CHAPTER V
DISCUSSION

Various types of insecticides, rodenticides and fungicides are being used extensively in the fields for increasing yield. However, irrational and indiscriminate usage had led to a widespread concern over the potential health hazards of these chemicals on human and animal health (Al Saleh, 1994). In view of increasing application of the fungicide mancozeb in agricultural practices, it was thought worthwhile to investigate the nature and extent of its effect on biochemical constituents in the body of Swiss albino mice.

Though in general mancozeb has low acute toxicity, yet it produces significant toxicological effects on various organs including carcinogenic and teratogenic activities, etc. Repeated administration of various doses of mancozeb at the rate 4.156 mg/kg body weight and 6.650 mg/kg body weight as well as the aqueous extract of Aloe vera at 400 mg/kg body weight and aqueous extract of Ocimum sanctum at 250 mg/kg body weight was administered for different time duration and comparisons of all end points in treated versus control group were determined to investigate the potential effects on health.

In the present study, “Studies on Herbal Protection against Mancozeb (Ethylene Bis Dithio Carbamate Group) Induced Toxicity in Albino Mice” various parameters were considered to see the possible toxic effects of mancozeb exposure and thereafter the possible therapeutic properties (curative or protective effects) of selected herbal plant extracts by assessing the tissues and blood samples. Hence to assess this, investigation on clinical signs of haematological parameters such as haemoglobin, RBC, TLC and ESR; the biochemical parameters such as protein, cholesterol and glucose were measured to investigate the effect of mancozeb in the body metabolism. The enzymological parameter such as Serum Glutamate Pyruvate Transaminase (SGPT) and Serum Glutamate Oxaloacetate Transaminase (SGOT) were determined to find out the effect of mancozeb on the hepatic system and other possible cellular damage in organs. The immunological parameters such as Differential Leucocyte Count, Immediate Type Hypersensitivity, and Delayed Type Hypersensitivity, albumin, globulin and Albumin / Globulin (A/G) ratio were measured to see the possible immunity problems. Such blood constituents serve as a valuable index to reflect the general health of the animals.
A large number of biological activities have been ascribed to *A. vera* and *O. sanctum* to explain its purported health benefits, including antimicrobial, anti-inflammatory, lipid and glucose lowering, analgesic, chemopreventive, antipyretic, immunostimulatory and antioxidant functions. A number of potentially active ingredients have been identified such as aloins A and B and 5-hydroxyaloin A, aloeresins, aloe emodin etc in aloe and eugenol, euginal, urosolic acid, alkaloids, flavonoids, polysaccharides, etc in tulsi.

However, much has yet to be determined about their mechanisms of action. Reportedly, *Aloe vera* does not have a single mechanism of action. It works like an Orchestra by the help of one of a polysaccharide (mannose molecules joined by beta I-4 linked chain), which acts as the conductor that leads a symphony composed of these 200 plus biologically active compounds. This conductor-orchestra relationship creates an infinite array of biological activities.

The leaf extract of *O. sanctum* modulates humoral immune response and lowers the uric acid levels and hence is considered as a potential anti-inflammatory agent. It is a good source of antioxidants and offer substantial protection against free radical induced damage. The mechanism of action of the anti-inflammatory effects of tulsi could be the cyclo-oxygenase and lipoxygenase pathways (Ahmed, et al. 2002). Presence of eugenol attributes to anti-oxidative property and is also thought to be responsible for inhibition of lipid per oxidation.

The mean sum of squares based on ANOVA of the various groups under pooled analysis over 3 years i.e., 2010-2013 for all the 15 parameters as mentioned above during 7, 15, 21 and 30 days of observation has been presented in Tables 2-62.

### 5.1. THE HAEMATOLOGICAL ASSAYS

#### 5.1.1. Haemoglobin gm percentage and RBC count per cubic millimeter

Haematological parameters are used in the assessment of protein quality and utilization. Alterations in the qualitative and quantitative composition are warning signals of impaired function and are first detectable and quantifiable responses to environmental changes (Stroev, 1989). Thus, haematological profile is an important index of the physiological state of an individual.
During the present study exposure to mancozeb decreased the **haemoglobin** (gram per decilitre) content in intoxicated mice in comparison to control mice. Highly significant (p<0.01) reduction in haemoglobin content as compared to control, was observed in Group (Gr) III (mancozeb with higher dose) and Gr II (mancozeb with lower dose) with severe deterioration in the former. This reduction in haemoglobin value may be due to excessive protein utilisation and breakdown of haemoglobin, the phenomenon observed during anaemia of various types, erythropoietin deficiency from kidney disease, nutritional deficiencies, bone marrow disorders, etc. When kidney histopathology was studied tissue degeneration was seen which might also suggest haemoglobin production deficiency.

In the present study, the decrease in **RBC count** (X 10^6 cubic millimeter) during exposure to mancozeb, was found to be highly significant (p<0.01) in intoxicated Gr II and III, with more decrease in the latter as compared to that of the control. This reduction might be due to the excessive cell destruction or decreased cell proliferation.

Collectively, the decrease in the erythrocyte count and haemoglobin content recorded in the present work indicates that mancozeb-treated mice were anaemic or there was homeostatic disturbance in the haemopoietic organs. The anaemia may be due to the inhibition of erythropoiesis and haemosynthesis and to an increase in the rate of erythrocytes destruction in haemopoietic organs. This is in accordance to the earlier findings (Yadav and Akela, 1993). According to Mahadevaswami, *et al.*, (2001) anaemia may be due to increased blood resulting from accelerated red cell destruction by haemolytic agents or rapid cell removal from an abnormality of cell shape or over-activity of the spleen or quantitative decrease in blood formation or quantitative decrease in marrow activity from deficiency in required substances. These results are in agreement with Demsia, *et al.*, (2007) which reported that rats fed with diets containing thiophanate-methyl fungicide showed a decreased RBC count and lower haemoglobin content.

Similar to this study, Bhatia, *et al.*, (1996) observed significant decline in RBC, haemoglobin content, etc. in malathion (0.1 mg) treated mice while Wael, (2012) found similar results in metalaxyl induced albino mice. Fathia, *et al.*, (2005) also showed decline in haemoglobin content and haematocrit values in dimethoate treated mice. Auletta, (1992) found that male dogs fed with thiophanate-methyl fungicide capsules showed decrease in their total erythrocyte counts as well as their haemoglobin and haematocrit values. Basir, *et al.*, (2011) noted that a blood analysis of rabbits treated with lambda-cyhalothrin revealed a significant decrease in red blood cell and haemoglobin concentration.
In the herbal treated groups (both simultaneous and after withdrawal) showed highly significant (p<0.01) changes in the haemoglobin values than control from day 7 to day 21. However, on day 30 highly significant (p<0.01) changes were only observed in groups II, III, VI, VII, IX and XI. In all the groups, the haemoglobin percentage was increasing as compared to that of Gr II and III. However, the value of haemoglobin returned to almost that of control in the rest of the groups with significantly better increase in Gr VIII. The ANOVA values between the treated groups as compared to the control showed highly significant (p<0.01) values.

In the herbal treated groups (both simultaneous and after withdrawal) highly significant (p<0.01) recovery changes in RBC count were also observed on all days of observations. However, in all these groups (Gr IV to XI) increase in the RBC count was seen as compared to that of Gr II and III demonstrating positive effect of the treatment. Highly increased value of RBC count was observed in Gr VIII on day 30 as compared to that of control than in the rest of the groups showing better recovery among the treated groups. The ANOVA values between the treated groups as compared to the control showed highly significant (p<0.01) values.

The results obtained during the present study are in harmony with others authors. There was an increasing RBC and WBC contents of *Clarias batracus* treated with aqueous extracts of *O. sanctum* which may be due to the effect of its bioactive principle and ascorbic acid to protect murine peritoneal macrophage from deleterious effect of nicotine and, simultaneously, help to restore their normal functions. *O. basilicum* enhanced RBC, haemoglobin in the common carp, *Cyprinus carpio* (Abasali and Mohammed, 2010). Iji, *et al.*, (2010) reported that the haemoglobin values of the rats administered with 100 mg/kg and 500 mg/kg *A. vera* gel were higher (p<0.05) than that of the control i.e. *A. vera* gel stimulated erythropoiesis in rats. This attribute may be due to the presence of thiamine, riboflavin, folic acid and other essential and non essential amino acids in the mucilaginous gel (Hamman, 2008). The polysaccharides have also been reported to stimulate erythropoiesis (Choi and Chun, 2003 and Ni, *et al.* 2004, Naveena, *et al.* 2011, Pecere, *et al.* 2000) because of its thiamine content.

Better results were obtained in the recovery group of *A. vera* (Gr VIII) than *O. sanctum*. Probably, the dose was effective against the after withdrawal of the low dose of mancozeb administered and was able to bring recovery from the damage done by mancozeb.
In the simultaneous treatment group also better results were seen in the *A. vera* group than *O. sanctum*.

Hence a perusal of data in Table 4 showed that variance for haemoglobin in all the days of observations were highly significant indicating appreciable results showing that the treatment given to mancozeb exposed mice with *A. vera* and *O. sanctum* was effective. Minimum difference was obtained in Gr VIII (D1/AV) on day 30 which was nearly towards the control value. Thus group VIII was best able to bring the haemoglobin value to the normal level among the other groups. Highly significant differences were observed for RBC in all the days of observation in Table 7. Minimum difference was obtained in Gr VIII (D1/AV) on day 30 indicating its highly appreciable effective nature in ameliorating the impact created by mancozeb in mice.

### 5.1.2. Total Leucocyte Count (TLC)

The TLC (cubic millimeter) in peripheral blood is an index of functional status of cellular defence in the body (Benjamin, 1978). In the present study, the TLC count showed highly significant (p<0.01) increase in the intoxicated Gr II and III, with more increase in the latter as compared to that of the control. Similar findings were reported by other authors. This may be due to the increase in the host immune response, both cellular and humoral defence of the body by increasing leukocyte mobilization to protect the mice against infection that might have been caused by chemical and also secondary infections, which may be contracted after the weakening condition of the mice.

Ismail and Huseyin, (2008) reported that methyl parathion during 28 day sub-acute exposure significantly increased WBC in rats suggesting elevation of tissue damage and organ toxicity. Wael, (2012), Choudhary and Joshi, (2002) and Johnson and Aderele, (1986) also found similar results in metalaxyl induced albino mice and oral chlordane induced mice respectively. Variable values were found in TLC count in dimethoate treated mice (Fathia, *et al.* 2005).

In the herbal treated groups (both simultaneous and afterwithdrawal) highly significant (p<0.01) changes were also observed on days 7 to 21. Highly significant (p<0.01) changes were observed in Gr II and III only on day 30. The present treatment normalized the levels of blood cell counts, and other indices in all groups and the TLC count was seen to
return towards that of the control value on day 30 in the treated groups of *A. vera* and *O. sanctum*. Significant relative decrease of TLC was seen in Gr VIII which neared to that of the control value. The ANOVA values between the treated groups as compared to the control showed highly significant (p<0.01) values. Thus, both the test drugs induce immune response against the intoxication.

Many researchers reported the role of ayurvedic drugs in TLC against exposure to toxicants. The present results are in harmony with other findings (Heba, *et al.* 2011 and Janeway, 2005). The effect of *A. vera* gel at 1000 mg/kg dose against sulphur mustard-induced systemic toxicity and skin lesions significantly decreased WBC count, and significantly increased RBC count and haemoglobin concentration (Anshoo, *et al.* 2005). Aloe exhibits anti-inflammatory property (Davis, *et al.* 1989) may be by inhibiting arachidonic acid pathway through cyclooxygenase (Vazquez, *et al.* 1996) and by amino acids such as phenylalanine and tryptophan. Barbaloin and other products of the phenylpropanoid pathway present in *A. vera* may act as antioxidants to inhibit free radical-mediated cytotoxicity (Cook and Samman, 1996). Since prostaglandins play an integral role in regulating both inflammation and immune reactions, *A. vera* may affect both of these systems by blocking prostaglandin synthesis.

Mediratta, (2002) and Abasali and Mohammed, (2010) showed the immunomodulatory potential of *O. sanctum* and other herbal immunostimulants respectively. *O. sanctum* oil was found to inhibit enhancement of the vascular or capillary permeability and leukocyte migration following inflammatory stimulus. Linolenic acid present in the oil could be responsible for this activity (Singh, *et al.* 1996; Singh and Majumder, 1999). The anti-inflammatory activity during chronic inflammation in rats by methanolic and aqueous suspension of *O. sanctum* was also reported by Godhwani, *et al.*, (1987) and Singh, *et al.*, (1997). The protective effects of *A. vera* and *O. sanctum* are most likely due to its antioxidant potential. The present treatment normalized the levels of blood cell counts, and other indices. The phagocytic activity of the blood leucocytes in *A. vera* and *O. sanctum* extract-treated group was significantly higher than the placebo group. This result supports the findings of enhanced phagocytic activity of leucocytes in rainbow trout by other medicinal herbs (Dugenci, *et al.* 2003; Haghighi and Sharif Rohani, 2013).

Better results were obtained in the recovery group of *A. vera* (Gr VIII) than *O. sanctum*. The dose probably was effective against the afterwithdrawal of the low dose of mancozeb administered and was able to bring recovery from the damage done by mancozeb.
Highly significant differences were observed for WBC in all the days of observation in Table 10. Minimum difference was obtained in Gr VIII (D1/AV) on day 30 indicating its highly appreciable effective nature in curing the impact created by mancozeb in mice.

5.1.3. Erythrocyte Sedimentation Rate (ESR)

The ESR (millimeter per hour) values indicate the infections present in the body. In the present study, the ESR values showed highly significant (p<0.01) increase in the intoxicated Gr II on day 21 and 30 and in Gr III on all days of observation. The values were higher in the latter group as compared to that of the control. ESR increases in all conditions of tissue breakdown, entry of foreign proteins in blood which was also evident in histopathological analysis. Changes in fibrinogen and globulins and increase in cholesterol also accelerates sedimentation while high albumin retards sedimentation which is similar to our finding in the present study. Similarly, ESR was found to increase in fungicide treated groups by Auletta, (1992) in male dogs. ESR and in the mercuric chloride treated fish Caspian brown trout (Salmo trutta caspius). This finding was similar to Atamanalp and Yanik, 2003; Atamanalp, et al., (2010) and Kumar, et al., (1999). Not much information is available on mancozeb effect of ESR on albino mice.

Decrease in the ESR values was seen in the herbal treated groups (both simultaneous and afterwithdrawal) with highly significant (p<0.01) changes on day 7 to day 21. Gr VIII showed improvement and decrease of ESR value from day 15 to 30 and showed better improvement as compared to the other treated groups with the control. Gr IV also showed low ESR values from day 21 to 30 while Gr V on day 30. The ANOVA values between the treated groups as compared to the control showed highly significant (p<0.01) values. Better results were obtained in the recovery group of A. vera (Gr VIII) than O. sanctum. Probably, the dose was effective against the afterwithdrawal of the low dose of mancozeb administered and was able to bring recovery from the damage done by mancozeb. Not much information is available on effect of A. vera and O. sanctum on mancozeb induced mice.

In Table 13, highly significant differences were observed for ESR in days 7, 15 and 21 of observation. Minimum difference was obtained in Gr VIII (D1/AV) on day 15, 21 and 30 indicating its highly appreciable effective nature in curing the impact created by mancozeb in mice. Least differences were also obtained in Gr IV on day 21. On day 30
significant differences was obtained on Gr II and III. Minimum differences were obtained in all the rest groups indicating significant effectiveness in treating mancozeb exposed mice the best group being Gr VIII.

The results of pesticides on different haematological parameters have been a matter of controversy and are still debatable. However, the type of insecticide, duration of exposure, dose and experimental animal and prevailing laboratory conditions determine the changes on haematological parameters.

5.2. BIOCHEMICAL ASSAYS.

It is an established fact that the carbohydrates, proteins, lipids and other structural components of tissues and the blood of living subjects are drastically influenced by insecticides (Bhatia, et al. 1973, Onikienko, 1966).

5.2.1. Total Serum Protein

Proteins play an important role by involving in the architecture and physiology of the cell and occupy a key role in the cell metabolism (Yeragi, et al. 2003). The protein synthesis is disturbed by pesticides. In the present study, the decrease of the total serum protein (gram/decilitre) was observed highly significant (p<0.01) in the mancozeb intoxicated Gr II and III on all days of observation, with severe depletion in the latter. The decline in the protein level indicates an acceleration of protein catabolism during mancozeb intoxication. Such loss of proteins was also reported by Richardson, (1981) due to pesticide toxicity. Naqvi, et al., (1986), Javid, (1989), Nizam, (1993), Saleem, et al., (1998), Ahmed, et al., (2000), Tabassum and Naqvi, (2001) also observed similar a reduction in total protein contents after pesticide application in different insects. Mancozeb leads to alterations in the content of proteins, carbohydrate, and amino acid metabolism leading to decrease of protein translation (Pedro, et al. 2009). The present finding confirms earlier findings. With the increase in the exposure period the activity of the body was found to reduce along with feeding and utilization of endogenous protein which causes reduction in the protein content (Ramlingam and Ramlingam, 1982; Nazeeul, et al.1991) or due to tissue destruction by necrosis or disturbance of cellular fraction and consequent impairment in protein machinery (Bradbury, et al. 1987). Such levels of change in plasma proteins such as fibrinogen and globulins usually take place during acute and chronic infections which usually occurs during toxic effects. The decline in the protein content in the present study indicates acceleration of
protein catabolism during mancozeb intoxication as well as disturbance in various metabolic activities and normal physiology of mice.

In the herbal treated groups (both simultaneous and after-withdrawal) highly significant (p<0.01) differences were also observed on days 7 to 30 as compared to the control value. Gr VIII and X showed good recovery of total protein content on day 30. The ANOVA values between the treated groups as compared to the control showed highly significant (p<0.01) values. This was also evident in histopathological and histochemical analysis. Acetylated glucomannan present in Aloe vera is primarily responsible for accelerating wound healing (Hamman, 2008; Ulbricht, et al. 2008). The anti-oxidative stress property helps in maintaining good health and in preventing the chances of the other biochemical diseases (Hannan, et al. 2006) which is present in both Ocimum sanctum and Aloe vera. Ocimum sanctum significantly found to increase the protein level in brain according to Tabassum, et al., (2009). Thus, better results were obtained in the recovery groups of Aloe vera and Ocimum sanctum with more curative properties in the former in being able to significantly increase the depletion of protein content in serum.

In Table 16, highly significant differences were observed for Total Serum Protein in all days of observation. The minimum difference was obtained in Group IV (D1+AV) and VIII (D1/AV) on day 15 and day 30 indicating its highly appreciable effective nature in curing the impact created by mancozeb in mice. The maximum increase of protein was seen in Recovery group of A. Vera Gr VIII indicating its effective nature in curing the depletion of protein during mancozeb intoxication.

5.2.2. Serum Cholesterol

Cholesterol is the precursor for steroid hormones and also for vitamin D, which is essential for regulation of calcium and phosphorous metabolism and bone growth. Cholesterol is essential for membrane synthesis.

During the present study, highly significant (p<0.01) increase in the cholesterol level (milligram/ decilitre) was observed in in the mancozeb intoxicated Gr II and III on all days of observation, with higher increase in the latter. The results confirm the finding of Sakr, et al., (2008) and Kluwe, (1981) and Ksheerasagar, et al., (2010). Wael, (2012) found increase in serum triglyceride and cholesterol levels in metalaxyl treated albino mice. Shivanandappa and Krishnakumari, (1981) reported that there was an increased serum cholesterol level in the rats exposed to benzene hexachloride suggesting increased synthesis and accumulation of
cholesterol in the liver, kidney and testis and impaired biliary function. This may be due to metabolic disorders like increase in lipogenesis probably due to acceleration of acetyl-CoA, the precursor of cholesterol biosynthesis or hepatic disorder like fatty liver and impaired biliary secretion, certain renal disorders like nephritis (Richmond, 1973). Dysfunction of steriodogenic function of leydig cells could also increase the level of cholesterol (Zine Kechrid, 2007). In the present study, increase in cholesterol might also be due to inhibition in the activity of enzymes involved in cholesterol break up thereby resulting in its deposition into cells.

In the herbal treated groups (both simultaneous and after withdrawal) highly significant \((p<0.01)\) differences were also observed on days 7 to 30 as compared to the control value. Gr IV, VI, VIII and X showed decrease in the cholesterol content on day 30, with highest significant decrease in cholesterol level in Recovery group of \(A.\ vera\) Gr VIII towards that of the control. Better results were obtained in the recovery groups than in simultaneous groups. The ANOVA values between the treated groups as compared to the control showed highly significant \((p<0.01)\) values. This was also evident in histopathological and histochemical analysis.

The antioxidant property of \(A.\ vera\) and \(O.\ sanctum\) due to the presence of flavonoids, triterpenoids, steroids, etc. (Naveena, 2011; Geetha and Vasudevan, 2004) which helps in lowering the cholesterol level. Similar reports of \(O.\ sanctum\) leaf extract reducing total cholesterol, triglyceride, phospholipids and total lipids in rats and rabbits were reported (Rai, 1997 b and Sarkar, et al. 1994). Aqueous extract of \(O.\ sanctum\) also provided significant liver and aortic tissue protection from hypercholesterolemia induced peroxidative damage (Geetha and Vasudevan, 2004). The dose dependent reduction in serum cholesterol of the treated fishes may be due to the levels of polyphenolic compounds present in the aqueous extract of \(Ocimum sanctum\) (Gupta, et al. 2002; Aswar and Joshi, 2010) along with the inhibition of cholesterol biosynthesis in the liver (Oyewo, et al. 2012). Aloe gel has cholesterol lowering properties and inhibits cholesterol absorption as reported by Tizard, et al., (1989), Rajasekaran, et al., (2005). Phytosterols, one of the major constituents of \(A.\ vera\) has been found to reduce visceral fat accumulation, improve hyperlipidemia and hyperglycemia (Misawa, et al. 2008). This reduction may be attributed to increased clearance and decreased production of the major transporters of endogenously synthesized cholesterol and triglycerides (Subbiah, et al. 2006). When given orally to animals, mannans have also been shown to inhibit cholesterol absorption and lower cholesterol (Sikarwar, et al.
2010). Subbiah Rajasekaran, *et al.*, (2006) reported that the presence of phenolic compounds and saponins in the gel extract of *A. vera*. Thus, the anti-oxidants present in the *A. vera* extract may be responsible, in part, for the anti-hyperlipidemic effect of the gel extract.

Highly significant differences were observed for Total Serum Cholesterol in all the days of observation in Table 19. Minimum difference was obtained in Group IV, VI, VIII and X on day 30 with the least value in Group VIII indicating its highly appreciable effective nature in curing the impact created by mancozeb in mice.

5.2.3. Blood Glucose

The major form of carbohydrate present in the cells of the body is glucose (Develin, 1992). During the present study, highly significant (*p*<0.01) decrease in the glucose level (milligram/decilitre) was observed in the mancozeb intoxicated Gr II and III on all days of observation, with severe depletion in the latter. This finding was in agreement with Zine Kechrid, (2007). This may be due to low thyroxine level caused by impaired thyroid function (Nebbia and Ferrero, 1991). According to Sood, *et al.*, (2000) the causes of hypoglycaemia were due to acute stress reaction or severe liver diseases and adreno-cortical insufficiency. Significant decrease in the levels of blood glucose in mancozeb treated rats was observed due to low thyroxine level and impaired thyroid function (Ksheerasagar, *et al.* 2010). In the present study this was also evident in histopathological and histochemical analysis of liver and kidney.

In the herbal treated groups (both simultaneous and after-withdrawal) highly significant (*p*<0.01) differences were also observed on days 7 to 21 as compared to the control value. Recovery of glucose level was seen in day 30 in the herbal treated groups except Gr VII with least recovery. Highest recovery was seen in the Recovery Groups of *A. vera* Gr VIII where the depletion of glucose was improving and nearing that of the control. In the present study, better results were again obtained in Recovery group of *A. vera* and least with simultaneous group of *Ocimum sanctum*. The ANOVA values between the treated groups as compared to the control showed highly significant (*p*<0.01) values. This was also evident in histopathological and histochemical analysis. Administration of *A. vera* at 100mg/kg inhibited the increase in body weight and also brought back the serum biochemical and haematological parameters towards the normal levels. (Naveena, *et al.* 2011). Overall blood biochemical parameters were brought to normal levels in broilers in the
experiment shown by Rozbeh Fallah, (2014). *O. sanctum* leaf extract contains eugenol which is effective in normalising the glucose content in blood (Prakash and Gupta, 2005).

Increased transaminase activity as observed in the present study also decreases the carbohydrate and protein content. Similar observations in biochemical constituents were also found by Raghavendra, *et al.*, (2010). The changes in the levels of protein, glycogen and total lipids with mancozeb treatment suggests either an increased catabolism of the biomolecules to meet the enhanced energy demand of animals under stress or their reduced synthesis due to impaired tissue function (Ivanova Chemishanska, 1982). Wael, (2012) reported increase in serum triglyceride and cholesterol levels while decrease in total protein and albumin levels in metalaxyl induced mice. Mahadevaswami, *et al.*, (2000) and Baligar and Kaliwal, (2001) have reported that mancozeb and carbofuran treatments have altered levels of protein, glycogen and total lipids in liver, uterus and ovary in intact and hematicastrated rats and mice.

Ethanolic *O. sanctum* leaf extract inhibits oxidative stress by reducing the extent of lipid and protein oxidation, lowering cholesterol, lactate dehydrogenase and alkaline phosphatise levels, and up-regulating antioxidant defences without affecting the glucose and urea levels in rats (Vats, *et al.* 2004). Eugenol present in *O. sanctum* is found to be effective on blood biochemistry (Prakash and Gupta, 2005). Similar results were shown by Gupta, *et al.*, (2005) with *A. vera*.

Thus, in Table 22 highly significant differences were observed for Serum Blood Glucose in all days of observation. Least improvement was seen in Simultaneous group of *O. sanctum* Group VII and highest recovery was seen in Recovery group of *A. vera* Gr VIII (D1/AV) on day 30 indicating its highly appreciable effective nature in curing the impact created by mancozeb in mice.

### 5.3. ENZYMEOLOGICAL ASSAYS

Aminotransfersases (Serum Glutamate Pyruvate Transaminase; SGPT and Serum Glutamate Oxaloacetate Transaminase; SGOT) are involved in amino acid metabolism and are the first enzymes to be used in diagnostic enzymology when liver damage occurs as an indicator of liver function (Whittby, *et al.* 1981; Kuchel and Ralston, 1988), because of their intracellular location in the cytosol. Toxicity affecting the liver, cause subsequent breakdown in membrane architecture of the cells leading to their spillage into plasma thereby increasing their concentration (Klassen and Plaa, 1966).
5.3.1. SGPT and SGOT

In the present study, highly significant (\(p<0.01\)) increase in the SGPT and SGOT (\(\mu\) moles/litre/minute) level was observed on all days of observation in the mancozeb intoxicated Gr II and III, with higher increase in the latter. The increase in the enzymes may be due to increased transamination for rapid breakdown of carbohydrates and proteins to compensate the increased energy crisis resulting from mancozeb intoxication which also suggests the decrease in these contents in the present study. The aminotransferases functions as a strategic link between carbohydrate and protein metabolism by converting \(\alpha\)-ketoglutarate acid and pyruvic acid on one hand and alanine and aspartic acid on the other hand (Knox and Greengard, 1965).

A significant rise in the enzymes could indicate probable liver damage such as hepatitis, cirrhosis, obstructive jaundice, etc. It markedly increases during hepatic necrosis and indicates infectious and toxic hepatitis as these enzymes are released in the blood circulation after mancozeb treatment. The toxicity is due to formation of a reactive metabolite trichlormethyl radical by microsomal fixed function oxidase which binds covalently to the macromolecules and induces peroxidative degradation of membrane lipids resulting in hepatotoxicity and subsequent increase in serum transaminase (Cabre, et al. 2000 and Kandasamy, et al. 2010 and Lavric, et al. 1990). According to Choudhary, et al., (2003); Barlas, (1996) and Srivastava, et al., (1989) such rise may be due to nephrotoxicity and hepatotoxicity causing permeability alterations and leakage of lysosomal enzymes and enhanced release of enzymes. Metalaxyl treatment in mice was found to increase the levels of transaminases probably due to fungicide-induced oxidative stress which leads to signs of toxicity (Dasgupta, et al. 2011). Similar findings were also documented by Wael, (2012) in mancozeb treated male rats suggesting cellular damage or increased permeability of plasma membrane for such rise.

This finding is in harmony with those reported by Srivastava, et al., 1989; Klassen and Plaa, 1966; Snow and Watson, (1973); Enan, (1983); Choudhary, et al., (2003). Begum, (2007) found the activity levels of SGPT and SGOT increased in liver and muscle tissues of *Clarias batrachus* when exposed to carbofuran. Murugesan, et al. (1999) also found that *Sarotherodon mossambicus*, when exposed to sublethal and lethal concentrations of carbaryl, showed adaptive elevation in the activity levels of serum transaminases enzymes, particularly in liver and muscle. Nephrotoxicity also increase the levels of these enzymes due
to altered permeability and leakage of lysosomal enzymes (Barlas, 1996; Wroblewski and La Due, 1955).

In the herbal treated groups (both simultaneous and after withdrawal) highly significant (p<0.01) differences were also observed on days 7 to 30 in SGPT values while on days 7 to 21 in SGOT values as compared to that of control value. Significant highest decrease was seen in day 30 in the herbal treated groups was seen in Gr VIII for SGPT values and SGOT values. Highest recovery was seen in the recovery group of A. vera and least with O. sanctum, though some improvement was seen in both where the increase of the above enzymes was decreasing and nearing that of the control. Thus, again better results were obtained in the recovery groups than in simultaneous groups. The ANOVA values between the treated groups as compared to the control showed highly significant (p<0.01) values.

O. sanctum leaf extract (10mg/kg body weight) significantly lowered the serum transaminases enzymes levels in mercury induced toxicity in albino mice (Sharma, et al. 2002) and exerts hepatoprotective effect in the models of predictable hepatotoxicity like paracetamol and carbon tetrachloride induced liver damage in rats (Bhargava and Singh, 1981 and Chattopadhyay, et al. 1992). Sen, et al., (1988) reported that the membrane stabilizing property of O. sanctum has been shown to be responsible for its hepatoprotective action. Though exact mechanism is not known its antioxidant property may be responsible for this action. Iji, et al., (2010) reported that A. vera gel also significantly reduced the plasma SGOT and SGPT and alkaline phosphatase of the treated rats. This might be because of the hepatoprotective and antioxidant properties of the gel extract. For example, Chadan, et al., (2007) reported that aqueous extract of A. vera significantly reduced hepatic damage induced by carbon tetrachloride in mice and reversed certain biochemical parameters. The hepatoprotective action was also attributed to preserving the metabolizing enzymes of the liver through an antioxidant activity (Chandan, et al. 2007). The reversal of SGOT and SGPT activity in A. vera-treated diabetic rats towards near normalcy is evidence of the prevention of cellular and tissue damage under diabetic conditions, which may further strengthen the optimized lipid metabolism in the liver of diabetic rats (Hearse, et al. 1979). The elevated levels of renal lipid contents in histochemistry observed in the present study are consistent with those reported previously.

In Table 25, highly significant differences were observed for SGPT in all days of observation. Minimum difference was obtained in Gr VIII to X and on day 30 and indicating
its highly effective nature in curing the impact created by mancozeb in mice with the best result being shown by Gr VIII. Highly significant differences were observed for SGOT in all the days of observation in Table 28. Minimum difference was obtained in Gr VIII and X on day 21 and Gr IV to XI on day 30 and with the least value in Gr VIII indicating its highly appreciable effective nature in curing the impact created by mancozeb in mice.

5.4. IMMUNOLOGICAL ASSAYS

One of the important mechanisms suggesting the deleterious effect of pesticides is by generating free radicals and derangement of antioxidant mechanisms.

5.4.1. Differential Leucocytes Count (DLC)

DLC (percent) helps in identifying the changes in the distribution of white cells and its role during infection, intoxication or treatment and the Differential Leucocyte Count in blood significantly expresses the immune response of the individual.

Polymorphocytes and eosinophils; lymphocytes and monocytes

In the present study, highly significant (p<0.01) increase in the counts (%) of polymorphocytes and eosinophils was observed to in the mancozeb intoxicated Gr II and III on all days of observation, with higher increase in the latter. Eosinophils count was fluctuating in the experimental study probably to compensate the Total Leucocyte Count (TLC). Polymorphocytes imparts resistance to the toxicant while eosinophils helps to kill the foreign or damaged cells the eosinophils are recruited from the circulation into inflammatory foci where they may modulate immune responses through an array of mechanisms. They have an important pro-inflammatory role in various immune disorders. Bortoletti, et al., (1989, 1992) demonstrated the role of cell- mediated immunity indicated by the increase of eosinophils and mast cells. Hence in the present study increase in the eosinophils and polymorphocytes count suggest their role in imparting immunity to fight against mancozeb toxicant. Increase in polymorphocytes was observed during leucocytosis as was seen during the present study.

In the herbal treated groups (both simultaneous and afterwithdrawal) highly significant (p<0.01) differences were also observed on days 7 to 21 in case of polymorphocytes while on day 7 to 15 in case eosinophils as compared to that of the control value . Decrease of polymorphocytes was seen in day 30 in the herbal treated groups except
Gr VII and XI of *O. sanctum*. This is due to suppression of bone marrow by drugs which decreases the neutrophils content. Highest recovery was seen for polymorphocytes in the recovery groups of *A. vera* Gr VIII where the counts of polymorphocytes was improving and nearing that of the control.

Better results in polymorphocytes values were obtained in the recovery groups than in simultaneous groups. Recovery of eosinophils was seen in day 30 in the herbal treated groups except Simultaneous group of *O. sanctum* Gr VI. Highest recovery was seen for eosinophils in the recovery groups of *Aloe vera* Gr VIII and good results were obtained in Recovery group *A. vera* IX and *Ocimum sanctum* Gr X where the counts of eosinophils was improving and nearing that of the control. Better results in eosinophils values were obtained in the recovery groups than in simultaneous groups in both *A. vera* and *O. sanctum* with best results of *A. vera*. The ANOVA values between the treated groups as compared to the control showed highly significant (p<0.01) values for all days except significant (p between 0.05 to 0.01) values for day 7 in case of eosinophilis.

In the present study, (highly significant, p<0.01) decrease in lymphocytes count (%) was observed in the intoxicated Gr II and III on all days of observation, with higher decrease in the latter. Similar observation was reported by Isaak, *et al.*, (1977). Bashir, (2011) noted that a blood analysis of rabbits treated with lambda-cyhalothrin revealed a significant decrease in lymphocytes. Bhagwant and Johri, 1986 found decrease in the lymphocyte count to compensate the neutrophils and eosinophils increase in TLC. The monocytes count (%) was fluctuating probably to compensate the TLC values. Significant (p between 0.05 to 0.01) increase was observed in the intoxicated Gr II and III on day 30 with higher increase in the latter. Slightly different results were obtained by Fathia, *et al.*, (2005) who found significant difference in neutrophils and lymphocytes as compared to control in dimethoate treated mice whereas monocytes, eosinophils and basophils showed no significant difference. Also Janeway, (2005) explained the reduction in the number of lymphocytes may be due to decreased production or rapid removal from circulation and subsequent destruction. The reduction of lymphocytes is indicative of immunosuppressive effects of organo-phosphates which may require other studies to assess the levels of immunoglobulins. Moreover, the results obtained by Goel, *et al.*, (2006) confirmed that, decrease in leukocyte counts following intoxication with chlorpyrifos could be attributed either to the slower rate of production of leukocytes or due to their inhibited release into the blood circulation.
In the herbal treated groups (both simultaneous and afterwithdrawal) highly significant (p<0.01) differences in lymphocytes count were observed on days 7 to 21 and day 30 for monocytes as compared to the control value. Increase in lymphocyte count in herbal drugs treated groups indicates the enhancement of cellular immunity. Recovery in the counts of lymphocytes was seen in day 30 in the herbal treated groups except Gr XI with highest recovery was in the Recovery group of A. vera Gr VIII where the counts of polymorphocytes was improving and nearing that of the control. However, recovery in the counts of monocytes was seen in day 30 in Gr V to Gr X while highest recovery was seen in the recovery groups of O. sanctum Gr X where the counts of monocytes was decreasing and nearing that of the control.

Better results were obtained in the recovery groups than in simultaneous groups with good results in O. sanctum group for monocytes while A. vera in lymphocytes. Both the drugs have significant role in immune response against the toxicant. According to Gopalakannan and Arul, (2006) increase in neutrophils in control fed fishes may be a non-specific immune response and increase in lymphocyte counts in herbal O. sanctum dose prepared diet fed fishes can be attributed to the specific immune response. The major reason for this enhanced concentration of lymphocytes and phagocytes in the experimental groups may be their participatory role in immune functions as observed by Kollner, et al., (2002). Mediratta, et al., (2002) reported the evaluation of immunomodulatory potential of O. sanctum seed oil. Lymphocyte proliferation with O. basilicum was studied by Gomez-Flores et al., (2008). Godhwani, et al.,(1988); Lamberkovics, et al., (1998) and Anuradha and Murrugasen, (2001). A large number of biological activities have been ascribed to Aloe vera to explain its immunostimulatory property. Acetylated glucomannan is primarily responsible for the gel’s mucilaginous properties (Hamman, 2008) and has been found in vitro and in animal studies to modulate immune function through macrophage activation and cytokine production. Another study suggests that A. vera could inhibit infectious diseases by stimulating the host defense mechanism, especially the phagocytic and killing activities of macrophages. (Tamura, et al. 2009). Fresh aloe gel has an anti-inflammatory effect (inhibits the synthesis of prostaglandin) which may be due to a combination of the substances magnesium silicate, bradykinase and the glucoprotein aloctin A (Wagner, 1993).

The present study shows that the effect of aqueous leaf extract of A. vera and O. sanctum on lymphocytes, eosinophils and neutrophils increased the counts in treated groups in comparison to the control at the end of the 15 and 30 days of studies. The results are
consistent with the results obtained by Alishahi, et al., (2010) who used common crap treated with dietary A. vera extracts.

Thus in Table 31, highly significant differences were observed for polymorphocytes in all days of observation. Gr VIII indicates its highly effective nature in curing the impact created by mancozeb in mice. Highly significant differences were observed for Lymphocytes in all the days of observation in Table 34. Highest recovery was shown by Gr VIII indicating its highly appreciable effective nature in curing the impact created by mancozeb in mice. Significant difference was observed for Monocytes on day 30 of observation in Table 37. Highest recovery was seen in Gr X indicating its highly appreciable effective nature in curing the impact created by mancozeb in mice. In Table 40, significant differences were observed for eosinophils on day 7 and highly significant differences were observed for eosinophils on days 15 to 30 of observation. Highest recovery was obtained in Gr VIII showing positive results of the herbal drugs. Overall Gr VIII and Gr V for monocytes indicate their highly effective nature in curing the impact created by mancozeb in mice.

1.4.2. Albumin/Globulin (A/G)Ratio

The globulin and albumin content also help to ensure this status as globulin is involved in antibody production. The Albumin/Globulin ratio helps to identify the immunological aspects of the individual.

In the present study, highly significant (p<0.01) decrease in albumin (gram per decilitre) content was observed on day 30 from day 7 in the mancozeb intoxicated Gr II and III, with higher values in the latter. Over all albumin level was seen to fall along with total protein content and consequently relative rise in globulin (gram per decilitre) was observed to compensate the total protein content in the mancozeb treated mice. However, the rise of globulin could not compensate the fall of albumin and consequently the level of protein fell. These results are in agreement with earlier workers in T-2 feed birds (Boonchuvit, et al. 1975). Fluctuating results in the globulin content was observed in the Simultaneous group of both A. vera and O. sanctum. Initially globulin was increasing till day 15 thereafter it falls. In the Recovery group of A. vera initially globulin decreases till day 15 thereafter it rises, while in O. sanctum (D1) group increase was seen throughout and in O. sanctum (D2) group fall was seen till day 21 and thereafter it increases. The value of globulin was relative to total protein and albumin. The hypoproteinemia and hypoalbuminaemia in the present study could be attributed to the reduction in feed consumption and hepatic damage, since liver is the
major organ of protein synthesis especially albumin, nephritic syndrome and protein loosing enteropathies, loss in urine, malaborption of protein from the alimentary canal or decreased formation in the liver owing to liver diseases. (Kaneko, et al. 1997).

The level of change in plasma proteins such as fibrinogen and globulins usually takes place during acute and chronic infections. This reduction could also be attributed to changes in the protein and free amino acid metabolism and their synthesis in the liver (Ncibi, et al. 2008; Li, et al. 2007). The increased loss of albumin takes place due to damaged glomeruli in case of renal failure detected by Venkatesan, et al., (2000). In harmony to the present finding Zahran, et al., (2005); El-Fiky, et al.,(1992); Zamanov, et al.,(1970); Prabhakaran and Kamble (1993); Prabhakaran,et al.,(2014) and Hassan, et al.,(1989) reported that albumin content was decreased while the globulins were increased and therefore A/G was decreased. These changes were related to the physiological state and the health of animals hence the insecticide may affect the gastrointestinal tract and induce the decrease in the absorption and assimilation of protein.

In the present study, highly significant (p<0.01) decrease in A/G ratio was observed on day 15 and 30 while significant (p between 0.05 to 0.01) decrease in A/G ratio was observed on day 7 and 21 in the mancozeb intoxicated Gr II and III, with higher values in the latter as compared to control. Fall in A/G ratio decreases total proteins due to low level of albumin. Reduction in total proteins occurs in oedema where more albumins is excreted in urine or low albumin due to haemorrhage, shock, negative nitrogen balance due to increased protein breakdown, acute infectious diseases, untreated hyperthyroidism. In severe liver disease albumin and total protein is low while globulin increases. Increase in globulin is due to chronic infections, rheumatoid arthritis, tuberculosis, kala azar, etc.

In the herbal drugs treated group (both simultaneous and afterwithdrawal) significant (p between 0.05 to 0.01) differences in globulin content were observed on days 7 as compared to the control value. The increase in the globulin content was gradually compensated during the treatment. The level of albumin was gradually increasing and along with globulin due to increase in total protein content and probably due to rise in antibodies suggesting the role of immunity to fight the toxicant exposure. Good recovery in day 30 was seen in the herbal treated groups. Significant (p between 0.05 to 0.01) differences were seen in day 7 and 21 in the A/G ratio while highly significant (p<0.01) differences were observed in day 15 and 30.The fall in the A/G ratio during the mancozeb exposure in Gr II and III was seen to improve after the administration of the herbal extract. In the Simultaneous group
ratio increases from day 7 to 30, in the Recovery group it increases till day 21 than declines on day 30 towards control level. The nearest value towards that of the control was seen in the recovery groups of *O. sanctum* Gr X.

Better results were obtained in the recovery groups than in simultaneous groups. Both albumin and globulin increases due to haemoconcentration and the ratio remain unaltered. Thus in the present study, overall albumin decreases and globulin increases whereas the A/G ratio falls in the mancozeb exposed group while in the herbal treated group globulin decreases along with increase in albumin and rise in A/G ratio.

Similar results using *O. basilicum* was reported by Abasali and Mohammed, (2010) reported in the common carp (*Cyprinus carpio*). In the present study, dietary supplemented *O. sanctum* extract group enhanced total plasma protein and globulin values in comparison to control group. Similar results were reported in rainbow trout fed with garlic, ginger, lipopolysaccharide (Awad, *et al.* 2009, Bilen, *et al.* 2010, Nya, *et al.* 2009 a, 2009 b) and in *Coggyria coggyria* by Bilen, *et al.*, (2011) which revealed that the humoral elements were triggered in the serum. Globulin is the main resource of immunoglobulin production, thus its enhancement in serum provide immunostimulatory potential (Sahu, *et al.* 2006). The observed differences in the serum albumin and globulin levels supported the explanation of the increase in serum total protein levels (Oyewo, *et al.* 2012). The overall results of the present study proved that the extract of *O. sanctum* induced the innate immunity of mice in all treated groups and suggest its protective ability through cellular and may be non cellular immune mechanisms, as evident from the enhanced haematological parameters such as RBCs and lymphocytes. The leaf of *O. sanctum* has been shown to contain water soluble phenolic compounds such as alkaloid, glycosides, saponin etc. (Gupta, *et al.* 2002 and Aswar, *et al.* 2010) that might act as a potential immunostimulant. However the active principle responsible for the immunostimulatory property observed in the present study has to be identified.

Our results show similar protective ability of *A. vera* which is also in harmony according to the findings of Haghighi, *et al.*, (2014). *A. vera* extract enhanced total plasma protein, albumin and globulin values in comparison with mancozeb treated groups. Nuzhat Sultana, *et al.*, (2013) demonstrated that rabbits after 30 days of *A. vera* dosing showed highly significant increase in A/G ratio in comparison to control animal group. Similar reports were cited by Maqsood, *et al.*, (2009) in *C. carpio* fingerlings treated with levamisole.
In the present study, albumin globulin ratio does not indicate significant differences in treated group in compare to control group. Serum proteins are various humoral elements of the non-specific immune system, measurable total protein, albumin and globulin levels suggest that high concentrations are likely to be a result of the enhancement of the non-specific immune response. Das, (2013) showed that the highest protein and globulin concentration and lowest A/G ratio was found in the group supplemented with O. sanctum extract than in the control group and since lower albumin-globulin ratio indicates the presence of more amounts of globulin this was also found in the group. In conclusion, supplementation of A. vera and O. sanctum in mice helps to enhance non-specific immune system in fish. The improvement in serum total proteins, albumin and globulin levels was found in groups that received low dose of mancozeb along with A. vera after its withdrawal when compared to control. Concerning the improvement in A/G levels in all treated rats was seen with low dose of mancozeb along with O. sanctum after its withdrawal when compared to control then high dose of mancozeb and O. sanctum. The effect of A. vera or O. sanctum during recovery treatment was higher in reducing the impact of mancozeb especially at low doses than that during simultaneous treatment and high dose of mancozeb.

Significant difference was observed for globulin on day 7 of observation in Table 43. Minimum difference was obtained in Gr II to VII; the least value was recorded in Gr IX indicating its highly appreciable effective nature in curing the impact created by mancozeb in mice. Highly significant difference was obtained for Albumin on day 30 while minimum difference was obtained in Gr IV to XI indicating its highly appreciable effective nature in curing the impact created by mancozeb in mice.

Significant difference was observed for A/G ratio on day 7 of observation in Table 46. Minimum difference was obtained in Gr IV, VI to VIII, X to XI.; the highest value was recorded in Gr VIII indicating its highly appreciable effective nature in curing the impact created by mancozeb in mice. Significant difference was also observed for A/G ratio on day 21 of observation with minimum difference recorded in Gr IV to XI. Highly significant difference was obtained for A/G ratio on day 15 and 30 with minimum difference being recorded in Gr IV to VIII and X on day 15 and Gr IV to VII and IX to XI indicating its highly appreciable effective nature in curing the impact created by mancozeb in mice.

5.4.3. Immediate Type Hypersensitivity (ITH) and Delayed Type Hypersensitivity (DTH) Reactions
Hypersensitivity refers to excessive undesirable or damaging, discomfort producing or fatal reactions caused by the normal immune system. Type I Hypersensitivity or Immediate or Anaphylactic Hypersensitivity involves in reactions associated with skin, eyes, gastro- intestinal tract, respiratory parts, etc. It is mediated by IgE along with basophils or mast cells. The reactions are increased by neutrophils and eosinophils. Allergens cause production of IgE which triggers the mast cells which releases the histamine, LT C4. Thus, mast cell degranulation, influx of neutrophils or eosinophils causes Immediate Type Hypersensitivity.

Type IV Hypersensitivity or Cell-Mediated or Delayed Type Hypersensitivity involves reactions in organs and tissues requiring 12 hour to develop involving cell mediated immune response. It is mediated by CD4 sometimes CD8 cytotoxic T- cells. Neutrophils increase during such reactions and are usually stimulated by haptons. It is affected by contact dermatitis or other allergens. During sensitization by hapten, Langerhans and other Antigen Presenting Cells become activated and migrate to the lymph node and present the antigen to naive T cells transforming it into hypersensitivity effector cells. Vasodilatation and, tissue damage, lymphocyte and macrophage infiltrate, granuloma, causes Delayed Type Hypersensitivity.

In the present study highly significant (p<0.01) increase in the Immediate Type Hypersensitivity and Delayed Type Hypersensitivity (millimeter) was observed in the mancozeb intoxicated Gr II and III on day 15 and 30, with higher values in the latter. During this study, neutrophils and eosinophils increase was observed which also justifies the increase in hypersensitivity reactions. Effects of mancozeb on the immune system are scarcely reported (Chung, et al. 2005) and not much references are available on this aspect in mice. However an increase in IgG, IgE and α2-macroglobulin serum levels was observed during occupational exposure to mancozeb (Vergova, et al. 1988). Colosio, et al., (2002) also suggested slight immunomodulating effect of mancozeb. Koch, (1996) reported that mancozeb causes allergic disease.

Thus, the present study indicates that mancozeb induces both ITH and DTH response in Swiss albino mice. The increase in the anaphylactic reactions indicates simulation of immune response by different leucocytes induced by the toxicant. Thus increase in the DTH values indicates that both humoral and cell-mediated immunity play role in mancozeb exposed mice.
In the herbal drugs treated group (both simultaneous and after-withdrawal) highly significant (p < 0.01) differences in ITH and DTH values were observed on days 15 and 30 as compared to the control value. The increase in the hypersensitivity values in Gr II and III was gradually decreased though was higher than control during the treatment suggesting immunostimulatory properties of the plant extracts. The decrease in the anaphylactic reactions in the herbal treated groups indicates recovery of simulated immune response or ameliorating or antagonising effects induced by the mancozeb toxicant. The values were nearing that of the control level after the treatment with herbal drugs. Better results were obtained in the recovery groups than in simultaneous groups with the highest recovery in Gr VIII i.e. A. vera was able to combat the hypersensitivity reactions and bring the body’s defence system to normal level. Not much data are available on immunological aspect on the response of A. vera and O. sanctum in mancozeb treated mice is reported.

The dose of A. vera may have stimulated IL-4 production therapy inducing synthesis of IgE. Similar role of A. vera were also reported by many researchers to demonstrate its immunomodulatory role. A. vera was found to stimulate both humoral and cellular immune response in the chickens (Akhtar, et al. 2011). Probably, acemannan induces the immune – stimulating activities by activating macrophages (Zhang and Tizard, 1996; Ramamoorthy, et al. 1996). Crude extracts of A. barbadensis gel protects DTH responses against suppression by UV radiation. Alternatively Aloe may contain several agents that act on DTH. (unknown Research article Mechanisms of Ultraviolet Induced Immune Suppression Strickland FM UT MD Anderson Cancer Center Crisp Data Base National Institutes of Health).

O. sanctum showed increased antibody production probably due to the release of mediators of hypersensitivity reactions and tissue responses to these mediators in the target organs as described by Godhwani, et al., (1988) and Shah and Qadry, (1989). Similar immunomodulatory role by O. sanctum were reported by Mediratta, et al., (1988); Mitra, et al., (1999); Anuradha and Murugesan, (2001) and Mukherjee, et al., (2005). O. sanctum seed oil (1ml/kg/day) was found to significantly antagonize the effect of lindane on DTH reaction demonstrating the ameliorating affect of lindane induced immunotoxicity and oxidative stress in subacute exposure. The O. sanctum seed oil was found to modulate both humoral and cell-mediated immune responsiveness by the GABAergic pathways (Mediratta et al. 2002 and Mukherjee, et al. 2005). O. sanctum seed oil not only has immune enhancing and antioxidant effects (Mediratta, et al. 2002, Gupta, 2006) but also significantly reduces the secondary antibody response on exposure to carbamate pesticides (Seth, et al. 2002;
Mediratta, et al. 2008). The use of aqueous and ethanolic extracts of leaves of *O. basilicum* Linn. showed strengthening of both specific and non-specific responses when DTH was assessed (Dashputre and Naikwade, 2010). Moreover, alcoholic and aqueous extracts of *O. sanctum* showed stimulatory effect on delayed type hypersensitivity (DTH) and significant improvement in humoral immunity in swiss albino mice (Vaghasiya, et al., 2010).

In the present study *A. vera* was found to give better results in Group VIII than *O. sanctum*. Better ITH response was obtained in the recovery group of *Aloe vera* than in simultaneous groups of both *A. vera* and *O. sanctum* indicating enhanced cellular immune response. Similar immunomodulatory effect of herbal drugs were studied by Abasali and Mohammad, 2010; Pangasa, 2005; Lambercovics, et al.1998; Godhwani, et al.1988 who proposed the role of herbal drugs in boosting immune response which explains the adaptogenic action of the plant. The results obtained by Atul N Chandua, et al., (2011) showed that *A. vera* extract produces stimulatory effect on the humoral and cell mediated immune response in the experimental animals by suppressing delayed type hypersensitivity reaction induced by SRBCs in mice. not much data is available on ITH response in mancozeb exposed albino mice and its cure by herbal plant extract.

In both ITH and DTH highly significant difference was obtained for on day 15 and 30 in Table 49 and 50. In both the cases significant results were obtained in Gr VIII indicating it’s highly appreciable effective nature in curing the impact created by mancozeb in mice.

5.5. Correlation analysis

A perusal of data in the tables 53-56 showed that variance for parameters in all the days of observations were significant indicating appreciable results. It also revealed that the treatment given to mancozeb exposed mice with *A. vera* and *O. sanctum* was effective.

In the present study, it was found that when there are positive correlation then related parameters increase or decrease proportionally, while when there are negative correlation then related parameters either increases or decreases in inversely proportional manner. When haemoglobin was correlated with other parameters it was seen to have positive correlation with RBC, protein, glucose, lymphocytes, and albumin which means that when haemoglobin decreases the other parameters also decreases while with WBC, ESR, cholesterol, SGPT, SGOT, polymorphocytes and eosinophils negative correlation was seen i.e. when haemoglobin decreases these parameters increases. Similarly, protein was
positively correlated with glucose, lymphocytes, albumin i.e. when protein decreases all the other parameters also decreases while negatively correlated with cholesterol, SGPT, SGOT, polymorphocytes and eosinophils i.e. when protein decreases all these parameters increases. The correlation analysis revealed the same results as the above results in the earlier findings.

5.6. Histopathological Tests

The histological assessment is indispensable for demonstration of hepatotoxic and nephrotoxic effect. The biochemical techniques serve to identify the mechanisms that bring about this effect. Severe alterations from the normal group was observed in Gr III treated with 6.650 mg/kg/body weight mancozeb than in Gr II treated with 4.167 mg/kg/body weight mancozeb. In the present study, mice when exposed to mancozeb showed general weakness, decreased feeding behaviour and inactivity. This finding is in agreement with Ivano-Chemishanska, (1969) who found anorexia and general weakness in animals exposed to manebl, zineb, and mancozeb.

The results of the mancozeb exposed mice in liver tissues shows severe damage and histopathological alterations as documented in the observation chapter page 168. The alterations from the control were severe in Gr III than Gr II. The SGOT and SGPT increase fall in albumin from renal or eternal loss of protein causes toxic damage to the liver. This causes degenerative changes in the cell (necrosis) or abnormal storage of fat (steatosis) affecting small groups of isolated parenchymal cells (focal necrosis), groups of cells located in zones (centrilobular, mid zonal or periportal necrosis) or virtually all the cells within hepatic lobule (massive necrosis). Altered hepatic cell membrane permeability can lead to increased enzyme activity in plasma. Ethylene thiourea the breakdown product of mancozeb was found to have hepatotoxic effects in male rats as reported by Pandey, et al.,(1990); Dikshith, et al.,1988; Subramaniam, et al.,(1991). Similar observations were made by Lavric, (1990) who indicated elevation of creatinin level in blood indicating impairment of kidney function by metalaxyl.

The kidney histopathology as documented in the observation chapter page 166 during mancozeb exposure in albino mice showed hypertrophied cells of tubular epithelium with patchy areas of mononuclear infiltrates in the cortical zone suggesting early tubular nephritis. The uriniferous tubules appeared deformed with spaces in between and glomerulus slightly disorganised. Degeneration of organelles, neutrophilic leucocytes, pycnotic changes in
nucleus, dilated blood vessels, lymphocytic infiltration with increased vacuolization were observed. Severe necrosis and congestion in lumen and arterioles was observed.

When aqueous extract of A. vera at 400 mg/kg/body weight and O. sanctum at 250 mg/kg/body weight was administered in Gr IV to XI, restoration of histopathological changes was seen towards the normal histological structure. Better healing was observed in Gr VIII and IX suggesting curative properties of A. vera and O. sanctum against mancozeb induced hepatic and renal toxicity. Concerning the hepatic histoarchitecture of the mancozeb treated animals there was an increased vacuolization of hepatocytes and focal necrosis in comparison to untreated normal controls. The congestion of the portal area, inflammatory infiltration increased in these animals. These observations indicated the marked changes in the overall histo-architecture of liver in response to mancozeb, which could be due to its toxic effects primarily by the generation of reactive oxygen species causing damage to the various membranous components of the cell. The necrotic conditions observed in liver of mancozeb treated animals are in correlation with the observed biochemical changes, wherein an increased level of lipid peroxidation was noticed.

After withdrawal of mancozeb and post treatment with A. vera and O. sanctum extract significantly prevented further damage to the kidney and liver tissue and helped in healing of tissue. Earlier lesions were significantly decreased in their severity and returning to their normal structure. Treatment with aqueous solution of herbal extracts for 30 days after withdrawing mancozeb, demonstrated better reversal of histopathological scores. The administration of A. vera and O. sanctum extract can be recommended as a concomitant supplementation to the routine therapy for the protection against severe tissue damage induced by the mancozeb.

The treatment with A. vera showed remarkable improvement as against the treatment by O. sanctum. The effect of toxins on kidney can be supplemented by antioxidants which are present in Aloe extract like alanine, an amino acid which displays antioxidant activity (Duke, 1985). Several reports on protective effect of the herbal extracts have been reported by many authors. Likewise Bolkent, et al., (2004) demonstrated kidney protection by A. vera from diabetes induced damage. Heggars, (1988) demonstrated the wound healing properties of A. vera. Vazquez, (1996) reported anti-inflammatory activity of extracts from A. vera gel. Aloe contains emodin, anthralin and aloin A which are found to have significant ameliorating effects against fungicide and pesticides (Duke, 1997). Histopathological studies also confirmed the curative efficacy of the water extract of A. vera against carbon
tetrachloride induced liver damage as indicated by reversal of centrilobular necrosis, macrovascular fatty changes and scattered lymphomononuclear cell infiltrate in hepatic parenchyma. (Iji, et al. 2010). The hepatoprotective action of the plant was attributed to the preservation of the liver enzymes through the antioxidant properties of the gel. In actual facts superoxide dismutase, catalase, β carotene, α tocopherol and other antioxidants have been isolated from the A. vera gel by several authors (Hammann, 2008). This was attributed to its anti-oxidative activities. Findings from this study also indicated that the gel extract increased plasma albumin confirming that chronic administration of Aloe for up to one month might not be toxic to the body. A. vera gel partially protected the lesions from severe damage in the histology of liver, spleen and skin sulphur mustard-induced systemic toxicity (Anshoo, et al., 2005).

Orafidiya, et al., (2001) reported that essential oil of Ocimum had significant effects on the function of kidneys, lungs, heart, testes and blood of rats and mice. Sharma, et al., (2002) found that oral administration of O. sanctum provides protection against mercuric chloride induced toxicity in Swiss albino mice. Eugenol present in the O. sanctum extract has significant ability as antioxidant and provides cure from body ailments. Yamamoto, et al., (2005) proved that Ocimum suppressed hepatic fibrosis and protected liver against parenchymal damage. Dasgupta, et al., (2007) found that O. basilicum increased the activity of xenobiotic metabolizing phase 1 and phase 11 enzymes, elevating antioxidant-enzyme response by increasing significantly the hepatic glutathione reductase, superoxide dismutase, and catalase activities, increasing glutathione content and decreasing lipid peroxidation and lactate dehydrogenase activity in the liver of mice.

Oral administration of hydro-ethanolic extract of O. sanctum leaves at 200 mg/kg in male Wistar albino rats gave protection against liver injury induced by paracetamol (Chattopadhyay et al., 1992). The cold water extract (3 g/100g, orally for 6 days) of O. sanctum was found to be effective against carbon tetrachloride (0.2 ml/100 g, subcutaneously) induced liver damage in albino rats (Bhargava and Singh, 1981). The ethanolic extract of Tulsi can protect the liver damage from anti-tubercular drugs in experimental rats (Ubaid, et al., 2003). Tulsi offered liver protection against various experimentally induced damages. These beneficial activities can thus promote cellular integrity of hepatocytes and other tissues of the body (Mesole, et al., 2012). Much data regarding the renal protective properties of O. sanctum against mancozeb induced mice are not available.
Hepatoprotective qualities of *A. vera* (Gbadejesin, *et al.*, 2009) and *O. sanctum* (Chaturvedi, *et al.*, 2007) were also reported by many authors. When the protective agent was given along with toxicant it was unable to suppress the toxic effect of the toxicant to a good extent. On the contrary, when the burden of the toxicant was removed probably the body’s own repair mechanism starts recovery which was further accelerated by the aqueous extract of *A. vera*. Better repair of damaged tissue was seen in low dose group of mancozeb in the recovery group than in the other groups. The post treatment is better than simultaneous treated group suggesting that the curative role of the herbal test drug is better than the protective role. Thus these herbal drugs having less side or no side effects can ensure cure or protection as also seen in the simultaneous group in the mancozeb exposed mice. A large number of biological activities have been ascribed to *A. vera* to explain its purported health benefits, including antimicrobial, anti-inflammatory, lipid and glucose lowering, antiproliferative, immunostimulatory, and antioxidant functions. A number of potentially active ingredients in the latex and gel of *A. vera* have been identified; however, much has yet to be determined about their mechanisms of action. Further studies are also required to determine the active properties of numerous other constituents and to explore the competitive or synergistic actions of particular combinations of ingredients.

However, not much work has been done on the protective effect of *Aloe vera* and *Ocimum sanctum* on mancozeb induced toxicity in mice, hence it was thought worthwhile to study on this aspect/direction. Out the two herbal drugs selected for treatment against mancozeb toxicity in albino mice *A. vera* afforded comparatively more significant amelioration. However, the amelioration was never upto the control level but was near to it. Thus, *A. vera* lowers high cholesterol, boosts oxygenation in blood, eases inflammation, protects from oxidative stress, nourishes the body with minerals, vitamins, enzymes and glyconutrients, stabilizes blood sugar, protects kidney from diseases.

### 5.7. Histochemical Tests

The results of the histochemical analysis were similar to the results as in the biochemical tests. The carbohydrate content in the kidney and liver tissues demonstrated decrease in the mancozeb expose group while improvement was seen in the herbal treated groups. The intensity of stain for the protein content in the kidney and liver tissues demonstrated decrease in the mancozeb exposed group while improvement was seen in the herbal treated groups. Likewise the lipid content decreased in the mancozeb exposed group.
while the results were better in the herbal treated groups. In all the cases better results were seen in the *Aloe vera* treated groups of Gr VIII than others. Ksheerasagar and Kaliwal, (2010) observed that treatment with mancozeb lead to decrease in protein and glycogen content in testes of mice. Awasthi, *et al.*, (1984) found elevated lysosomal enzymatic activity with decrease in protein and nucleic acid content in response to organophosphate insecticide with release of nucleases and proteases affecting nucleic acids and protein metabolism.

Leaves of *O. basilicum* are rich source of flavonoids which have been shown to possess various biological properties related to antioxidant mechanisms and provide protection against toxicity. The results of the present study indicated the beneficial role of *O. sanctum* on albino mice in augmenting the immunity, growth and survivality as evident from the enhanced haematological and biochemical parameters. Thus there is a great prospectus of using natural products including plant extracts in the treatment of various diseases in mice. The evaluated data from our study also suggests the use of *O. sanctum* as home remedy for controlling various diseases by increasing the immunity level in the human body. Further studies to evaluate about the safety and efficacy of the extracts in human are needed.

Analysis of the potential efficacy of *A. vera* in the treatment of particular disorders is complicated by differences in its preparations, their means of administration, and the animal model or study design employed in individual studies. Controlled in vivo toxicology and safety studies of its preparations in humans are also required. Another study suggests that *Aloe vera* could inhibit infectious diseases by stimulating the host defence mechanism (Tamura, *et al.* 2009). Mendonça, *et al.*, (2009) reported that simultaneous application of *Aloe vera* gel and microcurrent is excellent for the treatment of open wounds. Aloe extracts have equally been demonstrated to have antioxidant abilities in humans and animals (Rajasekaran, 2005; Kardosova and Machova, 2006; Loots, *et al.* 2007), whilst also protecting the liver, (the major detoxification organ) against injury (Can, *et al.* 2004, Chandan, *et al.* 2007) and improve liver enzyme functions that are associated with carcinogen metabolism (Singh, *et al.* 2003). Furthermore, no negative effects were observed on the markers of hepatic damage and also positively improved cholesterol status, which was presumably mediated by a control of lipid metabolism (Iji, *et al.* 2010). Naveena, *et al.*, (2011) reported that *Aloe vera* extract at the dose of 100 mg/kg inhibited the increase in body weight and abdominal circumference and also brought back the serum biochemical and haematological parameters towards normal levels.
Also, the results of the present study indicate that *Aloe vera* and *Ocimum sanctum* significantly reduces the toxic effects of mancozeb by altering the hepatic enzyme activities and thus can be considered a potential protective agent in conditions of fungicide mancozeb poisoning. However, in this present study *A. vera* showed much better recovery than *O. sanctum* against mancozeb induced toxicity in Swiss albino mice.