CHAPTER VI

SUMMARY

In the present world scenario the growing population, especially in India has increased the demand for agricultural productions. This along with the development of industries compels the use of harmful chemicals (fertilizers) and pesticides in large scale to solve the problem of food crises. The widespread and indiscriminate uses along with disposal of pesticides by farmers, large scale plantations, etc. have caused environmental contamination.

Some of these pesticides may be degraded into more toxic and resistant products (Fred Fishel, 1914) and use of such persistent products can lead to adverse by the process of biological magnification. At present there is a serious concern about environmental protection, human health, food safety and judicious pesticide use in agriculture.

The current study, “Studies on Herbal Protection against Mancozeb (Ethylene Bis Dithio Carbamate Group) Induced Toxicity in Albino Mice” is being performed on mancozeb, a fungicide of polymeric complex of zinc and manganese salts of ethylene-bis-dithio-carbamate (EBDC) group. A major toxicological concern with respect to mancozeb and other dithio-carbamates is its primary metabolite, ethylene-thiourea (ETU).

A study shows that mancozeb and its metabolites are capable of crossing the placental barrier and can produce DNA damage and initiate tumors in fetal cells as well as exhibit carcinogenic effects in test animals (Mehrotra et al., 1990). This fungicide may cause irritation of the skin, respiratory tract, and eyes (Houeto, et al., 1995).

Mancozeb has been shown to produce significant toxicological effects on thyroid (Kackar et al., 1997) & gonads in male and female rats (Mahadevaswami et al., 2000; Baligar and Kaliwal, 2001). It was found to exhibit tumor-initiating activity (Mehrotra et al., 1990) as well as teratogenic activity in rat (Lu et al., 1986). It also exhibited toxicity in chromosomes of bone marrow cells in mice (Kurinny et al., 1972). Due to complexities associated with synthetic pesticides, it is essential to use biocides and promote the use of herbal solutions to cure or minimize its effects (Worthing, 1987).
Since time immemorial medicinal plants have been used to cure or prevent diseases or to reduce toxic effects of toxicants. 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 with fewer side effects makes it suitable to use as alternative medicine. Several studies were conducted to observe the effect of herbal plant extracts in the treatment of various diseases induced by the pesticides.


The aqueous and ethanolic extracts of leaves 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 and significant improved humoral immunity (Vaghasiya, *et al.*, 2010). Basil has strengthening effect on the kidney and renal stone.

In the present investigation, the therapeutic effects of *Aloe barbadensis* Mill (*Aloe vera or guarpatha*) of Liliaceae and *Ocimum sanctum* Linn (*holy basil or tulsi*) of Lamiaceae family was assessed in the treatment of mancozeb induced health effects on albino mice using haematological, biochemical, enzymological, immunological, histochemical and histopathological parameters.
The main objectives of the present research work were -

- To study the haematological parameters, biochemical parameters, enzymological parameters, immunological parameters, histochemistry and histopathological parameters in control group (positive control) for finding normal values.
- To study the haematological parameters, biochemical parameters, enzymological parameters, immunological parameters, histochemistry and histopathological parameters in test group (negative control) for finding the toxic effects by the toxicant Mancozeb.
- To study the haematological parameters, biochemical parameters, enzymological parameters, immunological parameters, histochemistry and histopathological parameters in treated group during treatment of mancozeb intoxicated mice with herbal extracts after-withdrawal of mancozeb.
- To study the haematological parameters, biochemical parameters, enzymological parameters, immunological parameters, histochemistry and histopathological parameters in treated group during simultaneous administration of mancozeb and herbal extracts in mice.
- To analyze statistically the results obtained in all the above cases.

Methodology used for the present study is as follows-

1. Aqueous herbal extracts of Aloe vera (Aloe barbadensis Mill) by Bhaya, et al. (2008) method and Tulsi extract (Ocimum sanctum Linn) by Mahdi, et al. (2003) was prepared with little modification. Aloe vera was prepared at a dose of 400 mg/kg body weight and Ocimum sanctum at 250 mg/kg body weight.

2. The swiss albino mice, Mus albinus has been selected as the experimental animal after permission of ethical animal committee and were maintained following all the guidelines of anti cruelty laws.

3. Two doses 4.156 mg/kg body weight and 6.650 mg/ kg body weight of mancozeb were selected for the study. The mice were exposed to the toxicant by oral gavage for 6 days a week consecutively for 30 days.

4. Treatment with the selected herbal drugs was given by two ways:
   - Simultaneously along with the toxicant in both the two doses for 6 days a week consecutively for 30 days.
   - After-withdrawal of the toxicant after 30 days and administered in the same procedure herbal drugs was given using the same protocol for another 30 days.
5. After completion of requisite period the blood samples were collected from albino mice and assessment of the blood parameters were carried out. Likewise kidney and liver were dissected and preserved in 10% formalin for histological and histochemical observations in routine histological microtomy techniques.

6. Assessment of haematological, biochemical, enzymological and immunological tests were done in all the groups and analysed statistically using one way ANOVA.

In the present study, *Aloe vera* and *Ocimum sanctum* were found effective against mancozeb induced toxicity at the doses of 4.156mg/kg body weight and 6.650 mg/ kg body weight treatment. The results are summarized below:

**Haematological assay:**

The haemoglobin percentage values in mancozeb exposed groups were found to decrease as compared to the negative control value, with more decrease in high dose group. When the experimental mice were treated with different drugs, the haemoglobin value was found to increase.

The Red Blood Cell (RBC) count values in mancozeb exposed groups were found to decrease due to toxicity while in mice treated with different herbal drugs, the RBC count value were found increased as compared to those of positive control.

The White Blood Cell (WBC) count values in mancozeb exposed groups were found to increase while in experimental mice treated with different herbal drugs, the WBC count value was found to decrease as compared to those of positive control.

The Erythrocyte Sedimentation Rate (ESR) values in mancozeb exposed groups were found to increase while decrease in experimental mice treated with different drugs.

**Biochemical assay:**

The total serum protein values in mancozeb exposed groups were found to decrease while the experimental mice treated with different drugs the value was found to increase.

The total serum cholesterol values in mancozeb exposed groups were found to increase while the experimental mice treated with different drugs the value was found to decrease.

The blood glucose values in mancozeb exposed groups were found to decrease while the experimental mice treated with different drugs the results were found to improve.
**Enzymological assay:**

The Serum Glutamate Pyruvate Transaminase (SGPT) and Serum Glutamate Oxalate Transaminase (SGOT) values in mancozeb exposed groups were found to increase while the experimental mice treated with different drugs, the SGOT and SGPT values were found to decrease to that of control values.

The maximum improvement in all the above observations were shown by Recovery treated group of *Aloe vera* and least by Simultaneous treated group of *Ocimum sanctum*.

**Immunological assay:**

In mancozeb treated mice the differential leucocyte counts (DLC) of Neutrophils, Eosinophils, Basophils and Monocytes were found to increase while Lymphocytes count decreased as dose increases. In the experimental mice treated with different herbal drugs, the Neutrophils, Eosinophils, Basophils and Monocytes value was found to decrease while the Lymphocytes value increased. The maximum improvement of DLC was shown by Recovery Group of Aloe vera (Group VIII) and least by Simultaneous treated group of *Ocimum sanctum*.

Overall decrease in albumin and increase in globulin was observed in mancozeb exposed mice while when treatment was given with Aloe vera and Tulsi the reverse condition was observed. The A/G ratio in mancozeb exposed groups were found to decrease while increase in the experimental mice treated with different herbal drugs.

The Immediate Type Hypersensitivity (ITH) and Delayed Type Hypersensitivity (DTH) response in the mancozeb exposed albino mice increases as the duration of exposure increased while the values showed significant reduction during the treatment with therapeutic doses of *Ocimum sanctum* and *Aloe vera*. The ITH response in treated mice showed the humoral immunity against intoxication whereas the DTH response in treated mice showed the cellular immunity against intoxication.

Decrease of both ITH and DTH response in different experimental groups treated with herbal drugs demonstrates the immunomodulatory efficacy.

The maximum improvement in all parameters was shown by Recovery treated group of *Aloe vera* and least by Simultaneous treated group of *Ocimum sanctum*.

The **ANOVA** analysis showed high significance in haemoglobin, RBC, DLC, ESR, protein, cholesterol, glucose, SGPT, SGOT, polymorphocytes, lymphocytes, eosinophils
(except day 7), albumin (Day 30), globulin (except day 30), A/G ratio (except day 21), ITH and DTH. Significant results were seen in Monocytes (day 30), Eosinophils (day 7), A/G ratio (day 21).

The correlation analysis of the above study for haemoglobin depicted significant positive correlation was seen with RBC, protein, glucose, lymphocytes, albumin, globulin (day 7) and A/G ratio (day 21 and 30). Significant negative correlation was seen with WBC, ESR, cholesterol, SGPT, SGOT, monocytes and eosinophils.

During the present study RBC showed significant positive correlation was seen in with protein, glucose, lymphocytes, albumin, globulin (day 7) and A/G ratio (day 30). Significant negative correlation was seen with WBC, ESR, cholesterol, SGPT, SGOT, polymorphocytes, monocytes (days 7 and 30) and eosinophils.

WBC and ESR showed significant positive correlation with ESR, cholesterol, SGPT, SGOT, polymorphocytes, monocytes, eosinophils and A/G ratio (day 7). Significant negative correlation was seen with protein, glucose, lymphocytes, albumin (day 30), globulin (day 7) and A/G ratio (day 30). Cholesterol showed significant positive correlation with SGPT, SGOT, polymorphocytes, monocytes, eosinophils while significant negative correlation was seen with protein, glucose, lymphocytes, albumin negative correlation was seen with glucose, lymphocytes, albumin and A/G ratio (day 30).

Protein exhibited significant positive correlation with glucose, lymphocytes, albumin, globulin and A/G ratio (day 30) while negative correlation was seen with cholesterol, SGPT, SGOT, polymorphocytes, monocytes and eosinophils.

SGPT showed significant positive correlation with SGOT (in case of SGPT), polymorphocytes, monocytes (except day 15) and eosinophils while significant negative correlation was seen with lymphocytes, albumin (except day 15) and A/G ratio (day 21 and 30).

In case of SGOT significant positive correlation was seen with polymorphocytes, monocytes (except day 15), eosinophils and A/G ratio (day 7) while significant negative correlation was seen with lymphocytes (day 7), albumin (day 21) and globulin (day 7) and A/G ratio (day 21 and 30).

Polymorphocytes and lymphocytes showed reverse order of correlation. Polymorphocytes showed significant positive correlation with monocytes, eosinophils and significant negative correlation was seen with lymphocytes, albumin, globulin (day 7) and
A/G ratio (day 21 and 30). Lymphocyte showed significant positive correlation with albumin and globulin (day 7) and A/G ratio (day 30) and significant negative correlation was seen with monocytes and eosinophils.

Monocytes exhibited significant positive correlation with globulin (day 21) and eosinophils (day 30) while significant negative correlation was seen with albumin and A/G ratio (day 30). Eosinophils on the other hand showed no significant positive correlation while significant negative correlation was seen with albumin and A/G ratio (day 21 and 30).

Albumin exhibited significant positive correlation with globulin (day 7 and 30) and A/G ratio (except day 7) while no significant negative correlation was seen. Globulin showed no significant positive correlation while significant negative correlation was seen with A/G ratio.

**Histochemistry**

The qualitative analysis of histochemical demonstration in liver and kidney tissues for carbohydrates, proteins and lipids by Peroxide Acid Schiff, Mercuric Bromophenol Blue and Sudan Black III stains respectively, also justifies the quantitative biochemical results observed as above. The carbohydrate content decreased in the mancozeb exposed albino mice while during the treatment the content showed improvement. Similar observation was made with proteins. However, the lipid content showed increase in the mancozeb exposed groups while decrease in the herbal treated groups.

**Histopathology**

Mancozeb induced mice showed severe histopathological changes in the kidney and liver cells. Therapeutic doses of *Ocimum sanctum* and *Aloe vera* caused improvement of the damage induced by mancozeb, thereby bringing recovery of several pathological changes.

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 probably will be helpful in the field of chemotherapy and therapeutics. The results will help to find an effective treatment and preferably low cost alternative medicines (drugs) in general, against pesticidal health hazards among the public. Therefore, the main object of the present study was to focus on the applicability of herbal drugs, which can be used for curing the ill-effects of pesticides as well as for boosting the immune system for further prevention.
Overall better amelioration was seen in *Aloe vera* treated Recovery group (Gr VIII) as compared to *Ocimum sanctum* treated Recovery group. In the Simultaneous treated groups both the herbal extracts showed minimum. This probably suggests better curative property of the herbal extracts than preventive property. The low mancozeb exposed groups showed better recovery than the high mancozeb exposed groups possibly the dose of the herbal extracts were able to remove the burden of the toxicant from it.