I. INTRODUCTION

1.1. HERBAL

Nature products like plant, animal and minerals serve humans to maintain health, to treat and heal many ailments. A medicinal plant plays an important role to maintain a good health for every individual. The therapeutic potential of medicinal plants plays a significant role in health system for both animal and human all over the world. Herbal medicine is a triumph of popular therapeutic diversity. The world is now moving towards the herbal medicine to fight against the invading pathogens with fewer side effects. In 1970’s WHO encouraged the effective utilization of herbal an interest has shown in documenting the use of medicinal plants used by tribal from different parts of the world. We all are aware that India is one of the richest source of medicinal plants with many species. Interest in medicinal plants has increased enormously over the last two decades. The use of modern isolation techniques and pharmacological test helps to invent new active principle respond to various pharmacological activities. So a thorough knowledge is important with macroscopic and histological characters and chromatographic techniques. The quest for finding medicines at cheaper cost for ameliorating diseases has generated great interest in the studies of traditional system of medicines in India. About 80% of the world’s population relies on herbal medicines, and governments of 3rd world countries, unable to sustain a complete coverage with western-type drugs, have encouraged the rational development of traditional treatments. Modern research establishments now exist in many countries e.g. India, Pakistan, Saudi Arabia, China, Japan etc and their work is largely devoted to assess the value of thousands of ethnic remedies along lines acceptable to current medical thinking. One example in the medicinal aromatic and poisonous plants, Research centre which operates in association with the Pharmacognosy Department and College of Pharmacy, King Saud University, Riyadh, where indigenous medicinal plants are catalogued, cultivated and examined for pharmacognostical characteristics. Specimens are screened for various pharmacological activities and selected species investigated more fully for constituents, tissue culture possibilities, biological action, toxicity and anticancer activity. In the current search
examination for new therapeutically effective drugs with anticancer drugs, anti-malarial drugs and anti-hepatotoxic compounds are given importance in the research activities.

Even today in India there are 600 million people who are not covered or protected by any modern systems of medicine. There are about 20 large industries, 200 medium scale industries and 1200 small scale industries involved in the manufacture of medicines derived from the traditional systems of medicines in India with a turnover of more than 150 crore. Hence herbal drugs are valuable as well as precious gift nature to mankind. The herbal drugs have been used throughout the world have received greater attention in recent times, because of its diversity of curing diseases, safety and well tolerated remedies compared to the conventional medicines. The ability of herbal medicines to affect the body system depend on the chemical constituents that it contains.

The demand for the herbal remedies is increasing and is estimated that production of herbal drugs may be around Rs.4000 crore in 200 A.D. There is 1650 herbal formulation in the Indian market containing 540 major plants in them. Plants are directly used as medicines by a majority of cultures around the world, for e.g. Chinese medicine and Indian medicine. Asia has abundant medicinal and aromatic plant species, well documented traditional knowledge, a long-standing practice of traditional medicine, and the potential for social and economic development of medicinal and aromatic plants (MAPs). Herbal medicines flourished in Europe in the 17th century, then showed a decline with the scientific revolution. European immigrants brought to North America their own herbal medicine traditions as well as acquiring many from Native Americans. Two-thirds of entries in the first edition of the United States Pharmacopoeia (USP) published I 1820 were botanical substances.

Though the herbal drugs from the traditional systems of medicines have over the years contributed very useful drugs, it is not yet accepted by modern systems of medicines since; they lack the so called “Scientific validity of their therapeutic value”. Hence it was aimed by us to bring to treasures of these traditional systems of medicines to the modern systems of medicines by providing the necessary scientific validity.
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A herbal drug consists of a definite parts of single plant or mixture of plants which may be further processed through crushing, drying, powdering, etc, or extracting the juice either through pressure or by means of water at room temperature or by the application of heat. The product that is obtained is a very complex mixture of components that is belonging to different chemical classes, combined to give an effect, which is delivered from the synergistic or antagonistic effects of individual component. So the drug to be evaluated is a complex mixture both chemically from the point of view of individual bioactivity combining to produce a particular effect. The kind of information that is needed before a drug can be adequately evaluated can be classified as:

1. Raw material details.
2. Finished products or extracts.
3. Chemical identity and quality control.
4. Toxicity.
5. Therapeutic efficacy.

All parts of the plant have some use it can be the root, stem, leaf, stamens and pistils. In the neem plant the root and stem are used for fever, leaves for fever, flower for malaria, and oil from seeds to destroy lice. There are also many plants used for the same conditions. eg: Kalanelli, Karisalanganni, Tulasi, Ginger, Pepper, Periyanagai. According to the all India coordinated project on ethno-biology about 7500 wild plants are used for medicinal purpose by Tribes and 950 are found to be new claims and are worthy of scientific scrutiny. Most of these resources are obtained from the forest, markets, gardens and kitchens. Tribal communities not know the usefulness of the resources but also conserve them by suitable management tools. They take care not to pluck the premature plant or do not harvest off season or do not harvest if there is sparse growth of the plant species. The purely tribes restrict premature tapping of gum and resins from young plants. Indian people had an incredible knowledge of phytomedicine driven apparently by a tremendous passion for the study of medicinal plants. Indians obviously care for medicinal plants because they know much about them and have done much work on their applications. When there is imbalance in these humors they bring about diseases. Medicinal plants are used as raw materials for extraction of active constituents in pure form eg. Alkaloids like Quinine and Quinidine from Cinchona bark, Emetine from Ipecac.

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root, Glycosides from Digitalis leaves, Sennosides from Senna leaves, has precursors for synthetic vitamins or steroids and as preparation for herbal and indigenous medicines. Products such as ginseng, valerian and Liquorice roots are parts of the herbnals and health food market, as well as the food flavors, fragrance and cosmetic industries. By the classical system we refer to the codified systems such Ayurveda, Siddha, and Unani traditions homeopathy Chinese medicine, Aroma therapy, Naturopathy (yoga), Allopathy. They are characterized by institutionally trained practitioners. Medicine is not merely a science but an art as well. The science of medicine is of fundamental importance to man well being and survival. The three elements such as air, heat and water are known as humors. The allopathic system of medicine has adopted a number of plant derived drugs which form an important segment of the modern pharmacopoeia. Some important chemical intermediates needed for manufacturing the modern drugs are also obtained from plants. eg: Diosgenin, Solasodine, β-ionone\textsuperscript{14}, \textsuperscript{15}.

Our aim is to isolate the bioactive compound from a herbal compound. If no bioactive ingredient is identified, then we isolate the markens (chemical constituents’ which may not be bioactive but present in the plants). By this method even an inactive mark can help to give a lead regarding the concentration of related compounds. In another plant with similar ingredients the mark can be ignored and we can concentrate of the bioactive compound. The aim of the herbal plants is to standardize and formulate them. In India around 25000 effective plant based formulations are use in traditional and folk medicine. In the present scenario, the demand for herbal products is growing exponentially throughout the world and major pharmaceutical companies are currently conducting extensive research on lands materials for their potential medicinal value. Although the therapeutic effects of many herbs has not been scientifically proven, research continues to identify the active ingredients that may one day form the basis of drugs to fight diseases like cancer, aids, Diabetes mellitus, asthma, physiological disorders and many more chronic diseases\textsuperscript{16}. Herbal preparations alone or in combination with oral hypoglycemic agents sometimes produce a good therapeutic response\textsuperscript{17}.

After the scientific revolution which leads to development of the pharmaceutical industry, the synthetic drugs dominated. A number of medicinal plants, traditionally used
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for over 1000 years named Rasayana are present in herbal preparations of Indian traditional health care systems\(^9\). The practices continue today because of its biomedical benefits as well as place in cultural beliefs in many parts of world and have made a great contribution towards maintaining human health. These herbs and other compounds are used in varying proportions to remedy specific ills, and may be applied internally as infusions, topically as ointments, inhaled as smoke or pressed to the body as powders\(^9\).

Tribal healers in most of the countries, where ethno medical treatment is frequently used to treat cut wounds, skin infection, swelling, aging, mental illness, cancer, asthma, diabetes, jaundice, scabies, eczema, venereal diseases, snake bite and gastric ulcer, provide instructions to local people as how to prepare medicine from herbal. They keep no records the information is mainly passed on verbally from generation to generation. In order to make sure the safe use of these medicines, a necessary first step is the establishment of standards of quality, safety and efficacy. National Center for complementary and alternative medicine (NCCAM) has prioritized evaluating mechanisms, efficacy, and safety of botanical medicines through basic science studies, clinical research, and the establishment of dedicated botanical research centers. The first step towards this goal is the biological and phytochemical screening of plants extracts from traditional preparations used in popular medicine\(^9\). These herbal medicines derived from various plant extracts are being increasingly utilized to treat a wide variety of clinical diseases\(^9\). Addition, herbal medicines have a good potential in the emerging nutraceutical industry as these materials are often considered food, as well as medicine.

Some of the companies in India supplying herbal products, Dabur India Ltd. (India’s Largest Ayurvedic Medicine Supplier), Zandu Pharmaceutical Works, the Himalaya Drug Company, Charal Pharmaceutical, Vicco Laboratories, The Emami Group. Major requirements for establishing medicinal and aromatic plant based industries in developing countries are availability of natural forest resources capable of being sustainably harvested, initiation of systematic cultivation programmers, transfer of expertise on agronomical practices, harvesting and post harvest treatment. Training in methods of processing and quality control, actual processing with assistance from experts and NGO’s and international agencies, packaging and storage of finished products, marketing outlets.
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1.2. CANCER

Uncontrolled multiplication of the body’s own cells and spread within the body is Cancer or neoplasm. It is one of the major causes of death in the developed nations. There are hundred different types of cancers which can affect any part of the body\(^\text{22}\). Neoplasms are of two types, benign and malignant. The most important issue in cancer pathology is the distinction between benign and malignant tumors. A benign tumor, such as a common skin wart, remains confined to its original location, neither invading nor spreading to distant body sites. A malignant tumor however is capable of both invading surrounding tissue and spreading throughout the body via the circulatory or lymphatic systems. Adenomas are cancers that arise in the thyroid, the pituitary gland, the adrenal gland, and other glandular tissues. Cancers are often referred to by terms that contain a prefix related to the cell type in which the cancer originated and a suffix such as -sarcoma, carcinoma, or just -oma. Common prefixes include:

- **Adeno** - gland
- **Chondro** - cartilage
- **Erythro** - red blood cell
- **Hemangio** - blood vessels
- **Hepato** - liver
- **Lipo** - fat
- **Lympho** - white blood cell
- **Melano** - pigment cell
- **Myelo** - bone marrow

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- Myo - muscle
- Osteo - bone
- Uro - bladder
- Retino - eye

Tumors are further classified according to tissue of origin (e.g. lung or breast carcinomas) and the type of cell involved. eg. Fibro sarcomas arise from fibroblast. The four most common cancers are prostate, breast, lung and colon/rectum. Lung cancer, by far the most lethal, is responsible for nearly 30% of the cancer deaths.23

1.2.1 Causes of cancer

Substances that cause cancer, called as carcinogens, (e.g. the high incidence of lung cancer among cigarette smokers). Since the development of malignancy is a complex multi-step process, initiation and promotion. Many agents, including ionizing radiation, chemicals and viruses, have been found to induce cancer in both experimental animals and humans.

70 to 90% of all human cancers are attributed to environmental cause. Epidemiological studies identified specific chemical related tumors in certain occupations, i.e. radioactivity (lung cancer in uranium miners), copper and arsenic (Scrotal cancer in smelter workers), aromatic amines (bladder cancer in dye workers), asbestos (Pulmonary fibrosis and cancer in workers), and vinyl chloride (hepatic cancer in plastic industry worker).24 There are many different kinds of cancer. Cancer can develop in almost any organ or tissue, such as the lung, colon, breast, skin, bones, or nerve tissue.

There are many causes of cancer, including:

- Benzene and other chemicals
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- Drinking excess alcohol

- Environmental toxins, such as certain poisonous mushrooms and a type of poison that can grow on peanut plants (aflatoxins)

- Excessive sunlight exposure

- Genetic problems

- Obesity

- Radiation

- Viruses

Dietary factors play an important role in the prevention of cancers. Fruits and vegetables having flavonoids have been reported as cancer chemopreventive agents. Consumption of onions or apples are two major sources of the flavonol quercetin, is inversely associated with the incidence of cancer of the prostate, lung, stomach, and breast. In addition, moderate wine drinkers also seem to have a lower risk to develop cancer of the lung, endometrial, esophagus, stomach, and colon. The critical relationship of fruit and vegetable intake and cancer prevention has been thoroughly documented. It has been suggested that major public health benefits could be achieved by substantially increasing consumption of these foods. Several mechanisms have been proposed for the effect of flavonoids on the initiation and promotion stages of the carcinogenicity including influences on development and hormonal activities. Major molecular mechanisms of action of flavonoids are given as follows:

- Down regulation of mutant p53 protein
- Cell cycle arrest
- Tyrosine kinase inhibition
- Inhibition of heat shock proteins
- Estrogen receptor binding capacity
- Inhibition of expression of RAS proteins.
Mutations of p53 are among the most common genetic abnormalities in human cancers. The inhibition of expression of p53 may lead to arrest of the cancer cells in the G2-M phase of the cell cycle. Flavonoids are found to down regulate expression of mutant p53 protein to nearly undetectable levels in human breast cancer cell lines. Tyrosine kinase are a family of proteins located in or near the cell membrane involved in the transduction of growth factor signals to the nucleus. Their expression is thought to be involved in oncogenesis via an ability to override normal regulatory growth control. Drugs inhibiting tyrosine kinase activity are thought to be possible antitumor agents without the cytotoxic side effects seen with conventional chemotherapy. Quercetin was the first tyrosine kinase inhibiting compound tested in a human phase I trial. Heat shock proteins form a complex with mutant p53, which allows tumor cells to bypass normal mechanisms of cell cycle arrest. Flavonoids are known to inhibit production of heat shock proteins in several malignant cell lines, including breast cancer, leukemia, and colon cancer. Recently it has been shown that the flavanol epigallocatechin- 3-gallate inhibited fatty acid synthase (FAS) activity and lipogenesis in prostate cancer cells, an effect that is strongly associated with growth arrest and cell death.

1.3. DIABETES

Diabetes is a chronic disorder of carbohydrate, fat and protein metabolism characterized by increased fasting and post prandial blood sugar levels. The global prevalence of diabetes is estimated to increase, from 4% in 1995 to 5.4% by the year 2025. WHO has predicted that the major burden will occur in developing countries. Studies conducted in India in the last decade have highlighted that not only is the prevalence of diabetes high but also that it is increasing rapidly in the urban population. It is estimated that there are approximately 33 million adults with diabetes in India. This number is likely to increase to 57.2 million by the year 2025.

Diabetes mellitus is a complex metabolic disorder resulting from either insulin insufficiency or insulin dysfunction. Type I diabetes (insulin dependent) is caused due to insulin insufficiency because of lack of functional beta cells. Patients suffering from this are therefore totally dependent on exogenous source of insulin while patients suffering
from Type II diabetes (insulin independent) are unable to respond to insulin and can be treated with dietary changes, exercise and medication. Type II diabetes is the more common form of diabetes constituting 90% of the diabetic population. Symptoms for both diabetic conditions may include:

(i) high levels of sugar in the blood

(ii) unusual thirst

(iii) frequent urination

(iv) extreme hunger and loss of weight

(v) blurred vision

(vi) nausea and vomiting

(vii) extreme weakness and tiredness

(viii) Irritability, mood changes etc.

Though pathophysiology of diabetes remains to be fully understood, experimental evidences suggest the involvement of free radicals in the pathogenesis of diabetes and more importantly in the development of diabetic complications. Free radicals are capable of damaging cellular molecules, DNA, proteins and lipids leading to altered cellular functions. Many recent studies reveal that antioxidants capable of neutralizing free radicals are effective in preventing experimentally induced diabetes in animal models as well as reducing the severity of diabetic complications.

For the development of diabetic complications, the abnormalities produced in lipids and proteins are the major etiologic factors. In diabetic patients, extra-cellular and long lived proteins, such as elastic, laminin, and collagen are the major targets of free radicals. These proteins are modified to form glycoprotein due to hyperglycemia. The modification of these proteins present in tissues such as lens, vascular wall and basement
membranes are associated with the development of complications of diabetes such as cataracts, microangiopathy, atherosclerosis and nephropathy\textsuperscript{33}. During diabetes, lipoproteins are oxidized by free radicals. There are also multiple abnormalities of lipoprotein metabolism in very low density lipoprotein (VLDL), low density lipoprotein (LDL), and high density lipoprotein (HDL) in diabetes. Apart from this, advanced glycation end products (AGEs) are formed by non-enzymatic glycosylation of proteins. AGEs tend to accumulate on long-lived molecules in tissues and generate abnormalities in cell and tissue functions\textsuperscript{34,35}. In addition, AGEs also contribute to increased vascular permeability in both micro and macro vascular structures by binding to specific macrophage receptors. This results in formation of free radicals and endothelial dysfunction. AGEs are also formed on nucleic acids and histones and may cause mutations and altered gene expression.

Glucose is the most important source of metabolic energy for the majority of cells, particularly for some cells (e.g., neurons and erythrocytes) which are almost totally dependent on it. The brain requires a fairly stable glycemia in order to function normally. Concentrations of less than about 30 mg/dl or greater than about 300 mg/dl can produce confusion, unconsciousness and convulsions. Glucose may also be produced from noncarbohydrate precursors, such as pyruvate, amino acids and glycerol, by gluconeogenesis during starvation and intense exercise.

Blood glucose levels are not constant they rise and fall depending on the body’s needs, regulated by hormones. The blood glucose level can rise for three reasons: diet, breakdown of glycogen, or through hepatic synthesis of glucose.

Glycemia is the concentration of glucose in the blood. It is usually expressed in milligram per deciliter [mg/dl] or expressed in millimol per deciliter [M.mol/dl]. Glycemia fluctuates physiologically within a narrow range. Excessively low levels [e.g. a fasting glycemia of 70 mg/dl or below] are classed as hypoglycemia. These may result from poor diet, or as a side effect of diabetes medication.

Excessively high levels [e.g.250 mg/dl or more] are classed as hyperglycemia and are a particular threat to diabetes.
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70-100 mg/dl: Normal

101-125 mg/dl: Impaired fasting glucose

126 mg/dl and above: Risk of diabetes

The pancreas has both endocrine and exocrine function. The endocrine tissue is grouped together in the islets of langerhans and consists of four different cell type each with its own function. Alpha cells produce glucagon, Beta cells produce insulin, delta cells produce somatostatin, and PP cells produce pancreatic polypeptide. Several hormones are involved in the regulation of glucose metabolism, such as insulin, glucagon [secreted by the pancreas], epinephrine (adrenaline secreted by the adrenal glands) glucocorticoids and steroid hormones (secreted by the gonads and adrenal glands)\(^\text{36}\).

1.3.1 Diabetes Complications

1.3.1.1 Heart disease: People with diabetes have heart and blood vessel disease. Diabetes carries an increased risk for heart attack, stroke, and complications related to poor circulation\(^\text{37}\).

1.3.1.2 Kidney Disease (Nephropathy)/ Kidney Transplantation: Diabetes can damage the kidneys, which not only can cause them to fail, but can also make them lose their ability to filter out waste products. This is called nephropathy.

1.3.1.3 Eye complication: Diabetes can cause eye problems and may lead to blindness. People with diabetes do have a higher risk of blindness.

1.3.1.4. Diabetic Neuropathy and Nerve Damage: One of the most common complications of diabetes is diabetic neuropathy. Neuropathy means damage to the nerves that run throughout the body, connecting the spinal cord to muscles, skin, blood vessels, and other organs.

1.3.1.5 Foot Complications: People with diabetes can develop many different foot problems. Foot problems most often happen when there is nerve damage in the feet or when blood flow is poor.
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1.3.1.6. Depression: Feeling down once in a while is normal. But some people feel a sadness that just won’t go away. Life seems hopeless. Feeling this way most of the day for two weeks or more is a sign of serious depression.

Alloxan is an oxygenated pyrimidine derivative betacytotoxin and is known to induce diabetes mellitus in a wide variety of animal species through the damage of pancreatic β-cells resulting in a decrease in endogenous insulin release, which paves the way for Diabetes mellitus\(^{38}\). Alloxan has shown to induce free radical production and cause tissue injury. The pancreas is especially susceptible to the action of alloxan induced free radical damage. Alloxan-administered rabbits become hyperglycemic in a short period of time, followed by hepatic glucose overproduction. Reactive oxygen species (ROS) are important mediators of β-cell death during the development of DM. High glucose has been postulated to generate ROS and nitrogen species in numerous cell types. Generation of superoxide by high glucose is well described and arises principally via the mitochondrial electron transport chain. Another source of glucose-induced oxidative stress is via the polyol pathway where glucose is reduced to sorbitol by aldose reductase in a process that consumes NADPH. This will impair the NADPH-dependent generation of glutathione, an essential cellular antioxidant. Pancreatic islets exhibit greater susceptibility to damage by ROS compared to other tissues as a result of lower antioxidant defenses. Alloxan-induced free radical-mediated diabetes, led to a strain of rabbit with an elevated systemic as well as pancreatic ROS dissipation\(^{39}\). High ambient glucose can promote apoptosis, causing potential cellular damage as a result of hyperglycemia in diabetes\(^{40}\). Alloxan is one of the usual substances used for the induction of diabetes mellitus apart from streptozocin\(^{41}\). Insulin deficiency leads to various metabolic alterations in the animals viz increased blood glucose, increased cholesterol\(^{42}\), increased levels of alkaline phosphate and transaminases\(^{43}\).

1.4. HEPATO PROTECTIVE

1.4.1 Liver disorder

The liver is the largest organ in the body weighing 1200-1500 g. It is a key organ in regulating homeostasis within the body. Liver diseases are associated with distortion of
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these metabolic functions\(^{44}\). Although viruses are the main cause of liver diseases, the liver lesions arising from xenobiotics, excessive drug therapy, environmental pollution and alcoholic intoxication are not uncommon\(^ {45}\). Liver damage is always associated with cellular necrosis, increase in tissue lipid peroxidation and depletion in the tissue GSH levels. In addition serum levels of many biochemical markers like SGOT\(^ {46}\), SGPT, triglycerides, cholesterol, bilirubin, alkaline phosphatase, are elevated\(^ {47}\).

Every year about 20,000 deaths are found due to liver disorders. Thus to maintain a healthy liver is a crucial factor for overall health and well being\(^ {48}\). Thus, liver diseases remain one of the serious health problems and its disorders are numerous with no effective remedies\(^ {49,50}\). There is no rational therapy available for treating liver disorders and management of liver diseases is still a challenge to the modern medicine\(^ {51,52}\).

In this context, a resurgence of interest in medicine from natural sources (mainly plant products) is seen and there is tremendous hope that drugs of plant origin will have significantly lesser side effects than that observed with synthetic drugs while having comparable efficacy.

Acetaminophen is an antipyretic and analgesic drug, which is activated and converted by cytochrome P450 enzyme to toxic metabolite N-acetyl-para-benzoquinoneimine (NAPQI) at high doses. The reactive metabolite can be conjugated with cellular glutathione (GSH) to cause extensive GSH depletion which leads to the cellular necrosis\(^ {53,54}\). GSH removes free radical species such as hydrogen peroxide, superoxide radicals and maintains membrane protein thioles. The GSH depletion in hepatic mitochondria is considered the most important mechanism in the paracetamol induced hepatotoxicity. Reduced GSH level was depleted in paracetamol treated group may be due to conjugation of GSH with NAPQI to form mercapturic acid. Paracetamol toxicity is due to the formation of toxic metabolites when a part of it is metabolized by cytochrome P-450. Introduction of cytochrome\(^ {55}\) or depletion of hepatic glutathione is a prerequisite for paracetamol induced hepatotoxicity\(^ {56,57}\).

Normally, AST and ALP are present in high concentrations in liver. Assessment of liver function can be made by estimating the activities of serum ALT, AST, ALP and

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Bilirubin which are enzymes originally present in higher concentration in cytoplasm. When there is hepatopathy, these enzymes leak into the blood stream in conformity with the extent of liver damage. Due to hepatocyte necrosis or abnormal membrane permeability, these enzymes are released from the cells and their levels in the blood increases. ALT is a sensitive indicator of acute liver damage and elevation of this enzyme in non hepatic diseases is unusual. ALT is more selectively a liver parenchymal enzyme than AST. Silymarin reduced the pyknosis of hepatocytes when compared to paracetamol treated control.

Bilirubin is one of the most useful clinical clues to the severity of necrosis and its accumulation is a measure of binding, conjugation and excretory capacity of hepatocyte. Decrease in serum bilirubin after treatment with extract in liver damage induced by paracetamol, indicated the effectiveness of the extract in normal functional status of the liver.

![Fig.1.1. Schematic Metabolism of Acetaminophen (Paracetamol)](image)

Conjugation

Glucuronide ← Acetaminophen → Sulphate

Moiety (Non-toxic) P450 Moiety (Non-toxic)

N-acetyl-p-benzo-quinone imine (NAPQI) (TOXIC)

NAC glutathione

Cysteine and mercapturic acid

Conjugates (non-toxic)

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A number of chemicals including various environmental toxicants and clinically useful drugs can cause severe cellular damages in different organs of our body through the metabolic activation to highly reactive substances such as free radicals. The hepatotoxicity of CCl₄ has been reported to be due to the formation of the highly reactive trichloro CCl₃ free radical, which alters functions of endoplasmic reticulum and causes peroxidative degradation of lipid membrane of the adipose tissue leads to loss of metabolic enzymes located in the intracellular structures.

CCl₄ is one of such extensively studied environmental toxicant which is widely used as hepatotoxic in the experimental studies. Up to the present time, the etiology and treatment of most liver diseases are not known. The liver is the commonest site affected during the toxic manifestation of many drugs. Toxicity in liver due to CCl₄ and other chemicals is attributed to the toxic metabolites formed, responsible for the initiation of CCl₄ dependent lipid peroxidation, the nature of which is not yet unambiguously determined. The most likely candidate is the tri-chloro methyl radical. In the liver, CCl₄ is bio-transformed by the cytochrome P450-dependent mono-oxygenase system to produce the tri-chloro methyl free radicals, which in turn covalently binds to cell membrane and organelles to elicit lipid peroxidation. It has been evident that several phytoconstituents have the ability to induce microsomal enzymes either by accelerating the excretion of CCl₄ or by inhibition of lipid peroxidation induced by CCl₄. Water is retained in the cytoplasm of hepatocytes leading to enlargement of liver cells, resulting in increased total liver mass and volume as observed in the present study. The flavonoids constituents possess free radical scavenging properties. Liver secretions from CCl₄ treated control animal showed moderate degree of free fatty changes, mild congestions, connective tissue, proliferation and cirrhosis.

Silymarin is a flavonoid having three structural components Silibinin, Silydianine, and Silychristine extracted from the seeds and fruit of milk thistle Silybum marianum (Compositae). Silymarin has been reported to stimulate enzymatic activity of DNA-dependent RNA polymerase 1 and subsequent biosynthesis of RNA and protein, resulting in DNA biosynthesis and cell proliferation leading to liver regeneration only in damaged livers. Silymarin increases proliferating hepatocytes in response to FB1 (Fumonisin B1,
a mycotoxin produced by *Fusarium verticillioides*) induced cell death without modulation of cell proliferation in normal livers. The pharmacological properties of Silymarin involve the regulation of cell membrane permeability and integrity, inhibition of leukotriene, ROS scavenging, suppression of NF-κB activity, depression of protein kinase, and collagen production. Silymarin has clinical applications in the treatment of cirrhosis, ischemic injury, and toxic hepatitis induced by various toxins such as acetaminophen, and toxic mushroom.64

1.5. WOUND HEALING

Wound healing is a dynamic process of tissues restoration and reestablishing the injured skin and underlying tissues. It involves a systemic progression of events i.e. inflammation, angiogenesis, proliferation and collagen synthesis for final healing.65 Inflammation, which constitutes a part of the acute response, results in a coordinate influx of neutrophils at the wound site. Inspite of tremendous advances in the pharmaceutical drug industry, the availability of drugs capable of stimulating the process of wound repair is still limited. The search for natural remedies for healing has drawn attention to herbals66.

In wound healing mechanism following the migration of platelets, the first response cells, neutrophils and macrophages migrate to the wound. Numerous enzymes and cytokinins are secreted by macrophages and neutrophils. Among these TNF-α is the one which stimulate the angiogenesis, helps to build up the tissue granulation bed and thus has significant potential to improve the healing process. Plants may exert their affect by modulating the cytokine(s) secretions during different conditions. TNF-α is a major cytokine secreted by macrophages and neutrophils during the inflammation phase.67 Many studies indicate that plant products which are potential agents for wound healing are largely preferred because of the absence of unwanted side effects and their effectiveness.

Flavonoids and Triterpenoids are known to promote the wound healing process mainly due to their astringent and antimicrobial property, which seems to be responsible for wound contraction and increased rate of epithelialisation68,69. Flavonoids are known to
reduce lipid peroxidation not only by preventing or slowing the onset of cell necrosis but also by improving vascularity. Hence any drug that inhibits lipid peroxidation is believed to increase the viability of collagen fibrosis by increasing the strength of collagen fibers, increasing the circulation\textsuperscript{70}, preventing the cell damage and by promoting the DNA synthesis. Tannins, Flavonoids, triterpenoids and sequalterpenes are also known to promote the wound healing process mainly due to their astringent and antimicrobial property, which seems to be responsible for wound contraction and increased rate of epithelialization\textsuperscript{71}. The sequalterpene lactones are known to possess antioxidant property, which may also contribute to the wound healing process. Drugs which influence one phase may not necessarily influence another. Hence different models are used to assess the effect of various phases, which run concurrently, but independent of each other. Control group wound showed granulation tissue and fibroblast aggregation. Wound healing comprises of different phases such as contraction, epithelialization, granulation and collagenation. It normally involves an initial inflammatory phase followed by fibroblast proliferation, formation of collagen fibers and shrinking, occurring concurrently but independent of one another\textsuperscript{72}.

TNF-α, a macrophage derived cytokine, is also known to play a major role in the inflammatory phase of wound healing by enhancing angiogenesis\textsuperscript{73}. During the early phase of wound repair, TNF-α was predominantly expressed in polymorpho nuclear leukocytes suggesting a normal function of these cells in the initiation of wound healing. TNF-α inhibits collagen formation and hydroxyproline production which are essential for the final part of proliferation phase in wound healing\textsuperscript{74}.

1.6. ULCER

An ulcer is a discontinuity or break in a bodily membrane that impedes the organ, which is necessary for continuing its normal functions. Ulcer is caused due to the continuously exposure of gastric mucosa to potentially injurious agents such as acid, pepsin, bile acids, food ingredients, bacterial products (Helicobacter pylori) and drugs. These agents have been implicated in the pathogenesis of gastric ulcer, including
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enhanced gastric acid and pepsin secretion, inhibition of prostaglandin synthesis and cell proliferation growth diminished gastric blood flow and gastric motility\textsuperscript{75}.

Other possible cause is psychological stress can influence the development of peptic ulcers. A study of peptic ulcer showed that chronic stress was strongly associated with an increased risk of peptic ulcer, and a combination of chronic combination of chronic stress and irregular meal times was a significant risk factor. Such factors could range from natural causes (gastric cancer), infections (\textit{H. pylori}), life style (drugs – non steroidal anti inflammatory agents, alcohol, stress and cigarette smoking)\textsuperscript{76}.

In addition, stress, diet, smoking, drinking and other factors can cause ulcers by triggering excess acid secretion in the stomach. The H\textsubscript{2} blockers and anti-acids are used to treat up to 90\% of all ulcers and are the top-selling drugs in the USA. Yet these drugs are not very effective. The relapse rate for ulcers with H\textsubscript{2} blockers and antacids is about 50\% over 6 months and as high as 95\% over 2 years\textsuperscript{77}.

Decrease in gastric mucosal damage can be observed with the antioxidants and synthetic drugs including proton pump inhibitors, prostaglandins analogs, histamine receptor antagonists and cytoprotective agents are available for the treatment of peptic ulcer. But most of these drugs produce several adverse reactions including toxicities and even may alter biochemical mechanisms of the body upon chronic usage\textsuperscript{78}.

The goals of treating peptic ulcer disease are to relieve pain, heal the ulcer and prevent ulcer recurrence. Drug treatment of peptic ulcers is targeted at either counteracting aggressive factors (acid, pepsin, active oxidants, platelet aggravating factor “PAF” leukotrienes, endothelins, bile or exogenous factors including NSAIDs) or stimulating the mucosal defenses (mucus bicarbonate, normal blood flow, prostaglandins (PG), nitric oxide)\textsuperscript{79}.

1.7. DIARRHEA

Diarrhea is a symptom marked by rapid and frequent passage of semisolid or liquid fecal material through the gastrointestinal tract and involves both an increase in the motility of the gastrointestinal tract along with increased secretions and a decrease in the

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Absorption of fluid and thus a loss of electrolytes particularly Na\textsuperscript{+} and water\textsuperscript{80}. Diarrhea is also called loose motions.

Diarrhea is considered to be present if one of the following applies

(a) Stool weight of greater than 200 g per day.

(b) More than 2 stools per day for more than 30 days.

(c) More than 3 stools per day for more than 7 days.

(d) More than 3 stools per day, looser than usual, for more than 3 days .

(e) More than 3 stools per day, with a change in frequency or consistency.

The main causes of diarrhea are overeating or eating of wrong foods, putrefaction of food in the intestinal tract, fermentation caused by incomplete carbohydrate digestion, nervous irritability, use of antibiotic drugs, and excessive intake of laxatives. Intestinal infection is the most common cause of diarrhea worldwide. Causes of diarrhea include infectious agents such as viruses (rotavirus, norovirus, herpes simplex virus, etc), parasites (Giardia lamblia, Entamoeba histolytica and Cryptosporidium parvum), bacteria (Campylobacter, Salmonella, Vibrio cholera, Shigella and enterotoxigenic Escherichia coli etc.) certain medications such as antibiotics, anti cancer drugs and antacids containing magnesium; plant and animal toxins; GIT (gastro-intestinal) disorders and substances that increase gastrointestinal tract secretions. It can also be caused by the ingestion of poorly absorbable materials, or inflammatory and dysmotility problems of the gastro-intestinal tract\textsuperscript{81}. Mucosities or inflammation of the mucous membranes lining the alimentary tract is a potential adverse effect of chemotherapeutic agents, and diarrhea is the symptomatic result of mucosities of the large bowel\textsuperscript{82}. For some chemotherapy regimens, like 5-fluorouracil (5-FU) and IFL [Irinotecan (CPT-11; Camptosar), 5-FU and Leucovorin], diarrhea has been linked to excess hospitalizations and death\textsuperscript{83}.

Diarrhea may be accompanied by cramping abdominal pain, bloating, nausea, or an urgent need to use the bathroom and a person may have fever or bloody stools. Diarrhea of any duration may cause dehydration, which means the body lacks enough
fluid and electrolytes, affects the muscle activity, and other important functions. Signs of dehydration in adults are thirst, fatigue, dry skin and tongue, dark colored urine, dizziness, sunken eyeballs, etc and in infants and young children these are high fever, absence of tears when crying, dry mouth and tongue, sunken eyes, checks or soft spot in the skull and no wet diaper for 3 hours or more.

Castor oil causes diarrhea due to its active metabolite, Ricinolic acid, which stimulates peristaltic activity in the small intestine, leading to changes in the electrolyte permeability of the intestinal mucosa. Its action also stimulates the release of endogenous prostaglandin. This condition induces an increase in the permeability of the mucosal cells and changes in electrolyte transport, which results in a hypersecretory response (decreasing Na$^+$ and K$^+$ absorption), stimulating peristaltic activity and diarrhea. Thus the castor oil induced diarrhea demonstrates secretory diarrhea, since recinolic acid induces diarrhea by a hypersecretory response. Magnesium sulfate similarly causes an increase in the electrolyte secretion by creating an osmotic imbalance. It has also been demonstrated that it promotes the liberation of cholecystokinin from the duodenal mucosa, which increases the secretion and motility of small intestine and thereby prevents the reabsorption of sodium chloride and water.

1.8. INFLAMMATION

Inflammation is a local response of living mammalian tissues to the injury. It is a body defense reaction in order to eliminate or limit the spread of injurious agents. There are various components to an inflammatory reaction that can contribute to the associated symptoms and tissue injury. Edema formation, leukocyte infiltration and granuloma formation represent such components of Inflammation. Edema formation in the paw is the result of a synergism between various inflammatory mediators that increase vascular permeability and/or the mediators that increase blood flow. Several experimental models of paw edema have been described. The Carrageenan –induced paw edema model in rats is known to be sensitive to cyclooxygenase inhibitors and has been used to evaluate the effect of non-steroid anti-inflammatory agents, which primarily inhibit the cyclooxygenase involved in prostaglandin synthesis.
Carrageenan-induced hind paw edema is the standard experimental model of acute inflammation. The time course of edema development in Carrageenan-induced paw edema model in rats is generally represented by a biphasic curve. The first phase of inflammation occurs within an hour of Carrageenan injection and is partly attributed to trauma of injection and also to histamine and serotonin components. The second phase is associated with the production of bradykinin, protease, prostaglandin and lysosome. Prostaglandins (PGs) play a major role in the development of the second phase of inflammatory reaction which is measured at different hours. Histamine, 5-hydroxytryptamine and bradykinin are the first detectable mediators in the early phase of Carrageenan-induced inflammation\textsuperscript{86} whereas prostaglandins are detectable in the late phase of inflammation\textsuperscript{87}. First phase may be due to inhibition of the release of early mediators, such as histamine and serotonin, and the action in the second phase may be explained by an inhibition of cyclooxygenase and the continuity between the two phases is provided by kinins.

Inflammation plays an important role in various diseases, such as rheumatoid arthritis, atherosclerosis and asthma, which all show a high prevalence globally. During an inflammatory response, mediators, such as pro-inflammatory cytokines, including interleukin IL-1, Tumor necrosis factor (TNF), interferon (INF)-γ, IL-6, IL-12, IL-18 and the granulocyte-macrophage colony stimulating factors are released, this response is antagonized by anti-inflammatory cytokines such as IL-4, IL-10, IL-13, IFNα and the transforming growth factor. The nuclear factor-KB (NF-KB), transcription factor, also plays an important role in the inflammatory response by regulating the expression of various genes encoding pro-inflammatory cytokines, adhesion molecules, chemokines, growth factors and inducible enzymes such as cyclooxygenase-2 (Cox-2)\textsuperscript{88} inducible nitric oxide synthase (iNOS) and Cox-2 both stimulate the production of large amounts of pro-inflammatory mediators. In chronic inflammation, the negative regulatory mechanism appears to be dysfunctional. Although inflammation is primarily a protective response (against micro-organism, toxins or allergens, for example), inflammation that is chronic and uncontrolled becomes determined to tissues.

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Inflammation is an attempt to dispose off microbes, toxins or foreign material at the site of injury, to prevent their spread to other tissues, and to prepare the site for tissue repair in an attempt to restore tissue homeostasis. Inflammatory response has three basic stages. Vasodilatation and increased permeability of blood vessels, emigration (movement) of phagocytes from the blood into interstitial fluid and tissue repair.

Drugs which are in use presently for the management of pain and inflammatory conditions are either narcotics e.g. opioids or non-narcotics e.g. Salicylates and corticosteroids e.g. hydrocortisone. All of these drugs possess well known side and toxic effects. Moreover, synthetic drugs are very expensive to develop and whose cost of development ranges from 0.5 to 5 million dollars.

1.9 ANALGESIC

Pain has been defined by international Association for the study of pain (IASP) as an unpleasant sensory and emotional experience associated with actual or potential tissue damage. Pain is centrally modulated via a number of complex processes including opiate, dopaminergic, descending noradrenergic and serotonergic systems. The µ receptors stimulation is generally associated with pain relief and has been shown to be potent in regulating thermal pain. Non analgesic effects through the µ receptors include respiratory depression and most importantly for therapeutic consideration is its induction of physical dependence. Activation of µ2 opioid subtype leads to spinal analgesia and commonly causes constipation as adverse effect. So, analgesic action indicates a central action by binding with opioid receptors. Although opioids possess dependence and abuse liabilities, new drugs producing less euphoria at onset and withdrawal symptoms as the medication wear off would be more beneficial. Acute pain is frequently associated with anxiety and hyperactivity of the sympathetic nervous system (eg. Tachycardia increased respiratory rate and BP, diaphoresis, dilated pupils). Chronic pain does not involve sympathetic hyperactivity but may be associated with vegetative signs (eg. Fatigue and loss of appetite) and dressed mood.

1.10. CENTRAL NERVOUS SYSTEM
The field of behavioral pharmacology uses concepts and techniques derived from pharmacology and psychology for the study of interaction between drugs and behavior, the discovery of new compounds which act on CNS process will stimulate not only their clinical use but will also contribute useful information for the validation of animal models. Anxiety disorders are among the most common and prevalent forms of psychopathology. Although, anxiety is a normal emotional response for a fearful situation, individuals who suffer from anxiety disorders, show a greater sensitivity in both their physiological and behavioral reactions in a variety of situations. Decreased serotonergic and dopaminergic transmission and increased cholinergic transmission. These findings scientifically validated the traditional claim and suggested its valuable role in the treatment of various CNS disorders. BZD such as diazepam, work by increasing the efficiency of GABA which potentiates GABA\textsubscript{A} receptor activation\textsuperscript{95}. Saponins and flavonoids are responsible for sedative and hypnotic effect. Diazepam is a prototype of benzodiazepines which acts on GABA\textsubscript{A}ergic neurons in the brain and reduced out flow of the neurons causing a muscle relaxant. Inclined plane method was originally developed for testing curare like agents, later it has been used for testing both centrally and peripherally acting relaxants.