Anticancer activity of ethanolic extract of *Wattakaka volubilis* leaves on DAL induced lymphoma in mice with it's antioxidant effect

Dissertation Submitted to

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In Partial fulfillment for the award of the degree of

MASTER OF PHARMACY

IN

PHARMACOLOGY

Submitted by

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MAY-2012

EVALUATION CERTIFICATE

This is to certify that the dissertation work entitled **"Anticancer activity of ethanolic extract of *Wattakaka volubilis* leaves on DAL induced lymphoma in mice with it's antioxidant effect "** submitted by the student bearing **Reg. No:26103091** to "The Tamil Nadu Dr. M.G.R. Medical University", Chennai, in partial fulfillment for the award of degree of **MASTER OF PHARMACY** in **PHARMACOLOGY** was evaluated by us during the examination held on…….

Internal Examiner **External Examiner**

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DECLARATION

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I further declare that, this work is original and has not been submitted in part or full for the award of any other degree or diploma in any other university and the thesis is ready for evaluation.

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CONTENTS

ABBREVIATIONS

µg	-	Micro gram
mg/dl	-	milligram per decilitre
µl	-	Micro litres
ad libitum	-	unlimited, one's own pleasure
AAPH	-	2- amidino propanoic dihydro chloride
ANOVA	-	Analysis of variance
Conc.	-	Concentration
CSIR	-	Council of scientific and industrial research
CTC	-	Concentration test drug
cumm	-	Cubic millimeter
DAL	-	Dalton's ascites lymphoma
DCF	-	Dichloro fluorescin diacetate
DMEM	-	Dulbecco's modified eagle's medium
DNA	-	Deoxyribonucleic acid
DPPH	-	2, 2-diphenyl-1-picrylhydrazyl
EFSA	-	European food safety authority
FBS	-	Fetal bovine serum
Fl	-	Femto litres
FRAP	-	Ferric reducing antioxidant power
Hep G2	-	Hepato cellular carcinoma cells
i.p	-	Intra peritonial route of administration

MCH	-	Mean corpuscular haemoglobin
MCHC	-	Mean corpuscular haemoglobin concentration
MCV	-	Mean corpuscular volume
MDA	-	Muscular dystrophy association
Mg	-	Milli grams
mM	-	Milli molar
mmol/l	-	Milli molar/ litre
No.	-	Number
p.o	-	Oral route of administration
PBS	-	Phosphate buffer solution
Pg	-	Pico grams
ROS	-	Reactive oxygen species
s.c	-	Subcutaneous route of administration
TPTZ	-	2, 3, 5-triphenyltetrazol-2-ium chloride
TPVG	-	Trypsin phosphate versene glucose
TRAP	-	Total radical trapping antioxidant potential
ULCA	-	University of California, Los angels
U/L	-	Units per litre

1. INTRODUCTION

1.1 CANCER

Synonyms- carcinoma, malignant tumour, malignant neoplasm, C, big C, canker, carcinoma, corruption, disease, long illness, malignancy, sickness, tumor **(http://thesaurus.com/browse/cancer)**

Origin of the Word "Cancer"- The word cancer came from the father of medicine, Hippocrates, a Greek physician. Hippocrates used the Greek words, carcinos and carcinoma to describe tumors, thus calling cancer "karkinos." The Greek terms actually were words to describe a crab, which Hippocrates thought a tumor resembled. Although Hippocrates may have named "Cancer," he was certainly not the first to discover the disease. The history of cancer actually begins much earlier.

The First Documented Case of Cancer- The world's oldest documented case of cancer hails from ancient Egypt, in 1500 b.c. The details were recorded on a papyrus, documenting 8 cases of tumors occurring on the breast. It was treated by cauterization, a method to destroy tissue with a hot instrument called "the fire drill." It was also recorded that there was no treatment for the disease, only palliative treatment. There is evidence that the ancient Egyptians were able to tell the difference between malignant and benign tumors. According to inscriptions, surface tumors were surgically removed in a similar manner as they are removed today. **(http://cancer.about.com/od/historyofcancer/a/cancerhistory.htm)**

Definition of cancer- Cancer is a term used for diseases in which abnormal cells divide without control and are able to invade other tissues. Cancer cells can spread to other parts of the body through the blood and lymph systems.

Cancer is not just one disease but many diseases. There are more than 100 different types of cancer. Most cancers are named for the organ or type of cell in which they start - for example, cancer that begins in the colon is called colon cancer; cancer that begins in basal cells of the skin is called basal cell carcinoma**.**

Thus cancer is defined as the uncontrolled growth of abnormal cells in the body of a living organism these cells are also called as malignant cells. These cells form a mass called as tumours.

1). CLASSIFICATION:

The tumours can be of two types

a. Benign tumours
b. Malignant tumours

Benign tumours are generally noncancerous cells where as malignant tumours are cancerous and can invade nearby tissues which can break away and spread to other parts of the body.

Cancers are usually named using -carcinoma, -sarcoma or -blastoma as a suffix, with the Latin or Greek word for the organ or tissue of origin as the root.

For example, cancers of the liver parenchyma arising from malignant epithelial cells is called *hepatocarcinoma*, while a malignancy arising from primitive liver precursor cells is called a *hepatoblastoma*, and a cancer arising from fat cells is called as *liposarcoma*.

For some common cancers, the English organ name is used. For example, the most common type of breast cancer is called *ductal carcinoma of the breast*. Here, the *ductal* refers to the appearance of the cancer under the microscope, which suggests that it has originated in the milk ducts.

Benign tumours (which are not cancers) are named using -oma as a suffix with the organ name as the root. For example, a benign tumour of smooth muscle cells is called a *leiomyoma* (the common name of this frequently occurring benign tumour in the uterus is *fibroid*). Confusingly, some types of cancer also use the -oma suffix, examples including melanoma and seminoma.

Some types of cancer are named for the size and shape of the cells under a microscope, such as giant cell carcinoma, spindle cell carcinoma, and small cell carcinoma.

(**Kinzler *et al.*, 2002)**

2). TYPES OF CANCER:

Cancer types can be grouped into broader categories. The main categories of cancer include:

Carcinoma - cancer that begins in the skin or in tissues that line or cover internal organs.

Sarcoma - cancer that begins in bone, cartilage, fat, muscle, blood vessels, or other connective or supportive tissue.

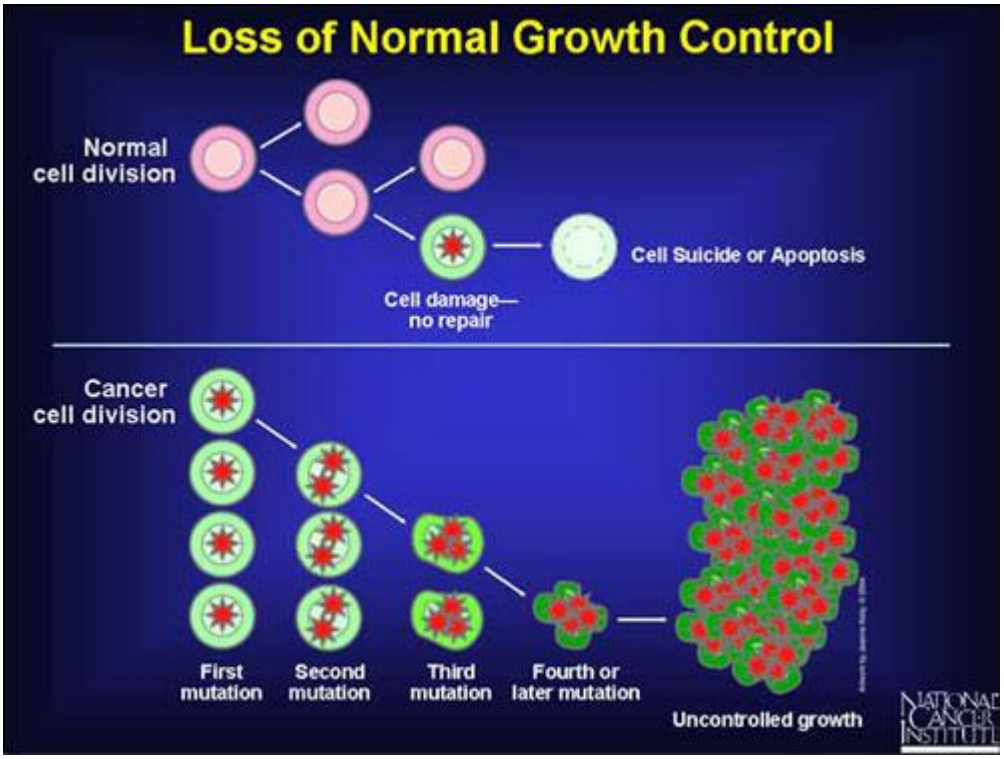
Leukemia - cancer that starts in blood-forming tissue such as the bone marrow and causes large numbers of abnormal blood cells to be produced and enter the blood.

Lymphoma and myeloma - cancers that begin in the cells of the immune system.

Central nervous system cancers - cancers that begin in the tissues of the brain and spinal cord.

3). ORIGINS OF CANCER:

All cancers begin in cells, the body's basic unit of life. To understand cancer, it's helpful to know what happens when normal cells become cancer cells.The body is made up of many types of cells. These cells grow and divide in a controlled way to produce more cells as they are needed to keep the body healthy. When cells become old or damaged, they die and are replaced with new cells.However, sometimes this orderly process goes wrong. The genetic material (DNA) of a cell can become damaged or changed, producing mutations that affect normal cell growth and division. When this happens, cells do not die when they should and new cells form when the body does not need them. The extra cells may form a mass of tissue called a tumor.



Not all tumors are cancerous; tumors can be benign or malignant.

- **Benign tumors** aren't cancerous. They can often be removed, and, in most cases, they do not come back. Cells in benign tumors do not spread to other parts of the body.
- **Malignant tumors** are cancerous. Cells in these tumors can invade nearby tissues and spread to other parts of the body. The spread of cancer from one part of the body to another is called metastasis.Some cancers do not form tumors. For example, leukemia is a cancer of the bone marrow and blood**.**

(http://cancer.about.com/od/historyofcancer/a/cancerhistory.htm)

2). CAUSES OF CANCERS

There are different kinds of cancers which can develop in almost any organ or tissue. The main causes of cancer are

a. Benzene and other chemicals
b. Drinking excess alcohol
c. Environmental toxins
d. Excessive exposure to sunlight
e. Genetic problems
f. Obesity
g. Radiation
h. Viruses

However many causes which may lead to cancer are unknown. The spread of cancer from one part to other part is called metastatis.

Free radicals compounds also cause damage to cells. These contain an unpaired electron in the outer nucleus of the cell. The free radicals generated from the oxygen are called ROS which can oxidise or reduce molecules.

The free radicals formed mainly by two sources in body i.e. endogenous sources (nutrient metabolism, aging process etc) and exogenous sources (smoke, radiation, air pollution, pesticides, chemicals etc)

3). SYMPTOMS

The symptoms of cancer are divided in to three groups:

a. Local symptoms- these are restricted only to the site of the cancer. This includes lumps, swelling, haemorrhage, ulceration and pain.
b. Metastatic symptoms- these are symptoms due to the spread of the cancer to other parts of the body. They include enlarged lymph nodes, hepatomegaly, splenomegaly, which can be felt in the abdomen, pain or fracture in the bones and neurological symptoms
c. Systematic symptoms - these occur due to distant effects of the cancer that are not related to direct metastatic spread which may include weight loss, fatigue, excessive sweating, anaemia and other symptoms which may be mediated by immunological or hormonal signals from the cancer cells.

All these symptoms are not diagnostic which occur commonly in patients not having cancer.Cancer is primarily an environmental disease with 90-95% of cases due to environmental factors and 5-10% due to genetics. **(Moscow *et al.*, 2007**)

4). PATHOPHYSIOLOGY OF CANCER

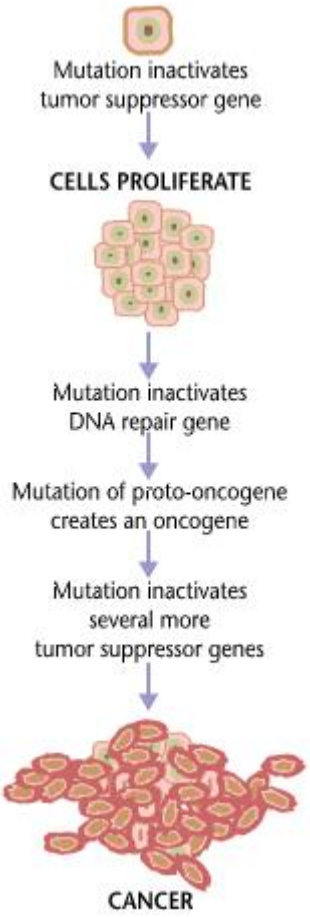


Figure – 1

In body there are cancer suppressor genes which get inactivated by mutation and these cells gets proliferated which further more inactivates the DNA repair genes which inactivates much more tumour suppressing genes which leads finally to cancer. **(Kim *et al.*, 2006)**

Cancer is caused by a series of mutations. Each mutation alters the behaviour of the cell. Cancer is fundamentally a disease where in there is a failure of tissue regulation in the body. In order to transform in to a cancer cell the genes which regulate the cell growth and differentiation must be altered.

The affected genes are divided in to two categories

a. Oncogenes
b. Tumour suppressor genes

Oncogenes are those genes which are responsible for the cell growth and reproduction. (**Croce, 2008**)

Tumour suppressor genes are vice-versa (i.e. Inhibit the cell division and survival). Malignant transformation can occur through formation of novel oncogens due to over expression or by under expression of tumour suppressing genes.

Genetic changes occur in different levels by different mechanisms. The gain or loss of a chromosome is due to error in mitosis or by mutations which change the nucleotide sequence of DNA.

According to the National Cancer Institute in 2010, the most common cancers (excluding non-melanoma skin cancers) are listed below.

Cancer type	**Estimated new cases**	**Estimated deaths**
Bladder	70,530	14,680
Breast (female-male)	207,090-1,970	39,840-390
Colon and rectal (combined)	142,570	51,370
Endometrial	43,470	7,950
Kidney (renal cell)	53,581	11,997

Leukemia	43,050	21,840
Lung (including bronchus)	222,520	157,300
Melanoma	68,130	8,700
Non-Hodgkin lymphoma	65,540	20,210
Pancreatic	43,140	36,800
Prostate	217,730	32,050
Thyroid	44,670	1,690

Some other cancers include brain cancer, hodgkin's lymphoma, testicular cancer, liver cancer, uterine cancer.

The three most common cancers in men, women and children are as follows:

- **Men**: Prostate, lung, and colorectal.
- **Women**: Breast, colorectal, and lung.
- **Children**: Leukemia, brain tumours, and lymphoma. **(Jermal *et al.*, 2011)**

5). SCREENING AND TESTING TO DETECT CANCER:

- Imaging Tests
- Laboratory Tests
- Other Testing Information

5.1) IMAGING TESTS:

A) Mammograms:

A fact sheet that defines screening and diagnostic mammograms. Discusses mammography screening guidelines and risk factors for breast cancer.

B) Find an FDA Certified Mammography Facility:

Find an FDA certified mammography facility near where you live or work.

C) Computed Tomography (CT):

A fact sheet that describes the CT scan procedure and technology and its uses in diagnosis and treatment.

5.2) LABORATORY TESTS:

A) Interpreting Laboratory Test Results:

A fact sheet that describes the role of screening and diagnostic laboratory tests. Includes a brief discussion of factors affecting the results.

B) Pap Test:

A fact sheet that describes the Pap test procedure, possible results, and the link between HPV and cervical cancer.

C) Pap Tests:

Provides information about the importance of routine Pap testing in preventing and detecting cervical cancer, when to get a Pap test, and what to expect during screening. For rarely- or never-screened women.

D) Prostate-Specific Antigen (PSA) Test:

A fact sheet that describes the PSA screening test for prostate cancer and explains the benefits and limitations of the test.

E) Tumor Markers:

A fact sheet that defines tumor markers and describes how they can be used to aid diagnosis and treatment.

5.3) OTHER TESTING INFORMATION:

A) Understanding Cancer Series: Gene Testing

This tutorial illustrates what genes are, explains how mutations occur and are identified within genes, and discusses the benefits of gene testing for cancer and other disorders.

B) Proteomics and Cancer:

This fact sheet describes proteomics (the study of proteins and how they respond to the needs of the body or in disease). This research may lead to new ways to diagnose cancer and identify the best treatments for individual patients.

C) What You Need To Know About™ Cancer Index:

Learn the symptoms of cancer - and symptoms of many cancer types - by viewing the online booklets listed here
.(http://www.cancer.gov/cancertopics/screening/types)

6). TREATMENT TYPES:

a) SURGERY:

Surgery can be used to diagnose, treat, or even help prevent cancer in some cases. Most people with cancer will have some type of surgery. It often offers the greatest chance for cure, especially if the cancer has not spread to other parts of the body.

b) CHEMOTHERAPY:

Chemotherapy (chemo) is the use of medicines or drugs to treat cancer. The thought of having chemotherapy frightens many people. But knowing what chemotherapy is, how it works, and what to expect can often help calm your fears. It can also give you a better sense of control over your cancer treatment.

c) RADIATION THERAPY:

Radiation therapy uses high-energy particles or waves to destroy or damage cancer cells. It is one of the most common treatments for cancer, either by itself or along with other forms of treatment.

d) TARGETED THERAPY

Targeted therapy is a newer type of cancer treatment that uses drugs or other substances to more precisely identify and attack cancer cells, usually while doing little damage to normal cells. Targeted therapy is a growing part of many cancer treatment regimens.

e) IMMUNOTHERAPY:

Immunotherapy is treatment that uses your body's own immune system to help fight cancer. Get information about the different types of immunotherapy and the types of cancer they are used to treat.

f) HYPERTHERMIA:

The idea of using heat to treat cancer has been around for some time, but early attempts had mixed results. Today, newer tools allow more precise delivery of heat, and hyperthermia is being studied for use against many types of cancer.

g) BONE MARROW AND PERIPHERAL BLOOD STEM CELL TRANSPLANT:

Here we offer a review of bone marrow transplants and other types of stem cell transplants that are used to treat cancer.

h) PHOTODYNAMIC THERAPY:

Photodynamic therapy or PDT is a treatment that uses special drugs, called photosensitizing agents, along with light to kill cancer cells. The drugs only work after they have been activated or "turned on" by certain kinds of light.

i) LASERS IN CANCER TREATMENT:

Lasers, which are very powerful, precise beams of light, can be used instead of blades (scalpels) for very careful surgical work, including treating some cancers.

j) BLOOD PRODUCT DONATION AND TRANSFUSION:

Transfusions of blood and blood products temporarily replace parts of the blood when a person's body can't make its own or has lost them from bleeding.

k) HORMONE REPLACEMENT THERAPY VS. HORMONAL TREATMENT:

In this therapy, doctors attempt to block the production of hormones that are feeding the growth of the cancer.

l) MOLECULAR TARGETED THERAPY:

Molecular targeted therapies may become a revolutionary change in the treatment of cancer**.**

(http://www.cancer.org/Treatment/TreatmentsandSideEffects/TreatmenTypes/index)

7). PREVENTION

The risk of cancer can be reduced by following methods

a. Healthy diet
b. Regular exercise
c. Limiting alcohol
d. Maintain healthy weight
e. Less exposure to radiation and toxic chemicals
f. Reducing sun exposure
g. Not smoking or chewing tobacco

(Thum *et al.*, 2007)

8). ALTERNATIVE TREATMENTS

Alternative cancer treatments include alternative medicine of non-related interventions that do not fit the regular western medicine and include mind–body interventions, herbal preparations, massage, acupuncture, reiki, electrical stimulation devices, and a variety of strict dietary regimens.

Alternative cancer treatments have never been shown to be effective at killing cancer cells in research studies, but remain popular in some cultures and religions.

9). PALLIATIVE CARE

Palliative care is a multidisciplinary approach to symptom management that aims to reduce the physical, emotional, spiritual, and psycho-social distress experienced by people with cancer. Unlike treatment that is aimed at directly killing cancer cells, the primary goal of palliative care is to make the person feel better as soon as possible. (**Astin *et al.*, 1998**)

10). HOME REMEDIES FOR CANCER AND TUMOURS

Turmeric - contains a therapeutic compound called curcumin, turmeric has been shown to fight cancer and tumourous growths.

Green Tea Extract - Green tea extract contains a chemical called ECGC, which neutralizes highly reactive free radicals that cause cell damage. Green tea extract also inhibits cell replication, a main feature of cancer.

Maitake Extract - from the Chinese mushroom, maitake extract aids in the destruction of cancer cells and combats the spread of tumours.

Red Clover - The flowers from this three-leafed herb contain four anti-tumor compounds. Also, the red clover contains large amounts of tocopherol, a powerful antioxidant that combats cell-destroying free radicals.

Other Important Nutrients for Cancer Prevention and Treatment

Quercetin - known as citrus bioflavanoid, quercetin may reverse abnormal cell growth and inhibit prostate cancer.

Beta Glucan - These are polysaccharides they can boost your immune system. Vitamin K and Vitamin C - taken together these vitamins might inhibit cancerous growths. Vitamin K has shown promise in treating liver cancer.

(Herbs for Cancer - Cancer herbs, mht, Herbal - Supplements-Guide.Com.)

1.2. INDIAN MEDICINAL PLANTS HAVING ANTICANCER ACTIVITY

Cancer is the leading cause of death in developing countries like India. As there is an enormous increase in the population day by day, the alternative therapy in the market is getting its glimpse. The cheap herbal drug treatment may highly be recommended to the rural and poor people to treat effectively the cancers of various type is an ideal choice. Based on that the siddha medicines are coming up in combination with metals and other essential supplements to improve the immune status of the cancer patients in India.

S.no	Name of the plant	Family	Parts used
1.	*Calotrophis gigantea*	Asclepiadaceae	Whole plant
2.	*Cajanus cajan*	Fabaceae	Leaves
3.	*Butea monosperma*	Fabaceae	Bark
4.	*Bauhinia variegate*	Caesalpinaceae	Root
5.	*Bacopa monnieri*	Scropulariaceae	Whole plant
6.	*Azadirachta indica*	Meliaceae	Bark
7.	*Asparagus racemosus*	Liliaceae	Root
8.	*Aphanamixis polystachya*	Meliaceae	Bark
9.	*Aloe barbadensis*	Liliaceae	Leaf juice
10.	*Alium cepa*	Liliaceae	Bulb
11.	*Acorus calamus*	Araceae	Rhizome
12.	*Cassia absus*	Caesalpinaceae	Leaves
13.	*Cassia auriculata*	Caesalpinaceae	Root

14.	*Cassia senna*	Caesalpinaceae	Leaves
15.	*Catunaregum spinosa*	Rubiaceae	Bark/Fruit
16.	*Citrullus colocynthis*	Cucurbitaceae	Root
17.	*Citrus medica*	Rutaceae	Root
18.	*Cissus quadrangularis*	Vitaceae	Whole plant
19	*Clerodendrum serratum*	Verbanaceae	Root
20	*Clerodendrum viscosum*	Verbanaceae	Leaves
21	*Crinum asiaticum*	Amaryllidaceae	Bulb
22.	*Daucus carota*	Apiaceae	Root
23.	*Embelia ribes*	Myrsinaceae	Fruit
24	*Flacourtia jangomos*	Flacourtiaceae	Bark/Leaf
25	*Jatropha curcas*	Euphorbiaceae	Leaves, seed, oils
26	*Kaempferia galanga*	Zingiberaceae	Rhizome
27	*Kaempferia rotunda*	Zingiberaceae	Tubers
28	*Lanata camara*	Verbanaceae	Whole plant
29	*Lens culinaris medikus*	Fabaceae	Seed
30	*Limonia acidissima*	Rutaceae	Fruit
31	*Macrotyloma uniflorum*	Fabaceae	Seed
32	*Mimosa pudica*	Mimosaceae	Whole plant
33	*Nicotiana tabacum*	Solanaceae	Leaves
34	*Operculina turpethum*	Convolvulaceae	Root
35	*Rhinacanthus nasuta*	Acanthaceae	Whole plant
36	*Salvadora persica*	Salvadoraceae	Bark, Leaf, Shoot, Fruit
39	*Symplocus cochinchinensis*	Symplocaceae	Bark
40	*Tylopora indica*	Asclepiadaceae	Root, Leaf
41	*Vernonia cinerea*	Asteraceae	Whole plant
42	*Vitex trifolia*	Verbanaceae	Leaf
43	*Zanthoxylum armatum*	Rutaceae	Bark,Fruit
44.	*Xanthium strumarium*	Compositae	Root

Hence many Indian medicinal plants have anticancer activities. Different parts of the plants used for anticancer activities.

(**Nair *et al.*, 2010), (Paolo Scartezzini and Ester Speroni, 2000), (Dhanamani *et al.*, 2011), (Sakarkar and Deshmukh, 2011)**

1.4. WHAT ARE FLAVONOIDS AND THEIR ROLE AS ANTICANCER AGENTS?

Flavonoids (or **bioflavonoids**) (from the Latin word *flavus* meaning yellow), also collectively known as **Vitamin P** and **citrin**, are a class of plant secondary metabolites or yellow pigments having a structure similar to that of flavones. According to the IUPAC nomenclature they can be classified into:

- *flavonoids*, derived from 2-phenylchromen-4-one (2-phenyl-1, 4-benzopyrone) structure (examples: quercetin, rutin).
- *isoflavonoids*, derived from 3-phenylchromen-4-one (3-phenyl-1,4-benzopyrone) structure
- *neoflavonoids*, derived from 4-phenylcoumarine (4-phenyl-1,2-benzopyrone) structure. (Flavanoids IUPAC compendium of chemical technology)

The three flavanoids classes above are all ketone-containing compounds, and as such, are flavanoids and flavonol.

Flavonoids are the most important plant pigments for flower coloration producing yellow or red/blue pigmentation in petals designed to attract pollinator animals.

Flavonoids secreted by the root of their host plant help *Rhizobia* in the infection stage of their symbiotic relationship with legumes like peas, beans, clover, and soy.

In addition, some flavanoids have inhibitory activity against organisms that cause plant disease e.g. *Fusarium oxysporum*.

1). SOURCES

Good sources of flavanoids include all citrus fruits, berries, ginkgo biloba, onions (particularly red onion), parsley, pulses, tea (especially white and green tea), red wine, sea buckthorn, and dark chocolate (with a cocoa content of seventy percent or greater).

Over 5000 naturally occurring flavanoids have been characterized from various plants.

Flavonoids (specifically flav**a**noids such as the catechins) are "the most common group of polyphenolic compounds in the human diet and are found ubiquitously in plants". Flavonols, the original bioflavonoids such as quercetin, are also found ubiquitously, but in lesser quantities. **(Brown, 1980**), **(Justesen *et al*., 2001)**

2). EFFECTS ON HUMAN HEALTH

Flavonoids might induce mechanisms that affect cancer cells and inhibit tumor invasion. In preliminary studies, UCLA cancer researchers proposed that smokers who ate foods containing certain flavanoids, such as catechins found in strawberries and green and black teas, kaempferol from brussel sprouts and apples, and quercetin from beans, onions and apples, may have reduced risk of obtaining lung cancer.

The widespread distribution of flavonoids, their variety and their relatively low toxicity compared to other active plant compounds (for instance alkaloids) mean that many animals, including humans, ingest significant quantities in their diet. Preliminary research indicates that flavonoids may modify allergens, viruses, and carcinogens, and so may be biological "response modifiers". In vitro studies show that flavonoids also have anti-allergic, anti-inflammatory, anti-microbial, anti-cancer, and anti-diarrheal activities.

3). ANTIOXIDANT ACTIVITY *IN-VITRO*

Flavonoids (both flav**o**nols and flav**a**nols) are most commonly known for their antioxidant activity. Additionally, at high experimental concentrations that would not exist in vivo, the antioxidant abilities of flavanoids in vitro are stronger than those of vitamin C & E.

4). WHAT DO THEY DO?

As antioxidants, some flavanoids such as Quercetin protect LDL cholesterol from oxidative damage.

The ability to limit inflammation gives the flavanoids their disease fighting potential. They actively prevent the release of histamine in the body - this is the agent responsible for most allergy symptoms like congestion.

Flavonoids also actively scavenge free radicals they tend to boost immune system functioning and strengthen the blood vessels, thereby increasing the blood flow.

Flavonoids exhibit potent anticancer effects, although the exact target of such inhibition has not been definitely established, given the recent evidence for a prominent role for reactive oxygen species (ROS) in carcinogenesis. It is tempting to speculate that flavanoids inhibit carcinogenesis due to ROS scavenging. However there have been a number of reports that directly contradict the potential role of flavanoids as antioxidants/anticancer agents.

Flavonoid and related compound are effective in scavenging DPPH radical, hydroxyl radical and in metal-chelating capacity. **(Irfan khan 2011)**

a). QUERCETIN

Quercetin, a flavanoids and more specifically a flavonol, is the aglycone form of other flavanoids glycosides, such as rutin and quercitrin, found in citrus fruit, buckwheat and onions. Quercetin forms the glycosides, quercitrin and rutin, together with rhamnose and rutinose, respectively.

Although there is preliminary evidence that asthma, lung cancer and breast cancer are lower among people consuming higher dietary levels of quercetin, the U.S. Food and Drug Administration (FDA), EFSA and the American Cancer Society have concluded that no physiological role exists. The American Cancer Society states that dietary quercetin "is unlikely to cause any major problems or benefits." **(Pawlknekt *et al.*, 2002**)

b). EPICATECHIN (EC)

Epicatechin may improve blood flow and has potential for cardiac health. Cocoa, the major ingredient of dark chocolate, contains relatively high amounts of epicatechin and has been found to have nearly twice the antioxidant content of red wine and up to three times that of green tea in vitro. **(Lee *et al*., 2003)**

1.5. PHYTOSTEROLS

Phytosterols (also called plant sterols) area group of steroidalcohols, phytochemicals naturally occurring in plants. Plants contain a range of phytosterols. They act as a structural component in the cell membrane, a role that, in mammalian cells, is played by cholesterol.

1). SOURCES

Phytosterols occur naturally in small quantities in vegetable oils, especially sea buckthorn oil (1640 mg/100g oil), corn oil (968 mg/100g), and soybean oil (327 mg/100g oil). One such phytosterol complex, isolated from vegetable oil, is cholestatin, composed of campesterol, stigmasterol, and brassicasterol, and is marketed as a dietary supplement. They are white powders with mild, characteristic odor, insoluble in water and soluble in alcohols. They have applications in medicine and cosmetics and as a food additive taken to lower cholesterol. **(Pennigton, 2005)**

2). USES

As a food ingredient or additive, phytosterols have cholesterol-lowering properties (reducing cholesterol absorption in intestines).

The FDA has approved the following claim for phytosterols "Foods containing at least 0.4 gram per serving of plant sterols, eaten twice a day with meals for a daily total intake of at least 0.8 gram, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease." **(Ostlund *et al*., 2003)**

Researchers in Uruguay who conducted a small case study suggested an association between dietary sterol intake and decreased risk for the development of gastrointestinal cancers, However, a subsequent large-scale study from the Netherlands reported that high dietary intake of plant sterols was not associated with a lower risk of colon and rectal cancers. **(Lunasin-Cholesterol Reducing Soy Peptide That's Natural and Beneficial.htm., Copyright 2011)**

Phytosterols demonstrated cholesterol lowering effects and reduction in coronary heart disease risks. Several studies have indicated that phytosterols may possess anticancer activity. Cytotoxicity of plant sterols particularly by stigmasterol against cancer cells were evidenced towards breast cancer cells **(Awad *et al.*, 2000a).** Moreover, cytotoxicity of plant sterols was also observed in colon cancers cells**. (Chong *et al.*, 2010)**

3). HOW DO THEY ACT ON CANCER

Phytosterols (PS) or plant sterols are structurally similar to cholesterol. The most common PS are beta-sitosterol, campesterol and stigmasterol. Epidemiologic and experimental studies suggest that dietary PS may offer protection from the most common cancers in western societies, such as colon, breast and prostate cancer. This review summarizes the findings of these studies and the possible mechanisms by which PS offer this protection. These include the effect of PS on membrane structure and function of tumor and host tissue, signal transduction pathways that regulate tumor growth and apoptosis, immune function of the host and cholesterol

metabolism by the host.**(Awad *et al.*, 2000), (Lunasin-Cholesterol Reducing Soy Peptide That's Natural and Beneficial. htm., Copyright 2011)**

1.5.1. BETA-SITOSTEROL

β-Sitosterol is one of several phytosterols (plant sterols) with chemical structure similar to that of cholesterol. Sitosterols are white, waxy powders with a characteristic odor. They are hydrophobic and soluble in alcohols.

1). SOURCES

It is widely distributed in the plant kingdom and found in *Nigella sativa*, pecans, *Serenoa repens* (saw palmetto), avocados, *Cucurbita pepo*(pumpkin seed),*Pygeum africanum*, cashew fruit, rice bran, wheatgerm, cornoils, soybeans, sea buckthorn, wolfberries, and *Wrightia tinctoria*.

2). USES

Alone and in combination with similar phytosterols, β-sitosterol reduces blood levels of cholesterol, and is sometimes used in treating hypercholesterolemia. β-Sitosterol inhibits cholesterol absorption in the intestine. When the sterol is absorbed in the intestine, it is transported by lipoproteins and incorporated into the cellular membrane. Phytosterols and phytostanols both inhibit the uptake of dietary and biliary cholesterol, decreasing the levels of LDL and serum total cholesterol. Because the structure of β-sitosterol is very similar to that of cholesterol, β-sitosterol takes the place of dietary and biliary cholesterol in micelles produced in the intestinal lumen. This causes less cholesterol absorption in the body.

One small study shows a positive effect on male hair loss in combination with Saw palmetto.

In Europe, β-sitosterol is used in herbal therapy, especially in the treatment of benign prostatic hyperplasia (BPH).

It is also used in Europe for the treatment of prostatic carcinoma and breast cancer, although the benefits are still being evaluated in the US. **(Berges *et al.*, 1995)**

1.6. SYNTHETIC DRUGS USED IN THE TREATMENT OF TUMOURS

Chemotherapy is the treatment of an ailment by chemicals especially by killing micro-organisms and other malfunctions of the body.

The majority of chemotherapeutic drugs can be divided in to alkylating agents, antimetabolites, anthracyclines, plant alkaloids, topoisomerase inhibitors, and other antitumour agents. All of these drugs affect cell division or DNA synthesis and function in some way.

Some newer agents do not directly interfere with DNA. These include monoclonal antibodies and the new tyrosine kinase inhibitors e.g. *imatinib mesylate* (*Gleevec* or *Glivec*), which directly targets a molecular abnormality in certain types of cancer (chronic myelogenous leukemia, gastrointestinal stromal tumours). These are examples of targeted therapies.

In addition, some drugs that modulate tumor cell behaviour without directly attacking those cells may be used. Hormone treatments fall into this category.

The different types of anti cancer agents used in chemotherapy are as follows

TYPES	**EXAMPLES**
Alkylating agents	Cisplatin, carboplatin and oxaliplatin
Anti-metabolites	Purines - azathioprine, mercaptopurine Pyramidines - 5-fluro uracil
Plant alkaloids	Vincristine, vinblastine, vindesine, vinorelbine
Podophyllotoxin	Etopside, teniposide

Taxanes	Docetaxel
Topisomerase inhibitors	Type 1 – irinotecan, topotecan Type 2- amasacrine, etopside
Cytotoxic antibiotics	Actinomycin, anthracyclines, belomycin
Others	Hydroxyurea, procarbazine, carboplatin,cisplatin
Drugs atering hormonal milieu	Prednisolone, tamoxifen, hydroxyl progesterone, fosfestrol, letrozole etc

1.7. STANDARD DRUG USED FOR THE PRESENT STUDIES

Fluorouracil (5-FU or f5U) is a drug that is a pyrimidine analog which is used in the treatment of cancer. It is a suicide inhibitor and works through irreversible inhibition of thymidylate synthase. It belongs to the family of drugs called antimetabolites.

1). MECHANISM OF ACTION

As a pyrimidine analogue, it is transformed inside the cell into different cytotoxic metabolites which are then incorporated into DNA and RNA, finally inducing cell cycle arrest and apoptosis by inhibiting the cell's ability to synthesize DNA.

It is an S-phase specific drug and only active during certain cell cycles. In addition to being incorporated in DNA and RNA, the drug has been shown to inhibit the activity of the exosome complex, an exoribonuclease complex of which the activity is essential for cell survival. **(Thripathi, 2008)**

2). USES

The chemotherapy agent 5-FU (fluorouracil), which has been in use against cancer for about 40 years, acts in several ways, but principally as a thymidylate synthase inhibitor, interrupting the action of this enzyme blocks synthesis of the pyrimidine thymidine, which is a nucleotide required for DNA replication.

Thymidylate synthase methylates deoxyuridine monophosphate (dUMP) into thymidine monophosphate (dTMP). Administration of 5-FU causes a scarcity in dTMP, so rapidly dividing cancerous cells undergo cell death via thymineless death.

Like many anti-cancer drugs, 5-FU's effects are felt system wide but fall most heavily upon rapidly dividing cells that make heavy use of their nucleotide synthesis machinery, such as cancer cells (other parts of the body with rapidly dividing cells include the cells lining the digestive tract).

Some of its principal uses are in colorectal cancer, and pancreatic cancer, in which it has been the established form of chemotherapy for decades (platinum-containing drugs approved for human use in the US since 1978 are also very well established). It is also sometimes used in the treatment of inflammatory breast cancer, an especially aggressive form of breast cancer.

5-FU is also used in ophthalmic surgery, specifically to augment trabeculectomy (an operation performed to lower the intraocular pressure in patients with glaucoma) in patients deemed to be at high risk for failure. 5-FU acts as an anti-scarring agent in this regard, since excessive scarring at the trabeculectomy site is the main cause for failure of the surgery.

Fluorouracil can be used topically (as a cream) for treating actinic (solar) keratoses and some types of basal cell carcinomas of the skin. It is often referred to by its trade names *Efudex*, *Carac* or *Fluoroplex*.

Due to Fluorouracil's toxicity and the fact that it can be manufactured using the same reaction as uracil, its precursor, 5-Fluoroorotic Acid, is commonly used in laboratories to screen against organisms capable of synthesizing uracil. **(Longley *et al.*, 2003)**

1.8. DIFFERENT METHODS FOR SCREENING OF NEW ANTICANCER MOLECULES

The main aims of screening methods are

a. To test the ability of a compound to kill cancerous cells.
b. To discriminate between replicating and non-replicating cells.
c. To find the potency of the drug
d. Effectiveness of drug and specific site of action

The different methods of screening are

1. In-vitro methods
2. In-vivo methods
3. Cell line methods

1). IN-VITRO METHODS

In-vitro testing is preferred to in-vivo testing for testing the potential of chemotherapeutic agents. In-vitro cultures can be cultivated under a controlled environment (pH, temperature, humidity, oxygen/carbon dioxide balance etc) resulting in homogenous batches of cells and reducing the mistakes.

Different types of in-vitro methods include

1. Tetrazolium salt assay(MTT)
2. Sulphorhodamine B assay
3. ^{3}H thymidine uptake
4. Dye exclusion tests
5. Clonogenic assay

6. Cell counting assay

These methods used to find different type of properties in culture cells and thus used to detect the therapeutic effect of the drug and potency. These tests are frequently used for screening of anti-cancer drugs:

S.NO	**ASSAY**	**PROPERTIES**
1	Tetrazolium salt assay(MTT)	Enzymatic properties
2.	Sulphorhodamine B assay	Protein content and synthesis
3.	^{3}H thymidine uptake	DNA content and synthesis
4.	Dye exclusion assay	Membrane integrity of cell
5.	Clonogenic assay	Clonogenic properties
6.	Cell counting assay	Cell divison

2). IN-VIVO METHODS

The in-vivo testing is performed on animals to test the efficacy of the drug on animals and to check the different parameters and compare them with the standard.

1. DMBA induced mouse skin papillomas, rat mammary gland carcinogenesis, oral cancer in hamster.
2. N-methyl, N- nitrosurea(NMU) induced rat mammary gland carcinogenesis.
3. NMU induced tracheal squamous cell carcinoma in hamster.
4. N, N diethyl nitrosamine (DEN) induced lung asenocarcinoma in hamster.

5. 1, 2 Dimethyl hydralazine (DMH) inducer colorectal adenocarcinoma in rat and mouse.
6. Azoxymethane (AOM) induced aberrant crypt foci in rats.
7. 1- Butyl-n-(4 hydroxy butyl)- nitrosamine induced bladder carcinoma in mouse.
8. 2- Methylcholanthrene induced fibrosarcoma tumor in mouse.
9. 3-Methylcholanthrene induced skin tumours in mouse.
10. Benzopyrene induced fore stomach tumours in mouse.

In all these in-vivo methods the cancer is induced by some chemicals and then the drug given by different routes and the efficacy measured and compared with that of the standard drugs and thus the activity of the drug found out statistically.

3). CELL LINE METHODS

In this method of studies the cancer cells are directly induced into the animal and propagated to induce cancer in the animals. The specified number of cells inoculated in to a sensitive mouse strains and tumor developed rapidly as compared to chemical carcinogen. It is much faster and less time consuming model than other methods.

Different types of cell line techniques are

1. Hollow fiber technique.
2. Use of kenografts.
3. Nude mouse models.
4. New born rat model.
5. Transgenic mouse model.
6. DAL induced mouse model. **(Gupta, 2008)**

a). DAL (DALTON'S ASCITES LYMPHOMA) MOUSE MODEL

Ascites is excess fluid in the space between the tissues lining the abdomen and abdominal organs (the peritoneal cavity). **(Ascites medline plus, A service of US national library of Medicine NIH national institute of health)**

Lymphomas are cancers that begin in the cells of the immune system

- It is a tumor cell line originally grown from a tumour of the thymus.
- It is propagated by growing as ascites tumour in mice.
- We can induce both ascites tumour and solid tumours using DAL cells.
- It is easy to maintain in vivo.
- It is not an immunogenic.

The solid tumours were obtained by injecting the DAL cell lines subcutaneously in to the mouse hind paw which gets increased in the mass and becomes solid. This cannot be propagated like the ascites lymphoma.

b). MAINTENANCE OF CELL LINES:

Dalton's lymphoma ascites tumour cell lines (DLA), originally obtained from Amala Cancer Institute, Thrissur, Kerala and was propagated as transplantable tumours in the peritoneal cavity of the mice were used for the study.

The tumour cell lines were maintained by serial peritoneal cavity I.P transplantation in mice.

The fully grown tumour cell lines were aspirated from mouse peritoneal cavity and mixed with PBS solution and mixed with tryphan blue solution. Taken that suspended solution and counted the number of cells present in one ml by using tryphan blue exclusion method and adjust the cell count to $1\times10^{6.}$ Then PBS solution was used and the cells were mixed well and were injected intraperitonially in to a new healthy mouse and thus the cancer got developed with in 10-15days of time.

2. LITERATURE REVIEW

2.1. TAXANOMY OF THE PLANT

Domain **:** *Eukaryota*- Whittaker &Margulis, 1978 - eukaryotes

Kingdom **:** *Plantae*- Haeckel, 1866 - Plants

Subkingdom **:** *Viridaeplantae*- Cavalier-Smith, 1981

Phylum **:** *Tracheophyta*- Sinnott, 1935 Ex Cavalier-Smith, 1998 – Vascular Plants

Subphylum **:** *Euphyllophytina*

Infraphylum **:** *Radiatopses*- Kenrick& Crane, 1997

Class **:** *Magnoliopsida*- Brongniart, 1843 - Dicotyledons

Subclass **:** *Magnoliidae*- NovKk Ex Takhtajan, 1967

Superorder **:** *Magnolianae*- Takhtajan, 1967

Order **:** *Magnoliales*- Bromhead, 1838

Family **:** *Asclepiadaceae*

Subfamily **:** *Magnolioideae*

Genus **:** *Wattakaka*- Linnaeus, 1753

Specific epithet **:** *volubilis* - Stapf

Botanical name **:** *Wattakaka volubilis* Stapf

2.2. SYNONYMS

1. *Asclepias volubilis* L. F.
2. Dregea Volubilis
3. *Dregea volubilis* (L. F.) Benth. Ex Hook. F.
4. Marsdenia Volubilis
5. *Marsdenia volubilis* (L. F.) Cooke
6. *Schollia volubilis* (L. F.) Jacq. Ex Steud.

(Frederick G. Meyer "Magnoliaceae". in Flora of North America Vol. 3. Oxford University Press. Online at EFloras.org)

2.3. VERNACULAR NAMES

Common name	:	Sneeze Wort, Cotton milk plant, Green milkweed climber, Green wax flower, Sneezing silk
Telugu	:	dugdhive
Tamil	:	koti-p-palai
Bengal	:	tita kunga
Assamese	:	Khamal lata
Hindi	:	akad bel, harandodi, nak-chikni
Malayalam	:	velipparuthi
Marathi	:	harandodi, nakhasikani
Telugu	:	dudipalatiga
Kannada	:	dugdhive
Bengali	:	tita kunga
Oriya	:	dudghika
Gujarati	:	malati

Sanskrit : hemajivanti

(http://www.flowersofindia.in/catalog/slides/Sneeze%20Wort.html)

2.4. GEOGRAPHICAL DISTRIBUTION

It is recorded from Nepal, India, Bangladesh, Sri Lanka, Indo-China, southern China, Taiwan, Peninsular, Maleshia, Java and the Philippines

2.5. DESCRIPTION

This is a large twing shrub,which can reach up to 10 m. tall.The young stems are densely hairy but later hairless.

The leaves are arranged opposite,simple and entire,broadly ovate,measuring 7-18.5 cm x 5-13 cm obtuse to truncate or subcordate at base,acuminate at apex,thinly leathery and pinnate veined.The petiole is 2.5-4.5 cm long,furrowed,hairless or hairy.The stipules are absent.

The inflorescence is umbrella-shaped cyme,which is situated between the petioles of a leaf-pair and many flowered.The peduncle is 2.5-7.5 cm long.The flowers are bisexuals,regular and 5-merous.The peducle is thin and up to 2.5 cm long.The sepal is on the inside,with 5 basal glands, 3 mm long segments and spreadings.The petal is bell shaped to rotate,12-16 cm in diameter, bright green , hairless and with obtuse segments.The stamens are inserted at the base of petals, with connate filaments, anther with short apical membrane overarching the stigma and with solitary pollinium in each anther cell.The corona scales are about 4 mm in diameter which are inserted in staminal tube. There are two superior ovaries, free 1-celled with two discoid stigma.

The consist of (1-) 2 ovoid- to lance shaped follicles which is 10-15 cm long, blunt to slightly acute finely longitudenaly ribbed and many seeded.

The seeds are with about 4 cm long coma

2.6. ECOLOGY / CULTIVATION

In Malaysian region,W. volubilis occurs in brushwoods and village-groves in lowlands.However in mainland Asia, it can be found in a variety of habitats including montane forests

(http://www.globinmed.com/index.php?option=com_content&view=article&id=62793:dregea-volubilis-lf-benth-ex-hookf&catid=368:d)

2.7. CHEMICAL CONSTITUENTS

Drevogenin D has been isolated from seeds; hydrolysate of seed extract yields drevogenins B, D, and P, D-cymarose, (+)methyl pachybioside, oleandrose, pachybiose and digitoxose. A new glycoside - dregoside A along with drevogenin A and drebbysogenin G has also been isolated from this plant. ***(http://www.mpbd.info/plants/wattakaka-volubilis.php)***

2.8. PARTS OF PLANTS GENERALLY USED

The leaves are much employed as an application to boils and abscesses. The roots and tender stalks are considered emetic and expectorant. The young roots are cut and the exuding juice is inserted into the nose to cause sneezing. ***(http://www.flowersofindia.in/catalog/slides/Sneeze%20Wort.html)***

2.9. THERAPEUTIC USES

Leaves used as application to boils and abscesses. Roots and tenderstalks used as emetic and expectorant. juice exuding from cut roots are inserted into the nose to cause sneezing. in South EastAsia used as antifebrile and emetic. used to treat hematemesis, sore throat, carbuncles, eczema, asthma and as antidote for poison.

(http://www.stuartxchange.org/GreenWaxFlower.html))

2.10. GENERAL VIEW OF HERBS

Now a day the era of everything is herbal and natural. Millions of "all natural" and "all herbal" health and dietary supplements are ruling the markets today.

The relationship between natural herbal healing and health is well established. Herbs are used for a wide range of health concerns. They are very useful for supporting body detoxification, supporting organs and systems, stress relief and building health.

For thousands of years traditional wisdom has dictated the preparation and use of natural healing herbs. When we work with the plants in partnership and respect, we are more likely to realize the benefits of natural herbal healing.

All herb properties can be placed in one of **three** general classifications:

Detoxification: When toxins accumulate in the system, they must be removed, or disease will manifest. Herbs that help detoxify can be used in teas and poultices. There are also herbs with specific properties that influence various parts of the body such as herbs with laxative properties cleanse the bowel diuretic herbs will cleanse the kidneys plants that have alterative properties will aid in detoxifying the blood, treat skin diseases, mucous congestion, lung conditions and constipation.

System Relief: There are many herbs that can counteract problems and relieve symptoms. Some of these actions include antibiotics, antiseptics, antispasmodics, aromatics, carminatives, sedatives, demulcents, diaphoretics, emollients, stimulants and styptics.

Building and Toning: Herbs to strengthen the body, improving the functions of the internal organs and strengthening the body's resistance to disease. These herbs will help with recovery from acute ailments, injury, surgery anemia, impotence, miscarriage and childbirth. They are also used for chronic diseases and emotional instability. Building and toning herbs acts as aphrodisiacs, astringents, cardiac tonics, digestives, diaphoretics, nerviness, nutritives, stomachic's, vulneraries.

2.10.1. ADVANTAGES OF NATURAL HERBS

- Natural herbs should be considered especially when we find that synthetic drug treatments are not helping you with our symptoms. A recent study showed that more and more people are turning to natural herbal remedies for treating their illnesses. This number is set to increase, as the benefits of using all natural substances as compared to synthetic drugs become more publicly known.
- The healing benefits from natural herbal remedies can be in only a few ways. One may choose to consume the herbs raw or you can take capsules containing herbs that are ground.
- Making tea out of natural herbal remedies is also very popular with many people. Herbal teas such as chamomile, dandelion, and many others are now widely sold.
- Another method of gaining from natural herbal remedies is to remove the essential oils found in the herb and use them as a type of ointment or in aromatherapy.
- Natural herbal remedies also tend to be less expensive than drug remedies. As with any type of medication, you need to ensure that it will not counteract any other medications that you may be taking.
- However, what is beneficial to know about natural herbal remedies is that they are much more well-tolerated by your body than synthetic drugs, hence fewer side effects were expected.
- Without a prescription, you can go to the health food store and buy whatever you want. However, it is always wise to consult with your doctor first before taking ANY medications.
- Potency may vary, depending on genetic variation of different strains of herbs, growing conditions, timing and method of harvesting, exposure to air, light and moisture over time and type of preservation.

It is your responsibility to take care of your own health. Hence, it is in our interest to be aware about all of the options that are available when it comes to treating various ailments and illnesses. Synthetic drugs are not the only option. So do take the time to learn about how natural herbal remedies can help in your specific ailment.

NOTE - Still there are no guarantees when using natural herbal remedies, although there are many who have reported good success with this form of treatment. Hence one will find that there are many forums discussing about the efficiency of some of the natural herbal remedies. Natural herbal remedies are not subject to the various tests that synthetic drugs go through because major pharmaceutical companies make more money promoting clinical drugs and would therefore channel more of their money and resources there.

If one finds themselves prone to getting side effects when you take synthetic drugs, natural herbal remedies may just be the solution that you need. **(Prema *et al.*, 2011), (Hallelujah 2009), *(Natural herbs used in treatment of cancer., livestrong_com)***

SCIENTIFIC REVIEW

- **Arun kumar R *et al.*, 2010** investigated the anti-hyperlipidemic and hypoglycemic activities of methanol leaf extract of Wattakaka volubilis in alloxaninduced diabetic rats. The extract was administered at doses of 50, 100, and 200 mg/kg and glibenclamide was administered at a dose of 5mg/kg for 28 days to animals with alloxan-induced diabetes. Serum glucose and lipid profiles were determined on days 7, 14, 21, and 28. At the end of 28 days, the oral administration of a graded dose (200mg/kg) of Wattakaka volubilis produced a significant reduction in serum glucose, total cholesterol, triglyceride, LDL, and VLDL levels and an increase in HDL levels from the regulation of insulin secretion and islet protection compared with diabetic control rats. An acute toxicity study was observed in plant extracts that did not reveal any behavioral changes or mortality even at a dose of 1000mg/kg. The present study suggests that W. volubilis leaves show significant anti-hyperlipidemic and hypoglycemic activities in diabetic rats.

- **Nandi *et al*.,2011** investigated to possess medicinal effects. In the present study, the dried leaf extract [methanol–water (1:1)] of W. volubilis designated as 'the extract' was evaluated for pharmacological activity in rats and mice. The anti-inflammatory activity was evaluated using acute, sub-chronic and chronic models of inflammation in rodents. The antipyretic and analgesic activities were evaluated in mice models. In the acute toxicity study, it was found that the extract was non-toxic up to 1 g/kg, i.p. The extract (50, 100 and 200 mg/kg, i.p.) was found to possess, anti-inflammatory, analgesic and antipyretic activities in a dose dependent manner and the effect was comparable with the standard drug,ibuprofen.The extract significantly inhibited the arachidonic acid-induced paw oedema in rats, indicating that the extract inhibited both the cyclo-oxygenase and lipo-oxygenase pathways of arachidonic acid metabolism. The extract also significantly enhanced the macrophage count in mice in a dose- and time-dependent manner. It is possible that the saponins present in the extract may be responsible for these activities.
- **Maruthupandian A *et al*.,2010** investigated for its antidiabetic effect in Wistar Albino rats. Diabetes was induced in Albino rats by administration of alloxan monohydrate (150mg/kg, i.p). The ethanol extract of Wattakaka volubilis at a dose of 150mg/kg of body weight was administered at single dose per day to diabetes induced rats for a period of 14 days. The effect of ethanol extract of Wattakaka volubilis leaf extract on blood glucose, plasma insulin, glycosylated haemoglobin, serum lipid profile [total cholesterol, triglycerides, low density lipoprotein - cholesterol (LDL-C), very low density lipoprotein - cholesterol (VLDL-C), and high density lipoprotein- cholesterol (HDL-C) serum protein, albumin, globulin, A/G ratio, serum enzymes [Serum glutamate pyruvate transaminases (SGPT), serum glutamate oxaloacetate transaminases (SGOT) and alkaline phosphatase (ALP)], lipoprotein peroxidation (LPO) antioxidant enzymes (catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH) ,glutathione peroxidase (GPx) and glutathione reductase (GR) were measured in the diabetic rats. The ethanol extract of Wattakaka volubilis leaf elicited significant reductions of blood glucose ($p<0.01$), lipid parameters except

HDL-C, serum enzymes and significantly increased HDL-C and antioxidant enzymes. The extracts also caused significant increase in plasma insulin ($p<0.01$) in the diabetic rats. From the above results it is concluded that ethanol extract of Wattakaka volubilis possesses significant antidiabetic, antihyperlipidaemic and antioxidant effects in alloxan induced diabetic rats.

- **Madhavan V *et al.*, 2010** investigated In the present study two botanical sources of Murva, viz. Wattakaka volubilis and Maerua oblongifolia, were tested for antipyretic activity by yeast-induced pyrexia in Wistar albino rats. Alcohol and aqueous extracts of both species significantly reduced the elevated rectal temperature in febrile rats within 30 min of their administration. The results of these studies support the traditional use of these two botanical sources of the drug Murva in the treatment of fever.
- **Moulisha Biswas *et al*.,2010** has investigated the use of petroleum ether extract of Dregea volublis fruits (PEDV) for antitumour effect in Ehrlich Ascitis Carcinoma (EAC) bearing swiss albino mice. 24 h after intraperitonial inoculation of tumour (EAC) cells in mice, PEDV extract was administered at 100 and 200 mg/kg body weight for 9 consecutive days. On 10^{th} day half of the mice were sacrificed and rest were kept alive for assessment of increase in life span. The antitumour effect of PEDV was assessed by tumour volume,tumour weight,viable and non-viable cell count,median survival time and increase in life span of EAC bearing hosts. Haematological profiles were investigated. PEDV showed significant ($p< 0.001$) decrease in tumour volume,tumour weight,viable cell count and increase in life span of EAC bearing mice. Haematological profiles were significantly ($p<0.001$) restored to normal levels in PEDV treated mice as compare to EAC control. Therefore from the present study it can be concluded that Dregea volubilis exhibit remarkable antitumour activity against Ehrlich Ascitis Carcinoma (EAC) in swiss mice.

- **Divya T S *et al*,2009** investigated for its anti-inflammatory and analgesic effects in animals. The extract showed a significant inhibition of carrageenan-induced rat paw edema and acetic acid-induced writhing in mice compared to the standard anti-inflammatory and analgesic drug, indomethacin. The extract also showed potent in vitro inhibition of FeCl2-ascorbic acid-stimulated mice liver lipid peroxidation.
- **Biswas M *et al*,2009** investigated for analgesic activity in swiss albino mice by inducing writhing reflex with acetic acid. The petroleum ether extract of the fruits of *Dregea volubilis*Benth led to isolation of a pentacyclic triterpenoid designated as taraxerol and characterized as D- friedoolean- 14-en, 3 ol [Figure 1]. In vitro analgesic activity has been shown by the isolated taraxerol. The compound taraxerol obtained from the petroleum ether extract of the fruits of*Dregea volubilis* showed analgesic activity in swiss albino mice.
- **Emdad Hossain *et al*.,2010** investigated for anti-inflammatory activity of methanolic extract of Wattakaka volubilis leaves with it's fractions and to declineate the possible mechanism of action for MEDV. The anti-inflammatory activity of MEDV alongwith it's petroleum ether and chloroform extract is evaluated in a carrageenan induced model for acute inflammation. The effect of MEDV on lipopolysacchariedes induced NO production was also studied. MEDV (100,200 and 400 mg/kg body weight) significantly reduced carrageenan induced paw edema,chloroform fraction was most potent (66%, $p <$ 0.001). MEDV was non-toxic up to 125 microgram/ml in mice peritoneal macrophages wherein it (0-100 microgram/ml) reduce liposacchariedes induced NO production. Thus MEDV possesses significant anti-inflamatory. Chloroform extract of MEDV showed best anti-inflammatory activity.
- **Shukla Amit Kumar *et al*.,2011** investigated to possess medicinal effects. In the present study, the dried root ethanol extract of *W. volubilis* designated as 'the extract' was evaluated for pharmacological activity in rats and mice. The anti-inflammatory and antipyretic activities were evaluated in rodents. The analgesic activity was evaluated in mice models. In the acute toxicity study, it was found that the extract was non-toxic up to 1 g/kg, p. o. The

extract (100, 200 and 300 mg/kg, p. o.) was found to possess, anti-inflammatory, analgesic and antipyretic activities in a dose-dependent manner and the effect was comparable with that produced by the standard drug, ibuprofen. The extract significantly inhibited the arachidonic acid-induced paw oedema in rats, indicating that the extract inhibited both the cyclo-oxygenase and lipo-oxygenase pathways of arachidonic acid metabolism.

- **Jeyachandran R *et al*.,2010** investigate the qualitative phytochemicals and antimicrobial properties of different solvent crude extracts of four Indian medicinal plants namely, Nerium oleander, Lippia nodiflora, Wattakaka volubilis and Wrightia tinctoria against various bacterial pathogens. The presence of steroids, triterpenoids, phenolic compounds, tannins, alkaloids, saponins, flavanoids and reducing sugars was indicated by the tests conducted. The plant extracts showed different diameters of inhibition zone ranging between 4 to 28 mm. Among themselves, methanol, ethyl acetate, hexane and petroleum ether extracts of N. oleander exhibited the highest inhibition zone against S. typhi. The in vitro antibacterial assay and preliminary phytochemical analysis may open way for complementary future investigations in identifying potentially useful properties of chemical and pharmacological importance

3. AIM AND OBJECTIVE

The main aim of the study is to evaluate the anti cancer activity of ethanolic extract of *Wattakaka volubilis* leaves on DAL induced lymphoma in mice.

Cancer is one of the dangerous, lethal and a major challenging disease to public health. To produce more potent and the site specific anti-neoplastic drugs, vast number investigations are going on to find out the potent and safer new anti-neoplastic drugs.

Most of the synthetic anti-neoplastic agents produce undesirable side effects. On the other side the plant sources are available as a potent and safer alternative for cancer treatment than compared to synthetic drugs. Thereby in the current study, natural plant source was selected rather than a synthetic drug. The drug is also cheaper than synthetic drugs. Being a natural herb the side effects may also be lesser than the synthetic drugs.

Wattakaka volubilis has large amounts of flavanoids, phytosterol compounds and many other active chemical constituents. (Preliminary phytochemical studies)

Flavanoids have a capacity to elevate the antioxidant levels and prevent the free radical reactions and it is also reported that flavanoids have an anti-cancer activity. Flavanoids are also called as free radical scavengers because of their activity on the free radicals and ROS. Hence the present study of *Wattakaka volubilis leaves* which have high amount of flavanoids content was choosen for evaluating the anti-cancer activity due to its free radical scavenging properties; since many studies done previously on flavanoid for antioxidant and anti-cancer activities. ***(*Gupta M *et al., 2004).***

The study was also supported by the anti-tumour effect of fruits of *Dregea volubilis* ***(*Moulisha Biswas *et al.,2010).***

Thus the present study was choosen with an objective of **anticancer activity of ethanolic extract of *Wattakaka volubilis* leaves on DAL induced lymphoma in mice with it's antioxidant effect.**

4. PLAN OF WORK FOR THE STUDY

1. Collecting the plant material .
2. Authentication for the plant specimen.
3. Drying of the leaves of the plant in shade and ground to a coarse powder.
4. Extraction by soxhlet extractor.
5. Acute toxicity studies of the extract.
6. Preliminary phytochemical studies of the extract.
7. Quantitative estimation of extract obtained.
8. Evaluation of *in-vitro antioxidant* effect.
9. *In-vivo anti-cancer* activity.

    a) Mean survival time and body weight

    b) Parameters

10. *In-vivo antioxidant* activity
11. Results of *in-vitro* and *in-vivo* studies.
12. Interpretation of the results.
13. Discussion.
14. Conclusion.

5. MATERIALS AND METHODS

5.1. COLLECTION OF PLANT AND AUTHENTICATION

The leaves of *Wattakaka volubilis* was collected personally from forest reserve area, kolli hills, Dt. Namakkal,Tamilnadu and was authentified by Dr. G. V. S. Murthy, Scientist 'F' & Head of Office, Botanical Survey of India, Southern Regional Centre, Coimbatore. (Ref. No. – BSI/SRC/5/23/2011-12/Tech.-844)

1). EXTRACTION OF LEAVES

The leaves of *Wattakaka volubilis* were collected and dried in shade for about a week until they were completely dried and powdered to a coarse powder. (Sieve No.- 40). Then the powder was extracted in petroleum ether using soxhlet apparatus for 30 hours at a temperature of 40-60° C to remove all of the fatty materials. Then the coarse powder was again dried and extracted using ethanol for

72hours at a temperature of 50° C. Then the extract was distilled to remove the excess of solvent using a simple distillation apparatus and concentrated.

5.2. PRELIMINARY PHYTOCHEMICAL TESTS OF ETHANOLIC EXTRACT OF *Wattakaka volubilis*

A) Test for carbohydrates

1) Molisch Test

A small amount of methanolic extract was treated with α-naphthol and concentrated sulphuric acid was added along the sides of the test tube. Purple colour or reddish violet colours obtained at the junction between two liquids indicates the presence of carbohydrates.

2) Fehling's Test

In this a small amount of extract was treated with equal quantity of Fehling's solution A and B is and heat gently in a water bath for few minutes, brick red precipitate was obtained which indicates the presence of carbohydrates.

3) Benedict's test

To 5 ml of Benedict's reagent, added 8 drops of extraction solution and mixed well, and boiled the mixture vigorously for two minutes and then cooled. Red precipitate indicates the presence of carbohydrates.

4) Barfoed's test

To the 5 ml of the Barfoed's solution added 0.5 ml of extract solution and mixed well and heated to boiling, red precipitate indicates the presence of carbohydrates.

B) Test for Alkaloids

About 2 ml of the extract was boiled for five minutes on a water bath with 10 ml of 10% hydrochloric acid. The extract was filtered and the pH of the filtrate was adjusted to about 6 by adding a few drops of dilute ammonia solution and tested with litmus paper.

Then to the filtrate was added with few drops of Dragendroff's, Mayer's and Wagner's reagent separately in the test tubes. A reddish brown, cream and reddish brown precipitate indicates the presence of alkaloids.

C) <u>Test for Steroids and Sterols</u>

1) Libermann Burchard test

The extract was dissolved in 2 ml of chloroform in a dry test tube and added 10 drops of acetic anhydride and 2 drops of concentrated sulphuric acid. The solution becomes red, which later was changes to blue and bluish green color indicates presence of steroids and sterols.

2) Salkowski test

Extract was dissolved in chloroform and added equal volume of conc. sulphuric acid. A bluish red cherry red and purple color in chloroform layer, and also green fluorescence indicates the presence of steroids and sterols.

D) <u>Test for Glycosides</u>

1) Legal's test

The extract was dissolved in pyridine sodium nitropruside solution and made alkaline. Pink or red color indicates the presence of glycosides.

2) Baljet test

The extract was added with, sodium picrate solution and Yellow to orange colour shows the presence of glycosides.

3) Borntrager test

A few ml of dilute sulphuric acid added to the extract solution, boiled, filtered and extracted the filtrate with ether or chloroform. The organic layer was removed to which ammonia was added. Pink, red or violet colour in organic layer shows the presence of glycosides.

4) Keller Killiani test

The extract was dissolved in acetic acid containing traces of ferric chloride and concentrated sulphuric acid was added. At the junction of liquid reddish brown color was produced which gradually becomes blue indicates the presence of glycosides.

E) Test for Saponins

About one gram of the powdered sample was boiled with 10 ml of distilled water for ten minutes. The samples were filtered while hot, cooled and the following tests were carried out.

1) Frothing

2.5 ml of the filtrate was shaken vigorously for 2 minutes with water. If any frothing was observed it indicates the test was positive.

2) Emulsification

2.5ml of the filtrate was shaken vigorously with a few drops of olive oil. If an emulsified layer obtained indicates a positive test.

F) Test for Flavanoids

A small quantity of the extract added with dilute sodium hydroxide and hydrochloric acid was also added. A yellow solution that turns colorless on addition of hydrochloric acid indicates the presence of flavonoids.

G) Test for Triterpenoid

In the test tube, 2 or 3 granules of tin was added, and dissolved in a 2ml of thionyl chloride solution and test solution was added. If any pink color was produced it indicates the presence of Triterpenoid.

H) Test for Protein and Amino acid

1) Biuret test

To the extract added 1 ml of 40% sodium hydroxide and 2 drops of 1% copper sulphate, if any violet color it indicates the presence of proteins.

2) Ninhydrin test:

Added 2 drops of freshly prepared 0.2% ninhydrin reagent to the extract and heated. A blue color indicates the presence of proteins, peptides or amino acids.

3).Xanthoprotein test

The extract, was added with 20% of sodium hydroxide any orange color produced indicates presence of aromatic amino acid.

I). Phenols and tannins

100 mg of extract was boiled with 1 ml of distilled water and filtered. The filterate was used for the test

1). Ferric chloride test

To 2 ml of filtrate, 2 ml of 1% ferric chloride was added in a test tube. The formation of bluish black color indicates the presence of phenolic nucleus.

2). **Lead acetate test**

To 2 ml of filtrate few drops of lead acetate solution was added in a test tube. Formation of yellow precipitate indicates the presence of tannins. **(Khandelwal, 2004)**

5.3. DETERMINATION OF TOTAL PHENOLS

Total soluble phenolic content of the extract was determined by using the Folin-Ciocalteu reagent and the values are expressed as equivalents of gallic acid. An aliquot (1 ml) of extract or standard solution of Gallic acid (20, 40, 60, 80 and 100 µg/ml) were mixed with 0.2 ml of Folin-Ciocalteu reagent.

After 5 min, 1 ml of 15% Na_2CO_3 solution and 2 ml of distilled water were added to the above mixture. A blank solution using distilled water was prepared. After incubation for 90 min at room temperature, the absorbance of the mixture was measured against blank at 750 nm. Samples were analyzed in triplicate and mean of three readings was used and the total phenolic contents were expressed as milligrams of Gallic equivalents per gram extract. **(Sreeramulu and Raghunath, 2010), (Padma *et al.*, 2011**)

5.4. DETERMINATION OF ACUTE ORAL TOXICITY (LD_{50}) OF ETHANOLIC EXTRACT OF *Wattakaka volubilis*

Table-1 Test substance details

Name of the test substance	Ethanolic extract of *Wattakaka volubilis* leaves
Color	Greenish black
Nature of the test substance	Liquid

Table – 2 Experiment protocol

Name of the study	Acute toxicity
Guideline followed	OECD 425 method-acute toxic class method
Animals	Healthy young adult Swiss albino mice, non-pregnant

Body weight	25-30 grm
Sex	Male
Administration of dose and volume	2000,550,1000 mg/kg body weight, single dose in 0.2ml
Number of animals	3 animals
Route of administration	Oral (by using mice oral feeding tube)
Vehicle	Carboxy methyl cellulose (CMC)

Table – 3 Housing and feeding conditions

Room temperature	22 ± 3°C
Humidity	40-60%
Light	12 hr light/12 hr dark cycle
Feed	Standard laboratory animal food pellets with water *ad libitum*

Table -4 Study period and observation parameters

Initial once observation	First 30 minutes
Special attention	First 1-4 h after drug administration
Long term observation	Up to 14 days
Direct observation parameters	Diarrhoea, sitting in the corners, sniffing excessively, standing on hind limbs

Additional observation parameters	Skin, fur, eyes and mucous membrane, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavior pattern etc.

If any death of animal was found it was recorded.

Study procedure

Acute oral toxicity was performed as per organization for economic co-operation for development (OECD) guideline 425 methods. The extract was administered in a single dose by gavages using specially designed mice oral needle. Animals were fasted 24h prior to dosing (water *ad libitum*). **(OECD Guideline for testing of chemicals 425**)

5.5. ESTIMATION OF *INVITRO* ANTIOXIDANT ACTIVITY OF ETHANOLIC EXTRACTS OF *Wattakaka volubilis* (L).

1). DPPH PHOTOMETRIC ASSAY:

$$AA\ \% = 100 - \{ [(ABS_{SAMPLE} - ABS_{BLANK}) \times 100] / ABS_{CONTROL}\}$$

DPPH radical scavenging activity was determined based on the ability of the antioxidant to scavenge the DPPH cation radical. Briefly, 100µl of sample extract or standard was added to 2.9 ml of DPPH reagent (0.1 mM in ethanol) and shaken vigorously. It was incubated in dark for 30 min at room temperature and the discoloration of DPPH was measured against blank at 517 nm. Percentage inhibition of the discoloration of DPPH by the sample extract was expressed as trolox equivalents.

2). **FERRIC REDUCING ABILITY OF PLASMA (FRAP) EXPRESSED AS A FUNCTION OF TIME:**

Absorbance = 0.274 x «M of Fe^{++} *+ 0.114* [R^2 = 0.974]

Ferric reducing antioxidant power (FRAP) was determined in sample extract according to method which was based on the ability of the sample to reduce Fe^{3+} to Fe^{2+}ions.

In the presence of TPTZ the Fe^{2+}- TPTZ complex exhibits blue color which is read at 593 nm. Briefly, 3 ml of working FRAP reagent was added to the appropriate concentration of sample extract. After incubation for 6 min at room temperature the absorbance was measured at 593 nm against Ferrous sulphate as standard. **(Ratnesh K Sharma *et al.*, 2009), (Sreeramulu and Raghunath**, **2010)**

3). ABTS RADICAL SCAVENGING ASSAY

Scavenging of 2, 2-azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid) Diammonium salt (ABTS) radical cation

$ABTS^{\cdot+}$ radical was freshly prepared by adding 5 ml of 4.9 mM ammonium per sulfate solution to 5 ml of 14 mM ABTS solution and kept for 16 h in dark. This solution was diluted with ethanol (99.5%) to yield an absorbance of 0.70±0.02 at 734 nm (0.05 ml taken and added with 2.9 ml) and the same was used for the assay.

950 µl of above adjusted ABTS radical solution was, taken and to it added about 50 µl of extract solutions (20, 40, 60, 80 and100µl) and the reaction mixture was vortexed for 10 sec. After 6 minutes the absorbance was recorded at 734 nm. Percentage inhibition was calculated from the formula.

(**Satish kumar *et al.*, 2008**)

Percentage inhibition= [1−(absorbance of test/absorbance of control)]×100

4). DCF/AAPH ASSAY (TRAP)

An azo initiator, AAPH, was used to produce peroxyl radicals, and the scavenging activity of ethanolic extract was monitored via the spectrophotometric analysis of 2, 7- dichlorofluorescin diacetate (DCF).

The activation of DCF was achieved by mixing 350 µl DCF (1mM in methanol) and NaOH (1.75 ml of 0.01N solution) and allowing the mixture to stand for 20 min before adding 18.25 ml of sodium phosphate buffer (25 mM, pH 7.2).

From the above reaction mixture 150µl solution was taken and 500 µl of sample taken in different concentration (20, 40, 60, 80 and 100µl) and made up to volume. To this solution added 25 µl of 600 mM AAPH (2- amidino proponic dihydro chloride, adjusted to a final concentration of 56 mM).

The reaction was initiated by adding the AAPH solution. After 10 min, the absorbance was read at 490 nm using a Specrophotometer. (**Yong cui *et al*., 2005)**

5) SUPER OXIDE SCAVENGING ABILITY

Nitro blue tetrazolium (NBT) (156 µM, NBT in 100 ml phosphate buffer pH 7.4) mixed well with 1ml of NADH (468 µM in 100mM of phosphate buffer pH 7.4). To this mixture added sample in different concentrations (20, 40, 60, 80 and 100 µl) and adjusted the volume to 100 µl and added 100 µl of PMS (phenazine metro sulphate 60 µM PMS in 100 µM of phosphate buffer).

Incubated the mixture for 5 min at 25^{o} C and the absorbance was measured at 560 nm.

6). NITRIC OXIDE SCAVENGING ABILITY

About 4 ml of sample solution at different concentrations (20, 40, 60, 80 and 100 µl) was taken and to this added 1 ml of sodium nitoprusside solution (5 mM) and incubated well for 2 hrs at 25^{o}C.

After 2 hrs the 2 ml of the above mixture solution was taken and mixed with 1.2ml of griess reagent(1% sulphanilamide + 5% of 0.1% napthyl ethylene diamine hydrochloride + phosphoric acid) and absorbace measured at 550 nm.

5.6. EVALUATION OF IN VIVO ANTICANCER ACTIVITY

Animals used

Inbred male or female Swiss albino mice of 2 months age, weighing 25 ± 5 grm, were used for the study. They were housed at room temperature of 22^{o} C under 12 hr light/12 hr dark cycle in the animal house. Mice were fed with commercial pellet diet and water *ad libitum* throughout the study. All animal procedures were performed after approval from the IAEC (institution of animal ethical committee) and in accordance with the recommendations for the proper care and use of laboratory animals. (Proposal No- 25MP15JUN11)

5.7.1. DAL-INDUCED ASCITIC ANTITUMOR MODEL

1). Adjust Cell Count

0.5 ml of 0.4% Tryphan blue, 0.3 ml of PBS and 0.2 ml of cell suspension were mixed and kept aside for 5 min and not more than 15 min. From this one drop of solution was taken on a neubar chamber and a cover slip was placed. This is placed on microscope and the viable, non-viable cells were counted using 10x power lens. Viable cells do not take color and appear white in color on blue background. Nonviable cells (dead cells) take blue color and give dark blue shading to the cells. The cell count was calculated using formula**.**

Cell count = No. of cells x Dilution factor × volume factor. (**Talwar, 1974)**

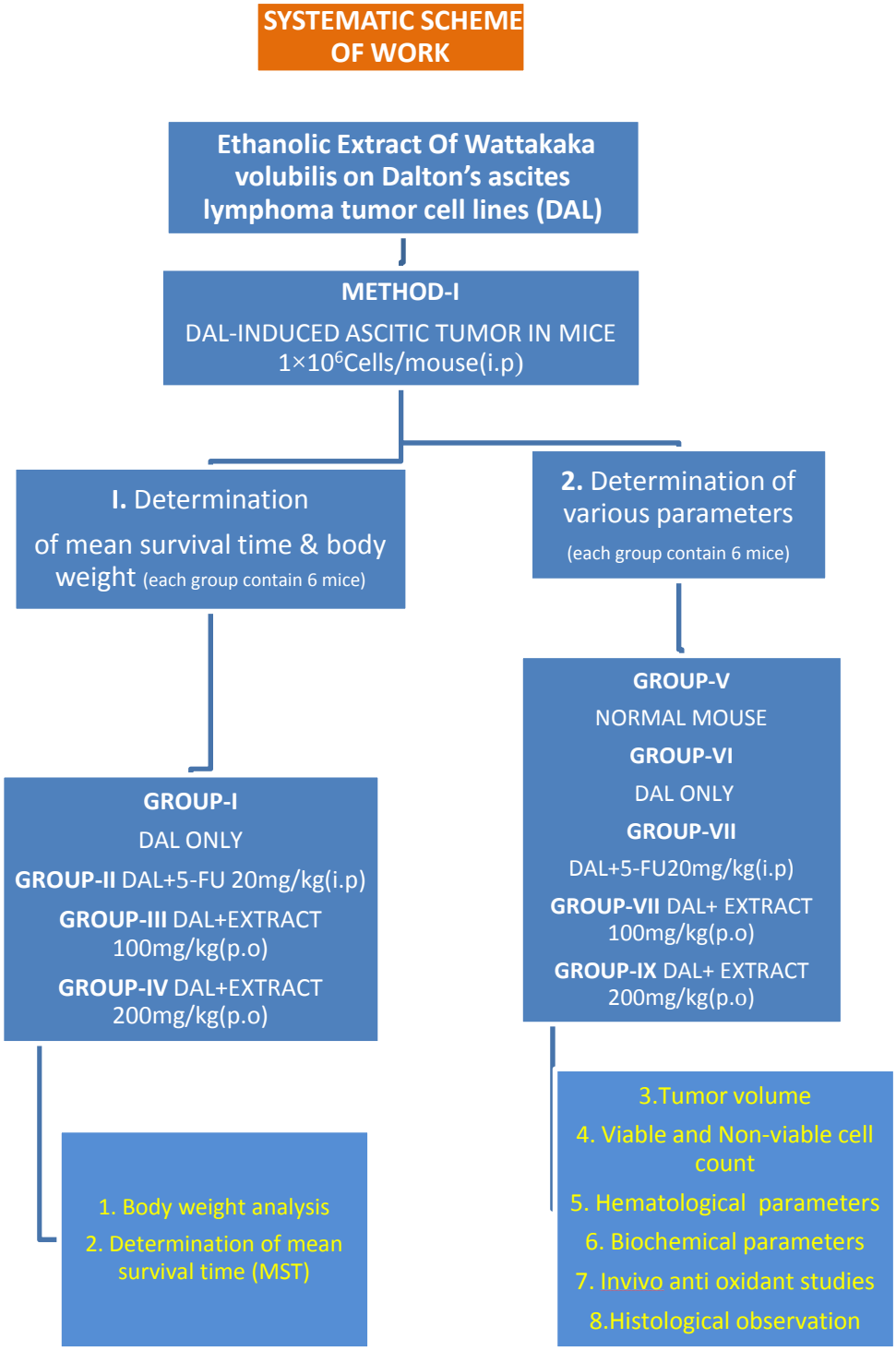


Chart-1 The chart shows the doses to different groups of animals (5-FU and Extract) which were fixed based on earlier works done on DAL. (*International journal of health research* 2008; 1(2): 79). The various parameters were also performed which were explained in detail below:

2). DAL-induced ascitic antitumor model:

The anti tumor activity of the ethanolic extract was determined by injecting DAL cell suspension (1×10^6 cells per mouse) in to the peritoneal cavity of the animals. The treatment was started after 24 hours of the tumor inoculation and continued once daily for 14 days and the antitumor efficacy of extract was compared with that of standard drug 5-FU (20mg/kg, i.p) and DAL control.

5.7.2. DETERMINATION OF BODY WEIGHT AND SURVIVAL TIME

1). Body weight analysis:

All the mice were weighed for every seven days, after tumour inoculation. The average gain in body weight was determined and recorded and a % decrease in body weight was calculated by the formula.

$$\% \text{ Decrease in body weight} = \frac{\text{Decrease in body weight}}{\text{Initial body weight}} \times 100$$

2). Mean Survival Time (MST):

After induction of the tumour, every day all the groups were checked for mortality & recorded. The no. days each mouse survived was noted. The mean survival time (MST) and percentage increase in life span (ILS %) was calculated by the formula.

$$\text{Mean survival time} = \frac{\text{1st Death + Last Death}}{2}$$

$$\text{ILS (\%)} = \left[\frac{\text{Mean survival of treated group}}{\text{Mean survival of control group}} - 1 \right] \times 100$$

5.7.3. DETERMINATION OF VARIOUS OTHER PARAMETERS

1). Determination of tumor volume

All the mice in this group were inoculated with tumour cells and the drug given after 24 hrs of inoculation. Treatment continued for 14 days and after14 days treatment the mice were dissected by anaesthetizing using diethyl ether and the ascitic fluid was collected from the peritoneal cavity. The volume was measured by taking it in a graduated centrifuge tube.

2). **Viable and nonviable cell count**

After 14 days of treatment animals were anaesthetized with diethyl ether. Then from the intraperitonial cavity 0.2 ml of cell suspension was aspirated out using a syringe and was mixed with 0.5 ml of 0.4% tryphan blue, 0.3 ml of normal saline or PBS and kept aside for 5 min and not more than 15 min. From this one drop of solution was taken on a neubar chamber and a cover slip was placed on the solution. The chamber was placed on a microscope and the viable, nonviable cells were counted under 10x power lens. Viable cells appear white in colour on blue background and nonviable cells (dead cells) take blue colour and give dark blue shading to the cells. The cell count was calculated using formula **.**

Cell count = No. of cells x Dilution factor × volume factor

Preparation of Blood Serum and Tissue Samples for Bio Chemical Studies

After 14 days of treatment the animals were fasted over night and anesthetized using diethyl ether. The blood sample was collected from eye (retro orbital bleeding) to maximum using a capillary tube. Then animal sacrificed by cervical dislocation and the intraperitonial fluid was taken and measured.

Blood was collected for estimation of hematological parameters (RBC, WBC, Differential cell count , Hb percentage, MCV, MCHC, Hematocrit, MCH, platelet count) and serum was separated from blood cells by centrifugation at about 2000 rpm for 30 min and used for estimation of different biochemical parameters

(SGOT, SGPT, Serum creatinine, serum triglycerides, alkaline phosphatase, albumin, total protein values.)

The mice were dissected after collecting the blood and the liver was removed. The blood and other body fluids were cleaned using a blotting paper and then cleaned in normal saline and transferred to ice cooled containers with 10% formalin solution.(histopathological studies). Some tissues were cleaned with normal saline and again wiped and were used for other parameters (*In-vivo* antioxidants).

5.7.4. ESTIMATION OF HAEMATOLOGICAL PARAMETERS

1). Enumeration of white blood cells

The total white blood cells were enumerated according to the method of John (1972) using Turk's fluid (WBC diluting fluid).

Using a white blood cell pipette of haemocytometer, blood was drawn up to 0.5 mark and WBC diluting fluid was taken up to mark II. The blood mixture was shaken and transferred onto the counting chamber (neubar chamber) and the cells were allowed to settle for 2 min the fluid should not get dried.

Using 10x objective the WBC's were counted uniformly in four larger corner squares. The cells were expressed as number of cells $x10^9$/L. (**John, 1972)**

2). Enumeration of red blood cells

Using a red blood cell pipette of haemocytometer, blood was drawn up to 0.5 mark and RBC diluting fluid was taken up to mark II. The mixture was shaken and transferred onto the counting chamber (neubar chamber). The cells were allowed to settle for 2 min the fluid does not get dried. Using 45x objective the RBC's were counted uniformly in the smaller 4 corner squares. The cells were expressed as number of cells $x10^{12}$/L or in $x\ 10^6$ / cu.mm. **(Text book of medicinal lab technology, 2007)**

3). Differential Leukocyte Count

Differential Leukocyte count was determined by the method of John (1972). Leishmann's stain 150 mg of powdered leishmann's stain was dissolved in 133 ml of acetone free methanol.

A blood film stained with leishmann's stain and examined under oil immersion lens and the different types of WBCs were identified. The percentage distribution of these cells was then determined. Smears were made from anticoagulant blood specimens and stained with leishmann's stain. The slides were preserved for counting the number of lymphocytes and neutrophils, per 100 cells were noted. The number of neutrophils was expressed as (%). **(John 1972)**

4). Estimation of Haemoglobin:

Haemoglobin was converted into acid haematin by the action of dilute hydrochloric acid. The acid haematin solution is further diluted with distilled water until its colour matches exactly with that of permanent standard. The Hb concentration is read directly from the calibration tube by comparing the color with the standard in the stand.

By using pipette 0.1 N HCl was taken up to the lowest marking in the Haemoglobinometer and blood was drawn up to 20µl in the sahli's pipette. The excess of blood on the sides of the pipette was cleaned by using a dry piece of cotton. Blown the blood into the acid solution in the graduated tube, rinsed the pipette well and stirred well to remove if air bubbles present. Mixed the solution and allowed the mixture to stand at room temperature for 10 minutes. The solution was then diluted using distilled water. Water was carefully added drop by drop and stirred until the color matches with that in the comparator in the haemoglobinometer. The lower meniscus of the fluid was noted and reading was noted in g/100ml. **(Sahli's acid hematin method)**

The haematological paramaeters like MCH, MCV, MCHC, Hematocrit, and Differential leukocytes were measured using automated method using instrument-celldyn 1700 haematology analyzer (3 part).

5.7.5. ESTIMATION OF SERUM BIOCHEMICAL PARAMETERS

SGOT, creatinine, triglycerides, albumin, total Protein, ALP etc were estimated using blood serum which was separated from blood by centrifuging at 10,000 rpm for 10 min and the supernatant solution taken and all the above biochemical parameters were performed which were estimated using semi-auto analyzer (Photometer 5010 $_{V5+}$) with enzymatic kits.

1). Estimation of SGOT (Serum glutamic oxaloacetic transaminase):

Alanine aminotransferase (ALAT) and aspartate amino transferase (ASAT) are the most important group of enzymes of aminotransferase. These enzymes act as catalyst in conversion of α-keto acids in to amino acids.

Increased levels of ALAT is found in the hepatobillary disease condition where as increased ASAT levels occur in damaged conditions of heart and skeletal muscles well as liver parenchyma. Parallel measurement of ALAT and ASAT is therefore applied to distinguish liver from heart or skeletal muscle damages.

The ASAT/ALAT ratio is used from differential diagnosis of liver diseases

L-Aspartate + 2-Oxaloglutarate $\xleftrightarrow{\text{ASAT}}$ L-Glutamate + oxaloacetate

Oxaloacetate + NADH + H^+ $\xleftrightarrow{\text{MDH}}$ D-Malate + NAD^+

Optimized UV- test used according to IFCC (International Federation of Clinical Chemistry and Laboratory Medicine).

Table- 5 Reagents of SGOT kit

Reagent-1	**Concentration**
TRIS buffer pH 7.8	80 mmol/l
L- Aspartate	240 mmol/l

MDH (malate dehydrogenase)	≥ 600 U/l
LDH (lactate dehydrogenase)	≥ 600 U/l
Reagent-2	**Concentration**
2-Oxaloglutarate	12 mmol
NADH	0.18 mmol
Good's Buffer pH 9.6	0.7 mmol/l
Pyridoxal-5-Phosphate	0.09 mmol/l

Assay procedure:

a) Mixed 800 µl of reagent-1 with 200 µl of reagent-2 in a 5 ml test tube.

b) To this, added 100 µl of serum.

c) Mixed well and took the absorbance measured immediately in the photometer and values were noted.

Normal range : < 37 µ/l

2).Estimation of Creatinine:

Creatinine levels were estimated by modified Jaffe Method. Creatinine forms a colored complex with picrate in alkaline medium. This rate of formation is measured.

Table - 7 Reagents of creatinine kit

Reagent 1	Standard creatinine (2 mg/100 ml)
Reagent 2	Picric acid solution
Reagent 3	Sodium hydroxide solution

500 µl of reagent-2 and 500 µl of reagent-3 were taken in a 5ml test tube. To this added 100 µl of serum and mixed well and read the sample in photometer.

Normal range: 0.6 -1.1 mg/dl.

3).Triglycerides

Determination of triglycerides (TG) alters enzymatic splitting with lipoprotein lipase. Quinoneimine used as an indicator which is generated from 4-aminoantipyrine and 4- chlorophenol by hydrogen peroxidase under the catalytic action of peroxidase.

Triglycerides $\xrightarrow{LPL}$ Glycerol + fatty acid

Glycerol + ATP $\xrightarrow{GK}$ Glycerol-3-phosphate+ ADP

Glycerol-3-phosphate +O_2 $\xrightarrow{GPO}$ Dihydroxyaceton phosphate + H_2O_2

$2H_2O_2$ + 4- Amino antipyrine + 4- chlorophenol $\xrightarrow{POD}$ Quinonelimine + HCL + $4H_2O_2$

Colorimetric enzymatic test performed using glycerol-3-phosphate-oxidase (GPO).

Table- 8 Reagents of Triglycerides in the kit

4-chloroPhenol	4 mmol/l
ATP	2 mmol/l
Mg^{2+}	15 mmol/l
Glycerokinase	> 0.4 Kμ/l
Peroxidase	> 2 Kμ/l
Lipoprotein lipase	> 4 Kμ/l
4-aminoantipyrine	0.5 mmol/l
Glcerol-3-phosphate- oxidase	> 1.5Kμ/l
Standard	(2.3 mmol/l)
Good's buffer pH 7.2,	50 mmol/ l

Assay procedure:

a) 1 ml (1000 μl) of reagent-1 was taken in a 5 ml test tube.
b) Added 0.01 ml (10 μl) of serum.
c) Mixed well and incubated at 37°C for 15 min.
d) Read the test sample under the photometer.

Normal Range: < 200 mg/dl **(Cole T.G. *et al.*, 1997)**

4). Total protein

Protein forms a colored complex with cupric ions in alkaline medium

Table- 9 Reagents of total protein in the kit

Reagent-1	Cupric sulphate(6 mmol/l) Potassium iodide (15 mmol/l)
Reagent-2	Protein (std) 6 g/100ml

Biuret method was followed for estimation

Preparation of test sample

1. Taken 1ml of reagent-1 in a5 ml test tube
2. To this added 0.02 ml of serum
3. Mix well and incubated at a room temperature for 15 min and Read the test sample in photometer.

Normal range: 3.2 to 4.2 g/dl. **(Weichselbaum, 1946)**

5). Alkaline phosphatese

Alkaline phosphatase (ALP), and hydrolytic enzyme act optimally at an alkaline pH. They are present in blood in numerous distinct forms which originate mainly from bone and liver

p-Nitro phenyl phosphate +water $\xrightarrow{\text{ALP}}$ Phosphate + *p*-Nitro phenol

Kinetic photometric test, according to the international Federation of clinical chemistry and laboratory Medicine (IFCC)

Table- 10 Reagents of Alkaline phosphatase in the kit

Reagent 1 :	Concentration
2-Amino-2-methyl-1-propanol pH 10.4	0.35 mol/l

Magnesium sulphate	2.0 mmol/l
Zinc sulphate	1.0mmol/l
HEDTA	2.0 mmol/l
Reagent 2 :	Concentration
p-Nitrophenylphosphate	16.0mmol/l

Assay method:

1. Taken 1000 µl of reagent-1 in a 5 ml test tube
2. To this added 250 µl of reagent-2 and mixed well
3. Add 20 µl of serum and mix well and take reading immediately using a photometer.
   Normal range: 53-128 µ/l. **(Soldin *et al.*, 1996)**

6). Estimation of Serum Total cholesterol (TC)

Principle

Determination of cholesterol was done after enzymatic hydrolysis and oxidation. The colorimetric indicator is quinoneimine, which is generated from 4-aminoantipyrine and phenol by hydrogen peroxide under the catalytic action of peroxidise (trinder's reaction).

$$\text{Cholesterol ester} + H_2 \xrightarrow{\text{CHO}} \text{Cholesterol} + \text{Fatty acid}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHO}} \text{Cholesterol-3-one} + H_2O_2$$

$$2H_2O_2 + \text{Amino antipyrine} + \text{phenol} \xrightarrow{\text{POD}} \text{Quinonelimine} + 4H_2O_2$$

Method

CHOD-PAP: enzymatic photometric test

Table- 11 Reagents of TC kit

goods buffer(pH 6.7)	50 mmol/I
Phenol	5 mmol/I
4-aminoantipyrine	0.3 mmol/I
Cholesterol esterase	>200 U/I
Cholesterol oxidase	>100U/I
Peroxidase	3 KU/I
Standard	5.2 mmol/I

Assay procedure

a. 1ml (1000 µl) of reagent-1 was taken in a 5ml test tube.

b. Added 0.01 ml (10 µl) of serum.

c. Mixed well and incubated at 37^0C for 5min.

d. Read the test sample in photometer.

Normal range: < 200 mg/dl

5.7.6. IN VIVO ANTI OXIDANT STUDIES

Preparation of tissue homogenate

The tissues were weighed and 10% tissue homogenate was prepared using phosphate buffer of pH 7.5. After centrifugation at 10,000 ×g for 10 min, the clear supernatant was used to measure thiobarbituric acid reactive substances (TBARS). (**Yong cui *et al.*, 2005)**

A. Enzymatic antioxidant activity:-

1). Estimation of Superoxide Dismutase (SOD) activity

Reagents:

1. Adrenaline
2. Carbonate buffer (pH10.2),
3. 0.1Mm EDTA

The activity of superoxide dismutase (SOD) was assayed by the method based on the oxidation of epinephrine adrenochrome transition by enzyme. The post-mitochondrial suspension of mice liver (0.5 ml) was diluted with distilled water (0.5 ml).To this chilled ethanol (0.25 ml) and chloroform (0.15 ml) was added. The mixture was shaken for 1 min and centrifuged at 2000 × g for 10 min.The PMS (0.5 ml) was added with PBS buffer (pH 7.2, 1.5ml). The reaction initiated by the addition of epinephrine (0.4 ml) and change in optical density (O.D.,min-1) was measured at 470 nm. SOD activity was expressed as U/mg of tissue. Change in O.D (min-1) at 50% inhibition to adrenochrome transition by the enzyme was taken as one enzyme unit. **(Bito *et al*., 2008)**

2). Estimation of Catalase (CAT) activity

Reagents:

1. Dichromate/acetic acid reagent (5% solution of potassium dichromate in acetic acid at 1:3 ratios)

2. 0.01 M Phosphate buffer, pH 7.0

3. 0.2 M Hydrogen peroxide

Catalase (CAT) was estimated by mixing 1.0 ml of 0.01 M phosphate buffer (pH 7.0), 0.1 ml of tissue homogenate and 0.4 ml of 2 M hydrogen peroxide. The reaction was stopped by the addition of 2.0 ml dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in 1:3 ratio). Then thebabsorbance was measured at 530 nm. CAT activity was expressed as µM of H_2O_2 consumed/min/mg protein. **(Sinha, 1972)**

3). Estimation of Glutathione peroxidase (GPx) activity

Reagents:

1. 0.32 M Phosphate buffer, pH 7.0

2. 0.8 mM EDTA

3. 10 mM Sodium azide

4. 3 mM reduced glutathione

5. 2.5 mM H_2O_2

6. 10% TCA

7. 0.3 M Disodium hydrogen phosphate

8. DTNB solution (40 mg of DTNB in 100 ml of 1% sodium citrate)

Procedure:

Glutathione peroxidase (GPx) was measured by mixing 0.2 ml 0.4 M phosphate buffer (pH 7.0), 0.1 ml 10 mM sodium azide, 0.2 ml tissue homogenized in 0.4 M phosphate buffer (Ph 7.0), 0.2ml reduced glutathione, and 0.1 ml 0.2 mM hydrogen peroxide. The contents were incubated for 10 min at 37° C, 0.4 ml 10% TCA was added to stop the reaction and centrifuged at 3200 × g for 20 min.The supernatant was assayed for glutathione content using Ellman's reagent (19.8 mg 5,5'-dithiobisnitrobenzoic acid (DTNB) in 100 ml 0.1% sodium nitrate). The activities were expressed as µg of GSH consumed/ min/mg protein. **(Pridaran Murugan, 2009)**

B. Non enzymatic anti oxidant activity

1). Estimation of reduced glutathione(GSH) activity

Reagents:

1. 10% TCA
2. 0.6 mM 5, 5'-dithiobis-2-nitrobenzoic acid (DTNB) in 0.2 M sodium phosphate
3. 0.2 M Phosphate buffer, pH 8.0

Reduced glutathione (GSH) was measured by mixing PMS of rat liver (720 µl) and 5% TCA to precipitate the protein content. After centrifugation at 10,000 × g for 5 min, the supernatant was taken. DTNB (5,5'-dithio-bis(2-nitrobenzoic acid) Ellman's reagent was added to it and the absorbance was measured at 412 nm. A standard graph was drawn using different concentration of standard GSH solution. GSH contents were calculated in the PMS of rat liver. **(Brahma N Singh *et al.*, 2009)**

2). **Estimation of Lipid Peroxidation**

Reagents:

1. Thiobarbituric acid 0.37%
2. 0.25 N HCl
3 .15% TCA

Lipid peroxidation in liver was estimated calorimetrically by measuring thiobarbituric acid reactive substances (TBARS). 0.1 ml of tissue homogenate was treated with 2 ml of TBA- trichloroacetic acid– hydrochloric acid reagent (0.37% TBA, 0.25 M hydrochloric acid and 15% TCA, 1:1:1 ratio), placed for 15 min in a water bath and then cooled and centrifuged at 3500 ×g for 10 min at room temperature, the absorbance of clear supernatant was measured at 535 nm against a reference blank. Values were expressed as mM/100 g tissue. (**Pridan Murugan *et al*.,2009**)

5.7.7. **HISTOPHATOLOGICAL TECHINIQUES**

Histopathology is the microscopical study of tissues for pathological alterations. This involves collection of morbid tissues from biopsy or necropsy, fixation, preparation of sections, staining and microscopical examination.

1). Collection of materials

Thin pieces of 3 to 5 mm, thickness were collected from tissues showing gross morbid changes along with normal tissue.

2). Fixation:

Kept the tissue in fixative for 24-48 hours at room temperature

The fixation was useful in the following ways:

a) Serves to harden the tissues by coagulating the cell protein,
b) Prevents autolysis,
c) Preserves the structure of the tissue, and
d) Prevents shrinkage

Common Fixatives: 10% Formalin

3). Haematoxylin and eosin method of staining:

Deparaffinise the section by xylol 5 to 10 minutes and remove xylol by absolute alcohol. Then cleaned the section in tap water and stained with haematoxylin for 3-4 minutes and again cleaned under tap water. Allow the sections in tap water for few minutes and counter stained with 0.5% eosin until section appears light pink (15 to 30seconds), and then washed in tap water. Blotted and dehydrated in alcohol and cleared with xylol (15 to 30 seconds). Mounted on a Canada balsam or DPX Moutant and kept the slide dry and remove air bubbles.

6. RESULTS AND DISCUSSION

6.1 Phytochemical Studies of Ethanolic Extract of *Wattakaka volubilis* Linn.

Preliminary Phytochemical Tests were performed using the ethanolic extract and various results were obtained which were as follows

Table 12.

S.N.	Phytochemical constituents	Extract
1.	Carbohydrates	+ve
2.	Alkaloids	+ve
3.	Steroids & sterols	+ve
4.	Glycosides	+ve
5.	Saponins	+ve
6.	Flavonoids	+ve
7.	Tannins	+ve
8.	Proteins & amino acids	-ve
9.	Phenols	+ve

+ve – present **-ve – absent**

Inference- From the preliminary Phytochemical studies it was confirmed that the ethanolic extract contains carbohydrates, alkaloids, steroids, glycosides, saponin, tannins, flavanoids, phytosterols, and phenols as active chemical constituents.

6.2. Determination of Total Phenols

Total phenol content of Ethanolic extract of *Wattakaka volubilis* Linn.

Table- 13

Sample	**Phenolic content(Gallic acid equivalents mg/100g**
Ethanolic Extract of *Wattakaka volubilis* Linn	50.79±0.19

Data was expressed in fresh weight basis and is presented as mean ±SD. Data subjected to statistical analysis (correlation between antioxidant activity and phenolic content) using GraphPad Prism 5 statistical package. P< 0.03.

Inference – The presence of phenols in the extract shows that the extract has anti-oxidant activity.

6.3. Toxicological evaluations of ethanolic extract of *Wattakaka volubilis* Linn*:*

Effect of ethanolic extract of *Wattakaka volubilis* on mice.

Table- 14

S.N	**Response**	**Mice 1(4 hrs)**		**Mice 2 (4 hrs)**		**Mice 3 (4 hrs)**	
		Before	**After**	**Before**	**After**	**Before**	**After**
1	Alertness	Normal	Normal	Normal	Normal	Normal	Normal
2	Grooming	Absent	Absent	Absent	Absent	Absent	Absent
3	Anxiety	Absent	Absent	Absent	Absent	Absent	Absent
4	Roaming	Normal	Normal	Normal	Normal	Normal	Normal
5	Sniffing	Normal	Normal	Normal	Normal	Normal	Normal
6	Tremors	Absent	Absent	Absent	Absent	Absent	Absent
7	Convulsion	Absent	Absent	Absent	Absent	Absent	Absent
8	Depression	Normal	Present	Normal	Present	Normal	Present
9	Gripping strength	Normal	Normal	Normal	Normal	Normal	Normal
10	Scratching	Present	Present	Present	Present	Present	Present

11	Defecation	Absent	Present	Absent	Present	Absent	Present
12	Writhing	Absent	Absent	Absent	Absent	Absent	Absent
13	Pupils	Normal	Normal	Normal	Normal	Normal	Normal
14	Urination	Normal	Present	Normal	Present	Normal	Present
15	Salivation	Normal	Normal	Normal	Normal	Normal	Normal
16	Skin colour	Normal	Normal	Normal	Normal	Normal	Normal
17	Lacrimation	Normal	Normal	Normal	Normal	Normal	Normal

Result: From acute toxicity study it was observed that the administration of ethanolic extract of *Wattakaka volubilis* Linn to mice did not cause any toxicity of extract (4 hrs) and mortality in the animals (14 days) up to 1000 mg/kg orally.

6.4. INVITRO ANTI OXIDANT STUDY

1). DPPH Radical scavenging activity:

% inhibition of DPPH radical scavenging activity of *Wattakaka volubilis* extracts

Table- 15

Sample	**DPPH(Troloxequivalents)**
Ethanolic extract of *Wattakaka volubilis Linn*	50.20±1.21

Data was expressed in fresh weight basis and is presented as mean ±SD. Data subjected to statistical analysis (correlation between antioxidant activity and phenolic content) using GraphPad Prism 5 statistical package. P< 0.0001.

2). FRAP Assay:

Table- 16

Sample	FRAP($FeSO_4$ Equivalents)
Ethanolic extract of *Wattakaka volubilis Linn*	46.10±7.32

Data was expressed in fresh weight basis and is presented as mean ±SD. Data subjected to statistical analysis (correlation between antioxidant activity and phenolic content) using GraphPad Prism 5 statistical package. P< 0.0002.

3). ABTS:

ABTS radical scavenging activity of ethanolic extracts of *Wattakaka volubilis*

Table- 17

SAMPLE	20(µg/ml)	40 (µg/ml)	60 (µg/ml)	80 (µg/ml)	100 (µg/ml)
Ethanolic extract	0.5685	0.4727	0.3130	0.1957	0.1240

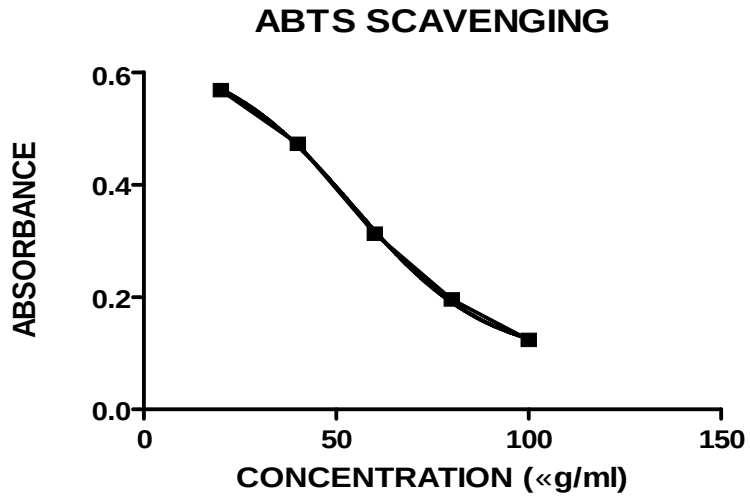


Figure-2

4). TRAP:

Absorbance of the ethanolic extract of *Wattakaka volubilis*

Table- 18

SAMPLE	Absorbance				
	20(µg/ml)	**40 (µg/ml)**	**60 (µg/ml)**	**80 (µg/ml)**	**100 (µg/ml)**
Ethanolic extract	0.2372	0.2598	0.2635	0.2722	0.2786

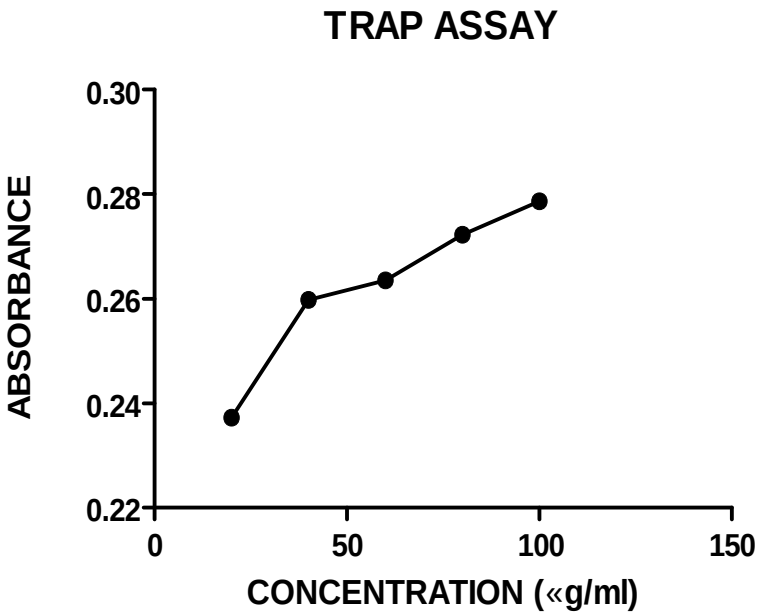


Figure- 3

5). SUPER OXIDE SCAVENGING (SOS)

Absorbance of the ethanolic extract of *Wattakaka volubilis*

Table- 19

SAMPLE	% of SOS Activity				
	20(µg/ml)	**40 (µg/ml)**	**60 (µg/ml)**	**80 (µg/ml)**	**100 (µg/ml)**
Ethanolic extract	32.63	46.54	51.68	55.08	56.59

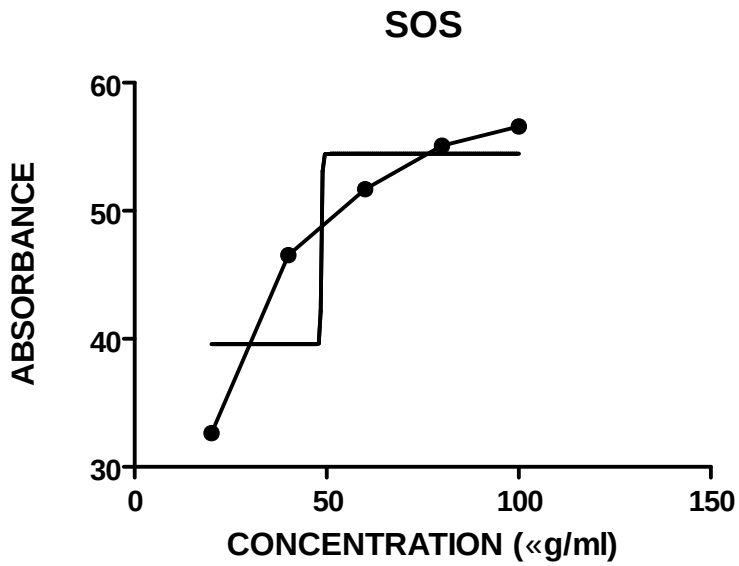


Figure- 4

6). NITRIC OXIDE SCAVENGING (NOS)

Absorbance of the ethanolic extract of *Wattakaka volubilis*

Table- 20

SAMPLE	% of NOS Activity				
	20(µg/ml)	**40 (µg/ml)**	**60 (µg/ml)**	**80 (µg/ml)**	**100 (µg/ml)**
Ethanolic extract	25.73	29.13	36.62	48.28	55.09

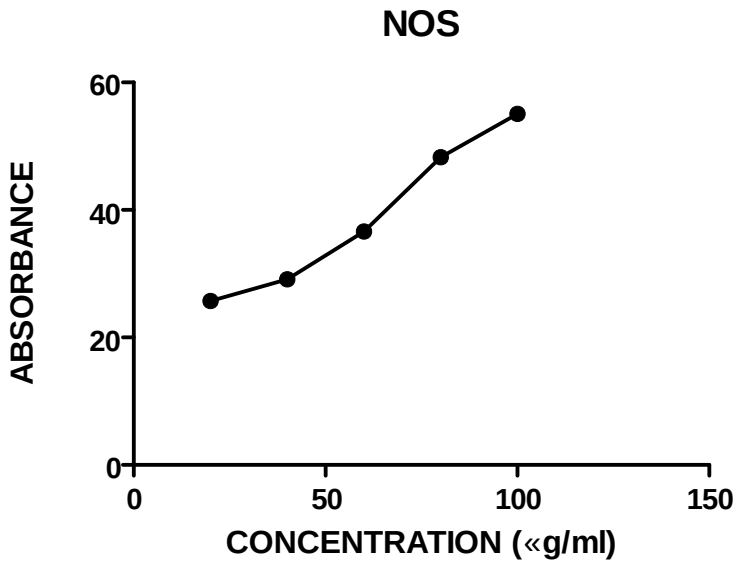


Figure- 5

6.5. DAL INDUCED ACITIC TUMOR

1). Body weight analysis

Effect of Ethanolic extract of *Wattakaka volubilis Linn* on body weight of mice challenged with Daltons lymphoma ascetic cells.

Table- 21

Experimental groups	**Body weight (gm)**			**Decrease in body weight from 7th day to 14th day (gm)**	**% Decrease in body weight**
	0 day	**7th day**	**14th day**		
ONLY DAL	24 ±1.211	31.5 ±1.455**	37.17 ±1.922***	--nil--	--nil--
DAL+5- FU (20mg/kg)	23.17 ±1.641	19.67 ±0.9545 ns	18.00 ±1.238 *	5.17	22.31
DAL + Extract (100mg/kg)	24.83 ±1.249	23.17 ±1.376 ns	20.17 ±1.046 *	4.66	18.76
DAL + Extract (200mg/kg)	28.83 ±2.023	26.17 ±1.493 ns	22.50 ±1.204*	6.33	21.17

Values are expressed as the mean ± S.E.M., (n = 6) on day 14 of the experiment; Statistical significance (p) calculated by one way ANOVA followed by dunnett's, P<0.05 calculated by comparing treated group with only 0 day of each group.

ns- Non significant

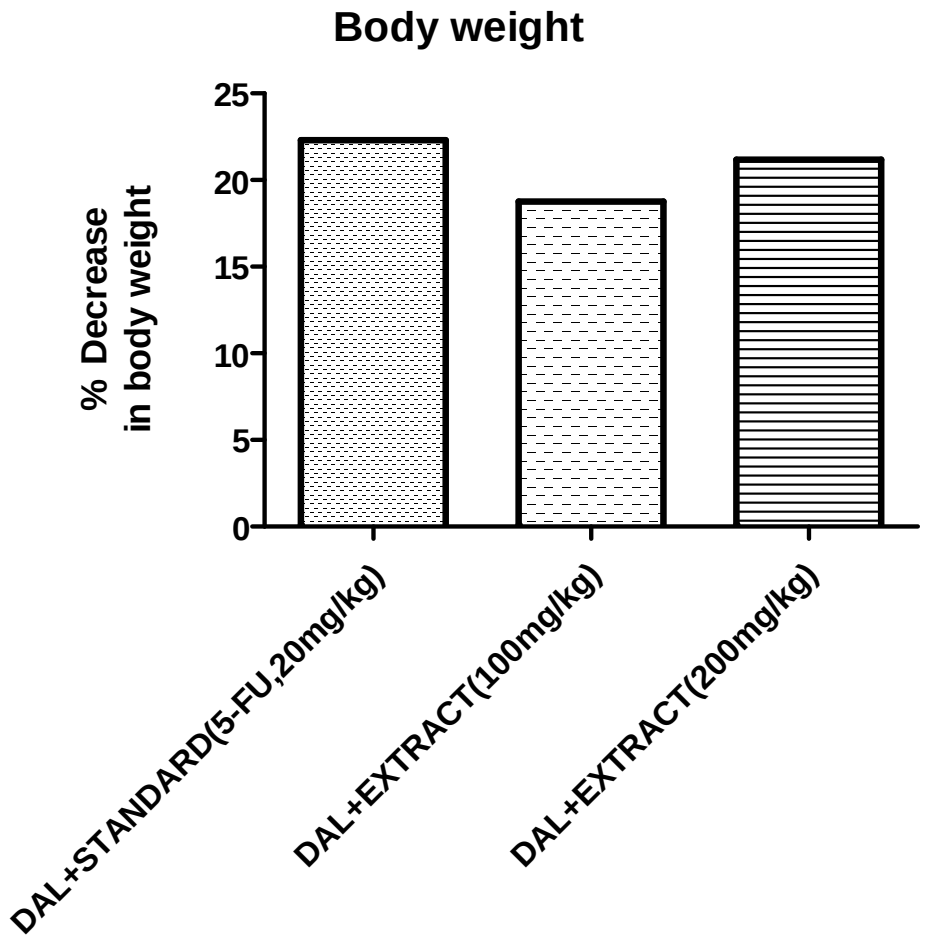
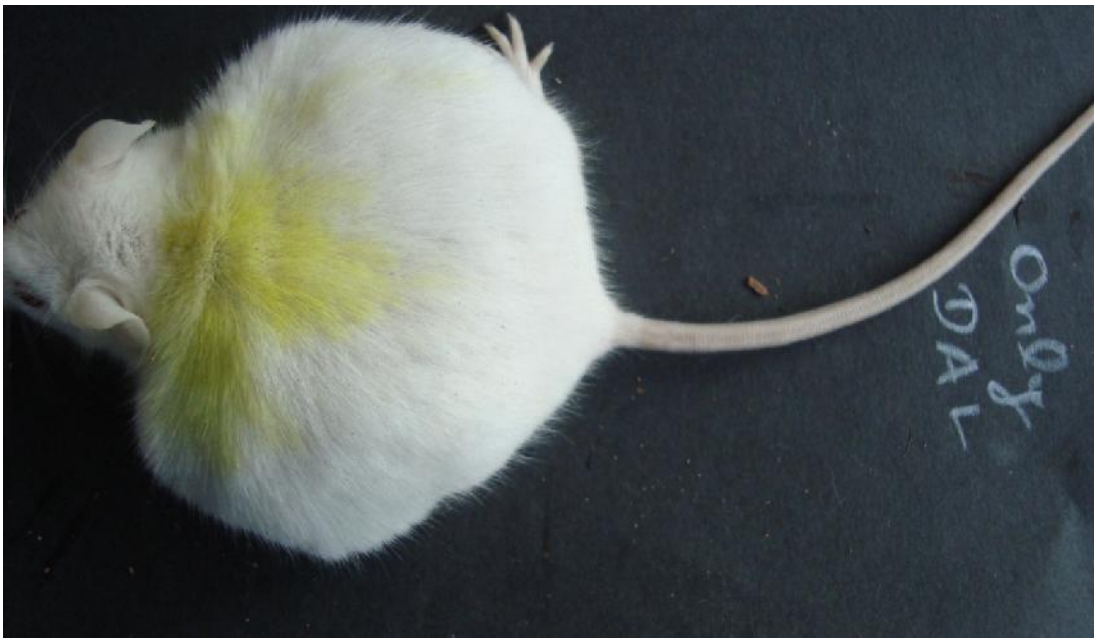
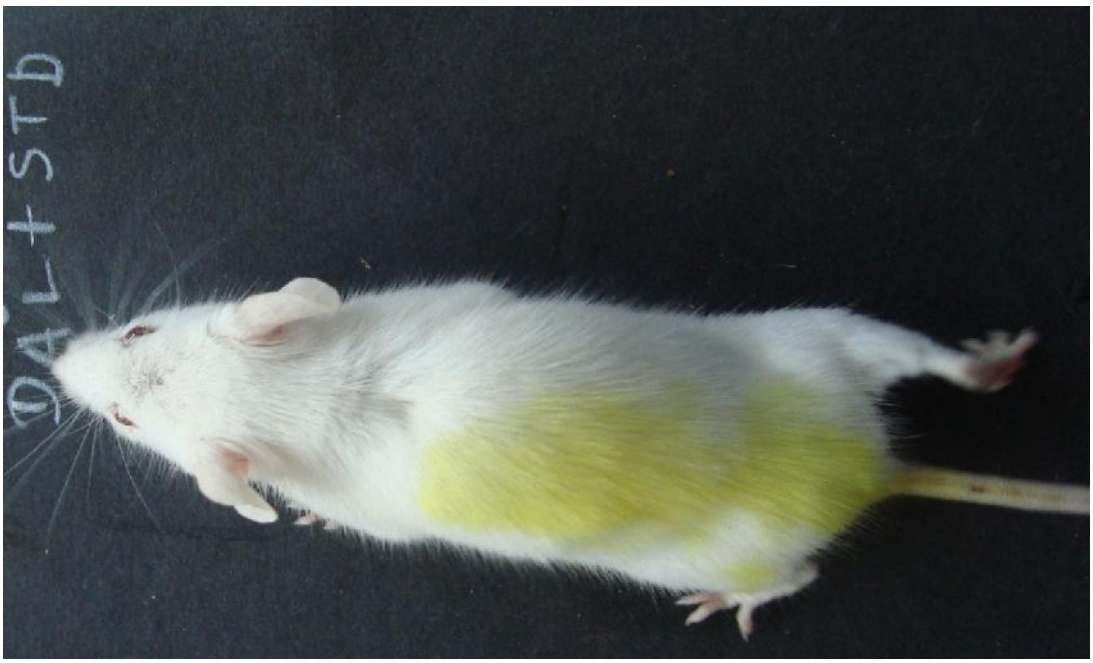

Body weight
% Decrease in body weight
25
20
15
10
5
0
DAL+STANDARD(5-FU,20mg/kg)
DAL+EXTRACT(100mg/kg)
DAL+EXTRACT(200mg/kg)

Figure- 6

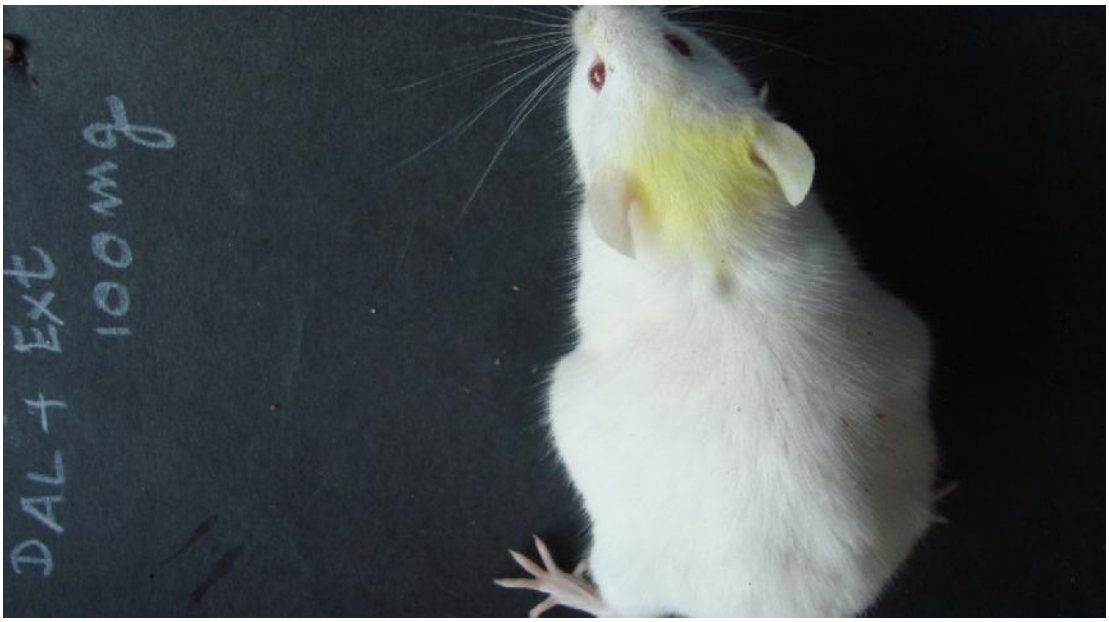
BODY WEIGHT ANALYSIS



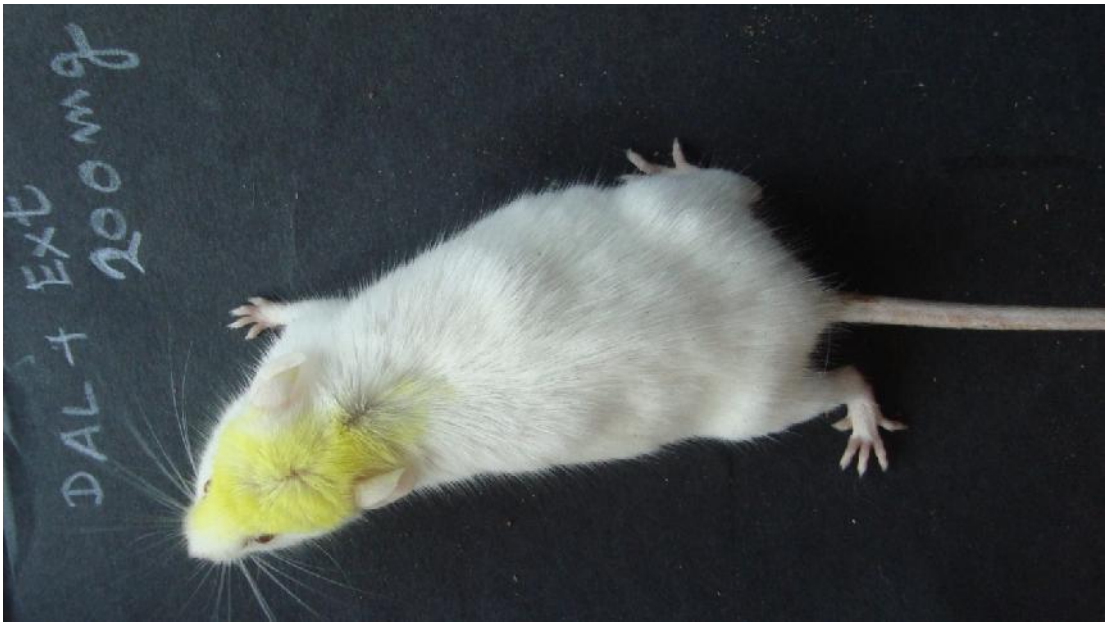
[A]



[B]



[C]



[D]

Figure- 7 [A] - Shows the mice with only DAL, [B] - Shows the mice with DAL+STD drug (5-FU 20mg/kg), [C] – Shows the mice with DAL+EXTRACT (100mg/kg), [D] – Shows the mice with DAL+EXTRACT (200mg/kg).

2). MEAN SURVIVAL (MST):

Effect of Ethanolic extract of *Wattakaka volubilis* Linn on survival time of mice challenged with Daltons lymphoma ascetic cells.

Mean survival time and percentage increase in life span of various groups

Table- 22

Experimental groups	Mean survival time in days	%ILS
ONLY DAL	19.83±1.470	--nil--
DAL + 5-FU, 20mg/kg	40.83±1.424***	105.90
DAL+ Extract (100mg/kg)	27.17±1.778*	37.01
DAL+ Extract (200mg/kg)	34.00±2.309***	71.45

Values are expressed as the mean ± S.E.M.; Statistical significance (p) calculated by one way ANOVA followed by dunnett's, $P < 0.0001$,

ns – non significant calculated by comparing treated group with only DAL.

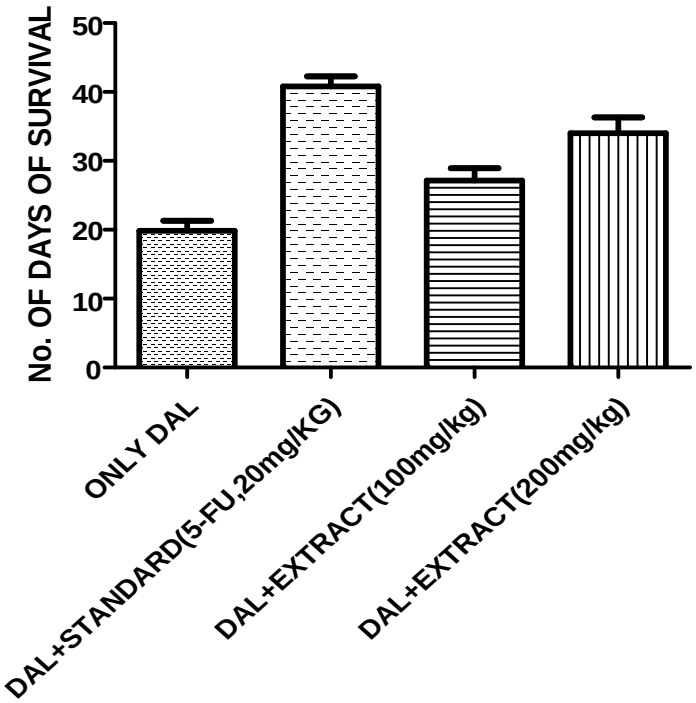


Figure- 8

3). EFFECT OF *Wattakaka volubilis* **ON TUMOR VOLUME**

Table- 23

Experiment groups	**ONLY DAL**	**DAL + 5-FU, (20mg/kg)**	**DAL + Extract (100mg/kg)**	**DAL + Extract (200mg/kg)**
Tumor volume (ml)	10.00 ±1.732	1.033 ±0.08819***	7.1 ±0.9504ns	3.9 ±0.2082**

Values are expressed as the mean ± S.E.M.; Statistical significance (p) calculated by one way ANOVA followed by dunnett's, p<0.0011,

ns– non significant calculated by comparing treated group with DAL group.

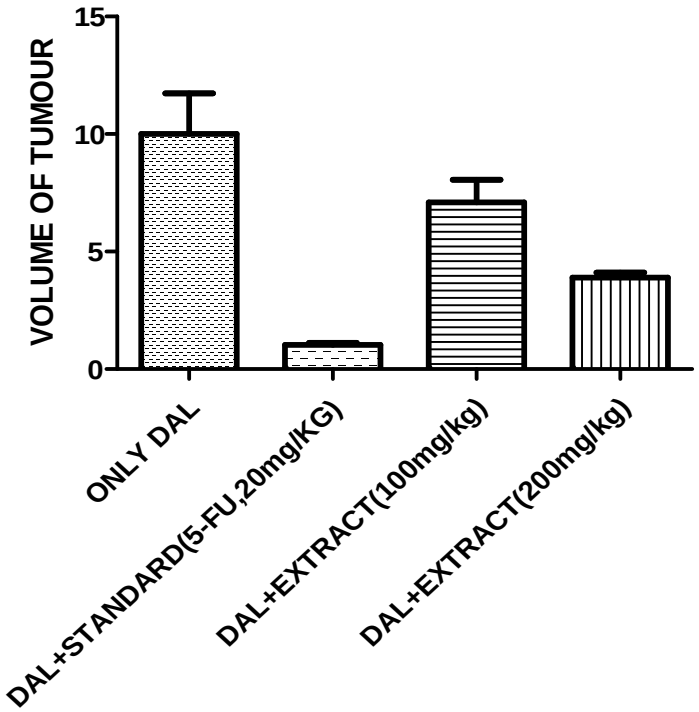


Figure- 9

4). EFFECT OF *Wattakaka volubilis* **NON-VIABLE CELL COUNT**

NON-VIABLE CELL COUNT

Table- 24

Experiment groups	ONLY DAL	DAL+5-FU, (20mg/kg)	DAL + Extract (100mg/kg)	DAL + Extract (200mg/kg)
Non-viable cell count	1×10^{6}	564.3±81.53	173.7±33.89**	222.7±13.62**

Values are expressed as the mean ± S.E.M., (n = 6) on day 14 of the experiment; Statistical significance (p) calculated by one way ANOVA followed by dunnett's

$P < 0.0034$,

ns – non significant calculated by comparing treated group with DAL control group.

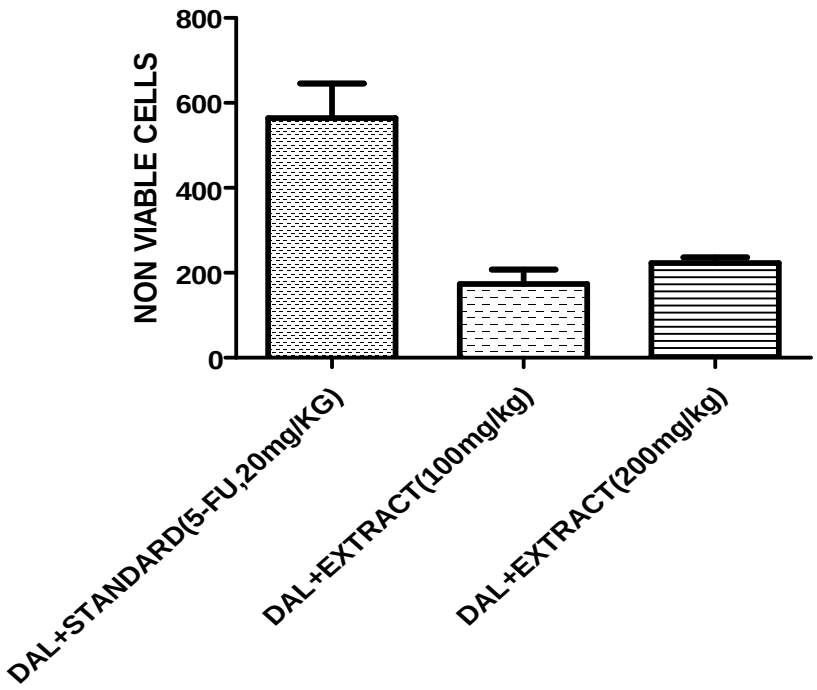
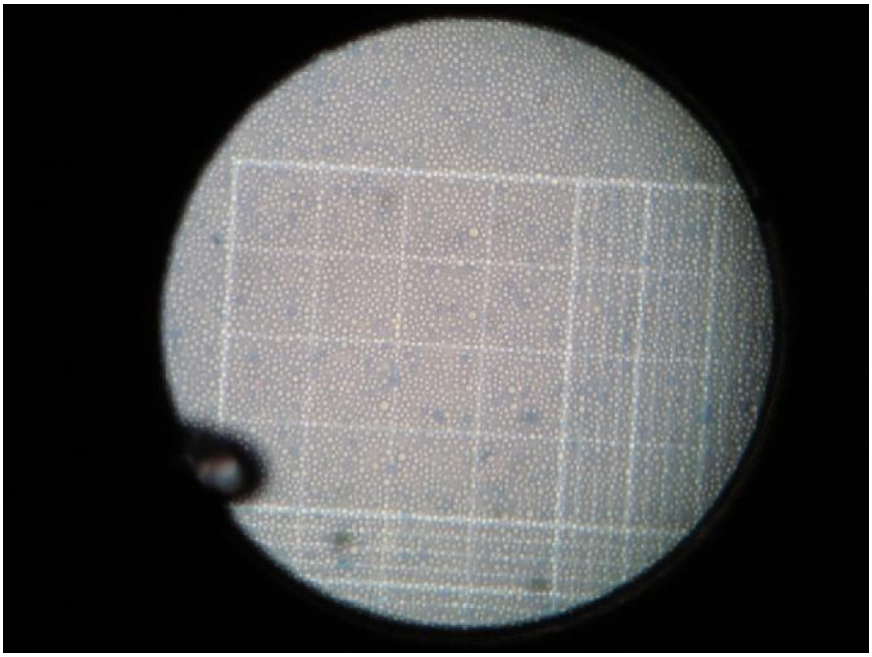
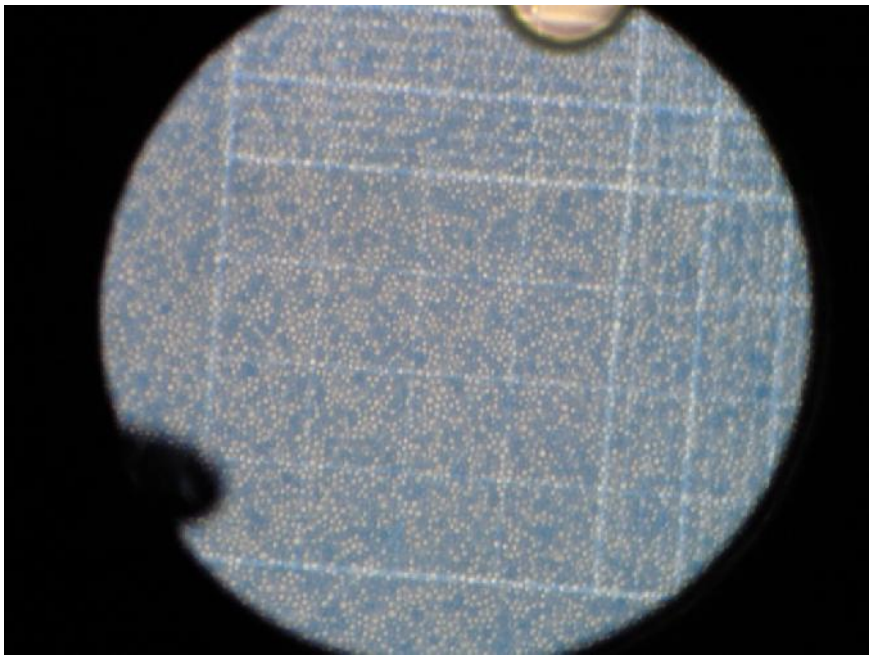


Figure- 10



[A]



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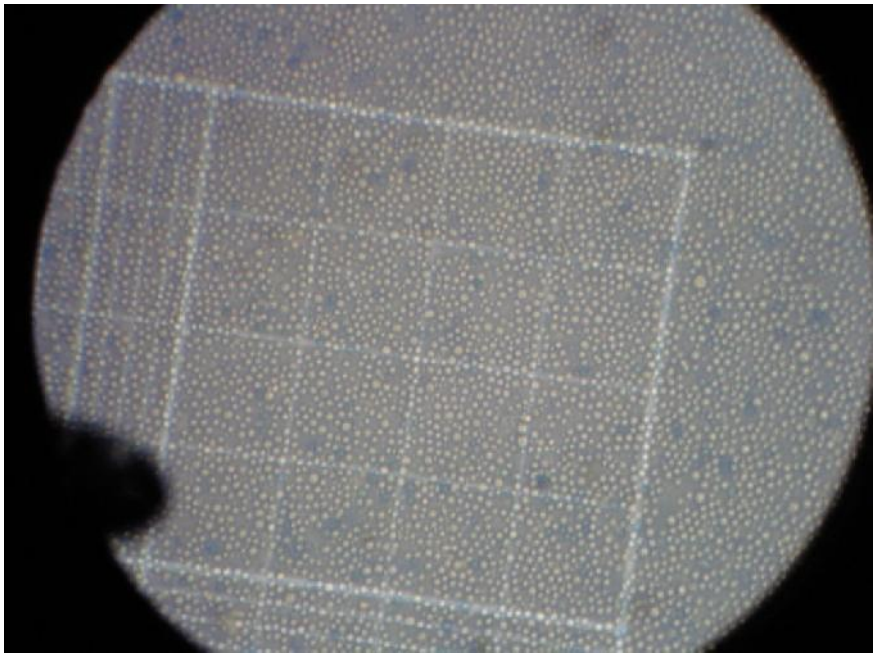
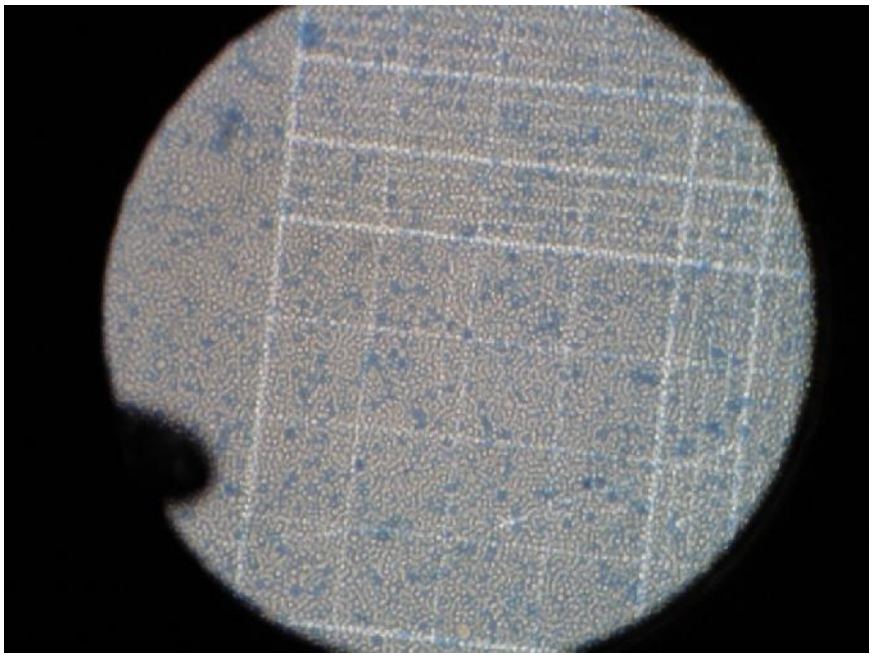


[C]



[D]

Figure-11 Effect of extract on Non-Viable Cell Count after 14 days treatment

[A]: only DAL; [B]: DAL + 5-FU, 20 mg/kg body weight; [C]: DAL + EXTRACT 100 mg/kg body weight; [D]: DAL + EXTRACT 200 mg/kg body weight.

6.7.1. EFFECT OF ETHANOLIC EXTRACT OF *Wattakaka volubilis* ON HEMATOLOGICAL PARAMETERS:

1). RED BLOOD CELLS (RBC):

RBC count of experimental groups

Table- 25

Experiment groups	NORMAL	ONLY DAL	DAL+ 5-FU, (20mg/kg)	DAL+ Extract (100mg/kg)	DAL+ Extract (200mg/kg)
RBC (million/mm^3)	5.217 ±0.07491	1.090 ±0.03742***	4.872 ±0.08360^{ns}	4.595 ±0.5749^{ns}	5.767 ±0.47 38^{ns}

Values are expressed as the mean ± S.E.M., (n = 6) on day 14 of the experiment P<0.0001, between tumor control and treated groups and

ns-non significant.

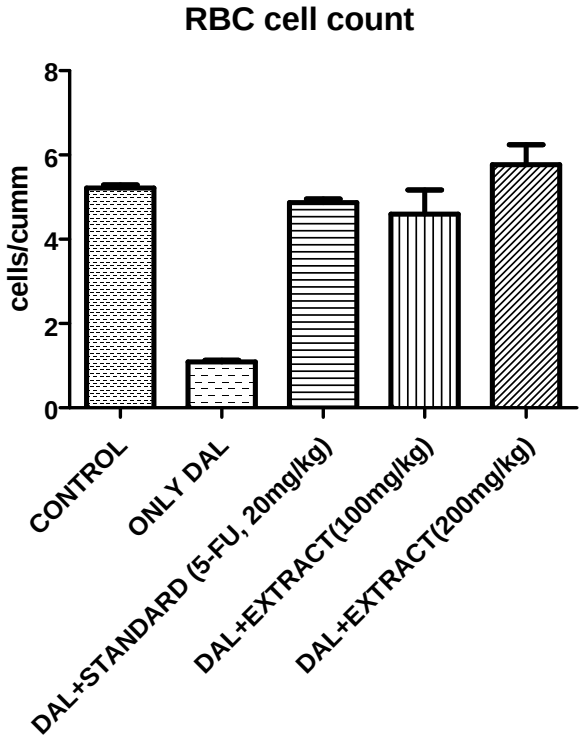


Figure-12

2). HAEMOGLOBIN

HAEMOGLOBIN values of the experimental animals

Table- 26

Experiment Groups	NORMAL	ONLY DAL	DAL+ 5-FU, (20mg/kg)	DAL+ Extract (100mg/kg)	DAL+ Extract (200mg/kg)
Hb (%)	15.50 ±0.2033	5.817 ±2.038***	12.57 ±0.6931ns	10.88 ±0.8167*	15.15 ±0.2680ns

Values are expressed as the mean ± S.E.M., (n = 6) on day 14 of the experiment; P<0.0001, between tumor control and treated groups.

ns-non significant.

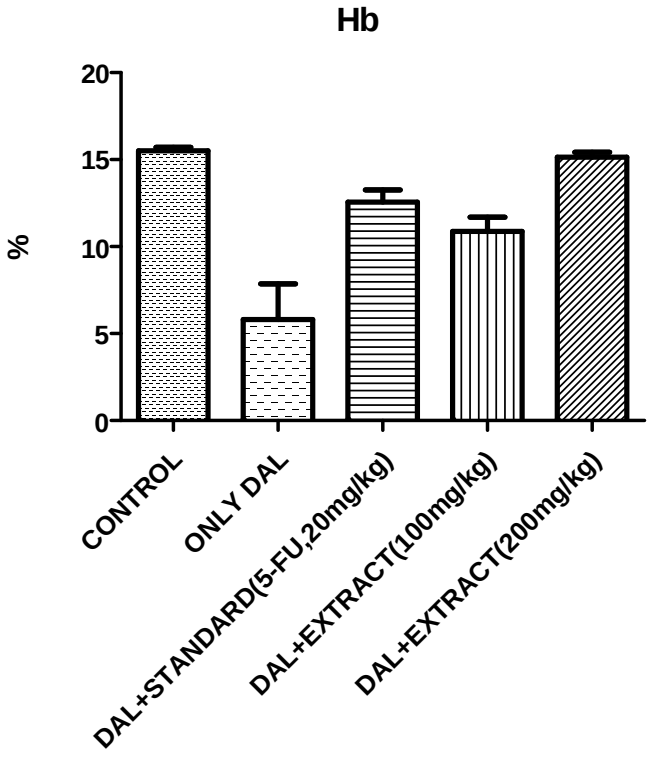


Figure- 13

3). HEMATOCRIT

HEMATOCRIT of the experimental animals

Table- 27

Experiment groups	NORMAL	ONLY DAL	DAL+ 5-FU, (20mg/kg)	DAL+ Extract (100mg/kg)	DAL+ Extract (200mg/kg)
Hematocrit (%)	43.72 ±0.7631	10.92 ±1.339***	34.98 ±0.3609**	28.88 ±0.5839***	36.77 ±3.443*

Values are expressed as the mean ± S.E.M., (n = 6) on day 14 of the experiment, P<0.0001, between tumor control and treated group.

ns-non significant.

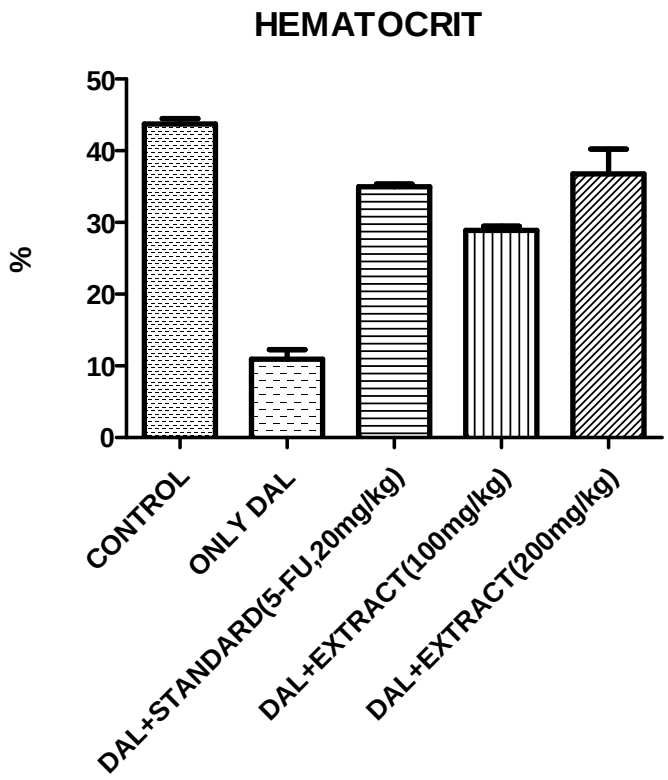


Figure- 14

4). MCV

The MCV values of the experimental animals

Table- 28

Experiment groups	NORMAL	ONLY DAL	DAL+ 5-FU, (20mg/kg)	DAL+ Extract (100mg/kg)	DAL+ Extract (200mg/kg)
MCV (fl)	43.30 ±0.2352	31.20 ±3.808**	42.33 ±1.022ns	34.24 ±2.527*	39.05 ±0.6158ns

Values are expressed as the mean ± S.E.M., (n = 6) on day 14 of the experiment P<0.0015, between tumor control and treated group.

ns-non significant.

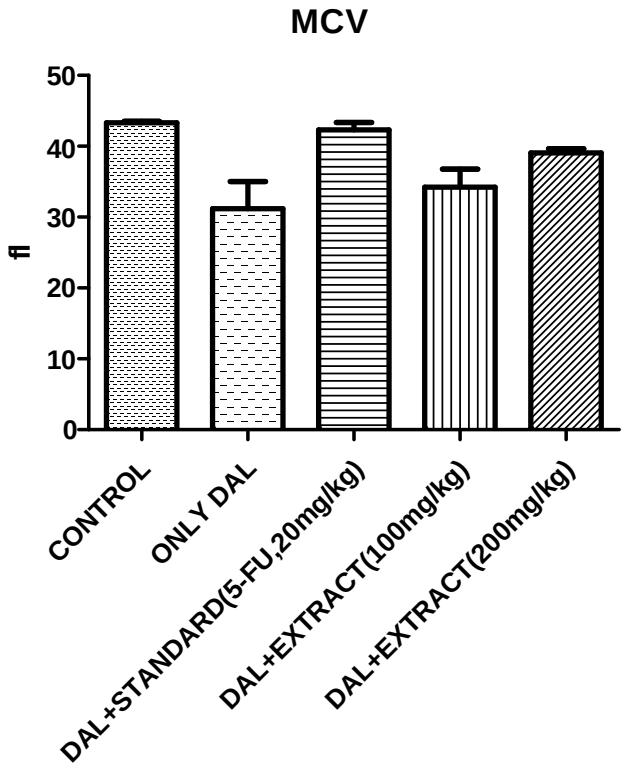


Figure- 15

5). MCH

The MCH values of the experimental animals

Table- 29

Experiment groups	NORMAL	ONLY DAL	DAL+ 5-FU, (20mg/kg)	DAL+ Extract (100mg/kg)	DAL+ Extract (200mg/kg)
MCH (pg)	29.47 ±0.4145	15.63 ±0.4944 ***	24.17 ±1.390 **	20.35 ±0.3528 ***	29.98 ±1.966 ns

Values are expressed as the mean ± S.E.M., (n = 6) on day 14 of the experiment P<0.0001, between tumor control and treated groups.

ns-non significant.

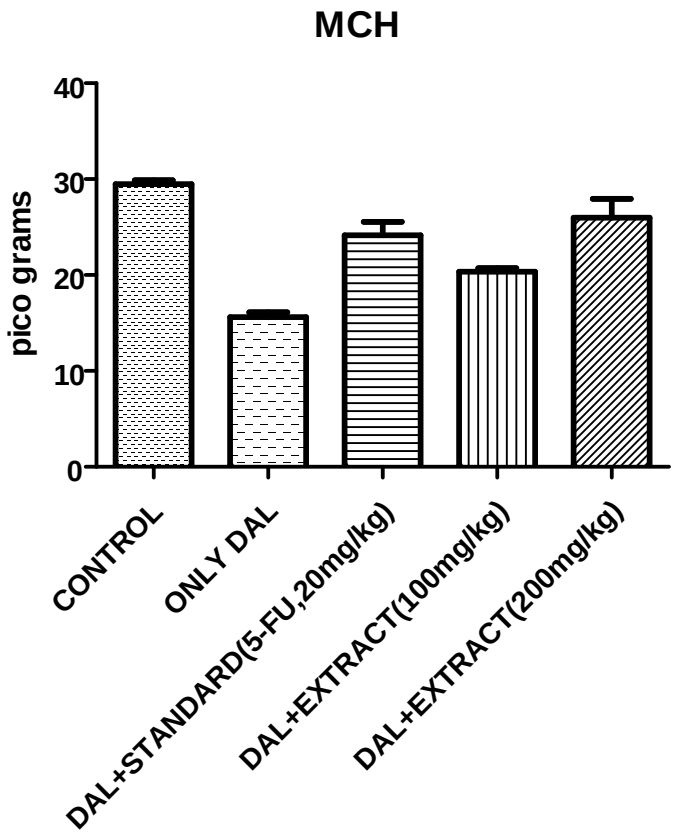


Figure- 16

6). MCHC

The MCHC values of the experimental animals

Table- 30

Experiment groups	NORMAL	ONLY DAL	DAL+ 5-FU, (20mg/kg)	DAL+ Extract (100mg/kg)	DAL+ Extract (200mg/kg)
MCHC (%)	31.50 ±0.3907	15.45 ±0.3452 ***	31.83 ±0.4096 ns	21.35 ±0.3547 ***	28.85 ±0.4958 ***

Values are expressed as the mean ± S.E.M., (n = 6) on day 14 of the experiment P<0.0001, between tumor control and treated group.

ns-non significant.

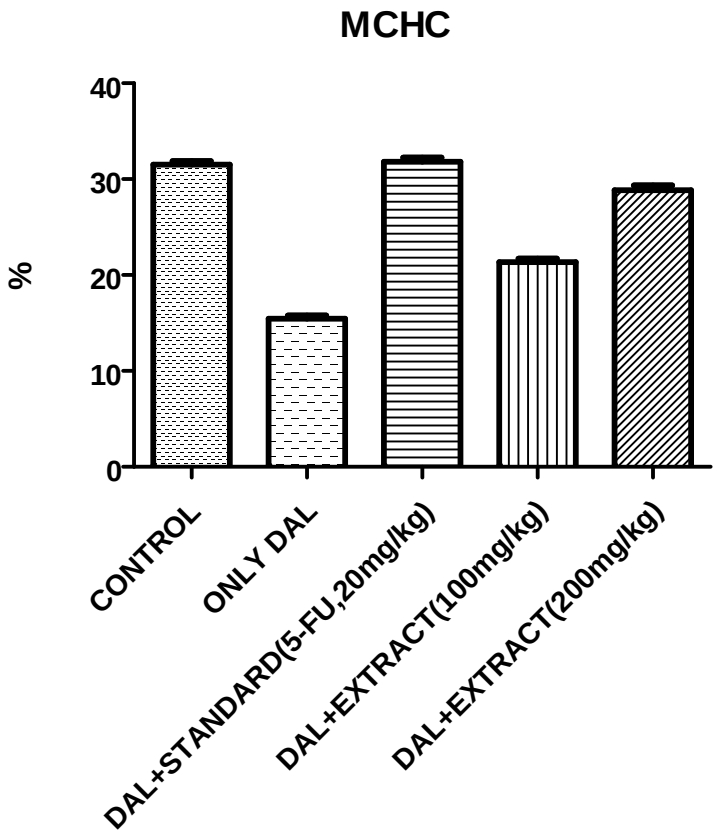


Figure- 17

7). WBC

WBC count of experimental groups

Table- 31

Experiment groups	NORMAL	ONLY DAL	DAL+ 5-FU, (20mg/kg)	DAL+ Extract (100mg/kg)	DAL+ Extract (200mg/kg)
Total WBC ($1\times10^3/mm^3$)	8890 ±535.4	27425 ±4289 ***	11967 ±757 ns	15360 ±2025 ns	16383 ±2541 ns

Values are expressed as the mean ± S.E.M., (n = 6) on day 14 of the experiment P<0.0002, between tumor control and treated group,

ns- non significant.

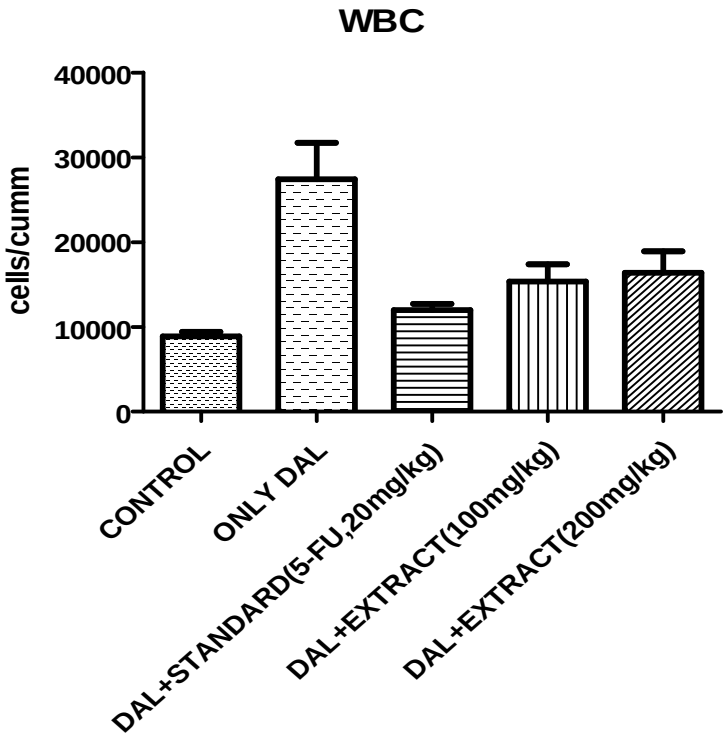


Figure- 18

6.7.2. SERUM BIOCHEMICAL PARAMETERS:

1). SERUM GLUTAMATE OXALOACETATE TRANSAMINASE (SGOT)

Serum SGOT Levels.

Table- 32

Experiment group	NORMAL	ONLY DAL	DAL+5-FU, (20mg/kg)	DAL+ Extract (100mg/kg)	DAL+ Extract (200mg/kg)
SGOT(u/l)	146±7.743	80.67 ±3.461 ***	110.2 ±0.7032 ***	65.50 ±3.374 ***	85.33 ±4.006 ***

Values are expressed as the mean ± S.E.M., (n = 6) on day 14 of the experiment; P<0.0001, between tumor control and treated groups.

ns-non significant.

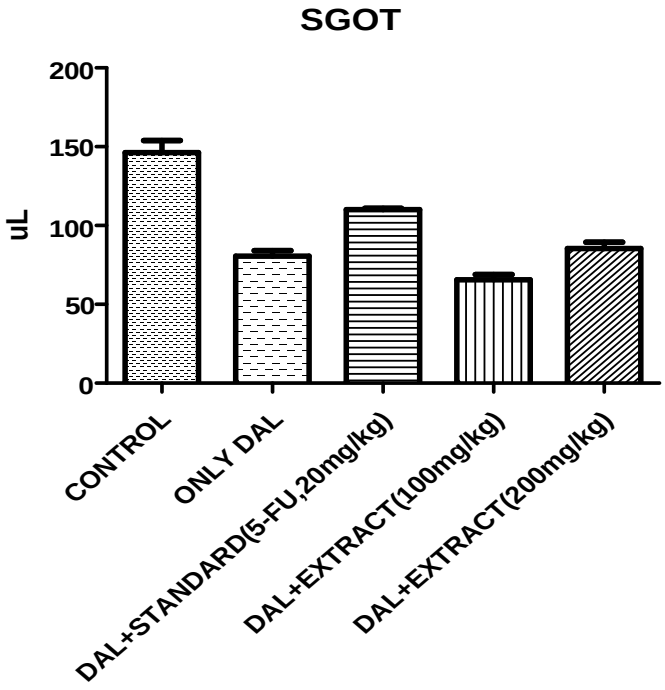


Figure- 19

2). Alkaline Phosphates (ALP)

Serum ALP levels

Table- 33

Experiment group	NORMAL	ONLY DAL	DAL+5-FU, 20mg/kg	DAL+ Extract (100mg/kg)	DAL+ Extract (200mg/kg)
ALP(u/l)	73.67 ±1.116	146.3 ±9.783 ***	83.67 ±15.18 ns	112.7 ±12.49 ns	93.5 ±12.89 ns

Values are expressed as the mean ± S.E.M., (n = 6) on day 14 of the experiment P<0.0011, between tumor control and treated group.

ns-non significant

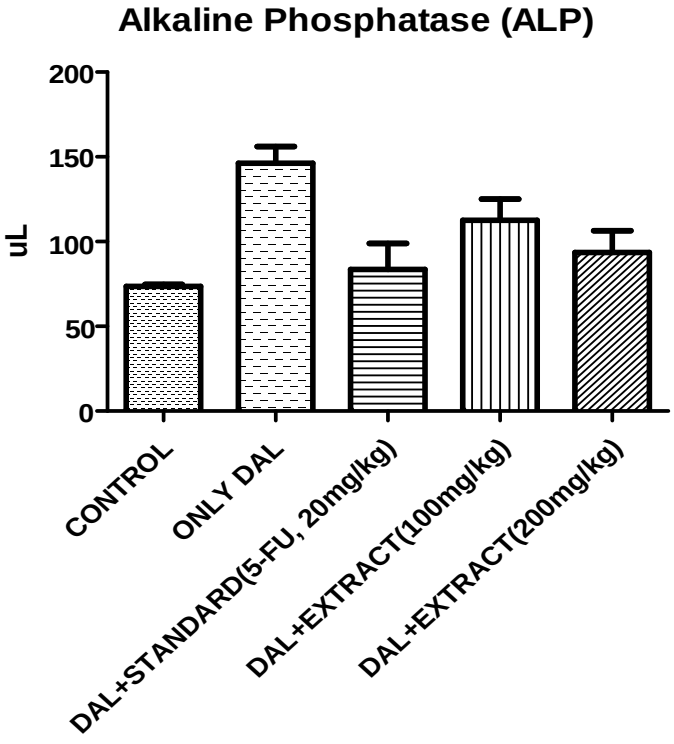


Figure- 20

3). UREA

Serum UREA values

Table- 34

Experiment group	NORMAL	ONLY DAL	DAL+5-FU, (20mg/kg)	DAL+ Extract (100mg/kg)	DAL+ Extract (200mg/kg)
UREA	49 ±3.134	26.30 ±1.232***	44.57 ±2.673ns	33.32 ±2.920 ***	41.10 ±1.794 ns

Values are expressed as the mean ± S.E.M., (n = 6) on day 14 of the experiment P<0.0001, between tumor control and treated group.

ns-non significant.

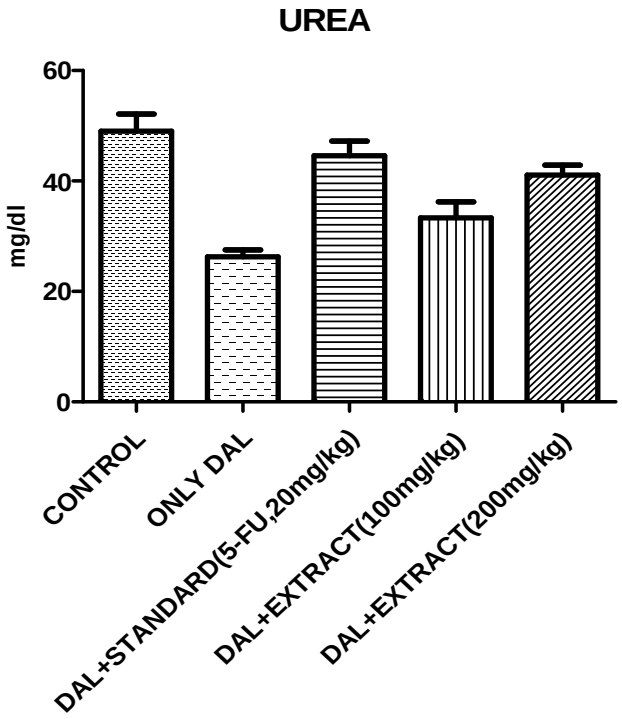


Figure- 21

4). URIC ACID

Serum URIC ACID values

Table- 35

Experiment group	NORMAL	ONLY DAL	DAL+5-FU, (20mg/kg)	DAL+ Extract (100mg/kg)	DAL+ Extract (200mg/kg)
URIC ACID	5.550 ±0.1384	3.267 ±0.5463 *	5.867 ±0.6781 ns	5.150 ±0.7702 ns	6.167 ±0.5584 ns

Values are expressed as the mean ± S.E.M., (n = 6) on day 14 of the experiment P<0.0136, between tumor control and treated groups.

ns-non significant.

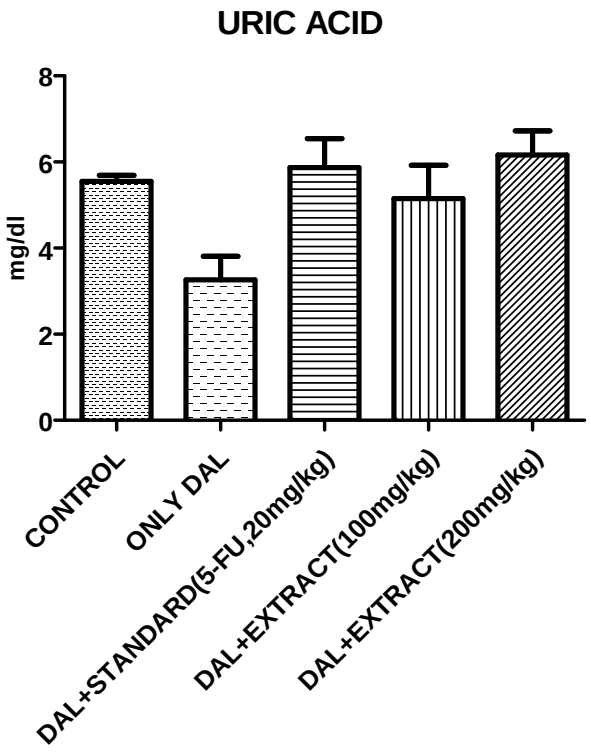


Figure- 22

5). BILURUBIN

Serum BILURUBIN values

Table- 36

Experiment group	NORMAL	ONLY DAL	DAL+5-FU, (20mg/kg)	DAL + Extract (100mg/kg)	DAL + Extract (200mg/kg)
BILURUBIN	0.4500 ±0.06708	0.1000 ±0.02582**	0.4167 ±0.06009 ns	0.2167 ±0.08724 ns	0.3000 ±0.08563 ns

Values are expressed as the mean ± S.E.M., (n = 6) on day 14 of the experiment P<0.0081, between tumor control and treated group.

ns-non significant.

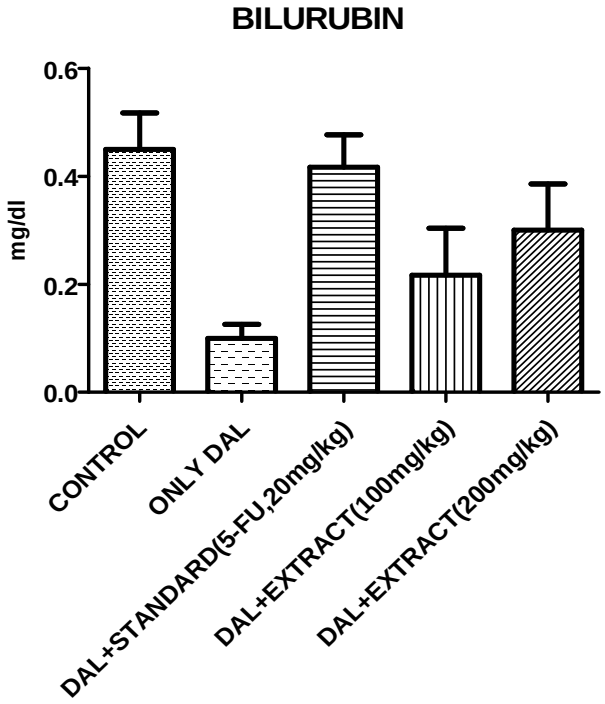


Figure- 23

6). TRIGLYCERIDES (TGL):

Serum TGL levels

Table- 37

Experiment group	NORMAL	ONLY DAL	DAL+5-FU, (20mg/kg)	DAL + Extract (100mg/kg)	DAL +Extract (200mg/kg)
TGL(mg/dl)	153.4 ±22.93	177.6 ±15.67^{ns}	48.05 ±4.622 **	144.4 ±31.30 ^{ns}	100.6 ±11.18^{ns}

Values are expressed as the mean ± S.E.M., (n = 6) on day 14 of the experiment, P<0.0007, between tumor control and treated group.

ns-non significant.

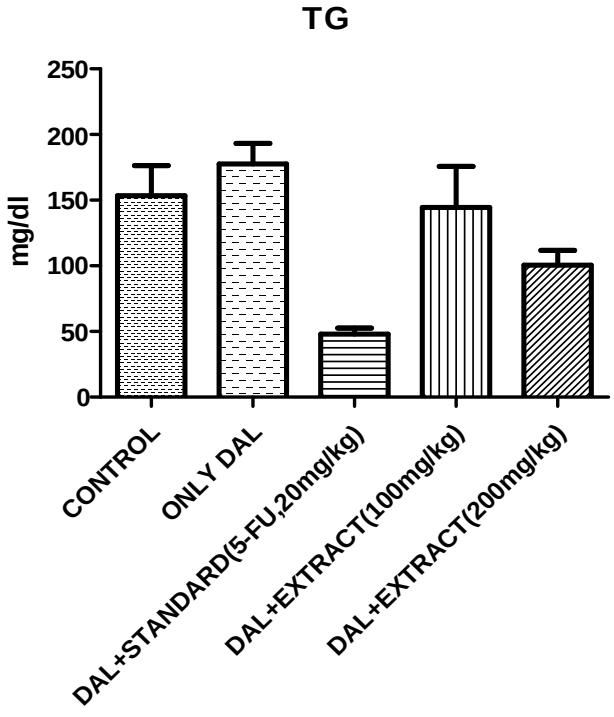


Figure- 24

7). CREATININE (CR)

Serum CR levels

Table- 38

Experiment group	NORMAL	ONLY DAL	DAL+5-FU, (20mg/kg)	DAL+ Extract (100mg/kg)	DAL+ Extract (200mg/kg)
CR(mg/dl)	0.3667 ±0.03333	0.4167 ±0.06009 ns	0.2167 ±0.04014 ns	0.3167 ±0.03073 ns	0.2000 ±0.03651 *

Values are expressed as the mean ± S.E.M., (n = 6) on day 14 of the experiment P<0.0038, between tumor control and treated group.

ns-non significant.

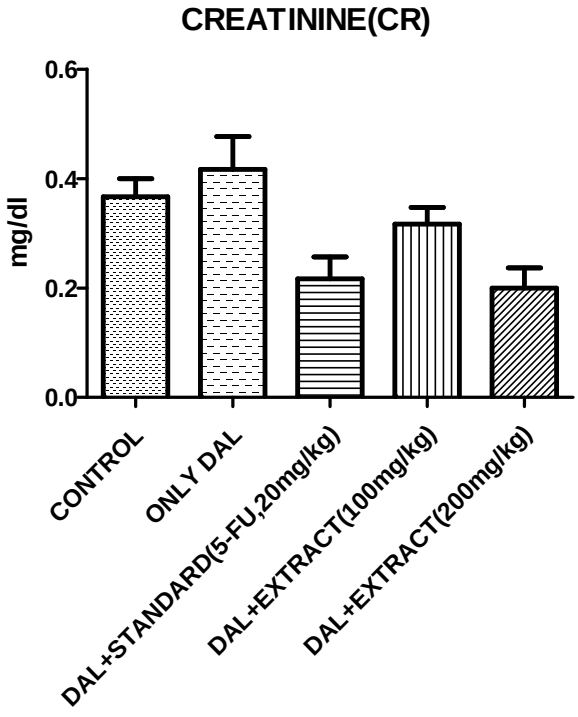


Figure- 25

8). TOTAL CHOLESTROL

Serum CHOLESTROL values

Table- 39

Experiment group	NORMAL	ONLY DAL	DAL+5-FU, (20mg/kg)	DAL +Extract (100mg/kg)	DAL +Extract (200mg/kg)
TOTAL CHOLESTROL	43.40 ±3.144	47.57 ±5.0 ^{ns}	25.57 ±0.5714 *	47.62 ±6.060 ^{ns}	41.25 ±2.043 ^{ns}

Values are expressed as the mean ± S.E.M., (n = 6) on day 14 of the experiment P<0.0027, between tumor control and treated group.

ns-non significant.

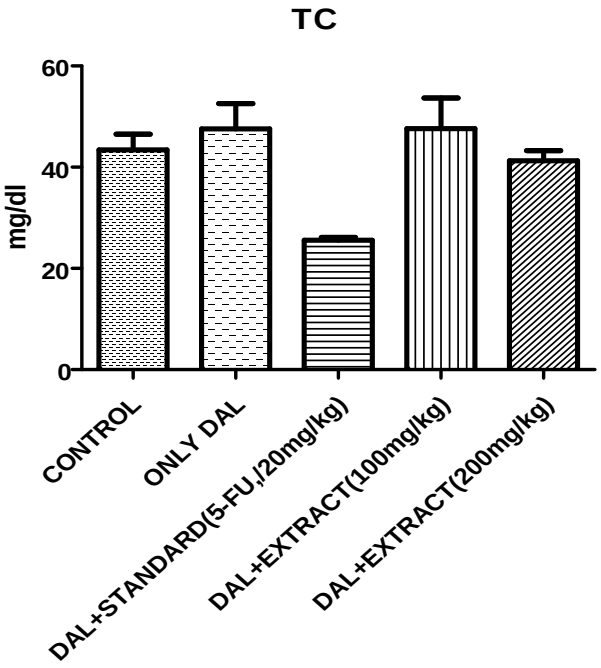


Figure- 26

6.7.3. IN-VIVO ANTI OXIDANT STUDIES:

A. ENZYMATIC ANTI-OXIDANT ACTIVITY

1). Total Protein (TP):

Table- 40 Serum TP level

Experiment group	NORMAL	ONLY DAL	DAL+5-FU, (20mg/kg)	DAL + Extract (100mg/kg)	DAL + Extract (200mg/kg)
TP(g/dl)	0.5260 ±0.00954	0.5190 ±0.0316 ns	0.5990 ±0.00517*	0.5800 ±0.00546 ns	0.5960 ±0.0122 *

Values are expressed as the mean ± S.E.M., (n = 6) on day 14 of the experiment P<0.0021, between tumor control and treated group.

ns-non significant.

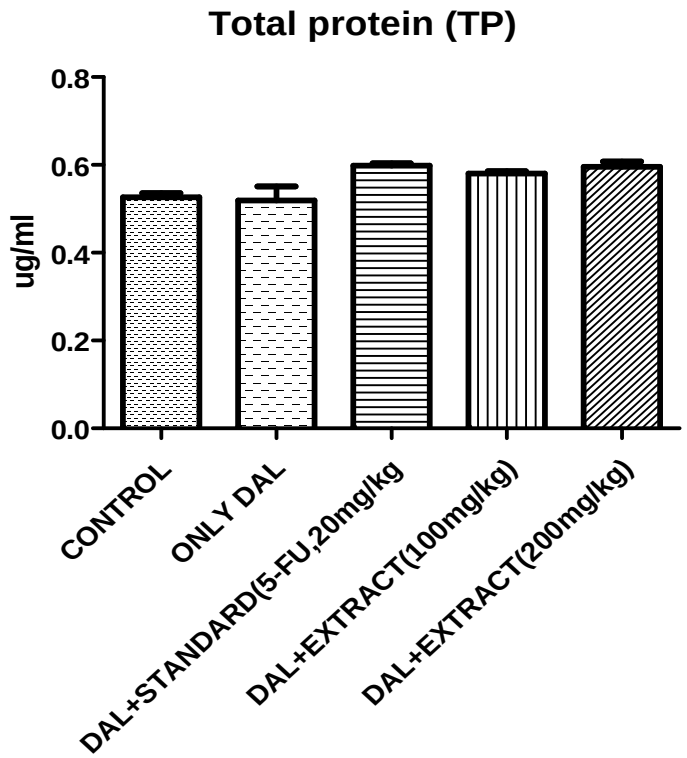


Figure- 27

2). ESTIMATION OF SUPEROXIDE DISMUTASE (SOD) ACTIVITY:

SOD levels of liver

Table- 41

Experiment group	NORMAL	ONLY DAL	DAL+ 5-FU, 20mg/kg	DAL +Extract (100mg/kg)	DAL +Extract (200mg/kg)
Liver	0.2060 ±0.00530	0.1520 ±0.00478***	0.1940 ±0.00387ns	0.1690 ±0.00699***	0.1890 ±0.00490ns

Values are expressed as the mean ± S.E.M., (n = 6) on day 14 of the experiment P<0.0001, between tumor control and treated group.

ns-non significant.

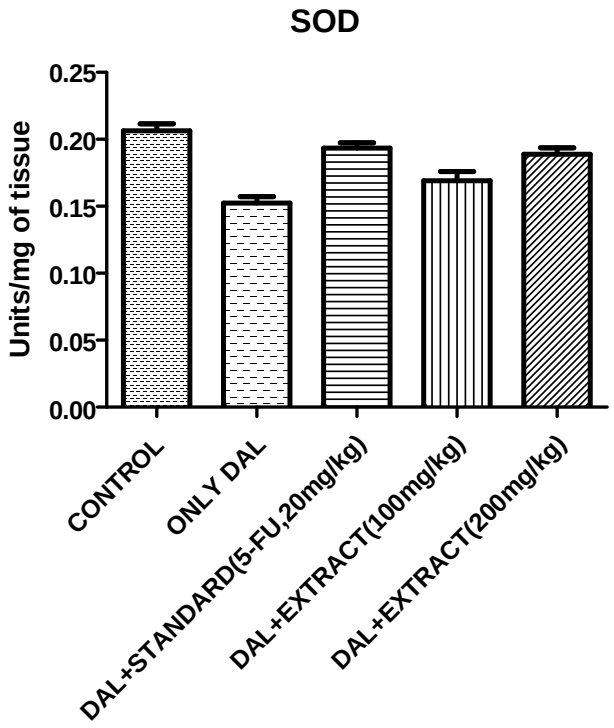


Figure- 28

3). CATALASE

Catalase levels of levels of liver

Table- 42

Experiment group	NORMAL	ONLY DAL	DAL+5-FU, 20mg/kg	DAL +Extract (100mg/kg)	DAL +Extract (200mg/kg)
Liver	0.8280 ±0.00909	0.7310 ±0.0195**	0.8400 ±0.0111 ns	0.7780 ±0.0201 ns	0.8380 ±0.0265ns

Values are expressed as the mean ± S.E.M., (n = 6) on day 14 of the experiment, P<0.0009, between tumor control and treated groups and ns-non significant.

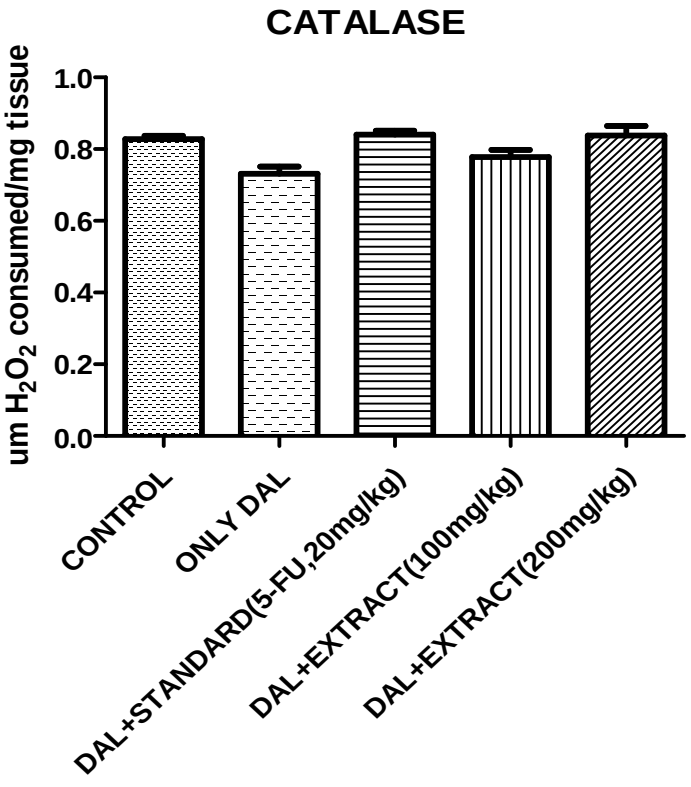


Figure- 29

4). ESTIMATION OF GLUTATHIONE PEROXIDASE (GPx) ACTIVITY:

GPx levels of liver

Table- 43

Experiment group	NORMAL	ONLY DAL	DAL+5-FU, (20mg/kg)	DAL +Extract (100mg/kg)	DAL +Extract (200mg/kg)
Liver	0.320 ±0.00480	0.250 ±0.0219*	0.299 ±0.00967 ns	0.262 ±0.0176 *	0.277 ±0.0142 ns

Values are expressed as the mean ± S.E.M., (n = 6) on day 14 of the experiment P<0.0194, between tumor control and treated group.

ns-non significant.

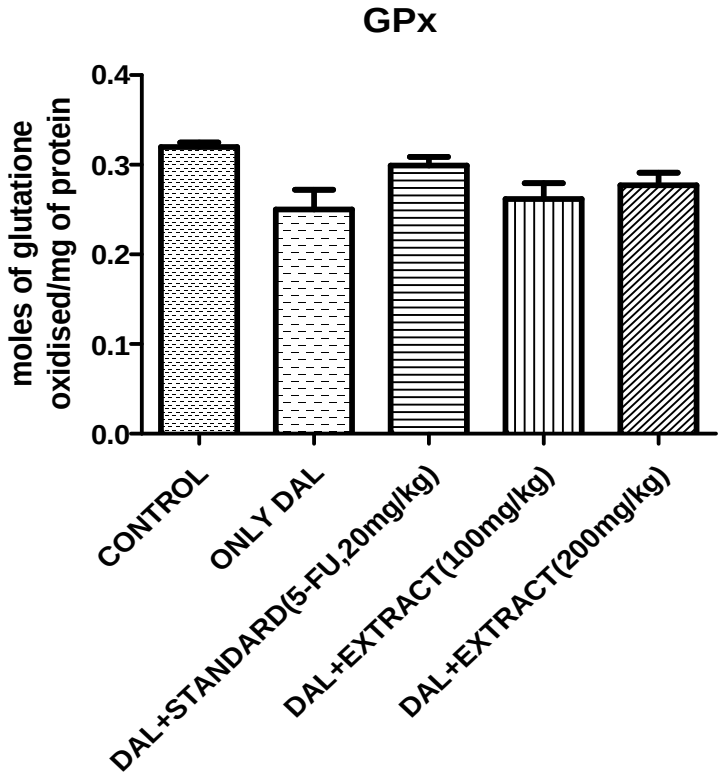


Figure- 30

B. NON-ENZYMATIC ANTIOXIDANT ACTIVITY

1). Estimation of reduced glutathione (GSH) activity:

GSH levels of liver

Table- 44

Experiment group	NORMAL	ONLY DAL	DAL+5-FU, (20mg/kg)	DAL +Extract (100mg/kg)	DAL + Extract (200mg/kg)
Liver	0.422 ±0.00408	0.273 ±0.0264 ***	0.393 ±0.0363 ns	0.328 ±0.0119 *	0.344 ±0.0298 ns

Values are expressed as the mean ± S.E.M., (n = 6) on day 14 of the experiment P<0.0026, between tumor control and treated group.

ns-non significant.

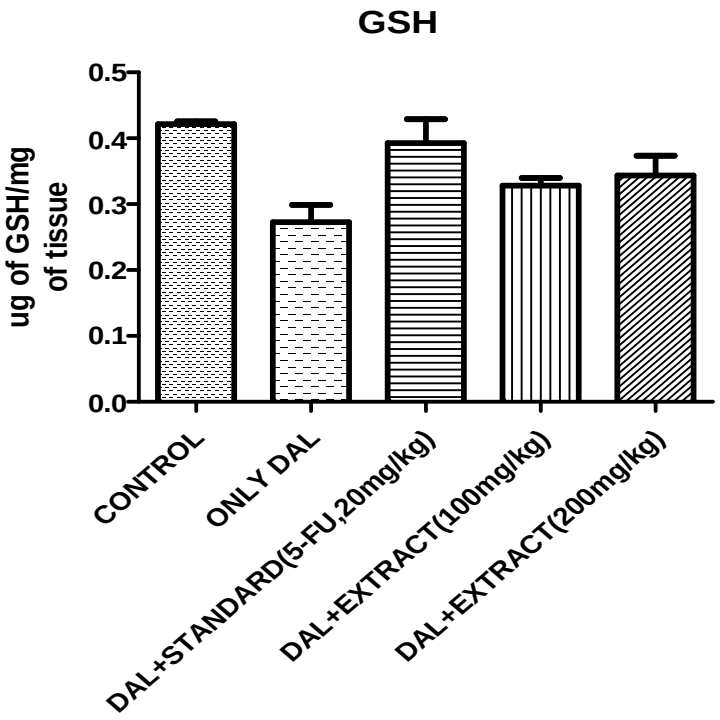


Figure- 31

2). ESTIMATION OF LIPID PEROXIDATION (LPO) ACTIVITY:

LPO levels in liver

Table- 45

Experiment group	NORMAL	ONLY DAL	DAL+5-FU, (20mg/kg)	DAL +Extract (100mg/kg)	DAL+ Extract (200mg/kg)
Liver	0.8020 ±0.02839	0.8469 ±0.05823 ns	0.5270 ±0.04364 ***	0.6526 ±0.03949 ns	0.5719 ±0.03959 **

Values are expressed as the mean ± S.E.M., (n = 6) on day 14 of the experiment P<0.0001, between tumor control and treated group.

ns-non significant.

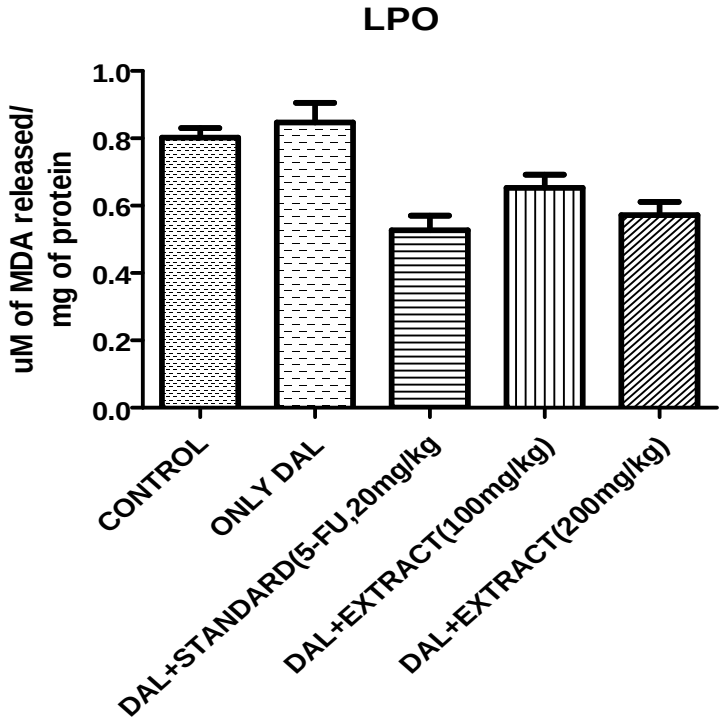
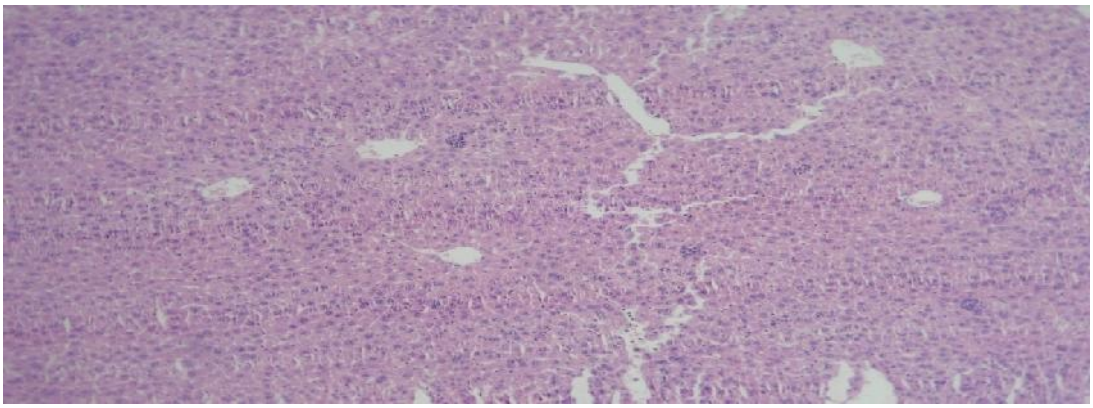
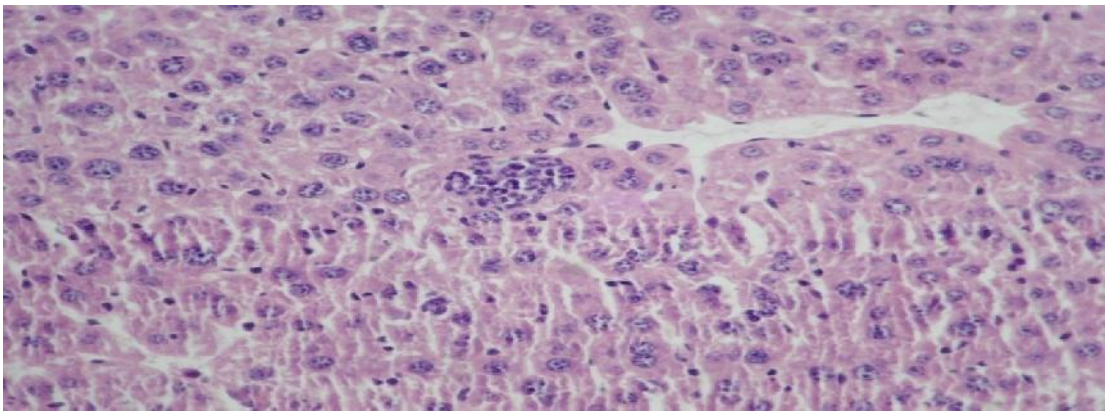


Figure- 32

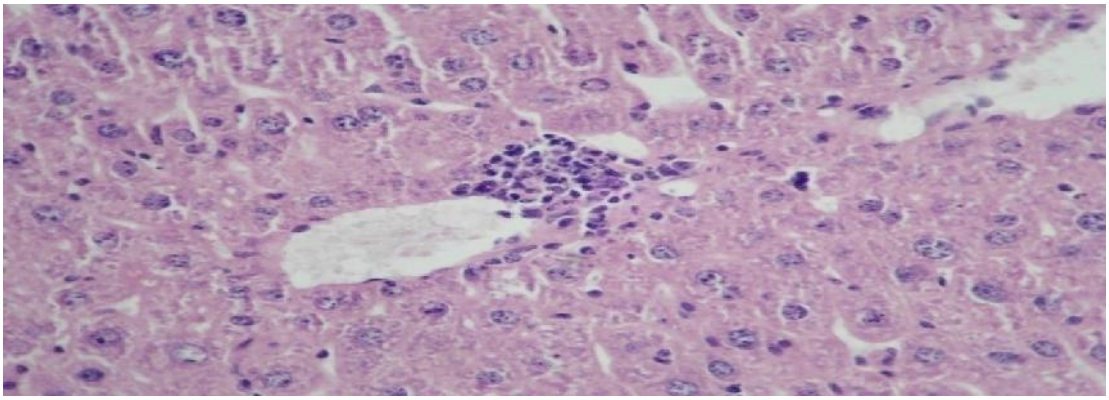
6.8. HISTOLOGY OF LIVER STAINED HAEMOTOXYLIN-EOSIN (×100) OF MICE FROM THE DIFFERENT GROUPS



[A]



[B]



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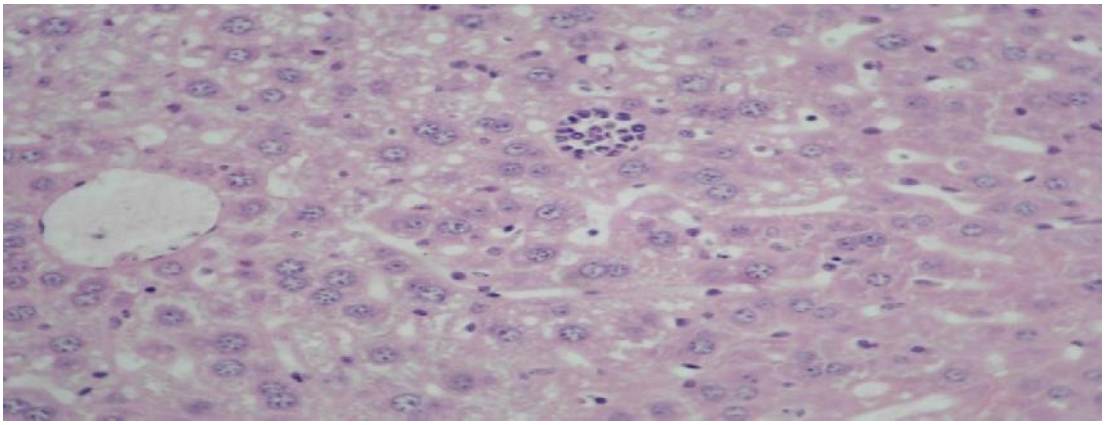
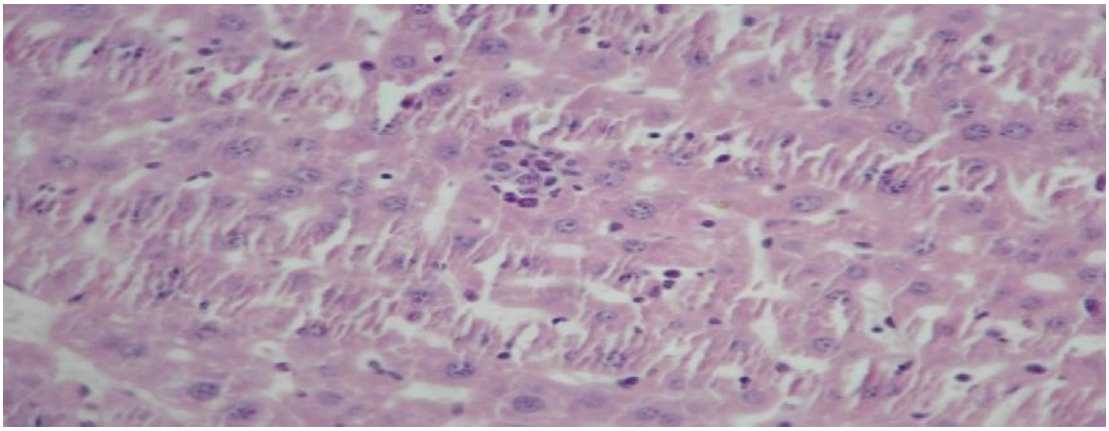


[D]



[E]

Figure- 33, [**A]**- shows normal liver, [**B]**- shows only DAL, [**C]**- shows DAL+ Standard (5-FU, 20mg/kg), [**D]**- shows DAL+ Extract (100mg/kg), [**E]**- shows DAL+ Extract (200mg/kg)

DISCUSSION

The **preliminary phytochemical studies** of ethanolic extract of *Wattakaka volubilis* (L.) indicated the presence of flavonoids, glycosides, phenols and triterpenoids. The observed antitumour, and antioxidant activities may be due to the presence of any of these compounds in *Wattakaka volubilis.*

Bioactive antioxidant levels:

Flavonoids are a group of effective antioxidants which are present abundantly throughout the plant kingdom. Flavonoid and related compound are effective in scavenging DPPH radical, hydroxyl radical etc and also have metal-chelating capacity Flavonoids are found to exhibit numerous biological activities like vasodilaton, anticarcinogenic, anti-inflammatory, antibacterial, immune-stimulating, antiallergic, and antiviral effects.

Phytosterols (PS) or plant sterols were structurally similar to cholesterol. The most common PS are β-sitosterol, campesterol and stigmasterol. The PS acts on membrane structure and function of tumor and host tissue, and also on signal transduction pathways. They regulate tumor growth and apoptosis, immune function of the host and cholesterol metabolism of the host.

Free radicals may be defined as any species that are capable of independent existence and possessing one or more unpaired electrons. These radicals can react with other molecules in different ways. The net effect is that radical donates its unpaired electron to another molecule and the other molecule then becomes a radical.

In-vitro antioxidants:

The **DPPH** assay method is based on the reduction of DPPH, a stable free radical. The free radical DPPH with an odd electron gives a maximum absorption at 517 nm (purple colour). As the odd electron of the radical becomes paired off in the presence of a hydrogen donor. e.g., a free radical scavenging antioxidant, the absorption strength is decreased and results in decolorization (yellow colour) with

respect to the number of electrons captured (**Blois, 1958**). More the decolorization more is the reducing ability. This test has been the most accepted model for evaluating the free radical scavenging activity of any new drug. The percent DPPH scavenging activities of *Wattakaka volubilis* extract was summarized in **(Table – 17)**

The decolorization of **ABTS** cation radical is an unambiguous way to measure the antioxidant activity of phenolic compounds. Thus the ability of a compound to scavenge ABTS radical can demonstrate oxygen radical absorbance capacity. Ethanol extracts of *Wattakaka volubilis* showed a potent ABTS radical scavenging activity. **(Table 19).** The absorbance was found to be increased with the increase in concentration.

FRAP assay measures the reducing ability of antioxidants against oxidative effects of reactive oxygen species. Electron donating anti-oxidants can be described as reductant or reducing agent and inactivation of oxidants by reductant can be described as redox reactions. In the current study ethanolic extracts of *Wattakaka volubilis* exhibited a significant scavenging activity in ferrous sulphate equivalents.

TRAP is the total radical trapping antioxidant parameter. The ethanolic extract of *Wattakaka volubilis* showed free radical scavenging activity which was found out by the absorbance of the sample at different concentrations**. (Table 20).** The max absorbace was observed in 100 µg/ml of extract.

NOS and SOS activity of ethanolic extract of *Wattakaka volubilis* were checked which showed a potent activity. The super oxide scavenging was found to increase with concentration of extract **(Table 21)** and max absorbance was found at 100 µg/ml of extract. The nitric oxide scavenging activity was also observed and the maximum absorbance was observed at 100 µg/ml of extract.

DAL-induced ascitic anticancer studies

The reliable criteria for judging the value of an anticancer drug is the prolongation of the life span of animals. In DAL tumor bearing mice, a regular rapid increase in ascitic tumor volume was observed. The DAL bearing mice co-administered with ethanolic extract of *Wattakaka volubilis* at 100 and 200 mg/kg body weight showed significant increment in the average life span of animals compared to that of the tumor control group. However, the percentage increase in body weight, tumor cell volume, and number of viable tumor cells were found to be significantly lesser than the tumor control animals, indicating the anticancer effect of the extract. (**Badami *et al.*, 2003**) These results could indicate either a direct cytotoxic effect of *Wattakaka volubilis* on tumor cells or an indirect local effect, which may involve macrophage activation and vascular permeability inhibition. Hence, the observed anticancer activity of *Wattakaka volubilis* leaves may be due to the cytotoxic properties.

The mean survival time of extract 100mg/kg and 200mg/kg were found to be significant to that of standard drug. The mean survival time of 200mg/kg was found to be more significant to that of standard drug**.** The % decrease in body weight was also observed for 14 days and was found to be significant in extract treated group with respect to standard drug; wherein 100mg/kg showed moderately significant and 200mg/kg showed highly significant to that of standard drug treated group..

The tumour volume was measured after 14days of treatment. In both of extract treated group, the tumour volume was lesser than that of the DAL control group. The tumour volume of 200mg/kg extract treated group was more significant to that of standard as compare to 100mg/kg extract treated group**.** The non viable cell count was also observed from the peritoneal volume and compared with that of standard. The 200mg/kg group showed moderate effect compared to that of standard and on the other hand the 100mg/kg extract treated group was less significant compared to standard.

Haematological parameters

In cancer chemotherapy the major problems are of myelosuppression and anaemia (**Price, Greenfield, 1958), (Hogland HC, 1982,**)

Anaemia encountered in tumour bearing mice is mainly due to reduction in RBC and Hb% and this may occur either due to iron deficiency or due to hemolytic or myelopathic conditions. However, the elevation of WBC levels may be due to its adverse effect on the haemopoietic system. (**Fenninger, Mider, 1954**)

The MCH, MCV, MCHC and Hematocrit values also gets affected based on the RBC count and Hb values.

In cancer condition the RBC, Hb and all other blood related parameters (MCH, MCV, MCHC and Hematocrit) gets decreased leading to anaemia and the WBC, platelet factors and other WBC related parameters gets increased which shows that the immune system of the animal is affected, and also shows the presence of infections in the body.

The RBC and all blood related parameters got decreased in all tumour control groups signifying the presence of tumour cells. The WBC and related levels were found increased in all the groups. The extract group 200mg/kg showed significant reversal in the parameters signifying the activity of extract and significant values compared with that of control. The values were almost equal to that of control group. The standard drug and extract 100mg/kg also showed significant activity in reversal of the parameter but moderately less significant than 200mg/kg group.

Biochemical parameters

The inoculation of DAL cells caused significant increases in the levels of TGL, TP, TC, ALP, and there is no significant change in the levels of ASAT and ALAT in the serum of tumor control group as compared to the normal group. The urea, uric acid and bilurubin levels found to be decreased in group than the normal levels.

The treatment with ethanolic extract of *Wattakaka volubilis* at 100 and 200 mg/kg body weight reversed these changes towards the normal levels. Most of the values were found to be significant. The results with standard drug treated group gave almost similar to that of the extract treated groups. (**Moss, Butterworth, 1974**).

Serum SGOT, ALP levels were found to be less for treated group 200mg/kg than that of the control and only DAL groups and also the standard drug. The urea, uric acid and TG levels of standard drug was found to be less than that of control and only DAL groups. The 100mg/kg showed moderately significant activity compared to that of standard drug group.

The bilurubin and TC values of the only DAL groups was very much less than that of the control group. The extract 200mg/kg group showed significant activity and the values almost similar to that of the normal groups**.**

The creatinine levels of the only DAL groups got increased and the extract 100mg/kg group showed significant values that nearly coinciding with that of the normal groups.

In-vivo Antioxidant studies

The antioxidant nature of *Wattakaka volubilis* was also evaluated by the in-vitro antioxidant studies. Plants with high total phenol content are known to possess strong antioxidant properties. The observed antioxidant activity may be due to the high phenolic, or flavanoid or may be due to phytosterol content of the extract.

It was observed that tumor cells produced more peroxides; when they proliferate actively after inoculation of tumor. This rise in peroxides indicated the occurrence of intensification of oxygen free radical production. Cells which are equipped with enzymatic antioxidant mechanisms play an important role in the elimination of free radicals.(**Lee*, et al.*, 2004**)

Lipid peroxidation mediated by free radicals considered being a primary mechanism of cell membrane destruction and cell damage. The oxidation of unsaturated fatty acids in biological membrane leads to reduction in membrane fluidity and disruption of membrane structure and function. MDA, the end product of lipid peroxidation was also reported to be higher in carcinogenous tissue than the non-pathological tissues. Increase in levels of TBARS indicate enhanced lipid

peroxidation leading to tissue injury and failure of antioxidant defense mechanism to prevent the formation of excess free radicals.

Both the extract treated groups 100mg/kg & 200mg/kg shows a significantly decrease in the liver TBARS and the values were significant to that of the normal animals**.** The lipid peroxidation values of standard drug were much more less than that of normal group.

The active role of GSH against cellular lipid peroxidation has been well recognized and thereby reduces the glutathione (GSH) activity. GSH can act either to detoxify the reactive oxygen species such as H_2O_2 or reduce lipid peroxidation. The increased the levels of glutathione content indicates antioxidant activity.

The GSH values of the standard drug treated group and extract 100mg/kg treated group were almost equal and are nearly significant with that of the normal group. The GSH values of the extract treated group 200mg/kg were highly significant with that of the control groups. (**Navarro *et al*., 1997**)

SOD is a chain breaking antioxidant and is found in all aerobic organisms. It is a metaloprotein widely distributed in all cells and plays an important protective role against ROS-induced Oxidative damage. The free radical scavenging system catalase, which are present in all major organisms in body of animals and human beings and is especially concentrated in liver and erythrocytes . Both enzymes play an important role in the elimination of ROS derived from the redox process of xenobiotic in liver tissues. It was suggested that catalase and SOD are easily inactivated by lipid peroxidase or ROS. In correlation it has been reported that DAL bearing mice shows decreased levels of SOD activity and this may be due to loss of MN^{++} SOD activity in liver and inhibition of catalase activity in tumor cell lines also reported.

In this study, catalase and SOD were significantly elevated by administration of extract 200mg/ kg which was almost significant with that of the control group. (**Kavitha, Manoharan, 2006), (Valenzuela, 1990**)

The TP level of only DAL group should be increased indicating the damage of the liver cells. The extract group 200mg/kg values were significant with that of the normal group**.**

Histopathological analysis

Histopathological observation of liver under a light microscope was done to observe the effect of ethanolic extract of *Wattakaka volubilis* on the anatomical integrity of the cells.

The liver of normal animal showed normal histological appearance and mild sinusoidal vein dilation, congestion with mild periportal and lobular inflammation.

The only DAL group showed congestion and dilatation of central vein and sinusoids, few portal tracts showing peroportal inflammation and foci show lobular necrosis with neutophils.

The animals treated with standard 5-FU at 20 mg/kg I.P shows hepatic tissue with mild sinusoidal and central vein dilatation and congestion with mild periportal and lobular inflammation.

The animals treated with ethanolic extract of *Wattakaka volubilis* at 100mg/kg and 200mg/kg body weight showed almost similar histological appearance of liver cells, except for a few lymphocytes and neutrophils in a particular laminated structure and were found to be quiet better than that of only DAL bearing group.

7. CONCLUSION

The present pre-clinical investigation was carried out to evaluate the anticancer activity of ethanolic extract of Wattakaka volubilis leaves in DAL induced lymphoma in mice with it's antioxidant effect.

It was found that there was significant increase in the life span with decreased tumour volume in all the extract treated mice. The haematological and antioxidant status got improved in DAL with extract treated groups.. The effect was also found to be dose dependent. The biochemical and histopathological studies supported the anticancer and antioxidant properties of the extract.

The antioxidant nature of *Wattakaka volubilis* leaf extract was confirmed by in-vitro antioxidant studies which showed significant results at various concentrarions.

The results of present investigation proved that ethanolic extract of *Wattakaka volubilis* leaf was found to be effective in inhibiting cancer growth by *in-vivo* screening and also provide some insights in to the possible mechanism by which *Wattakaka volubilis* leaf extract brings about inhibition in growth of DAL cells in mice. Thus these observations indicate that the plant leaf was having antioxidant property and also a protective effect against Dalton's ascites lymphoma.

The present studies also suggest the herb is cost effective and alternative in treatment of tumour and related disorders.

Further studies were essential to elucidate and evaluate both it's molecular mechanism of action and potential usefulness of Wattakaka volubilis as a alternative drug in cancer therapy.

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INTRODUCTION

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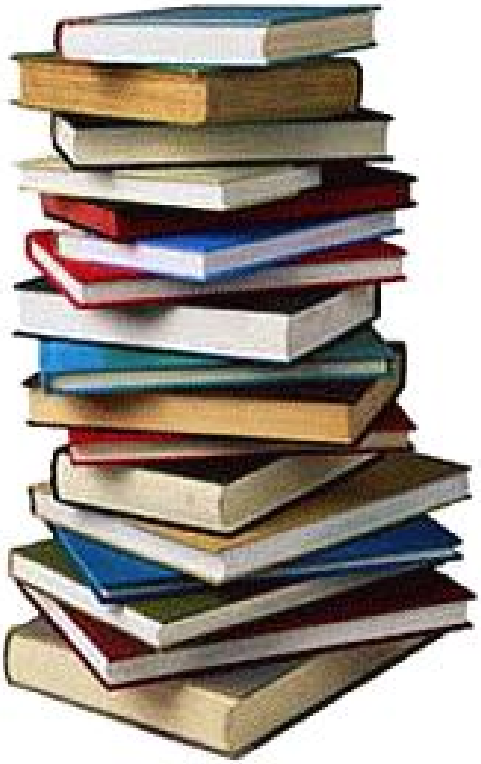

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