8.1 INTRODUCTION

Cancer is a group of disease which is described as growth of cells out of control. It is a disease where group of cells shows extreme growth without control (extreme cell division than normal limits), invasion (penetration), destroying neighbour healthy tissues and may also leads to metastasis (reaches to other location via blood and lymph in body). Many cancers leads to formation of tumor but some other cancer like leukemia does not form tumor\(^1\). Tumor has ability to develop and interfere with circulatory, nervous and digestive systems results to hormone release that change the functions of body. Tumors with limited growth and occur in one spot is considered as benign.

Cancer occurs to all age group people and there is a risk of developing cancer with increase in age\(^2\). Cancer is responsible for human death about 13% in 2007\(^3\). There are 100 types of cancer affecting different parts of body. Each type of cancer is specific by its own symptoms, causes and method used for treatment. In US, 0.6 million citizens die because of cancer.

8.1.1 Structural properties of tumor cell

Apart from loss of growth regulation, tumor cells also cause numerous structural changes. For example,

- Cell nucleus is normally round shaped but nucleus is lobate and irregular shaped to great extent in tumor cells.

- Tumor cells are also determined by very irregular karyotypes. The several chromosomes are varying from one cell to another cell than normal which leads to chromosome abnormalities.
• The normal cell tissues are extremely organized due to presence of several intracellular junctional contacts (eg, desmosomes and gap junctions) but in tumor cells only few such junctional contacts are present.

8.1.2 Functional properties of tumor cells

Tumor cells also differ functionally from non- tumor cells.

• Tumor cells are determined by unregulation of mitotic activity.

• Non- tumor cells exhibit inhibition of cell movement by contact. It means that movements are restricted when one cell contact with another cell. For example, cells will not move over one another. Like that, blockage of cell division occurs due to touching of cells each other and it is referred as contact inhibition of mitosis. Neither of these forms of contact inhibition occurs routinely in tumor cells routinely.

• In tumor cells, reorganization of cell surface with extracellular components (eg, fibronectin) and receptors (eg, integrins) occurs and usually varies in tumor and non- tumor cells.

• Finally, in laboratory if tumor cell kept in culture conditions, it happens to be immortal i.e., they grow and divide without control till proper nutrient is provided to medium. But non- tumor cells in culture have only limited capacity for cell division.

8.1.3 Frequency of cancer

In men, most common cancer is cutaneous melanoma, cancer of lung, bladder colorectal and prostate. For women, most common cancer is colorectal,
lung, non- Hodgkins lymphoma, breast and endometrium. In all cancer, there is a genetic material abnormality of cancer cell and its progeny.

Fig 8.1: Frequency of cancer

8.1.4 Causes and risk factors of cancer

The most cancer occurs at irregular intervals over time when some normal genes start mutating and that mutated genes will rapidly multiply leads to malignant. The mutations of these genes develop because of combination of various factors like heredity, environment and lifestyle.

8.1.4.1 Environmental risk factors

Growing older: Main risk factor of cancer is growing older which occur with age greater than 65 years. Sometimes it also occurs in all age group people including children.

Radiation: Increased radiation level leads to damage of cell which causes cancer. This kind of radiation comes from rays that enter the Earth's atmosphere
from outer space, radioactive fallout, radon gas, x-rays (repeated exposure) and other sources. Radioactive fallout can come from accidents at nuclear power plants or from the production, testing, or use of atomic weapons. People exposed to fallout may have an increased risk of cancer, especially leukemia and cancers of thyroid, breast, lung and stomach. Radon is a radioactive gas that you cannot see, smell, or taste. It forms in soil and rocks. People who work in mines may be exposed to radon. In some parts of the country, radon is found in houses. People exposed to radon are at increased risk of lung cancer.

UV radiation: UV rays from sun is directly associated with melanoma and some other types of skin cancer. These harmful rays of the sun cause premature aging and damage the skin. Artificial sources of UV radiation, such as sun lamps and tanning booths, also increase the risk of skin cancer.

Tobacco: Use of tobacco usually leads to death. More than 180,000 Americans die every year due to cancer caused by use of tobacco. The occurrence of lung cancer is increased due to great exposure of tobacco smoke regularly and cigarette smoking. Cigarette smoking also more likely leads to various types of cancer such as esophagus, larynx, mouth, cervix, pancreas, kidney and bladder and also increasingly develop cancers like prostate, liver, rectum, colon and stomach and sometimes leads to acute myeloid leukemia. Tobacco chewing also associated with cancers of throat, mouth and tongue.

Chemicals: Exposure for long period to chemicals like pesticides, cadmium, nickel, uranium, asbestos, benzene and radon can increase risk of cancer. Such carcinogen with another carcinogen, such as cigarette smoke can increase risk of cancer and other lung diseases.
Alcohol: Heavy drinkers of alcohol lead to high risk of developing cancers like liver, mouth, esophagus, throat and larynx. But in rare cases, even moderate drinking have the risk of breast cancer.

Diet: Diet with high cholesterol or fat is reported to be risk factors for seven various cancer types like uterus, prostate and colon. Obesity also associate with breast cancer in older women and also with cancers of ovary, uterus, colon, pancreas and prostate.

Physical activity/ overweight: Breast, uterus, colon, kidney and esophagus cancer are due to overweight or lack of physical activity.

Viruses: Infection of some bacteria or viruses increase the risk of causing cancer:

Approximately 85% of cancers are prevented by avoiding environmental risk factors though there is no change in gender, race, age and family history.

8.1.4.2 Hereditary risk factor

Cancer about 22% is due to hereditary i.e., abnormal gene is transferred to child from parent.

Genetics: It plays a major role in development of cancer. Extra precautions are important to take for family history of cancer like breast cancer. If cancer is genetic, there is possibility of transferring a mutated gene

Ethics groups: Few cancers are usually common in some ethical groups.

Family history: Ovarian, prostate, colon and breast cancer is associated with family history.
8.1.5 Types of cancer

Cancer is group of complex disease. Every cancer is very specific by its own way such as its growth and development of cells, the way it occurs in the body, its possibility of spreading and symptoms in which patients experience. All types of cancer come under any one of four broad categories.

8.1.5.1 Carcinoma

It constitutes about 80% of all cancer. In epithelial origin, there is a malignant neoplasm called carcinoma. This type of cancer develops in lining tissue of body organs like colon, breasts, ureter, nose, prostrate, penis and urinary bladder.
8.1.5.2 Sarcoma

This type of tumor occurs in fibrous tissue, muscle, cartilage and bone. The common types of sarcomas are Kaposi’s sarcoma and Ewing sarcoma.

Ewing sarcoma

During puberty, bone grows rapidly leads to Ewing sarcoma. It develops in pelvis or long bones of extremities but also in thigh bone (femur) and may also occur in trunk flat bones or skull. This tumor does not develop in black children.

Kaposi’s sarcoma

It is a malignant type tumor mostly involves patient’s skin suffering from AIDS because it develops rapidly in AIDS patients and also involves gastrointestinal tract, lungs, skin and other organs.

8.1.5.3 Leukemia’s

Leukemia’s refer to blood cancer or blood forming organs cancer. In leukemia, body produces excess of abnormal blood cells and mainly white blood cells in most leukemia’s. The leukemia cell varies in appearance than normal blood cells and does not function correctly. It may be either acute or chronic. Abnormal blood cells are blasts and remain immature and do not have normal functions in acute leukemia. The blasts number rapidly elevated thus on victim it forms an earlier and greater impact. Small number of blast cells present in chronic leukemia that are more mature so few normal functions will be carried out. Leukemia cells are abnormal cells that does not involved in fighting infections in body.
8.1.5.4 Lymphoma’s

Lymphomas are a type of cancer which affects lymphatic system which posses a large number of connected nodes and vessels which acts as body filters. Lymphatic system provides nutrients to tissue and blood and also involved in prevention of bacteria and any other foreign particles that enters bloodstream. More than 20 types of lymphoma are present e.g. Hodgkin’s disease and some other lymphomas grouped together known as non-Hodgkin’s lymphoma. Non-Hodgkin’s lymphoma can occur in single and group of lymph nodes or it may occur to another organ. It almost spread to all parts of body which includes spleen, liver and bone marrow. Non-Hodgkin’s lymphoma may occur with increases in age and most frequently occur in men than women.

The estimated number of new cases and deaths for individual cancer type is presented in following table.

Table 8.1: Types of cancer and their estimated new cases and deaths

<table>
<thead>
<tr>
<th>Type of cancer</th>
<th>Estimated new cases</th>
<th>Estimated deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukemia (All)</td>
<td>44,790</td>
<td>21,870</td>
</tr>
<tr>
<td>Bladder</td>
<td>70,980</td>
<td>14,330</td>
</tr>
<tr>
<td>Kidney Cancer</td>
<td>49,096</td>
<td>11,033</td>
</tr>
<tr>
<td>Breast cancer(male-female)</td>
<td>1,910 - 192,370</td>
<td>440 - 40,170</td>
</tr>
<tr>
<td>Endometrial</td>
<td>42,160</td>
<td>7,780</td>
</tr>
<tr>
<td>Non-Hodgkin Lymphoma</td>
<td>65,980</td>
<td>19,500</td>
</tr>
<tr>
<td>Rectal and Colon (Combined)</td>
<td>146,970</td>
<td>49,920</td>
</tr>
<tr>
<td>Lung (Including Bronchus)</td>
<td>219,440</td>
<td>159,390</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>42,470</td>
<td>35,240</td>
</tr>
<tr>
<td>Thyroid</td>
<td>37,200</td>
<td>1,630</td>
</tr>
<tr>
<td>Melanoma</td>
<td>68,720</td>
<td>8,650</td>
</tr>
<tr>
<td>Skin (Non melanoma)</td>
<td>&gt;1,000,000</td>
<td>&lt;1,000</td>
</tr>
<tr>
<td>Prostate</td>
<td>192,280</td>
<td>27,360</td>
</tr>
</tbody>
</table>
8.1.6 Mechanism of tumor formation

Mutation:

This is the tumor with loss or substitution or rearrangement of DNA in the cell. Tumors require no changes in genetic formation and develop without changes in cell genes.

Addition of new genetic material:

Involve addition and integration of new viral genetic material in cell genes as a result of infection by tumor producing virus.

Changed gene expression:

Involves no change in the integrity of the cell’s genetic information, moreover a persistent change in the way a cell expresses or uses that formation. This is called epigenetic.

8.1.7 Relationship between genes and cancer

Genes involved in cell replication is damaged which allows cell to reproduce without any restriction and finally spreads to neighboring tissues and build up the growth of cell all over the body. All types of cancer are genetic which is caused by altered genes. A mutation occurs in reproductive cells even if a small portion of cancer is inherited and passed from one generation to next and occurs throughout the body. Mistakenly if cells undergo cell division or injuries due to environmental agents like chemicals or radiations leads to random mutation occur in cells of body which results to cancer.
Cancer generally develops in single cell. The cell gradually moves from normal to malignant to metastatic and a chain of different steps is followed, every step is controlled by various gene or set of genes. Oncogenes generally increase the cell growth and if over expressed or mutated which can over flow cells with signals to continue dividing. The cell growth is usually restricted by tumor-suppressor genes but if it is missing or inactivated by mutation, it allows cell to grow and divide without control. DNA repair genes trigger cancer and other inherited disorders not by promoting growth of cell but it cause failure of correction of mistakes occur on copies of DNA itself leads to accumulation of mutations at thousand sites.
8.1.8 Treatment of cancer

Cancer can occur to anyone at all age and about 77% of cancers occur in people with age of 55 and even older. Treatment of cancer depends on age, cancer type, health status, cancer stage (percentage of spreading) and extra personal characteristics.

8.1.8.1 Surgery

The oldest familiar cancer treatment is surgery. Surgery is usually used for prevention, treatment, stage (determine the advancement of cancer) and cancer diagnosis. There is a possibility to cure the patients completely by surgical removal of cancer from the body if it is not metastasized e.g., removal of breast, prostate or testicle. It is not possible to delete all cancer cells if it is metastasized i.e. after spreading. Surgery also helps to control of symptoms like compression of spinal cord or obstruction of bowel.

8.1.8.2 Radiation therapy

Cancer is destroyed by passing high energy rays to cancer cells is called radiation therapy. It is done by damaging the DNA of cancer cells and prevents the cell to multiply. The high energy x-rays generated in special machine or high energy gamma rays emitted from radium is used for radiation therapy and high energy beams may cause damage to healthy tissues leads to undesirable side effects but now advanced technology is there so beams can be focused specifically to cancer cells. Radiotherapy is also used as independent treatment for shrinkage of tumors or destroying cancer cells [includes lymphoma and leukemia] and radiation therapy is also used in combination with other cancer treatment.
Radiation also leads to generation of free radicals due to indirect interaction with water and make up the cell volume to 80% that leads to damage of cell membrane proteins and other cell organelles. It is determined that X-ray induced is due to hydroxyl radical formation which as follows.

Ionizing radiation +H₂O → H₂O⁺ + e⁻

H₂O⁺ + H₂O → H₃O⁺ + OH⁻

OH⁻ → Cell damage

8.1.8.3 Chemotherapy

Chemotherapy is the treatment of cancer by chemicals⁶. Chemotherapy uses chemicals to eliminate cancer cells which interferes cell division by damaging DNA or proteins. It usually target all cells that are dividing rapidly including normal cells can be recovered from any damage induced by chemicals. Chemotherapy is usually used for treatment of cancers that are metastasized because medicine travels all over the body. This treatment is important for few forms of lymphoma and leukemia.

In recent years, treatment by chemotherapy for neoplastic disease is highly important. At present, at least 10 different neoplasms can be cured by chemotherapy in the majority of patients⁷. The era of chemotherapy of malignant disease was in the 1941 when Huggins demonstrated that the administration of estrogens produced cancer⁸.
Anticancer drugs

Anticancer drugs can be divided into three categories based on

Chemical drugs and resource of drugs

Biochemistry mechanism of anticancer action

Cycle or phase drug specificity.

8.1.8.3 (a) According to chemical drugs and drug resources

Drugs may be antimeabolites, alkylating agents, plant extracts, hormones, antibiotics and others.

Alkylating agents

The inhibition of reproduction is done by nitrogen mustards usually by irreversible binding with nucleic acids (DNA) by alkylation. DNA cannot able to replicate after alkylation so synthesis of proteins and other essential cell metabolites does not occur. As a result, reproduction of cell is inhibited so a cell at the end dies due to inability of maintenance of metabolic functions.

Antimetabolites

Antimetabolites are S-phase specific drugs which interfere with synthesis of DNA. These are structural analogues of essential metabolites. In this class drugs, dose-limiting toxicity is myelosuppression.

Antibiotics

All drugs in this class possess common mechanism of action and cause inhibition of proliferating cells of normal and neoplastic origin rapidly.
Hormones

Hormone antagonists are specific to various hormone dependent cancers (mainly prostate, endometrial and breast cancer). Estrogen antagonists are specifically used to treat breast cancer, whereas androgen antagonists are specific for prostate cancer. Corticosteroids are especially used to treat lymphocytic lymphomas and leukemia’s.

Examples: Progestins, Estrogens, Androgen, Glucocorticoids

Plant alkaloids

Tubulin binding agents

Vinca alkaloids - it prevents the assembly of microtubule. It prevents the formation of mitotic filaments for cell and nuclear division leads to cell arrestment in late G2 phase. E.g., Alkaloids derived from Vinca rosea (periwinkle plant) such as vincristin, vinblastine, vinorelbine and vindesine.

Taxanes enhance polymerization of tubulin in all aspects and this action is opposite to vinca alkaloids. E.g., Taxotere, paclitaxel.

Interfere ribosome function

Cephalotaxus Alkaloids : Homoharringtonine and Harringtonine

Platinum compound

Cisplatin – it is efficient to neoplasm and it binds to guanine in DNA and RNA by hydrogen bonding leads to unwinding and shortening of DNA helix.

Carboplatin – it is approved only secondary drug to ovarian cancer.
8.1.8.3 (b) According to biochemistry mechanism of anticancer action

Directly influence structure and function of DNA

Antibiotic - Mitomycin C, Alkylating agent - Cyclophosphamide Thiotepa,
Inhibitor of topoisomerase - Podophyllotoxin and camptothecine and Platinum - Cis-platinum

Block nucleic acid biosynthesis

Antimetabolites:

Purine antagonist - mercaptopurine, Pyrimidine antagonist – fluorouracil,
Folic acid antagonist – methotrexate and Ribonucleoside diphosphate reductase antagonist - hydroxyurea,

Interfere with protein synthesis and function

Interfere with ribosomal function - harringtonines, Antitubulin - taxanes and
vinca alkaloids and Influence the supply of amino acid - L-asparaginase which binds
with tubulin and destroys the spindle leads to arrestment of mitosis.

Interfere transcription and block RNA synthesis

Doxorubicin binds with DNA to block RNA production.

Influence homeostasis hormone

It blocks the actions of sex hormones by binding to hormone receptors
results to tumor growth inhibition. E.g., Androgens and androgen antagonistic
drug, glucocorticoid drug, inhibitor of aromatase - anastrazole, aminogluthathimide,
progestogen drug, estrogens and estrogen antagonistic drug, gonadotropin
releasing hormone inhibitor - goserelin and leuprolide.
8.1.8.3 (c) According to phase or cycle specificity of drug

Cell cycle specific agent (CCSA)

Cell cycle nonspecific agent (CCNSA)

Cell cycle specific agent (CCSA)

CCSA drugs act during cell cycle specific phase. E.g., topoisomerase inhibitors, antimetabolites specific to S phase, taxanes, vinca alkaloids specific to M phase and bleomycin specific to G2 phase

Cell cycle nonspecific agent (CCNSA)

CCNSA drugs are active all over the cell cycle. E.g., Platinum Compounds, antibiotics and alkylating agents

Fig 8.4: Summary and mechanism and site of action of chemotherapy
8.1.8.4 Biological therapy

One type of biological therapy is a targeted therapy which blocks tumor biological processes that allows the tumor to grow. Some type of therapy will block the supply of blood to tumor leads to starved and finally die due to lack of blood. Biological therapies also prepare the immune system of body to fight against cancer.

Biological therapy includes vaccines, monoclonal antibodies, interleukins, antibiotics, gene therapy, interferons, colony stimulating factors and non-specific immunomodulatory agents.

8.1.8.4 (a) Cancer vaccines

The biological therapy which is used currently under study is cancer vaccine. New vaccines are developed by researchers which give hope to patient’s immune system to identify cancer cells. Vaccines for cancer (therapeutic vaccines) are prepared to treat cancers and after diagnosis of cancer, the person is injected by therapeutic cancer. These vaccines block the growth of existing tumors and eliminate the cancer cell which is not killed by previous treatments. Even small amount of tumor can be completely eradicated by cancer vaccines. Therapeutic vaccines are used to treat many types of cancer like lung, kidney, pancreas, breast, prostate, colon, rectum, brain, leukemia and lymphoma.

On other side, prophylactic vaccines are given before development of cancer to healthy persons. These vaccines cause stimulation of immune system to attack the causing viruses. Previously, clinical trials of cancer vaccine involve
mainly melanoma patients. Prophylactic vaccines are also studied to prevent cancer of liver and cervix.

8.1.8.4 (b) Gene therapy

Gene therapy is an advance technique in treating cancer by gene manipulation i.e., alters the genetic material (DNA and RNA) of patient’s cell to fight disease. Various clinical trials for gene therapy are studied for different cancer type but routine clinical use of gene therapy has not yet approved.

8.1.8.4 (c) Laser therapy

Laser therapy is most commonly used for treatment of cancer occur on body surface or internal organ lining (superficial cancer) like cancer of Basel cell skin and also initial stages of few cancer type like vulvar, penile vaginal, non-small cell lung cancer and cervical cancer. Lasers also used to destroy or shrink a tumor which blocks the patient’s esophagus or trachea and also remove tumors or colon polyps which blocks stomach or colon. Laser therapy can combine with other cancer treatment like radiation therapy, surgery or chemotherapy but it may also use alone.

8.1.8.4 (d) Photodynamic therapy (PDT)

Photodynamic therapy also uses lasers to treat cancer. Certain drugs in photodynamic therapy called photosensitive agents or photo sensitizer is injected to the body of patient. Here laser light is used for activation of photosensitive agent and for destroying cancer cells PDT is also used in by combining with various other cancer therapies such as hyperthermia, chemotherapy, radiation therapy etc.
8.1.8.4 (d) Hyperthermia

Hyperthermia is used to treat cancer by exposing the body tissue to high temperature (up to 113°F). Previous studies reveal that exposure to high temperature damage and kill the cancer cells but generally minimum injury will occur to normal healthy tissues. Hyperthermia kills cancer cells and damages the proteins and structures within the cancer cells and may also cause shrinkage of tumors.

8.2 MATERIALS AND METHODS

8.2.1 Plant material

The Decalepis hamiltonii root used for study was purchased from a plant supplier in Chennai, Tamil Nadu, India. Root was authenticated taxonomically at Plant Anatomy and Research Center, Chennai, Tamil Nadu, India.

8.2.2 Preparation of extract

The Decalepis hamiltonii root was dried in shade, crushed to coarse powder. Using soxhlet apparatus the root was extracted with petroleum ether (60 - 80°C) to remove fat and then with 90% methanol. Under reduced pressure the solvent was evaporated and obtained filtrate was used for further studies.

8.2.3 Animals

Swiss albino male mice weighing about 20 to 25 g obtained from JSS college of Pharmacy, Ooty and sanctioned by animal ethical committee and proposal number was JSSCP/IAEC/M.Pharm/PH.COOG/06/2009-10. The animals were kept in microloan boxes in controlled environment (12 h dark and light cycle and temperature 25±2°C) and fed with water ad libitium and standard pellet diet.
8.2.4 Maintenance of DLA cell lines

Dalton cells were obtained from JSS College of Pharmacy, Ooty. DLA cells were allowed to grow in peritoneal cavity of mice by injecting $10^6$ cells. During log phase on 15th day of tumor, the cells were aspirated aseptically from developed tumor mice transplantation using 18 gauge needles by removing fluids from peritoneal cavity. Then the fluid was washed in phosphate buffer saline three times and the cell pellet was re-suspended in PBS. Tumor cell was counted using trypan blue dye exclusion method in hemocytometer. Cell suspension was diluted to get $2 \times 10^6/0.1$ ml.

8.2.5 Short term in vitro anticancer activity\textsuperscript{11}

Short term in vitro anticancer activity was done by using Daltons Lymphoma Ascites. This test relies on breakdown in membrane integrity which was determined by uptake of dye such as (trypan blue, erythoridine and nigerin) to which cell was normally impermeable [Appendix 20]

8.2.6 In vitro cytotoxicity activity by MTT assay method\textsuperscript{12}

Methanolic extract of Decalepis hamiltonii root was subjected to in vitro cytotoxicity activity by MTT assay method using two types of cell lines like Vero cell line and A-549 cell line (cancerous cell line) [Appendix 21]

The ability of cell to survive against toxic effect forms the basis for many cytotoxicity assays. MTT assay was done according to assumption of dead cells or their products that do not caused reduction of tetrazolium. This method usually depends on both the activity of mitochondria per cell and number of cell present.
8.2.7 In vivo anticancer activity

Swiss albino male mice were divided into 5 groups of 6 mice in each group. All groups were inoculated with 2x10^6/0.1 ml DLA cells intraperitoneally except control normal group.

Group I : Normal group
Group II : Cancer control
Group III : Cancer mice received cyclophosphamide (25 mg/kg ip)
Group IV : Cancer mice received 200 mg/kg of methanolic extract of Decalepis hamiltonii.
Group V : Cancer mice received 400 mg/kg of methanolic extract of Decalepis hamiltonii.

After 24 hours of inoculation of DLA cells, treatment was started daily once for 14 days. After 14 days, mice were sacrificed and blood samples were collected from all mice for evaluation of biochemical and hematological parameters.

8.2.8 Anti-tumor activity measurements

The anti-tumor activity was measured in DLA bearing mice by the following parameters.

1. Analysis of body weight
2. Percentage increase in life span (% ILS)
3. Mean survival time (MST)
4. Hematological parameters
5. Biochemical estimation

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8.2.8.1 Body weight analysis

Body weight was recorded at day 0 (when experiments starts) and regularly on every 7th day (weekly interval) during treatment period. Average body weight and percentage decrease in body weight was calculated by the formula

\[
\text{% Decrease of body weight} = \frac{\text{Gain in control body weight} - \text{Gain in treated group body weight}}{\text{Gain in control body weight}} \times 100
\]

8.2.8.2 Determination of MST and %ILS

At end of the experiment, effect of methanolic extract of Decalepis hamiltonii root on growth of tumor was regularly checked by recording mortality rate daily for 5 weeks (MST) and % increase in life span (%ILS) was calculated\textsuperscript{13}

\[
\text{MST} = \frac{\text{Day of 1st death} + \text{Day of last death}}{2}
\]

\[
\text{% ILS} = \frac{\text{MST of treated group} - \text{MST of normal control}}{\text{MST of normal control}} \times 100
\]

8.2.8.3 Hematological parameters

The activity of standard drug and methanolic extract of Decalepis hamiltonii root on hematological parameters of DLA tumor model was determined. The estimation of hemoglobin content and RBC count\textsuperscript{14} and WBC count\textsuperscript{15} were done by standard procedures.
8.2.8.4 Biochemical estimations

The effect of methanolic extract of root of Decalepis hamiltonii and standard drug on various biochemical parameters like aspartate transaminase, alanine transaminase and total protein was determined.

Assay of serum marker enzymes

Aspartate transaminase was assayed by Reitman and Frankel method\(^\text{16}\) [Appendix 6]

Alanine transaminase was assayed by Reitman and Frankel method\(^\text{16}\) [Appendix 7]

Determination of Protein

Serum and hepatic proteins were assayed by Lowry method\(^\text{17}\) [Appendix 8].

8.2.8.5 Histopathological studies

The rats were sacrificed by cervical decapitation and liver tissues were collected, washed in normal saline and fixed for 24 h in 10\% formalin and then dehydration was done by alcohol. Liver tissues were cleaned and embedded in paraffin block and then cut about 3-5 mM sections. Liver tissues were stained with routine hematoxylin - eosin dye and viewed under light microscope. Morphological changes like fatty changes, lymphocytes inflammation or cell necrosis were observed\(^\text{18}\).

8.2.9 STATISTICAL ANALYSIS

Statistical analysis was conducted by using one way variance analysis (ANOVA) followed by Duncan’s multiple range test. The values are mean ± SD of six rats in each group. Statistical significance was considered at \(p < 0.05\).
8.3 RESULTS

8.3.1 Short term in vitro anticancer activity

The DLA cultures were exposed to different concentrations of methanolic extract of Decalepis hamiltonii for 3 hrs at 37°C. The viability of cultures was estimated by trypan blue dye exclusion method. The results showed that the extract does not reduce the viability of the DLA cells even at highest concentration (1000 µg /ml) tested.

8.3.2 In vitro cytotoxicity by MTT assay method

The cytotoxic effect of methanolic extract of Decalepis hamiltonii root was tested on Vero cell line and A-549 cell lines by MTT method. The CTC50 value of methanolic extract of Decalepis hamiltonii root for Vero cell line and A-549 cell lines were 60 µg/ml and 215 µg/ml respectively. The methanolic extract of Decalepis hamiltonii showed good cytotoxicity against cell lines (Table 8.2).

Table 8.2: CTC\textsubscript{50} value of methanolic extract of Decalepis hamiltonii

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Cell line</th>
<th>Methanolic extract (CTC\textsubscript{50})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Vero</td>
<td>60 µg/ml</td>
</tr>
<tr>
<td>Cancer</td>
<td>A-549</td>
<td>215 µg/ml</td>
</tr>
</tbody>
</table>

8.3.3 In vivo anticancer activity

8.3.3.1 Mean survival time and % ILS

In DLA control group, MST and % ILS was decreased (Table 8.3 and Fig 8.5). On administration of 200 mg and 400 mg of methanolic extract of root of Decalepis hamiltonii increased the mean survival time and % increase life
span in DLA control group. Treatment with 400 mg of methanolic extract of Decalepis hamiltonii showed increase in mean survival time significantly as compared to 200 mg of methanolic extract of Decalepis hamiltonii root.

Table 8.3: Effect of methanolic extract of Decalepis hamiltonii root on MST and % ILS of DLA bearing mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>MST</th>
<th>% ILS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group II</td>
<td>21.4 ± 0.58a</td>
<td>-</td>
</tr>
<tr>
<td>Group III</td>
<td>32.4 ± 0.40b</td>
<td>41.94</td>
</tr>
<tr>
<td>Group IV</td>
<td>26 ± 0.63c</td>
<td>17.11</td>
</tr>
<tr>
<td>Group V</td>
<td>31 ± 0.48b</td>
<td>30.20</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of six rats each group. Values not sharing a common superscript differ significantly at P≤ 0.05 by DMRT. (Group II - Cancer control; Group III - Cyclophosphamide; Group IV - MEDH (200 mg/kg); Group V - MEDH (400 mg/kg).

Fig 8.5: Effect of MEDH on MST and % ILS of DLA bearing mice
8.3.2.2 Body weight analysis

Effect of methanolic extract of Decalepis hamiltonii on average body weight in DLA bearing mice was showed in Table 8.4 and Fig 8.6.

Table 8.4: Anticancer effect of methanolic extract of Decalepis hamiltonii on body Weight of DLA bearing mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Average body weight in gms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>30.0 ± 0.90a</td>
</tr>
<tr>
<td>Group II</td>
<td>40.4 ± 0.74b</td>
</tr>
<tr>
<td>Group III</td>
<td>30.2 ± 1.24a</td>
</tr>
<tr>
<td>Group IV</td>
<td>38.5 ± 1.07c</td>
</tr>
<tr>
<td>Group V</td>
<td>32.8 ± 1.02a</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of six rats each group. Values not sharing a common superscript differ significantly at P≤ 0.05 by DMRT. (Group I - Normal; Group II - Cancer control; Group III - Cyclophosphamide; Group IV - MEDH (200 mg/kg); Group V - MEDH (400 mg/kg).

Fig 8.6: Anticancer effect of MEDH on average body weight of DLA bearing mice
The average body weight was increased significantly in DLA control as than normal control group. On administration of 200 mg and 400 mg of methanolic extract of Decalepis hamiltonii showed the reduction in body weight of DLA bearing mice. Hence treatment with methanolic extract of Decalepis hamiltonii at dose 400 mg was considered to be more potent than with 200 mg of extract.

Since cancer control animals were survived up to 21.4 days, percentage decrease in body weight was calculated on 20th day. Antitumor effect of methanolic extract of Decalepis hamiltonii on weekly body weight analysis on DLA bearing mice was showed in Table 8.5 and Fig 8.7

Table 8.5: Anticancer effect of methanolic extract of Decalepis hamiltonii on weekly body weight analysis of DLA bearing mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Weekly body weight in gms</th>
<th>0 day</th>
<th>7 day</th>
<th>14 day</th>
<th>21 day</th>
<th>28 day</th>
<th>32 day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group II</td>
<td></td>
<td>27.8± 1.28</td>
<td>30.6± 1.20</td>
<td>35.4± 0.81</td>
<td>40.5± 0.64a</td>
<td>All animals dead</td>
<td>All animals dead</td>
</tr>
<tr>
<td>Group III</td>
<td></td>
<td>28.4± 0.67</td>
<td>28.2± 0.80</td>
<td>32.2± 0.73</td>
<td>33.2± 0.80b</td>
<td>33.0± 0.12</td>
<td>31.67±0.33</td>
</tr>
<tr>
<td>Group IV</td>
<td></td>
<td>27.8± 0.58</td>
<td>29.6± 1.16</td>
<td>34.0± 0.94</td>
<td>38.2± 0.96c</td>
<td>All animals dead</td>
<td>All animals dead</td>
</tr>
<tr>
<td>Group V</td>
<td></td>
<td>27.4± 2.15</td>
<td>28.6± 1.86</td>
<td>33.0± 1.64</td>
<td>32.8± 1.06b</td>
<td>31.6± 0.86</td>
<td>All animals dead</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of six rats each group. Values not sharing a common superscript differ significantly at P≤ 0.05 by DMRT. (Group II - Cancer control; Group III - Cyclophosphamide; Group IV - MEDH (200 mg/kg); Group V - MEDH (400 mg/kg).
8.3.2.3 Hematological parameters

The RBC count and hemoglobin content of DLA bearing mice were decreased significantly than normal control group. Treatment of methanolic extract of Decalepis hamiltonii at dose of 200 mg and 400 mg/kg bwt increased the RBC count and hemoglobin level to nearly normal levels. Total counts of WBC were significantly higher in DLA bearing mice as compared with control normal group.

Table 8.6: Effect of methanolic extract of Decalepis hamiltonii root on hematological parameters of DLA bearing mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>RBC/(cumm)</th>
<th>WBC/(cumm)</th>
<th>Hb (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>12.03 ± 0.30a</td>
<td>7.64 ± 0.56a</td>
<td>14.46 ± 0.43a</td>
</tr>
<tr>
<td>Group II</td>
<td>7.33 ± 0.4b</td>
<td>16.48 ± 0.53b</td>
<td>8.7 ± 0.74b</td>
</tr>
<tr>
<td>Group III</td>
<td>10.59 ± 0.28c</td>
<td>8.32 ± 0.39b</td>
<td>13.9 ± 0.44a</td>
</tr>
<tr>
<td>Group IV</td>
<td>9.69 ± 0.66d</td>
<td>10.96 ± 0.27d</td>
<td>10.16 ± 0.20c</td>
</tr>
<tr>
<td>Group V</td>
<td>10.25 ± 0.48c</td>
<td>9.44 ± 0.29a,c</td>
<td>12.94 ± 0.4a,c</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of six rats each group. Values not sharing a common superscript differ significantly at P≤ 0.05 by DMRT. (Group I - Normal; Group II - Cancer control; Group III - Cyclophosphamide; Group IV - MEDH (200 mg/kg); Group V - MEDH (400 mg/kg).
On administration of methanolic extract of Decalepis hamiltonii at the dose of 200 mg and 400 mg/kg reduced the WBC count than DLA mice. Treatment with 200 mg/kg of methanolic extract of Decalepis hamiltonii also recovered these altered parameters towards normal though treatment with 400 mg/kg was found to be more effective (Table 8.6 and Fig 8.8 & 8.9).
8.3.2.4 Biochemical parameters

Biochemical estimations indicated that significantly elevated the activities of serum alanine transaminase, aspartate transaminase and level of total protein in DLA bearing mice with respect to normal control.

Table 8.7: Effect of methanolic extract of Decalepis hamiltonii root on biochemical parameters of DLA bearing mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>ALT (IU/L)</th>
<th>AST (IU/L)</th>
<th>Total Protein (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>32.2 ± 0.58&lt;sup&gt;a&lt;/sup&gt;</td>
<td>85.6 ± 0.81&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.4 ± 0.24&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group II</td>
<td>50.8 ± 0.86&lt;sup&gt;b&lt;/sup&gt;</td>
<td>103.6 ± 1.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.4 ± 0.24&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group III</td>
<td>33.6 ± 0.58&lt;sup&gt;a&lt;/sup&gt;</td>
<td>88.8 ± 0.73&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.4 ± 0.24&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group IV</td>
<td>40 ± 1.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>87 ± 0.70&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.4 ± 0.24&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group V</td>
<td>35.6 ± 1.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>82.6 ± 1.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.6 ± 0.24&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of six rats each group. Values not sharing a common superscript differ significantly at P≤ 0.05 by DMRT. (Group I - Normal; Group II - Cancer control; Group III - Cyclophosphamide; Group IV - MEDH (200mg/kg); Group V - MEDH (400mg/kg).

However on administration of methanolic extract of Decalepis hamiltonii at the dose of 200 mg and 400 mg/kg and standard drug significantly reduced the activities of serum alanine transaminase, aspartate transaminase and total protein as compared to cancer control group indicated the protection of tumor cell induced hepatotoxicity by methanolic extract of Decalepis hamiltonii (Table 8.7 and Fig 8.10 & 8.11).
Fig 8.10: Effect of MEDH on activities of AST and ALT of DLA bearing mice

Fig 8.11: Effect of MEDH on total protein of DLA bearing mice
8.3.2.5 Histopathological studies

The histopathological observation of liver section of control and experimental animals were showed in Plate 8.1. Control animals showed normal liver architecture with intact central vein, well preserved hepatocytes, prominent nucleus and nucleolus [Plate 8.1 (a)] whereas DLA induced mice showed loss of infiltration of cells, disturbed hepatocytes, nucleus and vacuoles [Plate 8.1 (b)]. However, mice treated with methanolic extract of Decalepis hamiltonii at the dose of 200mg and 400mg/kg bwt and cyclophosphamide (25mg/kg) showed almost normal hepatocellular architecture [Plate 8.1 (c), (d) and (e)].
Plate 8.1: Histopathology of liver

(a) Group I  (b) Group II

(c) Group III  (d) Group IV

(e) Group V

Group I: Normal control; Group II: Cancer control; Group III: Cyclophosphamide;
Group IV: 200mg MEDH and Group V: 400mg MEDH
8.4 DISCUSSION

The investigation was done to determine the antitumor activity of methanolic extract of Decalepis hamiltonii root of DLA tumor mice. A promising evidence for prevention of cancer today was chemoprevention which was defined as use of natural or synthetic agents to prevent cancer development in humans\textsuperscript{19}. Several natural products were used as anticancer agents and also served as leading compounds for further research. A several bioactive compounds and their derivatives proved to inhibit carcinogenesis in variety of experimental systems involving initiation, promotion and progression\textsuperscript{20}. Vegetables, herbs and plants were used in traditional and folk medicine and they were currently accepted as one of the important sources for drug discovery and development of chemoprevention of cancer\textsuperscript{21}.

In Indian traditional medicine system, various plants were used for treatment of tumors but most of them were not evaluated scientifically\textsuperscript{22}. But there was a lot of scope occurs to identify potent anticancer plants. There was a risk of developing life threatening toxicity of host by using chemotherapeutic drugs. Several studies were conducted to minimize the side effects after treatment with the chemotherapeutic agents using different modalities\textsuperscript{23}. Various natural products and their derivatives were already in use like Paclitaxel, Vinblastine and Etoposide play a important roles in chemotherapy of cancer\textsuperscript{24,25}.

The well developed anticancer agent should have the capacity to kill or weakened the cancer cells without any excessive damage to normal healthy cells. In cancer cells, some plant products may induce apoptosis but not in normal cells.
So it was necessary to examine the plant apoptotic inducers that may be either as crude extracts or active isolated compounds. However plant photochemical was used as capable drug source for population in the World and various plant derived drugs were involved in clinical use extensively\textsuperscript{26}.

A recent study revealed that the methanolic extract of Decalepis hamiltonii at the dose of 400mg/kg bwt significantly increased the life span of mice as compared to DLA control. The important criteria for the ability of all anticancer drugs were the prolongation of life span\textsuperscript{27}, inhibition of gain of average body weight and decreased in WBC from blood\textsuperscript{28}. The methanolic extract delayed the cell division which suggested the reduction in DLA volume and increased in survival time of mice which proved the antiproliferative effect of the extract\textsuperscript{29}. Methanolic extract of Decalepis hamiltonii at the dose of 400 mg/kg considerably elevated the mean survival time of tumor possessing mice.

The cytotoxicity of chemotherapeutic agents has effects on malignant and also on rapidly dividing cells. This was the reason for mucosal ulceration, hair loss and suppression of hemopoiesis. Myelosuppression and anemia (reduced hemoglobin)\textsuperscript{30} were the important problems occurred in chemotherapy of cancer. Reduced hemoglobin content, increased WBC count and reduced RBC count were noticed in DLA control mice. In present study, oral administration of methanolic extract of Decalepis hamiltonii root restored the hemoglobin content, RBC and WBC count to nearly normal levels which proved the hematopoietic protecting activity without induction of myelotoxicity (main side effects of cancer chemotherapy). It was observed that improvement in hematological profile of
tumor bearing mice after treatment with extract may be due to action of different phyto constituents present in it.

In ascites carcinoma, anemia occurred mainly due to deficiency of iron either by myelopathic or hemolytic conditions which resulted in reduction of RBC count\textsuperscript{31}. Reduction of hemoglobin content was common in cancer patients and cause unfavorable effect on physical functioning and variables life quality such as cognitive function and fatigue. It was found that low hemoglobin levels were associated with decreased survival in cancer patients and also in solid tumors\textsuperscript{32}. The present results were coincided with the fact that cancer has been well described for many years as a cause of microangiopathic hemolytic anemia and thrombocytopenia\textsuperscript{33}.

Compelling evidence was found recently that immune response was affected in oral squamous cell carcinoma (SCC) patients\textsuperscript{34}. Previous studies showed that the growing burden of tumor was connected with worse changes in immunity. The leucocytes of the innate immune system, including neutrophils, macrophages and NK cells, infiltrated the tumor site for a multipronged killing response\textsuperscript{35}. The significant increased neutrophils and WBC in tumor possessed mice was due to that these cells appear at sites of infection at first which leads to released of proteases and chemokines which in turn persuades both specific and non-specific effectors cells of immune system\textsuperscript{36}. Against neighboring cells, they may also release toxic granules which suggested potential anti-tumor activity\textsuperscript{37}.

The wisdom of dietary traditional practices was supported by biochemical, physiological and pharmacological studies. Several beneficial biological functions
were exerted by phytochemicals derived from vegetables and fruits. The presence of carbohydrates, phytosterols, fixed oils, phenolic compounds, saponins, proteins, tannins and flavonoids were revealed in preliminary phytochemical screening of Decalepis hamiltonii. Flavonoids were showed to have antimalignant and antimutagenic effect\(^3\). Flavonoids also possessed chemopreventive role in cancer by acting on signal transduction in angiogenesis and cell proliferation\(^3\). The anticancer activity and cytotoxicity of methanolic extract of Decalepis hamiltonii root was mainly due to the presence of flavonoids.

The presence of phytosterols may involve in cell membrane incorporation, activity of membrane-bound enzymes and alteration of membrane fluidity. It also caused alteration in signal transduction in pathways which leads to growth of tumor and stimulation of apoptosis in tumor cell lines. They also have the ability to improve proliferation of T-cell and lymphocyte of human peripheral blood in vitro thus suggested the stimulation of function of immune system\(^4\).

Significant elevation in the activities of aspartate transaminase, alanine transaminase and level of protein indicated the hepatocellular damages by several agents. Inoculation of DLA causes hepatotoxicity to some extent which was proved in biochemical estimation of these parameters. The presence of tumor in experimental animals or humans was reported to affect various functions of vital organs mainly liver even there was no interference of tumor site directly with functions of organ\(^4\). Treatment with methanolic extract of Decalepis hamiltonii root returned the elevated biochemical parameters to nearly normal range which indicated the protection of hepatotoxicity induced by tumor cell.
Antitumor activities have been reported in several plant species\textsuperscript{42,43} however, up to now only few researches have done investigations for this traditionally used plant in identifying their mechanism and guaranteeing in future its therapeutic and scientific use. The best approach for isolation of anticancer lead compounds from medicinal plants was plant selection on the basis of ethno medical knowledge and the selected plant was to be tested for their efficacy and also for safety\textsuperscript{44}.

However, further investigation is necessary to prove its worthwhile potential effect of Decalepis hamiltonii root in treatment for tumor. Further studies are in progress for characterization of active principles and for elucidation of mechanism of action of Decalepis hamiltonii root.
8.5 REFERENCE


