Mother Nature stands as an inexhaustible source of novel chemotypes and pharmacophores (Brahmachari, 2009). Nature’s terrestrial flora and fauna have formed the basis of sophisticated traditional medicine systems that have been in existence for thousands of years. Such an intrinsic dependence of human beings on “Nature” invoked tremendous interest in the scientific world, which ultimately led to the isolation of a vast number of chemical agents with multipurpose medicinal potentials. Natural product based scaffolds find key importance in drug discovery as well as in optimizing chemical diversity for human use. This chemical diversity and unique biological activities of a wide variety of natural products have propelled many discoveries in chemical and biological sciences, and provided therapeutic agents to treat various diseases and also offered leads for the development of valuable medicines (Newman et al., 2003).

The modern tools of chemistry and biology, in particular allow scientists to detail the exact nature of the biological effects of natural compounds on the human body, as well as to uncover possible synergies, which holds much promise for the discovery of new therapies against many devastating diseases as herbal products symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment. Herbs are now staging a comeback and herbal ‘renaissance’ is happening all over the globe. It has been estimated that about 40% of medicines have their origin from natural products whose chemical potentials are however, still largely unexplored (Patwardhan and Vaidya, 2004). The use of herbal drugs is escalating in the form of complementary and alternative medicine (CAM) due to the devastating side effects that modern medicine has offered to the human community. This phenomenon has been mirrored by an increasing attention to phytomedicines as a form of alternative therapy by the health professionals (Dev, 1999).

The world health organization (WHO) has long recognized and drawn the attention of many countries to the ever increasing interest of the public in the use of medicinal plants and their products in the treatment of various ailments. Consumption of natural products reduces the risk of developing pathological conditions, including cancer, hepatic dys-functioning, nervous system disorders, cardiovascular, genetic, and inflammatory diseases (Jurenka, 2009). Various plant derived drugs such as morphine, aspirin, salicin, digoxin, quinine and pilocarpine are still in use (Borris, 1996).
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The pragmatic optimism currently placed on natural products in search of new drugs and lead molecules employs herbal remedies in pharmacy practice in India and other developing countries. India has been identified as one of the top twelve mega biodiversity centers of the world. It has a rich source of medicinal plants and a large number of plant derived extracts are used against diseases in various systems of medicine such as Ayurveda, Unani and Siddha. In India, thousands of species are known to have medicinal value and the use of different parts of several medicinal plants to cure specific ailments has been in vogue since ancient times (Tiwari, 2008). Recent technological advancement and the development of new methods have revolutionized the screening of natural products and offer a unique opportunity to re-establish natural products as major source of drug leads.

The impact of natural products on the development of the pharmaceutical industry is unabated. Medicinal plants work by combining three properties which are enlisted as curative, preventive and nutritive that offers the human body with all necessary strength and vigour to cope with the disease and facilitate the action of the curative agents in the herbal drug. A large number of traditional medicinal plants have been screened and used for treating and preventing various chronic diseases, such as liver diseases, diabetes, cancer, cardiovascular diseases, aging, and other degenerative diseases (Rawat 1994; Yarbro et al., 2005). Of various ailments, liver disease is a major health problem in India. Hepatic viral infections especially are of serious concern among cases of liver disease. It is estimated that 4.7% of the population of India is a carrier of hepatitis B virus (HBV). Liver disease is considered to be the fourth important cause of mortality in the most productive period of life (15-45 years). It is estimated that 15 - 40% of those affected eventually form serious complications, such as cirrhosis or liver cancer. Hepatitis C also remains significant as a cause of liver disease.

There is a prevalence rate of 26% in South India and 10-15% in North India with 16-20% accounting in Mumbai, which is considered as the financial capital of India. Global mortality prevalence due to liver cancer associate to about 5,98,000 deaths while 8-10 million deaths are estimated due to other liver diseases such as cirrhosis, cholestatis, fatty liver, hepatic encephalopathy, fulminant hepatic failure and chronic hepatitis (Handa and Sharma, 1990). Inspite of tremendous strides in modern medicine there are hardly

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any drugs that stimulate liver function, offer protection to liver from damage or regeneration of hepatic cell (Achuthan et al., 2003).

This statistics with perplexing report warrant the immediate necessity of studies to ensure the effectiveness of available formulations to reduce morbidity and mortality rate due to hepatic complications.

1.2 FACTS ON LIVER PHYSIOLOGY

Liver is the largest gland and is a vital organ. It is the metabolic “engine room of the body”. Human liver development begins during the third week of gestation and does not achieve mature architecture until about 15 years of age. It reaches its largest relative size, 10% of fetal weight, around the ninth week. It is about 5% of body weight in the healthy neonate. The liver is about 2% of body weight in the adult. It weighs around 1400g in an adult female and about 1800g in the male. It is a soft pinkish brown triangular organ. It is located in the right side of the upper abdomen below the diaphragm.

The lobes of liver are organized into liver lobules which form the functional unit of liver. Each lobule is surrounded by connective tissue partition called Glisson’s capsule. The capsule contains branches of portal vein, hepatic artery and bile ducts. Each lobule has a central vein at its center. The spaces between hepatic chords are called as sinusoids which are lined by endothelial cells. Some of these endothelial cells phagocytose dead RBC, bacteria and foreign particles. These phagocytic cells are called as the Kupffer’s cells. These cells are surrounded by bile capillaries or canaliculi which unite peripherally to form bile duct. Blood flows centripetally from branches of portal vein and hepatic artery to central vein via sinusoids but bile flows centrifugally from bile canalicule to bile ducts.

1.3 FUNCTIONS OF LIVER

It is pivotal to the body and plays an astonishing array of vital functions in the maintenance and performance of the body (Scott Treadway, 1998). The liver being an extremely active organ performs several important functions to maintain several processes of the body. It plays the centre role of action in synthetic, metabolic, excretion and detoxification functions like bile secretion, glycogenesis, glyconeogenesis,
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glycogenolysis, lipogenesis, deamination, ureogenesis, phagocytosis, synthesis of enzymes, coagulants and anticoagulants. Liver also functions as a store house of blood and aids in storage of iron, copper and vitamins.

1.4 FACTORS INDUCING HEPATOTOXICITY

The liver plays a central role in transforming and clearing chemicals and is susceptible to the toxicity from many extrinsic agents and intrinsic factors. Certain medicinal agents, when taken in overdoses and sometimes even when introduced within therapeutic ranges, may injure the organ. Other chemical agents, such as those used in laboratories and industries also induce hepatotoxicity. Such a vital organ, essential for human existence must be well protected and must be well maintained which is sometimes prone to damages and diseases. Whilst mankind is exposed to a vast array of foreign compounds through environmental exposure, consumption of contaminated food or exposure to chemicals in the occupational environment and is often abused by these environmental toxins, poor eating habits, alcohol consumption, prescribed and over-the-counter drug usage, which can damage and weaken the liver and eventually lead to hepatitis, cirrhosis, alcoholic liver diseases which will affect the structure and function of the liver as well as its physiological role such as the biotransformation of lipophilic compounds into water soluble derivatives.

1.4.1 Drug induced hepatotoxicity

Drug induced liver injury is responsible for 5% of all hospital admissions and 50% of all acute liver failures. Chemicals and drugs produce a wide variety of clinical and pathological hepatic injury. More than 900 drugs have been implicated in causing liver injury and it is the most common reason for a drug to be withdrawn from the market.

Hepatotoxicity and drug induced liver injury also account for a substantial number of compound failures, highlighting the need for drug screening assays that are capable of detecting toxicity early in the drug development process. Drug induced liver injury is of frequent occurrence since the role played by the liver to remove the toxic substances from the portal circulation makes it susceptible to the persistent attack by offending foreign compounds resulting in liver dysfunction (Arundel and Lewis, 2007).

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Drugs can cause liver diseases in many ways. Some drugs are directly injurious to the liver while others are transformed to chemicals that can be injurious to the liver directly or indirectly. The common drugs that induce toxicity to the liver are acetaminophen, statins, nicotinic acid (niacin), amiodarone, methotrexate, antibiotics administered for treatment of tuberculosis, tylenol and rifampicin- A broadline antibacterial drug and Non-steroidal anti-inflammatory drugs (NSAID).

1.5 HEPATITIS – AN OVERVIEW

The word hepatitis comes from the ancient Greek word hepar (root word hepat) meaning 'liver', and the Latin “itis” meaning inflammation. Hepatitis means injury to the liver with inflammation of the liver cells. It is not a condition, but is often used to refer to a viral infection of the liver. A group of viruses known as the hepatitis viruses cause most cases of liver damage worldwide. Hepatitis can also be due to toxins (notably alcohol), other infections or from autoimmune process. Hepatitis may be classified into two types namely: acute and chronic.

1.5.1 Acute hepatitis

Acute hepatitis may be caused by viral sources such as Vir-A through E, non-viral sources such as Leptospira, Q-fever, alcohol intake, toxins such as CCI₄, drugs such as paracetamol, antitubercular drugs, in-pregnancy, auto immune conditions such as SLE and in metabolic diseases such as Wilson’s disease. Initial features are of nonspecific flu-like symptoms, common to almost all acute viral infections and may include malaise, muscle and joint aches, fever, nausea or vomiting, diarrhea, and headache.

More specific symptoms, which can be present in acute hepatitis from any cause include: profound loss of appetite, aversion to smoking among smokers, dark urine, yellowing of the eyes and skin (i.e., jaundice) and abdominal discomfort.

1.5.2 Chronic hepatitis

Chronic hepatitis may be caused by viral sources such as Vir-B, D and C. However, non viral sources are generally not accounted for the cause of chronic hepatitis.

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But serious cases of chronic hepatitis due to intake of drugs such as methyldopa, nitrofurantoin, isoniazid, ketoconazole have been reported. Features may be related to the extent of liver damage or the cause of hepatitis. Symptoms are similar to that of acute hepatitis. Jaundice can be a late feature and may indicate extensive damage. Other features include abdominal fullness from enlarged liver or spleen, low grade fever and fluid retention (ascites). Extensive damage and scarring of liver may lead to weight loss, easy bruising and bleeding tendencies.

1.5.3 Acute alcoholic hepatitis

Ethanol, mostly in alcoholic beverages, is a significant cause of hepatitis. Usually alcoholic hepatitis comes after a period of increased alcohol consumption and is characterized by a variable constellation of symptoms, which may include feeling unwell, enlargement of the liver, development of fluid in the abdomen ascites, and modest elevation of liver biochemical parameters. Alcoholic hepatitis can vary from mild with only liver test elevation to severe liver inflammation with development of jaundice, prolonged prothrombin time, and liver failure.

1.5.4 Hepatic cancer

It is also called hepatocellular carcinoma. When cancer originates in the liver it is called primary cancer. Cancer may also spread to the liver by metastasis. Risk factors of primary liver cancer include hepatitis, cirrhosis or scarring of the liver and low birth weight.

1.5.5 Hepatitis virus – An overview

There are five main types of hepatitis that are caused by a virus, A, B, C, D, and E - plus types X and G. Of the above mentioned virus, A, B and C are found to be responsible for higher mortality and morbidity rates. Hepatitis A viral infection (HAV) otherwise named as infectious hepatitis is seen to be transmitted by faecal or oral route as a food borne infection. It has an incubation period of 15 to 45 days. HAV antibodies (IgG class) are detectable in the patients’ serum after the onset of the symptoms.

Hepatitis B otherwise known as serum hepatitis is transmitted by blood products and other body fluids. It has a longer incubation period between 40 and 180 days. Patients
untreated may develop fulminant hepatitis. Hepatitis C is considered to be as non A and non B type hepatitis is seen to affect about 170 million people globally. Hepatitis C is a blood borne virus and routes of transmission include intravenous drug use, mother to infant transmission, unsafe medical practices, high risk sexual behaviour, and blood transfusion. Chronic hepatitis C is in most patients a benign viral infection, but a minority of patients develop liver cirrhosis and may suffer from complications due to cirrhosis or die from it.

1.5.6 Stages of hepatic injury

Hepatic disorders have far reaching consequences, given the critical dependence of other organs on metabolic functions of the liver. Liver injury and its manifestations tend to follow characteristic patterns. In some instances the diseased process is primary to the liver. In others, the hepatic involvement is secondary, often to some of the most common diseases in humans, such as cardiac decompensation, alcoholism and extra hepatic infections with progression of diffused disease or strategic disruption of circulation or bile flow. In hepatic injury five general responses are read namely: hepatic inflammation, hepatic fibrosis, cirrhosis, hepatic degeneration and liver failure.

1.6 MODERN DRUGS IN TREATMENT OF LIVER DISEASE AND THEIR SIDE-EFFECTS

Standard treatment for hepatitis includes interferon α and ribavarin. The treatment is expensive and is associated with significant side effects. Ribavirin may cause anemia and should not be consumed by heart patients and should not be administered in patient with hypertension and breathing problems. Interferon α is administered for preventing viral multiplication. The most common side effects of interferon α -2a or α -2b therapy is a flu-like reaction with fever, fatigue, irritability, chills, headaches, and muscle aches. Most patients relapse and have recurrent liver inflammation after treatment is withdrawn (Howard Worman, 2007).

Cytokines control the patho-physiology and progression of liver diseases. Interleukin-10 is a cytokine that down regulates the pro-inflammatory response and has a modulatory effect on hepatic fibrogenesis. There was no effect on viral load, but the
serum alanine transaminase levels are reported to be normalized (Nelson et al., 2000). Antibiotics such as aminoglycosides, cephalosporins, chloramphenicol, clindamycin were also used in the treatment of liver diseases. These drugs are inadequate and inconsistent and they cause liver damage. Thus, these synthetic drugs that are in vogue in the management of various liver disorders provide only temporary relief but with serious side effects. Hence, the need of the hour is to develop an alternative medicine which can restore liver function efficiently and effectively.

1.7 HERBAL THERAPY IN HEPATOPROTECTION

Medicinal plants exert two types of actions on the hepatobiliary system: choleretic and cholagogue. Plants with choleretic properties increase the secretion of bile, reduce the congestion of the liver and promotes digestion. These are helpful in conditions such as hepatitis. Plants with cholagogue properties ease the emptying of the bile stored in the gall bladder to the duodenum. Cholagogue plants prevent the gall bladder andOddi of sphincter spasms, by alleviating pain and encouraging the proper functioning of the biliary system. They are used for biliary dyskinesia, biliary dyspepsia and cholelithiasis (George, 2000).

The use of natural remedies for the treatment of liver diseases has a long history and medicinal plants and their derivatives are still used all over the world in one form or another for this purpose. Numerous herbs namely Glycyrrhia glabra, Silybum marianum, Curcuma longa, Piper longum, Picrorhiza kurroa, Phyllanthus amarus, Tephrosia purpurea, Andrographis paniculata etc., have been accepted globally as effective hepatoprotective agents and are widely used in India to cure hepatic impairment. Such liver protective plants contain a variety of chemical constituents like phenols, coumarins, terpenes, glycosides, alkaloids and xanthenes namely phyllanthin, glycyrrhetic acid, taxifoline, silymarin, cucurmin, piperine, urosolic acids, quercitin which are considered to constitute to the hepatoprotective potential of these plants.

About 40 commercial polyherbal formulations reputed to have hepatoprotective action are being used in India. It has been reported that 160 phytoconstituents from 101 plants possess to have hepatoprotective activity (Ajay Kumar Gupta and Neelam Misra, 2006). Many such formulations are sold in Indian market claiming to possess medicinal
activity over liver dysfunction. However, management of liver disorders is still an intriguing problem. Due to the known side effects of approved pharmaceuticals, patients often turn to alternative medicine which is considered “natural” and “healthy”. Medicinal plant parts should be authentic and free from harmful materials like pesticides, heavy metals, microbial or radioactive contamination, etc (Kamboj 2000).

WHO encourages, recommends and promotes traditional/herbal remedies in National Health Care programmes because these drugs are easily available at low cost, safe and people have faith in them. The WHO assembly in number of resolutions has also emphasized the need to ensure quality control of medicinal plants. It is very important that a system of standardization is established for every plant medicine in the market because the scope for variation in different batches of medicine is enormous. Some of the standardization tests for herbal medicines are macro and microscopic examination, detection of foreign organic matter, determination of ash, moisture and extractive values, qualitative and quantitative chemical evaluation and toxicology studies (Ritch, 2000).

Globally there is a positive trend towards holistic health, drug discovery and therapeutics. Combining the strengths and knowledge base of the traditional systems which possess the power of combinatorial sciences, with a high thorough biological screening will provide new leads and will reduce time, money and toxicity which are considered to be the three major hurdles that exist in the field of drug development. Herbal medicines are highly valuable, as they have been effectively tested for thousands of years on people. Herbal knowledge database allows drug researchers to start from a well-tested and safe botanical material. There are many more plants that are available in the rich repository of Indian pharmacopoeia that has not been much explored and updated. But they need to be scientifically validated to ensure their ability to conserve their therapeutic effectiveness in the formulated form.

Therefore, the prime objective of the present study is to select a medicinal plant which can be used as an effective agent in combating against hepatic disorders/damage; and may not have side effects to other organs of the body. Taking all these facts into account, the present work focuses to develop an eco-friendly and human compatible hepatoprotective herbal drug from the leaves of Hiptage benghalensis collected from
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Kolli hill, a part of Eastern Ghats, Tamilnadu, India and screened for its pharmacological potential as per the WHO guidelines.