CHAPTER 1
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Living in a world of inadequately controlled environmental pollution and expanding therapy with potent drugs, the Liver, which is the key organ of metabolism and excretion is continuously exposed to a variety of xenobiotic and therapeutic agents.

It is being felt that many environmental factors, chemicals, drugs and contaminated food affect the liver physiology up to certain extent, which may lead to other secondary physiological changes. Surprisingly, there are not many liver protective drugs available for the treatment. Diet quality and life habits are the only remedy. An actual curative therapeutic agent has not yet been found. In fact, most of the available remedies rather support or promote the process of healing or regeneration of the liver. The drugs available in the modern system of medicine are the corticosteroids and immunosuppressive agents which bring about only symptomatic relief and in most cases have no influence on the disease. Further, their use is associated with the risk of relapses and danger of side effects.

Causative agents for HCC (Hepatocellular Carcinoma) have been studied along two general lines. First, there are those agents that have been found to be carcinogenic in experimental animals, particularly rodents, and which are thought to be present in the human environment. Second, epidemiologic associations of hepatoma with various other human diseases have been identified. Probably the best studied and most potent ubiquitous natural chemical carcinogen is aflatoxin B₁, a product of the Aspergillus Fungus. Aspergillus Fungus mold and aflatoxin products are found in a variety of stored grains, particularly in hot humid parts of the world where grains such as rice are stored in Unrefrigerated conditions (Linsell, 1979). In the months after the monsoon in southeast Asia, most village based grains can be seen to be covered by a white layer. This layer contains high levels of aflatoxin and is consumed after months by most of the villagers. Data of aflatoxin contamination in food stuffs correlate well with incidence rates in
Africa and to some extent in China. In hyperendemic areas of China even farm animals such as ducks have Hepatic cellular carcinoma.

The most potent hepatocarcinogens appear to be natural products that occur in the environment and are synthesized by plants, fungi and bacteria. In large area of the world, rice toxins are eaten, as are the Senecio plants and bush teas containing pyrrolizidine alkaloid, tannic acid, safrole and, in the pacific the cycad plants. Although hepatocarcinogens for rodents are used as medicinal compounds for humans like Sex hormones, Oxazepam, Thiouracil, Phenobarbital, Aurothioglucose etc., there is little evidence that any except the sex hormones have an important role in human hepatocarcinogenesis. A considerable literature exists on the hepatocarcinogenicity of anabolic steroids and the induction of benign hepatomas by estrogens (Henderson et al; 1983). Although estrogens are capable of causing HCC in rodents as promoting agents, an epidemiologic association in humans has never been shown. Complete carcinogens inducing HCC include aflatoxin B1 and, for hepatic angiosarcoma, vinyl chloride, thorotrast, and possibly inorganic arsenic compound. In an Industrial society, a large number of environmental pollutants, particularly pesticides and insecticides, are known rodent carcinogens, (US Department of Health and Human Services, Sixth annual report of Carcinogens, 1991).

Hepato Cellular Carcinoma is the most common primary malignancy of the liver and most common cancer. About 75% of all Hepatocellular carcinomas (HCCs) worldwide are associated with cirrhosis, in addition one of three other factors: hepatitis B virus (HBV) infection (Basley et al; 1981), hepatitis C virus infection (Ikeda et al; 1984). Cirrhosis is the end result of chronic hepatocellular necrosis, inflammation, and fibrosis are defined morphologically as a diffuse process with septal fibrosis and regenerative nodules (Anthony et al; 1977). The strong correlation between cirrhosis and HCC suggests that they may be due to a common cause or that the development of cirrhosis involves the same steps or mechanisms as hepatocarcinogenesis.
Hepatitis B Virus (HBV) infection is a public health problem of global importance (Tiollais, et al; 1985). It has been estimated that 5% of the world population is persistently infected by this virus. In South-East Asia and Central Africa, more than 10% of the population are HBV carriers, chronic active hepatitis and cirrhosis are major causes of mortality. Moreover epidemiologic studies have clearly shown the importance of HBV in hepatocellular carcinoma (HCC), one of the most common cancers in the world (Beasley et al; 1984). Among individuals who are chronically infected by HBV, 50% will develop HCC. The etiology of HCC appears to be multifactorial, and several events seem to be necessary for malignant transformation to occur; Besides Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV) infection and chemicals, many inherited and acquired conditions are associated with the development of hepatocellular carcinoma. Based on these results, many timely preventive or therapeutical interventions are currently applied to reduce the likelihood of HBV-infected persons developing HCC and to improve patients' survival rate. (Ohnishi et al; 1987, Paterson et al; 1994).

Preclinical studies in mice and rats provide essential information about pharmacokinetics and provide a basis for rational schedule development for the new drugs in humans. Pharmacokinetic information in animals can also provide a rational basis for dose escalation in humans. Collins and associates have hypothetised that dose limiting toxicity in mice and humans are function of drug exposure, as measured by the under drug concentration (Collins et al; 1990).

**Natural Product in Modern Medicine :-**

Life, disease and decay are inseparable. From his first awakening Man has sought to fight and control disease. He turned to nature for inspiration and guidance. During thousand of years of early human existence, many natural materials, by instinctive intuition of trial and error, got taken in use for combating human ailments. As ideas of different tribes, communities and cultures permeated each other, the use of these
materials or similar ones got wide spread, occasionally intermixed with religions, rituals or magic. Ancient health sciences, like Ayurveda of India, depended heavily on natural products. In India, Ayurveda is still an active and important system of medicine. Many herbal preparations found their way in the pharmacopoeias of different countries.

It is obvious that these traditional medicines which have withstood the test of time, must be effective. With the introduction of modern scientific methods and techniques, the validity of claims of many of these could be substantiated. With the advent of chemotherapy, several of these preparations got supplemented by more effective or less toxic synthetic drugs. There is a vague general feeling that nowadays bulk of the drugs in use are man-made.

About 600 commercial preparations with liver protecting activity are available all over the world. In India about 33% of herbal formulation are available for liver ailments and these preparations represent a variety of combinations out of 100 Indian medicinal plants belonging to about 40 families investigated as liver protecting drugs by Handa. S.S. et al; (1986).

A number of plants have been shown to posses hepatoprotective property. We studied the effect of aqueous extract of *Andrographis paniculata* on extreme liver damaging conditions like hepatic tumors. (Trivedi N. and Rawal U. M, 1998).

*Andrographis paniculata* (kalmegh), is used extensively in the Indian traditional system of medicine as a hepatoprotective and hepatostimulative agent. The aqueous extract of the leaves of this plant has been used for treatment of various liver disorders and jaundice. The herbs are well-known under the name of 'kalmegh' and forms the principle ingredient of a household medicine called 'alui' which is extensively used in Bengal. The macerated leaves and juice together with certain spices are made into little globules, which are prescribed for infants to relieve griping irregular stools and loss of...
appetite. The roots and leaves have also the reputation of being a febrifuge, tonic and anthelminic. In general debility, dysentry and certain forms of dyspepsia associated with gaseous distension of the bowels the decoction or infusion of the leaves have been used with satisfactory results.

*Andrographis paniculata* is one of the nineteen species of the genus Andrographis (N.O.Acanthaceae) available in India. It is commonly seen with its allied spices “Andrographis echiodes Needs” in the forest areas of Rajura, Wirur, Kanhargaon, Elgurtola, Ettapali, Repan Palli, Mesa, Troba, Sondhu, Lakkarkote etc in Chandanpur of Maharashtra state. The fresh and dried leaves of *Andrographis paniculata* and the juice extracted from the shrub are official in Indian pharmacopoeia.

**Botanical Characteristics and Habitat :-**

*Andrographis paniculata*, is known by various names: the West Indies as ‘Rice Bitters’ (Sanskrit : Kirata; Hindi; Kirayat; Bengali : Kalmegh; Marathi : Oli-Kirayat; Gujarati : Kariyatu; Tamil : Nilavaembu). *Andrographis Paniculata* is an annual shrub of a very bitter taste, found in the plains of India, West Indies and Ceylon. It grows erect to a height of 1-3feet, in moist and shaddy places with glabrous leaves and whitish flowers, spotted with rose purple colour. It is often seen cultivated in gardens or else seen growing wild along fences.

**Chemistry :-**

The herb, *Andrographis paniculata* is the main source of the bitter principle : Andrographolide (Yield : 1% on dry basis). Boorsma, 1896 was probably the first to isolate this colourless crystalline compound, C_{20}H_{30}O_{5}, m.p.23i; (decomp.); αD - 126° named as ‘Andrographide’. Andrographolide, Andrographiside and Neoandrographolide are active anti oxidants(Koul et al; 1994).
Andrographis paniculata (Acanthaceae) are sometimes referred as “big Chirata”. Andrographis paniculata (Kalmegh) consisting of the dried leaves and tender shoots of the plant forms the principal ingredient of the traditional medicine known to be effective against variety of disorders. Among the different constituents isolated from Andrographis paniculata is Andrographolide. Andrographolide has been reported to possess activities like reduction in hexabarbitual or phenobarbital sleeping time by Chaudhari S.K. (1978) and Handa et al; (1990). The effects of Andrographis paniculata extract and Andrographolide inhibition of drug metabolizing enzymes has reported by Choudhury et al; (1987). Andrographolide has been reported to antihepatotoxicity besides its choleretic and anticholestatic effects in animal by Shukla et al; (1992).

Other diterpenoids viz, Andrographiside and Neoandrographolide have also shown pronounced protective effect against liver injury caused by hepatotoxins (Kapil et al; 1993). The effect of the diterpenes i.e., andrographolide, andrographiside and neoandrographolide from Andrographis paniculata was investigated on the hepatocellular antioxidant defense system and lipid peroxidation in CCI4 treated mice by Koul et al; (1994).

Hepatocarcinogenesis induced by chemicals in laboratory animals has been used as a model system for human hepatocarcinogenesis and neoplastic development in general for more than half a century (Schmidt, 1924) was the first to describe the experimental induction of a hepatocellular neoplasm in mouse that had received scarlet red per os in an unsuccessful attempt to stain fat in vivo. It was, however, Sasaki and Yoshida, (1935) who produced a high incidence of Hepatocellular carcinoma (HCC) by the systematic feeding of rats with the carcinogenic compound of scarlet red, the azo dye O-aminoazotoluene, that established chemicals hepatocarcinogenesis as a main tool in cancer research. A large number of additional chemical hepatocarcinogens belonging to various chemical classes have been identified by Wogan (1976), Preussmann (1978), Montesano et al; (1994), Pitot et al; (1994), and Schwarz (1995). In animal experiments, the rodent liver has been shown to represent a favoured target tissue for chemical
carcinogens (IARC, 1972-1996). Although rodents continue to remain the preferred animal species for studying chemical hepatocarcinogens, a broad spectrum of other species including primates are also susceptible to the chemical induction of hepatic neoplasia (Stewart 1975, Bannasch 1983, Thorgeirsson et al; 1994, Jones et al; 1996).

In addition to the link between chemical structure and biologic effects, the metabolism of chemical hepatocarcinogens (Wogan 1976, Preussmann, 1978, Montesano et al; 1994, Pitot et al; 1994) and dose-response relationships (Druckrey 1967, Peto et al; 1984) have been studied in great detail. These areas are important for extrapolation from the results of carcinogenesis bioassays in laboratory animals to humans, and observations on the pathogenesis of experimental liver tumors are of particular interest (Aterman, 1995 and Bannasch, 1996). Nearly all types of primary liver tumors known from human pathology can be produced by chemicals in laboratory animals, especially in rats (Stewart 1975, Popper et al; 1977, Bannasch et al; 1990). The malignant variants of these neoplasma have been classified as Hepatocellular carcinomas, cholangiocellular carcinomas, angiosarcomas, and perisinusoidal (Ito) cell sarcomas. Sequential cellular and molecular changes preceding these neoplasma have been observed and analysed in several experimental models of chemical hepatocellular carcinogenesis (Bannasch 1994, Jones et al; 1996).

A large number of compounds representing many classes of chemicals have been shown to induce liver neoplasma in laboratory animals (Wogan 1976, Preussmann 1978, Montesano et al; 1994, Pitot et al; 1994, Schwarz 1995).

The earlier studies have shown the potential carcinogenic hazards of technical grade hexachloro cyclohexane (Nagasaki et al; 1971, 1972). Ito et al; (1973) have reported that α-isomer of hexachlorocyclohexane is mainly associated with the induction of liver tumours in mice. Other isomers of hexachlorocyclohexane neither have synergistic nor antagonistic effects on the induction of liver tumours by α-hexachlorocyclohexane. These
investigations were limited to histological and ultrastructural studies (Ito et al; 1973) and species, sex and stain specific tumorogenic effects of a diet containing the Hexachlorocyclohexane (Nagasaki et al; 1975).

The works on these pesticides in NIOH, Ahmedabad was initiated to confirm the earlier reports and to evaluate their chronic effects in laboratory animals by administering orally. It was found that BHC had a more potent hepatocarcinogenic effect than DDT (Nigam et al; 1981). The experimental animals study suggests that chronic exposure to this insecticides can lead to tumour induction, besides causing a reduction in the average life span and effects on the liver i.e. fatty infiltration and other associated liver disorders (Kashyap et al; 1979, Nigam et al; 1981, 1984, 1986 and).

On the basis of above findings, the BHC model was selected for the hepatocarcinogenicity and the drug *Andrographis paniculata* (aqueous extract of leaves of *Andrographis paniculata*) was administered to it.

The rationale behind selecting BHC model was that the inducement of liver tumour is faster than other carcinogenic substance as well there is no reversal of such tumors after prolonged treatments with BHC.

There are number of toxic chemicals causing liver damage, which are classified as hydrocarbons or substituted of hydrocarbons, the salt of certain metals, antimitotic agents, enzyme inhibitors and variety of drugs. Radiomimetic anti mitotic agents, such as 1, 4-dimethane sulphonyloxy butan (busulphan, myleran), used in man for chronic myeloid leukaemia (Solomon et al, 1955, Sallmann, 1957; Podos and Canellos, 1969).

The natural radiomimetics are nitrogen mustard triethylene melanin (TEM), 4-(P-dimethyl amino-styryl) quinoline-used in malaria treatment (Conklin et al; 1963,
Christenberry et al.; 1963). Mimosine, a cytotoxic agent derived from the leaves and seeds of levcaena glavca, and from mimosalpudica are used as therapeutic agents. Similarly *Andrographis paniculata* also can be considered as a radiomimetic plant because it emits some amount of radioactivity. When studied under Geiger muller counter and scintillation counter. After observing such activity of AP it was decided to study the extract of this radiomimetic plant leaves for extreme hepatic tumours conditions in mice.

The aim of the present study is to investigate the influence of the aqueous extract of *Andrographis paniculata* (AP) on the liver damage reaching up to carcinogenic condition. It was of interest to see whether supplementation of this plant extract has any effect in ameliorating the severe liver damage injuries caused by BHC in mice model. Therefore in the light of the above mentioned investigations, the work embodied in this thesis has been undertaken to study certain aspects involving the morphological, histomorphological, ultrastructural and biochemical analysis.

The work embodies the following aspects:

1. Morphological studies
2. Detailed histomorphology of the liver by light microscopy of all the below mentioned groups.
3. Ultrastructural study of below mentioned group.
4. Biochemical studies in the below mentioned groups:

   - Group I – Control group
   - Group II – BHC Treated group
   - Group III – BHC with AP Treated group (Supplemented Group)
   - Group IV – AP Treated group