CHAPTER 5

SUMMARY & CONCLUSION
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The present study was carried out to investigate the influence of the aqueous extract of *Andrographis paniculata* (AP) on severe liver damage leading to carcinogenic condition. It was of interest to see whether supplementation of this plant extract was ameliorating the severe liver damage injuries caused by BHC (Hexachlorocyclohexane) in mice model.

**BHC (Hexachlorocyclohexane) as a liver carcinogenic substance:**

Healthy adult male albino mice (*Mus musculus*) were administered BHC at a dose of 500ppm/kg food for 1 to 8 months duration to investigate the time-related changes in the liver metabolism.

The BHC experimental model forms an irreversible liver tumor in male mice. The sequential changes were very well observed in this model. The casualty of the animal was almost nil in the subscribed dose of BHC. There were no general changes in the behaviour pattern of the animals treated with BHC.

Grossly neoplastic nodules were observed only after 6 months of BHC administration, and the maximum number of tumours were observed after 8 months of BHC treatment indicating dose-response relationship.

Liver weight expressed as % body weight showed increasing trend in experimental groups as compared to control.
The critical period of the prescribed dosage in mice is 4 months as it induced irreversible cellular changes in the liver by that time. This finally leads to the development of hepatocellular carcinoma.

BHC induced liver lesions may be evaluated in terms of hyperplastic areas and hyperplastic nodules were labeled as early lesions and trabecular cell carcinoma considered as advanced lesion. The hyperplastic or neoplastic nodules observed after 4 months of BHC exposure consisted of basophilic cells mainly and histologically these nodules indicated permanent cellular alteration which led finally to tumour development. Histologically most of the liver tumours were identified as trabecular cell carcinoma.

Transmission electron microscopic studies revealed that BHC administered liver showed various changes like the presence of abundant glycogen and excessive accumulation of fat. The Proliferation of smooth endoplasmic reticulum with structural changes in rough endoplasmic reticulum takes place. At the later stage of BHC exposure the hepatocytes showed that mitochondria size and shape were changing and other related changes were also observed.

The BHC treated animals in Group – II resulted in decline in the protein level. This reduced protein level probably indicates an alteration in its synthesis.

Enzyme SGPT and SGOT are liver marker enzymes. In this study these enzymes showed a significant increase due to BHC toxication. Whenever liver hepatocytes are damaged or killed, the enzyme gets released into the blood.

The increased activity of acid phosphatase (ACPase) due to BHC, could also because of increase synthesis of lysosomal enzyme in response to increase cell degeneration and pathological liver injuries.
The BHC treatment caused a significant increased in alkaline phosphatase (ALP) activity in the liver. The activity of ALP is mainly associated with the bile duct canaliculi and is involved in the passive transport to bile.

During the BHC treatment period, the G6Pase and phosphorylase activity was significantly declined. This suggests interruptions of glycogenolysis due to BHC administration.

Decreased activity of mitochondrial enzyme SDH and ATPase were due to BHC treatment. The reduced SDH activities indicate reduction in aerobic oxidation, which could be in a response to reduced O$_2$ transport to tissue. ATPase is a marker enzyme for mitochondria and it was also reduced. Ultrastructural studies show mitochondrial swelling, dysfunction and lack of cristae structure. Biochemical studies indicate that there are deplecation in the ATPase and SDH activity. Thus both the results of the ultrastructural changes and biochemical parameters show clear interdependency.

The OCT activity was significantly increased by treatment of BHC in liver. This shows a severe hepatic damage.

The decreased level of glutathione found in the liver indicates its increased utilization for the rapid oxidation of the toxicant. This can be due to the stress imposed by BHC.

Decreased activities of GSH, GR, GSH-Px, SOD and CAT in BHC treated mice suggest their increase towards the susceptibility of oxidative injury.

The BHC treated animals also showed a significant increase in lipid peroxidation and depletion of GSH.
Activity of GST has shown an increased in the BHC treated group. This is indicative of increased stress due to toxin in the liver tissue, probably such condition occurs only in carcinogenic damage.

Y-Glutamyl transpeptidase (y-GTP) is the most sensitive indicator of liver disease and is a useful marker in patients with liver metastases. The y-GTP increased in the present study due to BHC toxication.

**Supplementation of Andrographis paniculata (AP) :-**

The BHC treated animals were also given an aqueous extract of *Andrographis paniculata* at a dose of 12 mg/kg body weight/day orally, from 1 to 8 months. BHC with supplementation of AP brought out recovery in various BHC in use alterations.

The histological changes observed in 1 to 4 months of AP supplementation emphasize that the recovery of the damaged liver, which was due to BHC toxicity, was almost to a normal condition. This recovery was also reflected in the neoplastic nodules formation.

In an ultrastructural study, it was observed that the AP supplemented group showed considerable recovery corresponding to the BHC treated group. A significant recovery was obtained in the BHC induced alteration by the supplementation of AP. This might be due to an additive action of this drug.

The result reveals AP supplementation showed an increased protein level. One can mention that AP plays a protective role in defence mechanism.
SGPT and SGOT were decreased with protective treatment of *Andrographis paniculata* in Group III animals of the present study. These observations confirm improved liver function in protected mice.

There was a recovery of ALP, y-GTP and ACPase in AP supplemented group, and the level of ALP and ACPase showed a significant decline due to AP supplementation. The y-GTP is a liver marker enzyme and this enzyme was also significantly decreased. These observations suggest that AP is a more effective drug on acute hepatic condition leading even to carcinogenic condition.

On the basis of changes in the activity of phosphorylase and G-6-pase in liver of AP supplemented group III, it seems that AP provides certain amount of protection and corrects the disfunctions. As such the increase in activity of phosphorylase and G-6-pase also suggests the improved glycogenolysis in liver.

On AP supplementation in Group III there was an increase in the liver ATPase and SDH activities, besides ultrastructural micrograph of liver also shows no mitochondrial swelling and disfunction. From these findings it can be mentioned that AP play a significant role in reversal of damage caused by BHC treatment on liver.

The OCT activity was significantly decreased in AP supplemented group III compared to BHC alone group II. This suggests that there was a remarkable recovery in the liver function of AP supplemented group.

This study indicates significant decrease in LPO activity and significant increase in GSH level in the liver of AP supplemented (group III) animals. This suggests accelerating the repair mechanism of damaged cell membranes. This
certainly indicates that AP contains certain substances, which are capable of preventing LPO, a natural deleterious process.

Increase activities of GSH, GR, GSH-Px, SOD and CAT in AP supplemented animal as compared to BHC treated animals, suggested that the antioxidant effects elucidated by *Andrographis paniculata* (AP) is possible due to their ability to activate antioxidant enzymes that catalyze the reaction of oxidants.

Thus the result of this study indicates that supplementation of *Andrographis paniculata* can be beneficial particularly if it is administered at an onset of liver damage.

However, the recovery was not upto the level of control ones but at the same time recovery was much more compared to only BHC treated animals. Significant recovery was obtained in the BHC induced alterations by the supplementation of AP. This might be due to an additive/synergistic action of this drug.

It may be mentioned over here that the *Andrographis paniculata* has a radiomimetic property as observed under gamma scintillation counter. Such properties are used for regulating the cytotoxic effects on the tissue. The two important properties of this plant i.e. radiomimetic and antioxidant, gives it valuable importance for the treatment of liver damage to the extent of carcinogenic (tumour) condition.