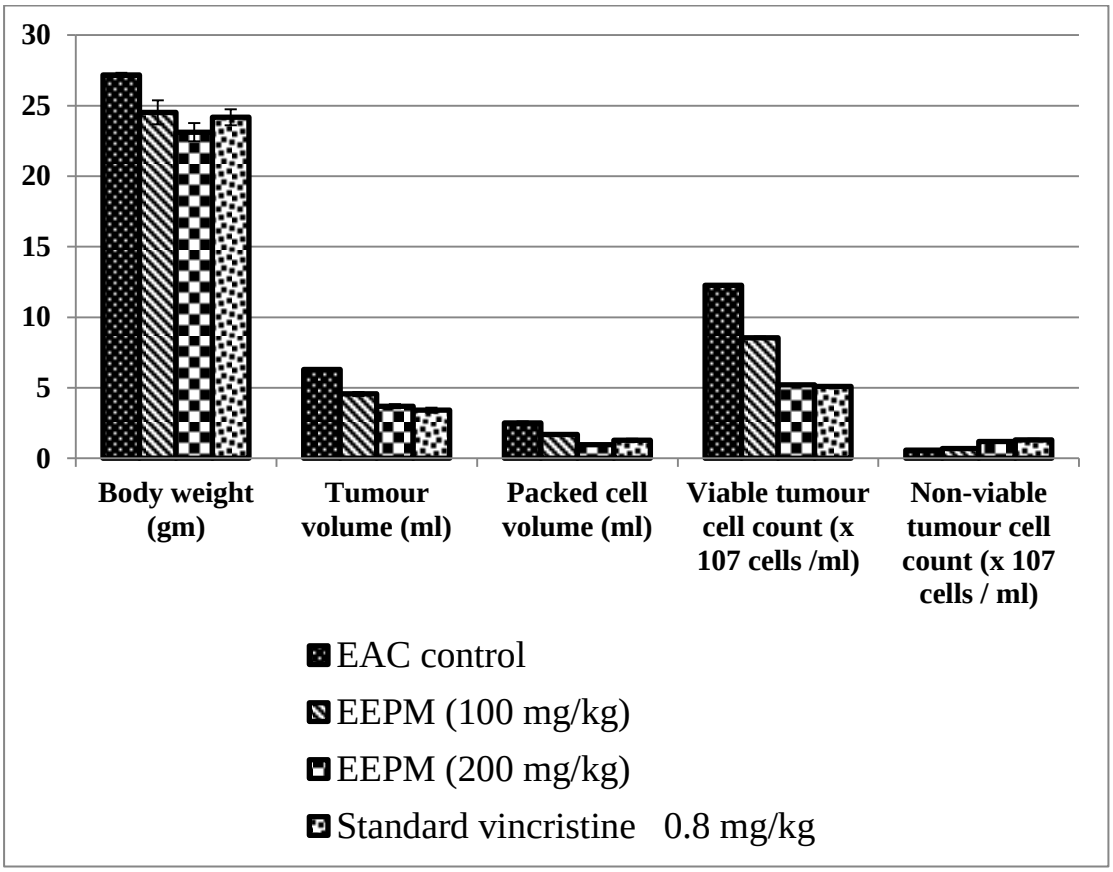


Table No.6 Effect of EEPM on tumour volume, packed cell volume, viable and non-viable tumour cell count of EAC bearing mice

Parameters	EAC control	EEPM 100 mg/kg	EEPM 200 mg/kg	Standard vincristine 0.8 mg/kg
Body weight (gm)	27.18±0.15	24.52±0.85*	23.12±0.65*	24.18±0.56*
Tumour volume (ml)	6.28±0.04	4.56±0.15*	3.69±0.14**	3.41±0.18***
Packed cell volume (ml)	2.51±0.10	1.68±0.08*	0.96±0.07**	1.27±0.11*
Viable tumour cell count (x 10^7 cells /ml)	12.26±0.09	8.54±0.08**	5.21±0.05***	5.09±0.05***
Non-viable tumour cell count (x 10^7 cells / ml)	0.57±0.07	0.69±0.05*	1.19±0.05*	1.29±0.09*

All the values are expressed as mean ±SEM, n =8 in each group. Values are significantly different from standard (Vincristine). *P < 0.05; **P < 0.01; ***P < 0.001.

Figure No.2. Effect of EEPM on tumour volume, packed cell volume, viable and non-viable tumour cell count of EAC bearing mice



7.2.2. Effect on haematological parameters

The findings are shown in table no.21, wherein the hemoglobin content in EAC control mice (9.80 g%) were significantly decreased when compared with normal mice. The hemoglobin content in EAC-treated mice increased (100 mg/kg: 10.55 and 200: mg/kg 11.67 g%) in EEPM-treated groups. Moderate changes in the RBC counts were observed in the EEPM-treated mice. The total WBC counts were significantly increased in EAC bearing mice as compared to normal mice; whereas, EEPM treatment, at the dose of 100 and 200 mg/kg, significantly reduced the WBC count as compared to control.

In differential leukocyte count, the % of lymphocyte decreased while the % of neutrophiles increased in EAC control; in EEPM-treated mice, the % changes in the lymphocyte levels were found to be increased while the % changes in the neutrophiles were found to be decreased significantly, as compared with EAC control.

Table No.7. Effect of EEPM on haematological parameters of EAC bearing mice

Parameter	Normal saline 0.5 ml/kg	EAC control 2 X 10^6 cells / mice	EAC + EEPM 100 mg/kg	EAC + EEPM 200 mg/kg	EAC Cell + Vincristine 0.8 mg/kg
Haemoglobin (gm)	12.58±0.52	9.57±0.15	10.08±0.15	11.56±0.18	11.970.05*
Total RBC million/mm^3	6.84±0.28	3.85±0.051	4.95±0.15	5.68±0.17	5.89±0.059
Total WBC (thousand/mm^3)	7.54±0.054	2.59±0.06*	15.65±0.04	12.58±0.02	9.54±0.057
Lympocytes %	79.57±0.25	35.65±0.87*	50.25±0.89	64.65±0.47	61.52±0.41
Neutrophils %	1.81±0.03	0.88±0.025	1.08±0.07	1.42±0.36	1.61±0.021
Monocytes %	31.54±0.25	54.23±0.17*	46.16±0.07	41.32±0.04	39.56±0.52

Figure No.3. Effect of EEPM on haemoglobin in EAC bearing mice.

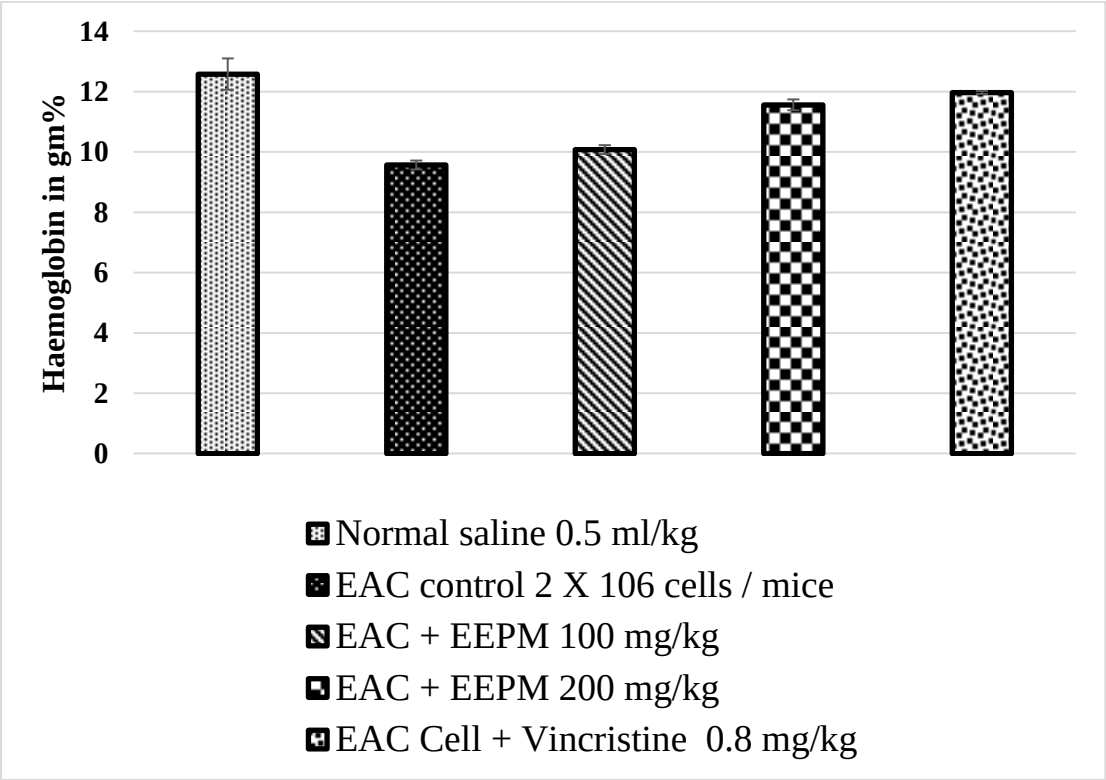


Figure No.4. Effect of EEPM on total RBC in EAC bearing mice

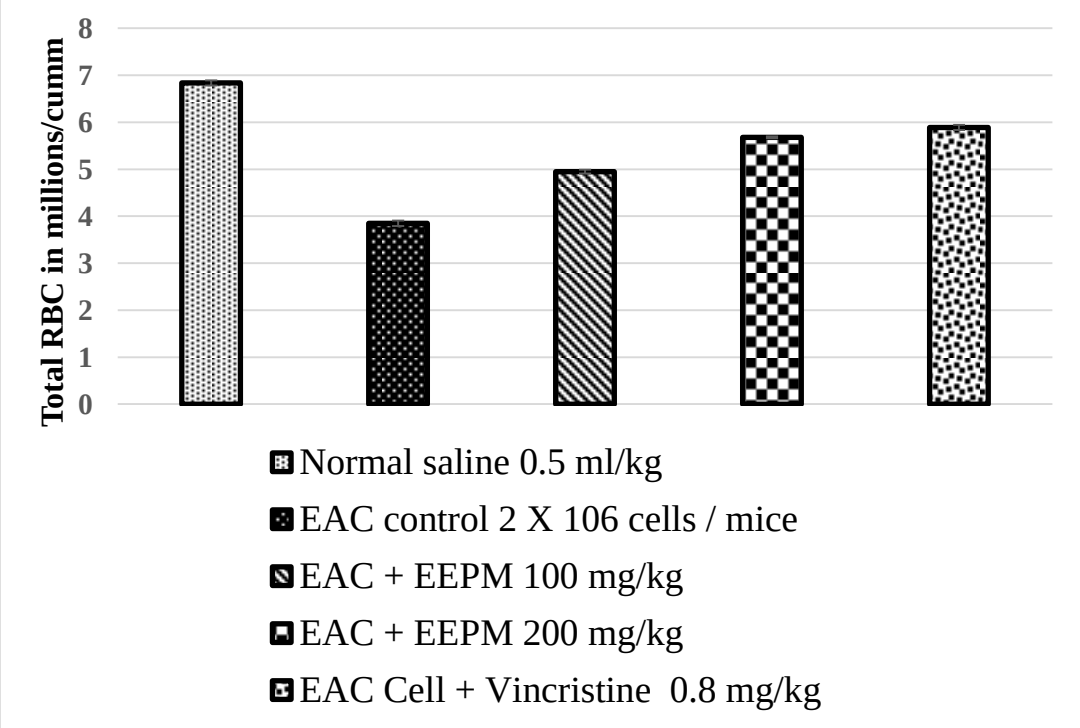


Figure No.5. Effect of EEPM on total WBC in EAC bearing mice.

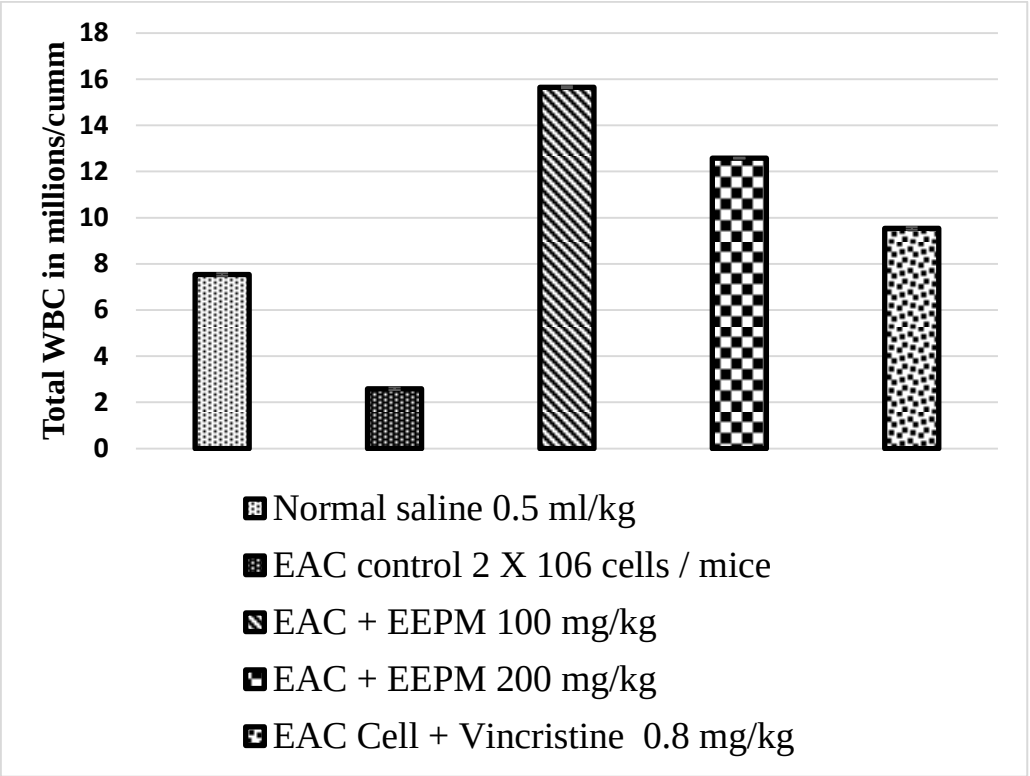
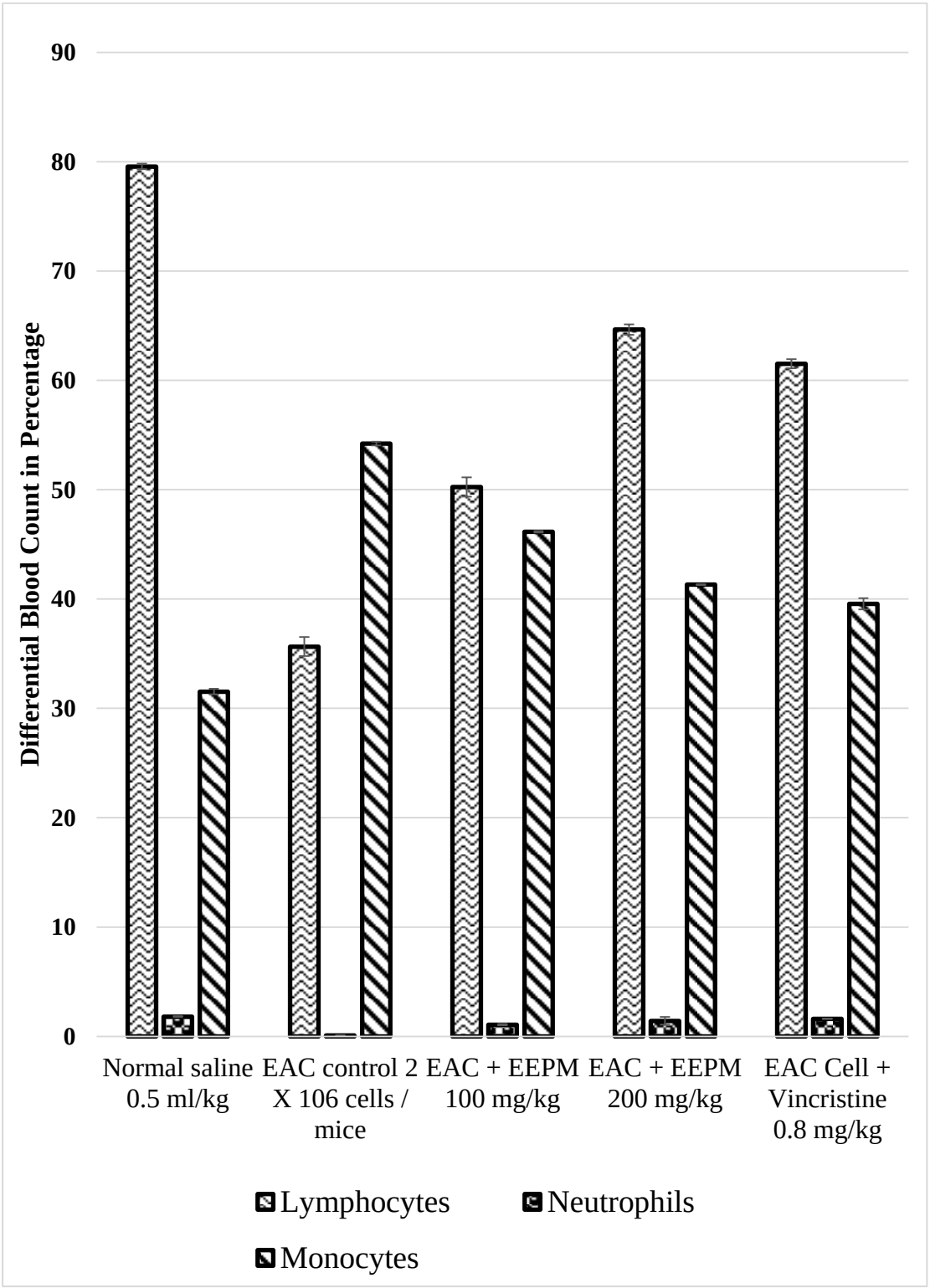


Figure No.6. Effect of EEPM on differential WBC in EAC bearing mice.



7.2.3. Effect of EEPM on biochemical parameters

The findings are shown in table no. 8. The results on the studies on the biochemical paramenters indicated that the perturbations in the levels of antioxicdant enzyme levels were normalized by EEPM in a dose dependent manner.

Table No.8. Effect of EEPM on on different biochemical parameters in EAC bearing mice.

Parameter	Normal saline 0.5 ml/kg	EAC control 2 X 10^6 cells / mice	EAC + EEPM 100 mg/kg	EAC + EEPM 200 mg / kg
LPO (n moles MDA/gm of tissue)	0.81±0.02	1.24±0.09*	1.02±0.20	0.92±0.01
CAT (units /mg tissues)	2.78±0.72	1.61±0.15*	1.96±1.20	2.15±0.04
GSH mg/gm of tissue	2.42±0.03	1.62±0.12	1.89±0.30	2.12±0.23
SOD units/mg of protein	4.43±0.43	3.23±0.22	3.47±0.24	3.92±0.22
Protein content (gm/100 ml)	14.56±0.69*	19.54±0.76	18.74±0.96	18.36±0.16

All the values are expressed as mean ± SEM, n =5. EAC control group compared with normal. Experimental group, compared with EAC control. Values are significantly different from compared groups. ANOVA, *P < 0.05; **P < 0.01; ***P < 0.001.

Figure No.7. Effect of EEPM on on different biochemical parameters in EAC bearing mice

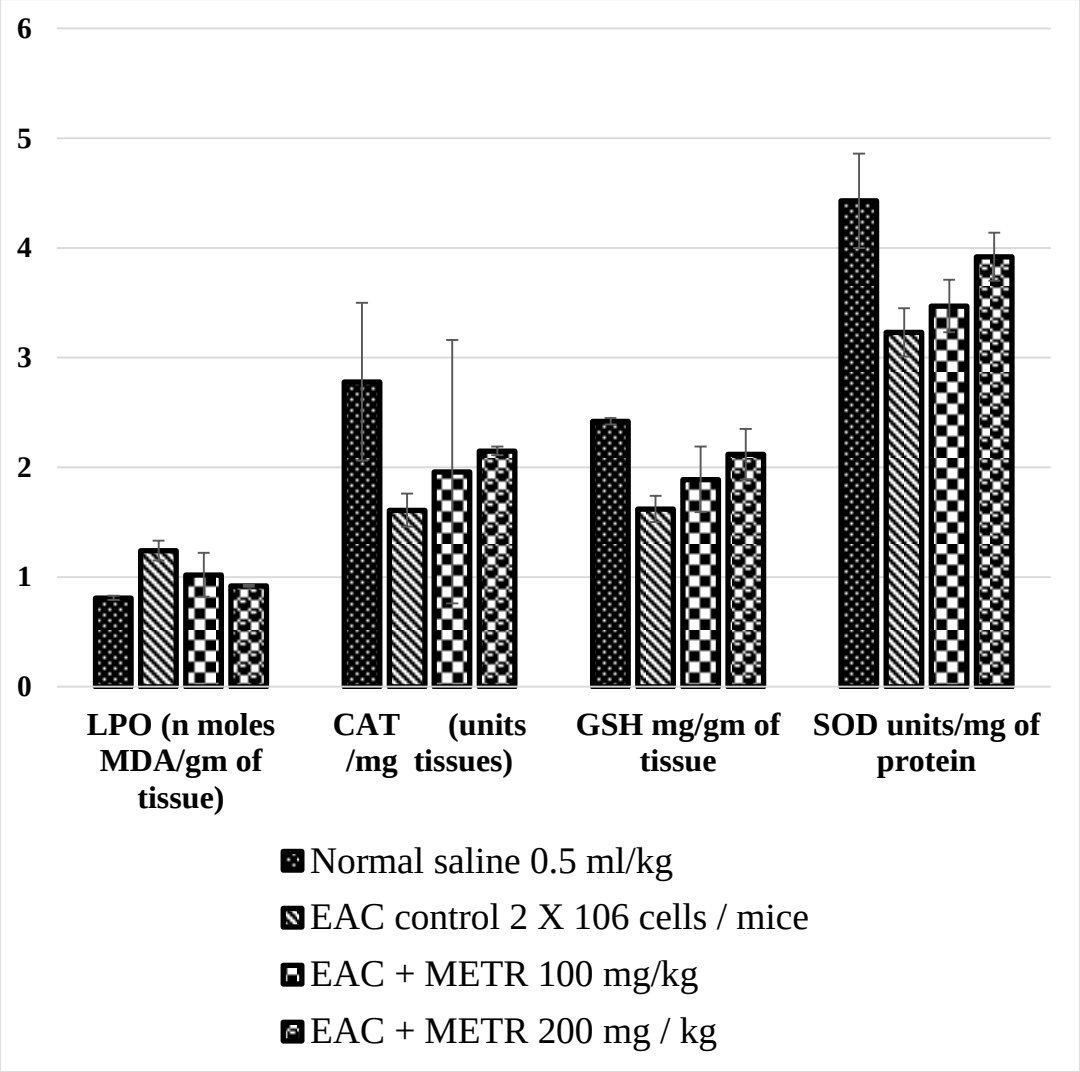
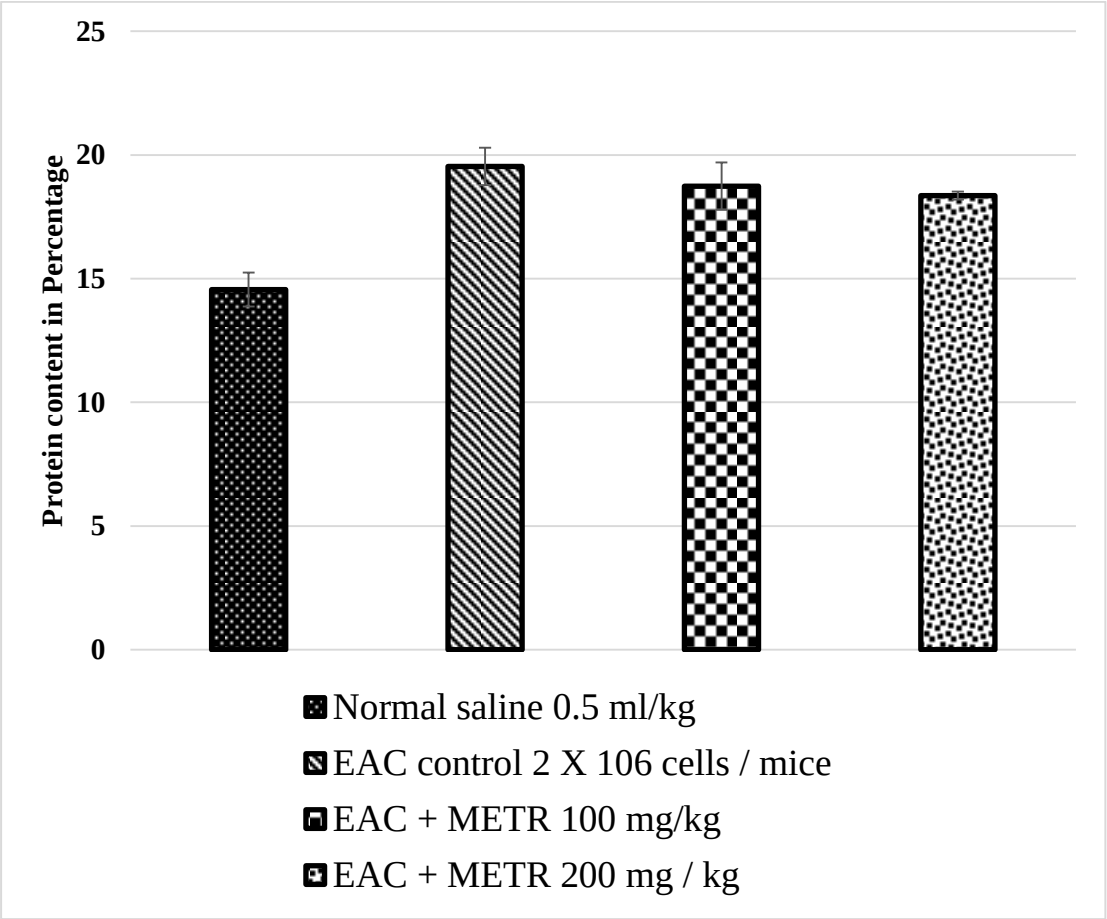


Figure No.8. Effect of EEPM on on protein content in EAC bearing mice



8. DISCUSSION

The present study was carried out to evaluate the anticancer effect of EEPM in EAC bearing mice. The EEPM-treated mice, at the dose of 100 and 200 mg/kg, significantly increased the survival time, inhibited the tumor volume, packed cell volume, tumor cell count and brought back the hematological parameter to more or less normal levels. The extract also restored the hepatic lipid peroxidation and antioxidant enzymes such as a glutathione content, SOD, and catalase (CAT) in tumor bearing mice to near normal level.

In EAC bearing mice, a rapid increase in ascites tumor volume was noted. Ascites fluid is the direct nutritional source for tumor cells and a rapid increase in ascetic fluid with tumor growth would be a means to meet the nutritional requirement of tumor cells[105].

Treatment with EEPM inhibited the tumor volume, tumor cell count, and increased the percentage of tryphan blue positive stained dead cells in tumor bearing mice. The reliable criteria for judging the value of any anticancer drug are the prolongation of the life span of animals.[106] The EEPM treatment decreased the ascetic fluid volume, viable cell count and increased the percentage of life span. It may be concluded that EEPM may act by decreasing the nutritional fluid volume and arresting the tumor growth, increases the life span of EAC bearing mice.

Usually in cancer chemotherapy the major problems that are being encountered are myelosuppression and anemia.[107,108] The anemia encountered in tumor bearing mice is mainly due to reduction in RBC or hemoglobin percentage, and this may occur either due to iron deficiency or due to hemolytic or myclopathic conditions[109]. Treatment with EEPM restored the hemoglobin content, RBC and WBC count more or less to normal levels. This indicates that EEPM may possess protective action on the hematopoietic system.

Lipid peroxidation, an autocatalytic free radical chain propagating reaction, is known to be associated with pathological condition of cell. Malondialdehyde (MDA), the end product of lipid peroxidation, was reported to be higher in cancer tissue than in non-cancerous organ[109]. A major target of reactive oxygen species (ROS) is cell membrane, due to the high content of polyunsaturated fatty acid. ROS mediated lipid

peroxidation causes damage to cellular DNA, membrane structure and inhibition of functions of several enzymes and alteration in the immune system. In cancer, enormous production of free radicals in the system has been reported. A close relationship between free radical activity and neoplastic transformation has been shown. Antioxidants play a vital role in scavenging reactive oxygen species and protect the cells from oxidative damage. In EAC bearing mice the level of lipid peroxidation was significantly increased. Administration of EEPM at the dose level of 200 mg/kg animal groups significantly inhibited as the elevated level of lipid peroxidation as compared with control animals.

Glutathione (GSH), a potent inhibitor of neoplastic process plays an important role in endogenous antioxidant system that is found particularly in high concentration in liver and it is known to have key function in protective process. Excessive production of free radicals resulted in oxidative stress, which leads to damage of macro molecules for example, lipid peroxidation *in vivo*.[110] It was also reported that the presence of tumors in the human body or in experimental animals is known to affect many functions of the vital organs, especially in the liver, even when the site of the tumor does not interfere directly with organ function .[111] The present study indicated depletion in GSH contents in the EAC control group, which was found to be significantly increased following treatment with EEPM in comparison to control groups and this may be attributed to the antioxidant and free radical mechanism.

The functions of the SOD and CAT activities, present in all oxygen metabolizing cells, is to provide a defence against the potentially damaging reactivity of superoxide and hydrogen peroxide. Inhibitions in the activities of SOD and CAT as a result of tumor growth were also reported[112]. Similar findings were observed in the present investigation with EAC bearing mice. The administration of EEPM at the doses 100 and 200 mg/kg significantly altered status of SOD and CAT levels in a dose dependent manner as compared with control.

Cells are also equipped with enzymatic antioxidant mechanisms that play an important role in the elimination of free radicals. SOD, CAT and glutathione peroxides are involved in the clearance of superoxide and hydrogen peroxide (H_2O_2) radicals. SOD catalyses the diminution of superoxide into H_2O_2, which has to be eliminated by glutathione peroxidase and or catalase[113]. In correlation, it has been

reported that a decrease in SOD activity in EAC bearing mice which might be due to loss of Mn^{2+} containing SOD activity in EAC cells and the loss of mitochondria, leading to a decrease in total SOD activity in the liver.[114] A small amount of catalase in tumor cell was reported.[115] The inhibition of SOD and CAT activities as a result of tumor growth was also reported.[114] Similar findings were observed in the present investigation with EAC bearing mice.

The administration of EEPM at different doses significantly increased the SOD and CAT levels as compared to that of DAL control group.

It was reported that plant derived extracts containing antioxidant principles showed cytotoxicity towards tumor cells[116] and anticancer activity in experimental animals.[117] Anticancer activity of these antioxidants is either through induction of apoptosis[118] or by inhibition of neovascularization.[119] The implication of free radicals in tumors is well documented.[120, 121] The free radical hypothesis supported the fact that the antioxidant and anticancer principles present in the extract.

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