CHAPTER – I
INTRODUCTION

The rapid population growth and economic development in our country leads to extensive agriculture, uncontrolled growth of urbanization and industrialization. All these activities are degrading the environment by destructing natural habitats. This is one of the major causes of environmental degradation in India. The Population Reference Bureau estimated India’s population to be 1033 millions in mid 2001 which will increase to 1.26 billion by the year 2016. The projected population indicates that India will be the first most populous country in the world by 2050 (Population Reference Bureau, 2001), which will be an alarming situation. India having 18% of the world’s population on 2.4% of its land area leads to water shortages, soil exhaustion, deforestation, air and water pollution, increasing demand for food with decreasing agricultural land.

![Flow Chart depicting population and pesticide effect](image)

**Figure 1: Flow Chart depicting population and pesticide effect**

There is an increasing food shortage in India, as the per capita food production is declining along with the loss of agricultural lands due to ever increasing population. This calls for need in improving agricultural productivity for which we depend on pesticides and other chemicals even though they are a source of concern because of their side effects (Figure 1).
At present, there is serious concern about environmental protection, human health, food safety and judicious pesticide use in agriculture. The population growth, combined with the diet demands of populace, is expected to double the world food demand by 2050; henceforth global pesticide production will be 2.7 times higher in 2050 than in 2000, exposing humans and the environment to considerably higher levels of pesticides (Tilman, 2001).

The widespread use of large number of harmful chemicals and pesticides by farmers, large plantations and the general public to solve the problem of food crises causes environmental contamination. It is estimated that the 68% of such contamination is a result of agricultural usage, followed by 17% of industrial and commercial uses and 15% of home and garden application (Majid, 2005).

1.1. ENVIRONMENTAL HAZARDS OF PESTICIDES

The environmental impact of pesticides is often greater than what is intended. Over 98% of sprayed insecticides and 95% of herbicides reach a destination other than their target species, including non-target species, air, water and food. In India, pesticides are commonly used and there are large occurrences of related health problems. Persistent pesticides can lead to greater adverse effects in the food chain and human beings by biological magnification (Figure 2).

The release of pesticides into the environment may give rise to different consequences. Its application directly to water bodies for weed control or indirectly as a result of run-off from soil or other routes may not only build-up pesticides in water, but may also cause air pollution through evaporation. Some of these pesticides may be degraded by the action of sunlight, water, other chemicals or microorganisms. Though, its degradation process usually leads to the formation of less harmful residue yet in some instances it can produce more toxic products. The pesticides can also become resistant to degradation and remain unchanged in the environment for long periods of time (Fred Fishel, 1914).

Animals may be poisoned by pesticide residues that remain on food after spraying, like when wild animals enter sprayed fields or nearby areas shortly after spraying. Poisoning from pesticides can travel up in the food chain. Some pesticides can bioaccumulate or build up to toxic levels in the bodies of organisms that consume them over time. When living creatures with accumulated pesticides in their tissue (bioaccumulation) are eaten by a higher organism the pesticide enters the latter’s tissue and can reach higher levels in the food chain than it is actually present in the environment.
Similarly, humans who are at the top of the food chain may be exposed to these high levels when such foods with bio-accumulated pesticides and other organic chemicals are consumed (Fred Fishel, 1914). Hence there is an urgent need to answer the ill effects of pesticides. We should find alternatives in some herbal treatments to cure the effects of pesticides naturally.

The public awareness in developing countries is extremely poor regarding the adverse effects of pesticides. Some of the commonly used pesticides are Dichlorodiphenyltrichloroethane, endosulfan (a and b, fenvalerate, lindane, malathion, parathion, diclofam, dieldrins, carbamates (mancozeb, maneb), etc. In India, 147 pesticides are registered for use at present and the tolerance limits of only 50 have been evaluated. This indicates that we are at risk not only from the commonly used pesticides with known tolerances but also from large number of pesticides that enter the fields, food commodities and the market without their maximum permissible limits beings known.

Hence, an integrated approach involving toxicology, epidemiology, physiology and behavioural sciences is essential for the proper assessment of human hazards related to pesticide exposure. There are various groups of pesticides which are widely used in fields among which carbamates are currently considered for toxicity studies. According to Gupta, (1984) carbamates have 15% usage.

Carbamate pesticides affect the nervous system by disrupting reversibly an enzyme that regulates acetylcholine, a neurotransmitter. Short-term exposure can produce muscle twitching, headache, nausea, dizziness, loss of memory, diarrhoea and slowed heartbeat.
Long-term exposure can produce delayed neurotoxicity, such as tingling and burning in the extremities which can progress to paralysis. Damage to the liver, kidney, immune system and bone marrow may also occur. Some carbamates are also suspected carcinogens. The Ethylene-Bis-Dithio-Carbamate group belongs to this class of compounds and are widely used in agricultural fields.

1.2. ETHYLENE-BIS-DITHIO-CARBAMATE (EBDC) PESTICIDES

The Ethylene-Bis-Dithio-Carbamate (EBDC) pesticides are organic compounds derived from carbamic acid (NH₂COOH) i.e. esters of N-methyl carbamic acid. Ethylene-Bis-Dithio-Carbamate pesticides are regarded as fungicides controlling wide range of diseases of vegetables, fruit, commercial sod, and field crops. The toxic effects of these chemicals were studied in various animal models. They generally persist from only a few hours to several months in the environment. They have been fatal to large numbers of birds on turf and in agriculture, and negatively impacted breeding success in birds. They are cholinesterase inhibitors in their mode of action; however their inhibitory effect on cholinesterase is generally brief.

Members of this chemical family include mancozeb, maneb, metiram, and nabam. Mancozeb is a coordination product of zinc ion and manganese salts of ethylene bis-dithiocarbamate (EBDC) group. These fungicides may cause irritation of the skin, respiratory tract, and eyes. In 1987, the Environmental Protection Agency (EPA) conducted a special review of the ethylene bis-dithio-carbamate compounds because of concerns about the potential health effects from dietary exposure to residues left on food crops and occupational exposure.

The current study will be conducted on mancozeb, a fungicide, which is commonly in use for foliar application and seed-treatment in agriculture (Worthing, 1991). Mancozeb is extensively used in agricultural fields (agronomy), vegetable crops, homes, gardens, large scale plantations in Madhya Pradesh and India against variety of foliar fungal diseases and many different purposes including protection of seed grain during storage and germination. This pesticide is better than other dithiocarbamates due to their low acute toxicity and short environmental persistence. Mancozeb is one of the oldest molecules and is still being accepted by farmers for more than 50 years in crop control. This wide spectrum fungicide protects against more than 100 fungi. The market consumption is still growing for this chemical for more than 50 years. The all India consumption market of mancozeb is about 8000-9000 metric tons while in Madhya Pradesh alone the consumption is 100-125 metric
tons (Source: The market survey of Indofill Chemicals Ltd, one of the major manufacturers). It is mainly used in cultivation of potato, tomato, grapes, cumin, apple, peas, garlic, etc. Seeing to this large scale usage of mancozeb in India and Madhya Pradesh, the molecule was selected for the present study.

However, several evidences have been emerging supporting the toxic nature and detrimental effects of mancozeb in both humans and experimental animals (Bindali and Kaliwal, 2002; Axelstad, et al. 2011). Several reports have been published regarding its toxicity however many aspects are still not clear and further investigations are required.

1.2.1. Mancozeb

Mancozeb is an ethylene-bis-dithiocarbamate (polymeric) complex with zinc and manganese salt. The formulation of 80% wettable powder contains 16% Mn$^{2+}$ and 2% Zn$^{2+}$. Mancozeb is classified as a contact fungicide with preventive activity. It inhibits enzyme activity in fungi by forming a complex with metal-containing enzymes including those involved in production of adenosine triphosphate (ATP). Mancozeb is used to protect many fruits, vegetables, nuts and field crops against a wide spectrum of fungal diseases, including potato blight, leaf spot, scab (on apples and pears), and rust (on roses). It is also used for seed treatment of cotton, potatoes, corn, sunflower, sorghum, peanuts, tomatoes, flax, and cereal grains.

1.3. MANCOZEB AND ITS HAZARDS

1.3.1. Environmental Fate of Mancozeb

Mancozeb has negligible vapour pressure hence it has low potential to volatilize into the air. The identified hydrolysis degradates are Ethylenethiourea (ETU), Ethylene Urea (EU) and Ethylene Bis-isothiocyanate sulfide (EBIS). Hydantoin is found as additional degrade at pH 9. One possible degradation route to form Ethylene Bis-isothiocyanate sulfide is via the oxidation of ethylene bisdithiocarbamate; the major metabolites being Ethylenethiourea and Ethylene Urea. Under aerobic conditions, the metabolites breakdown further to produce CO$_2$. A possible mechanism for degradation of mancozeb in the presence of water is the conversion of ethylene bisdithiocarbamate to Ethylene Thiuram Disulfide (ETD) and subsequent degradation to Ethylene Bis-isothiocyanate sulfide (Engrst and Schnaak, 1970; Aldridge and Magos, 1978) which may ultimately degrade to Ethylenethiourea or ethylenediamine (EDA). The possible metabolic pathway is represented in Figure 3.
Ethylenthiourea is a suspected human carcinogen but it may be quickly photo oxidized to Ethylene Urea and subsequently to glycine in water with a half-life of less than 3 days (Ross and Crosby, 1973; Cruickshank and Janrow, 1973 and Houeto, et al. 1995). Ethylenthiourea has a persistence of 5-10 weeks and a half-life of less than two days in non-sterile soil.

The primary concern with mancozeb is its spontaneous degradation to Ethylenthiourea in the presence of water and oxygen.
1.3.2. Toxicity to Humans

Mancozeb has been shown to produce significant toxicological effects on thyroid and gonads in male and female rats, carcinogenic and teratogenic activity in rats, etc. Mancozeb is a cholinesterase inhibitor and can therefore affect the nervous system. Symptoms of exposure include fatigue, headache, blurred vision, and nausea. At high doses, exposed persons experience convulsions, slurred speech, confusion and slowed heartbeat. In lower doses, mancozeb can also cause a skin rash if the chemical has contact with the skin. In one study, a vineyard worker developed a rash on the forearm as well as inflammation of the eyelids after handling seedlings which had been treated with mancozeb.

A major toxicological concern with respect to mancozeb and other dithio-carbamates is its primary metabolite, Ethylene Thio Urea, known to cause thyroid and carcinogenic...
effects in test animals. Many studies dating back to 1980 showed that mancozeb can cross the placental barrier and induce or increase tumour incidence.

1.4. SELECTED MEDICINAL HERBS

Since time immemorial, medicinal plants have been used to cure or prevent diseases or toxic effects. The ability of plants to synthesize a wide variety of chemical compounds used to perform important biological functions and strengthen immune system or to defend against predators makes it suitable to use as alternative medicines. Moreover it has fewer side effects than synthetic drugs. At least 12,000 such compounds have been isolated so far; a number estimated to be less than 10% of the total available (Tapsell, et al. 2006; Lai and Roy, 2004).

Chemical compounds present in plants mediate their effects on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs; thus herbal medicines do not differ greatly from conventional drugs in terms of how they work. This enables herbal medicines to be as effective as conventional medicines (Tapsell, et al., 2006 and Lai and Roy, 2004). Thus some of the most powerful and well-known medicinal plants are Aloe vera and Ocimum sanctum in this connection.

The medicinal uses of A. vera are amazing, as the benefits of its remedies are truly significant. However, more and better trial data are needed to define the clinical effectiveness of this popular herbal remedy more precisely (Oogler, 1999; Feily, 2009).

1.4.1. Therapeutics of Aloe Vera

Aloe vera is a boon to mankind from nature. It is used in traditional Indian medicine for constipation, colic pain, skin diseases, worm infestation, and infections (Heber, 2007). It is also used for hypertension (Lans, 2006) and for the treatment of type 2 Diabetes mellitus ( Coronado, et al. 2004). In Chinese medicine, it is often recommended in the treatment of fungal diseases (Heber, 2007). In Western society, A. vera is one of the few herbal medicines in common usage, and it has found widespread use in the cosmetic, pharmaceutical and food industries. It boosts immune system, relieves arthritis and rheumatism, reduces peptic ulcers, heals wound (Davis, 1989). It is antipuritic, hypoglycaemic, hypolipidemic. Bolkent, (2004) observed its positive effects on kidney in type II diabetic rats Can, et al. (2004) saw same effects in liver of type II diabetic rats. Fresh A. vera juice has a very interesting property. It enhances the growth of human cells in cell cultures and accelerates the healing of damaged tissue.
Clinical trials of *A. vera* have been conducted to prove it as a detoxifier, anti-septic, anti-diabetic (Bunyapraphatsara, *et al.* 1996). It has anti-inflammatory (Langmead, 2004), abortifacient, regenerates hair follicle and relieves pain. A recent study indicates that aloin and aloe-emodin may be a key constituent responsible for the anti-inflammatory effects of aloe (Park, *et al.* 2009).

Research on standardized methodological quality is, therefore, needed to identify which *A. vera* components, individually or in combination, exhibit therapeutic properties and the exact mechanisms by which they act. The application of aloe gel on burns quickly relieves pain, reduces the severity of the burn, accelerates healing and prevents scar formation (Danhof, 1987). The whole-leaf extract of *A. vera* helped in reducing total cholesterol, low-density lipoproteins, and triglycerides (Nasiff, 1993). Bunyapraphatsara, *et al.*, (1996) and Yongchaiyudha, *et al.*, (1996) studied that *A. vera* gel reduces blood glucose and serum triglycerides in *Diabetes mellitus*. *A. vera* and other plants that contain anthraquinones have an antiseptic effect against a number of bacteria and fungi (Steinegger and Hansel, 1988; Duke, 1997) and is very useful in the treatment of gastro-enteritis and peptic ulcers (Feily and Namazi, 2009).

Some results suggest that oral administration of *A. vera* in mice is effective on wound healing, reduce tumours and leishmania parasitemia by greater than 90% in the liver, spleen, and bone marrow.

1.4.2. Therapeutics of *Ocimum Sanctum*

Traditionally *O. sanctum* (tulsi) has been in use for thousands of years in Ayurvedic medicine for various types of wound healing. It is considered an adaptogen, balancing the processes of the body and allowing it to adapt to stressful situations. It promotes longevity (Voogelbreinder, 2009). Tulsi is used as common antidotes against colds, headaches, stomach disorders, inflammation, heart disease, poisoning and malaria. Different parts of the plant have been recommended for the treatment of bronchitis, diarrhoea, dysentery, skin disease, arthritis, cough, eye disease, etc. It also reduces blood glucose and cholesterol levels (Bhattathiry, 2011). Eugenol present in high levels in *O. sanctum* acts as a painkiller. Tulsi has anti-inflammatory properties. It helps in many skin disorders and is effective in skin rashes, insect bites and itching.

*O. sanctum* leaves act as nervous tonic and help to sharpen memory. It is beneficial in indigestion, intestinal parasites and constipation. Tulsi acts as a cardiac tonic and purifies
blood. The *O. sanctum* L. has also been suggested to possess anti-fertility, anti-cancer, anti-diabetic, antifungal, antimicrobial and anti-spasmodic actions. Basil has strengthening effect on the kidney and renal stone. The aqueous and methanol suspension of tulsi has shown to inhibit acute as well as chronic inflammation in rats. It was noted that tulsi extracts could significantly reduce the oedema when compared with the saline treated control (Singh and Majumdar, 1997; Kumar, 2013). *O. sanctum* has been found to be a powerful adaptogenic and antistress agent, helpful in preventing and reducing stress: mental, emotional, physical, and environmental stress. The immunostimulant capacity of *O. sanctum* may be responsible for the adaptogenic action of plant (Godhwani, *et al.* 1988; Singh, 1978; Bhargava, 1981; Srivastava, 1984). Aqueous and ethanolic leaf extracts of *Ocimum basilicum* Linn revealed strengthening of both specific and non-specific immune responses (Dashputre and Naikwade, 2010). It showed stimulatory effect on delayed type hypersensitivity (DTH) and significant improved humoral immunity (Vaghasiya, *et al.* 2010).

The pharmacological effects of *Ocimum sanctum* (tulsi) and *Aloe barbadensis* (*Aloe vera*) leaf extract possesses numerous healing properties and are easily available. These plants are hence selected for the present study to see its effect in the treatment of mancozeb induced health effects on albino mice (*Musculus albinicus*) for a stipulated time, exposure and dose level.

Hence the present study, “Studies On Herbal Protection Against Mancozeb (Ethylene Bis Dithio Carbamate Group) Induced Toxicity In Albino Mice” was undertaken to understand certain toxic nature of the fungicide mancozeb in albino mice and to see the possible healing or curative or protective effect of the selected herbal drugs against it.

### 1.5. AIM AND OBJECTIVES

The objectives of the present investigation will be to carry out a comprehensive study on the protective response of the selected herbal extracts against mancozeb induced toxicity in Swiss albino mice. The proposed research work will be emphasized on the following points:

1. To study the haematological parameters, biochemical parameters, immuno-logical parameters, in control group for finding normal values.
2. To study the haematological parameters, biochemical parameters, immuno-logical parameters in test group (positive control) for finding the toxic effects by the toxicant mancozeb.
3. Preparation of aqueous herbal extracts.

4. To study the haematological parameters, biochemical parameters, immuno-logical parameters in treated groups during treatment of mancozeb intoxicated mice with herbal extracts after withdrawal of mancozeb.

5. To study the haematological parameters, biochemical parameters, histochemistry and histopathological parameters and immunological parameters in treated group during simultaneous administration of mancozeb and herbal extracts in mice.

6. To study the histochemistry and histopathological parameters in kidney and liver in all the above groups.

7. Comparative study to find the result along with statistical analysis by finding significant value (p < 0.05).

The proposed investigation has a great significance in the field of public health in combating the side effects generated by the use of pesticides. It will probably be helpful in the field of chemotherapy and therapeutics. The results will surely help to find low cost and effective treatment of mass in general against pesticide induced health hazards in human being.