SUMMARY AND CONCLUSION

5.1 SUMMARY

The present investigation describes the potent antioxidant activity of ethanolic extract of *Piper cubeba* fruits in rats which helps in preventing hepatic toxicity and hence appears to possess the hepatoprotective activity against carbon tetrachloride and ethanol induced hepatic injury. Proton radical scavenging action shows the mechanism of oxidation. Due to proton donating ability the capacity of DPPH radicals was decreased due to reduced absorbance at 517 nm, hence the Piper cubeba fruit extract shows the antioxidant activity (Ganapathy et. al., 2007; Adesegun et. al., 2009). The dose required of Piper cubeba fruit extract is 1.5-fold greater than ascorbic acid to scavenge DPPH, hence the extract was less active for scavenging radicals than ascorbic acid. In the reducing power assay, Fe3+/ ferricyanide complex to the ferrous form is reduced in presence of antioxidants.

Hence, the reducing capacity of drug shows the antioxidant property and increase in absorbance indicates increase in reducing power (Umamaheswari et. al., 2008; Adesegun et. al., 2009). The dose required for Piper cubeba fruit extract is 2.0 fold greater than dose of ascorbic acid to produce same reducing power.

Hydrogen peroxide is intracellular precursor of hydroxyl radicals produce very toxic effect to the cell (Ganapathy et. al., 2007; Adesegun et. al., 2009). Piper cubeba fruit extract scavenged hydrogen peroxide which may be attributed to the presence of phenolic groups that could donate electrons to hydrogen peroxide, thereby neutralizing it into water (Jafri et. al., 1999). The dose required for Piper cubeba fruit extract is 1.5-fold greater than dose of ascorbic acid to scavenge hydrogen peroxide. The results of in vitro antioxidant tests of Piper cubeba fruit shows strong free radical scavenging activity, which also produces useful activity against hepatotoxicity produced by CCl4. The Piper cubeba fruits extract showed the reduction in levels of
SGPT and SGOT, stabilized and repaired plasma membrane and hepatic tissue damaged by CCl₄. The serum levels of transaminases, healing of hepatic parenchyma and restoration of hepatocytes goes to normal (Thabrew et al., 1987). The Piper cubeba fruits extract restrain the increased level of cholesterol and triglyceride, also produced inhibition of increased ALP activity with reduction of increased bilirubin shows the possibility of prevention of biliary dysfunction in rat liver during hepatic damage with CCl₄. A major defense mechanism involves the antioxidant enzymes like SOD, CAT and GSH which convert active oxygen molecules into non-toxic compounds. Enhanced lipid peroxidation results when free radicals are overwhelmingly formed. Results revealed reduced LPO as indicated by significant decrease in MDA level in extracts treated groups. Simultaneously, significant increase in GSH, SOD and CAT content of liver suggested antioxidant activity of Piper cubeba fruits extracts and silymarin. Scavenging of free radicals is known to be one of the major antioxidation mechanisms to inhibit the chain reaction of LPO (Constantin et al., 1990). The acute toxicity studies were carried out according to OECD guidelines. Piper cubeba is nontoxic up to 2000 mg/kg as indicated by observations and hence, the doses were selected i.e. 250 mg/kg and 500 mg/kg for the hepatoprotective evaluation against carbon tetrachloride (CCl₄) induced hepatotoxic effect.

Further, the hepatoprotective effect of Piper cubeba fruits extract was confirmed by histological examination of the liver tissue of control and treated animals. The histological architecture of liver sections of carbon tetrachloride treated group showed fatty degeneration of hepatocytes, however administration of Piper cubeba fruits extract treated group almost normalized to the level of the Silymarin treated groups, showing its potent hepatoprotective effects. The administration of ethanolic extract of Piper cubeba fruits exposed significant protection in hepatocyte regeneration against the toxic effect of carbon tetrachloride. Hence, the histological examination of Piper cubeba fruits extract treated group showing hepatoprotective effects and it supported to biochemical investigations. Carbon tetrachloride induced hepatic injuries are commonly used models for the screening of hepatoprotective drug and the extent of hepatic damage is
In hepatotoxicity, a depression in total protein is observed due to the defect in protein biosynthesis similar to our results. This is due to the disruption and disassociation of polyribosomes from endoplasmic reticulum following CCl₄ administration (Das et. al., 2005). Administration of fruit extract at a dose of 250 mg/kg and 500 mg/kg body weight prevented this change. This indicates that Piper cubeba possibly promotes the assembly of ribosomes on endoplasmic reticulum to facilitate uninterrupted protein biosynthesis. The results indicate that triglycerides (TG) increase in CCl₄ induced fatty liver. It is well known that CCl₄ administration induce an increased synthesis of fatty acids as well as decreased release of hepatic lipoproteins due to impair in β-oxidation of fatty acids (Sun et. al., 2009). The accumulation of TG in liver of CCl₄ treated rats is not due to the interference with the TG formation by the liver, but due to the inhibition or destruction of TG secreting mechanism (Sun et. al., 2009). Administration of fruit extract at a dose of 250 mg/kg and 500 mg/kg body weight prevented this change, suggesting that Piper cubeba probably improve β-oxidation of fatty acids. Serum activities of catalase (CAT) are the most sensitive enzymatic index in liver injury caused by Reactive Oxygen Species (ROS) and oxidative stress (Sanmugapriya et. al., 2006). CAT is a haemoprotein; it protects the cells from the accumulation of

assessed by the level of released cytoplasmic alkaline phosphatase, transaminases, triglycerides and bilirubin in circulation (Adesegun et. al., 2009). It is well documented that CCl₄ are biotransformed under the action of microsomal cytochrome P-450 of liver to reactive metabolites (Larrey et. al., 2000). These free radicals bind covalently to unsaturated lipid membrane, provoking a sharp increase of lipid peroxides followed by pathological changes such as elevated levels of serum marker enzymes like ALT, AST and ALP; decreased total protein, increased levels of total and direct bilirubin, serum triglycerides accumulation, depletion of GSH, increased LPO, and finally hepatocyte damage (Sies, 1999). This suggests that, CCl₄ induces liver injury by sharing a common property of free radical mechanism. The ability of a hepatoprotective drug to reduce the injurious effects or to preserve the normal hepatic physiological mechanisms that have been disturbed by hepatotoxin is the index of its protective effect (Suja et. al., 2004).
H$_2$O$_2$ by dismutating it to form H$_2$O and O$_2$ (Oh SI, et. al., 1997). A reduction in the activity of this enzyme is associated with the accumulation of highly reactive free radicals, leading to deleterious effects such as loss of integrity and function of cell membranes (Bhakta et. al., 1999). Administration of CCl$_4$ leads to generation of peroxy radical, O$_2^-$ which is associated with inactivation of CAT enzyme. This probably explains the significantly reduced activities of CAT observed by us in rats challenged with CCl$_4$. In rats receiving CCl$_4$ and Piper cubeba extract the activity of CAT is significantly higher than in CCl$_4$ control rats, and very similar to the values noted in normal rats. This suggests that Piper cubeba can reduce ROS that may lessen the oxidative damage to the hepatocytes and improve the activities of the liver antioxidant enzymes, thus protecting the liver from CCl$_4$.

The evaluated hepatoprotective activity could be related with the modification in antioxidant status of the liver tissue. As in the both in vivo models i.e. carbon tetrachloride and ethanol induced hepatotoxicity, ethanolic extract of *Piper cubeba* fruits has demonstrated decreased levels of serum enzyme like Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phophatase (ALP), Total bilirubin (Tbil) and Direct bilirubin (Dbil), whereas increased level of Total protein (TP) was observed. Furthermore, ethanolic extract of *Piper cubeba* fruits showed decreased levels of liver tissue MDA and rise in the levels of antioxidant enzymes glutathione and catalase which further indicates ethanolic extract of *Piper cubeba* fruits has potent antioxidant activity that helps in preventing hepatic toxicity. The results of the present investigation suggests that the *Piper cubeba* fruits had shown hepatoprotective activity against carbon tetrachloride and ethanol induced hepatic injury could be due to the cell wall membrane stabilization, hepatic cell regeneration and, activation of antioxidant enzymes like catalase.

Diabetes is categorised as a chronic metabolic disorder characterized by higher blood glucose levels due to the impaired or less secretion of insulin or insensitivity of pancreatic cells to secrete insulin (American Diabetes Association, 2005). The available therapy for the treatment of diabetes
mellitus includes insulin and a range of oral anti-diabetic agents. In addition, certain medicinal plants have been reported to possess anti-diabetic activity. Similarly, *Piper cubeba* fruits also possess several bioactivities and, hence it is used in traditional medicinal systems. However, its antidiabetic activity has not been well investigated and established so far. The aim of this further study therefore, was to investigate the antidiabetic activity of *Piper cubeba* fruits in alloxan-induced diabetic rats using oral administration of ethanolic extract of *Piper cubeba* fruits.

In this experimentation, ethanolic extract of *Piper cubeba* fruits had been tested in experimentally induced diabetes in rats. Treatment of alloxan hydrate had shown significant elevation of serum blood glucose when assessed on day 14 and on day 21. However, *Piper cubeba* extract significantly attenuated the alloxan induced elevated levels of serum blood glucose in dose dependent way. Serum blood glucose levels in *Piper cubeba* extract treated group at a dose of 250 mg/kg and 500 mg/kg on day 14 and on day 21 was comparable with Pioglitazone treated group suggesting the antidiabetic potential of *Piper cubeba* fruits.

Further, alloxan treated rats had revealed significant elevation of serum total cholesterol on day 14 and on day 21 when compared to vehicle treated control group. *Piper cubeba* extract treatment significantly attenuated these effects in dose dependent manner. Similarly, serum total cholesterol levels in *Piper cubeba* extract treated group at a dose of 250 mg/kg and 500 mg/kg on day 14 and on day 21 was comparable with Pioglitazone treated group.

Further, we assessed effect of alloxan treatment on serum HDL cholesterol levels. Alloxan treatment significantly depleted HDL cholesterol when tested on day 14 and 21. Nevertheless, treatment of *Piper cubeba* extract significantly attenuated alloxan induced effects in dose dependent manner. In addition, administration of alloxan hydrate toxin in rats has demonstrated significant rise of triglycerides on day 14 and 21 when compared to vehicle control group. However, treatment with *Piper cubeba* extract significantly inhibited alloxan hydrate induced elevation of serum triglycerides at a dose of 250 mg/kg and 500 mg/kg on day 14 and on day 21 and the effect was
comparable with Pioglitazone treated group.

The results of this investigation demonstrated that at a dose of 500 mg/kg, ethanolic extract of *Piper cubeba* fruits shown significant antidiabetic activity. The results are inline with previous report that demonstrated that ethanolic extract of *Piper cubeba* fruits at at dose of 400 mg/kg augmented the antihyperglycemic action of pioglitazone in rats (Gayasuddin MM et. al., 2011). To find out the underlie reason of antidiabetic activity, phytochemical investigation was carried out. Phytochemical screening of the ethanolic extract of *Piper cubeba* fruits showed the presence of alkaloids assessed by using Wagner’s Test, Hager’s Test, Dragendorff’s Test. Presence of glycoside was evaluated by Keller Killiani Test, Legal’s Test, and Borntrager’s Test. Carbohydrates have been tested using Molisch’s Test, Barfoed’s Test and Benedict’s Test. Saponins were tested using Foam Test, Haemolysis Test and Bromin water Test. Flavonoides steroids and terpenoids were evaluated by using Ferric chloride Test, Alkaline Reagent Test, Salkowski Test, Liebermann Burchard Test, and Lead Acetate Solution Test. The results demonstrated the presence of phytoconstituents in the fruits of *Piper cubeba* such as saponins, glycoside, alkaloids and terpenoids and these chemicals primarily appears to be responsible for the significant antidiabetic activity. In addition, ethanolic extract of *Piper cubeba* fruits significantly decreased blood glucose level, total cholesterol, and triglyceride levels, and elevated the level of HDL cholesterol that are the markers of liver enzymes and lipid profile. This study has provided scientific evidence for the safe use of ethanolic extract of *Piper cubeba* fruits by traditional healers in the treatment of diabetes. However, the exact nature of the active chemical compounds responsible for antidibetic potential needs further investigation.

Hepatic cells play a part in various metabolic actions. In tissue, at greater concentrations asparate aminotransferase (AST) and alkaline aminotransferase (ALT) were available in cytoplasm. In the liver injury, the disturbances in transport function of hepatocytes which leads to plasma membrane leakage, hence enzyme level in serum are increased. Due to hepatotoxins, in liver injury the excretion of bile is imperfect which leads to levels of bile in serum
are increased. The levels of TB, DB and LDH are raised in drug-induced liver toxicity.

The hepatotoxicity produced by CCl₄ leads to the enzymatic activation which releases CCl₃ radicles in to free state results in to disruption of structure and function of lipid and protein macromolecule in cell membrane. The levels of ALP, SGOT, SGPT, TB, DB and LDH are increases is the predictable indicator of liver injury. The balance in the levels of ALP, SGOT, SGPT, serum bilirubin and LDH by herbal drug is the basic indication in the development of functional position of the liver cells. The significant reduction in levels of ALP, SGOT, SGPT, TB, DB and LDH was observed in groups of animals treated with Piper cubeba.

The prolonged consumption of ethanol which creates injury to the liver cells. The plasma as well as organelle membrane destruction due to ethanol consumption. Due to ethanol consumption the levels of serum AST, ALT, ALP and albumin are increased. Due to consumption of ethanol leads to fatty infiltration and cirrhosis. Ethanol enhances production of lipid peroxidation during microsomal metabolism. Through activation of cytochrome P-450 2E 1 enzymes which leads to generation of free radicals like H₂O₂, OH (hydroxy free radicals), O₂ (superoxide), and CH₃CHOH (hydroxy ethyl free radicals). The considerable decrease in levels of ALP, SGOT, SGPT, TB, DB and LDH was observed in groups of animals treated with Piper cubeba.

The liver is the second major organ and metabolise xenobiotic in human body. Number of poisonous chemicals and drugs which produces Liver injury. Now a day’s number of Investigators studied with traditional medicines for development of new drugs for hepatitis. In the present study, we have used two different models one is carbon tetrachloride (CCl₄) and another is ethanol for induction of liver damage, and investigated the activity of plant extract whether toxicity produced by hepatotoxicants could decrease efficiently.

In Initial phytochemical investigations of Piper cubeba fruits ethanolic extract shown the presence of alkaloids, carbohydrates, saponin, steroid, terpnoids,
tannins and flavonoids. In contemporary formal of information, chemical
constituents of the *Piper cubeba* fruit extract, it’s not possible to characterise
liver protective activity to dynamic values among which observed in ethanolic
extract of fruits of *Piper cubeba*. Though, alkaloids (Rathi A et al, 2008),
flavonoids (Yoshikawa M et al, 2003), glycosides (Yang H et al, 2005),
saponins (Matsuda H et al, 1997) and tannins (Kinoshita S et al, 2007) are
identified to own a liver protective effect in animals.

On the basis of acute toxicity studies, the ethanolic extract of *Piper cubeba*
is safe up to 2000 mg per kg. As per OECD guidelines no. 425, the doses
were selected i.e. 250 mg per kg and 500 mg per kg. The assessment of
*Piper cubeba* fruits extract against carbon tetrachloride (CCl₄) induced
hepatotoxic effect, Ethanol induced hepatotoxic effect and Alloxone hydrate
induced diabetes by using dose of 250 mg per kg and 500 mg per kg
(OECD, 2001).

The different pathological indexes Free radicals show a confident role
(Uمامahesware M et al, 2008). Free radicals having a variety of effects in
human body system have attracted researchers. Therefore, causes of certain
diseases and aging these mechanisms may be important. Usage of antioxidant
may decrease oxidative stress level and in reducing or inhibiting growth of
difficulties accompanying by means of diseases (Sundararajan R et al,
2006). Free radicals produce their action by reactive O₂ species scavenging or
shielding antioxidant fighting mechanisms (Uമamahesware M et al,
2008).

The scavenging action of Proton radical is one of the oxidation mechanism.
Determination of decreasing competence of DPPH radicals with decreasing its
absorbance at 517 nm, hence the antioxidant action of ethanolic extract of
*Piper cubeba* fruits is depends on its proton giving ability (Ganapathy S et
al, 2007, Adesegun SA et al, 2009). For scavenge DPPH, the 1.5 fold dose of
*Piper cubeba* fruits extract required than ascorbic acid. The ability of
scavenge radicals by *Piper cubeba* fruits extract was less than ascorbic acid.
In reducing power analysis, the sample reduced Fe^{+++} / ferricyanide compound to ferrous custom in the existence of antioxidants which shows ability of compounds shows indicator of latent antioxidant assets and increasing in absorbance which indicates increase in reducing power (Umamaheswari M et al, 2008, Adesegun SA et al, 2009). For the same reducing power the requirement of dose of Piper cubeba fruits extract is 2.0-fold greater compared to ascorbic acid. While the ascorbic acid shows greatest reducing power, our results showed the Piper cubeba fruits is electron giver and can retort with free radicals, alter to better stable molecules and sack radical chain reaction.

Hydroxyl radical is most toxic and reactive amongst ROS and its half-life is very short than other free radicals. O_2 derived -OH radicals laterally with additional evolution metal Fe^{++} ion causes ruin of deoxyribose into malondialdehyde turns pink chromogen with TBA (Sundararajan R et al, 2006, Adesegun SA et al, 2009). For scavenge hydroxyl radicals, the dose of Piper cubeba fruits extract required 1.5-fold greater than ascorbic acid. At physical pH of solution of Sodium nitroprusside instinctively creates nitric oxide, nitric oxide interact with O_2 to yield nitrite ions which shows characteristics absorption at 546 nm (Adesegun SA et al, 2009, Rajeshwar Y et al, 2005). Scavenger of nitric oxide competes with oxygen which leads to production of nitric oxide is reduced, therefore the Piper cubeba fruits extract shows antioxidant activity. For scavenge nitric oxide radical, the dose of Piper cubeba fruits extract required 1.5-fold greater than curcumin.

H_2O_2 is not principally responsive to greatest naturally essential particles, but intracellular herald of – OH radicals and very toxic (Ganapathy S et al, 2007, Adesegun SA et al, 2009). Piper cubeba fruits scavenged hydrogen peroxide due to occurrence of phenolic groups that might give electrons to H_2O_2, thus counteracting in water (Jafri MA et al, 1999). For scavenge H_2O_2 radical, the dose of Piper cubeba fruits extract required 1.5-fold greater than ascorbic acid.
From in vitro antioxidant experimental analysis, it was observed that *Piper cubeba* possesses strong free radical scavenging activity which might utilise favorable action in contrast to pathological changes in the Liver initiated by CCl₄ and ethanol.

The commonly used models are Carbon tetrachloride induced hepatic injuries and ethanol induced hepatic injuries for investing of liver protecting drug, the degree of a liver injuries was evaluated by released cytoplasmic level alkaline phosphatase, Bilirubin in passage, Triglycerides and Transaminases (Adesegun SA et al, 2009). In most of the documents it is stated that CCl₄ / Ethanol are biotransformed under the action of microsomal cytochrome P-450 of liver to reactive metabolites (Larrey D, 2000). These free radicals bind covalently to unsaturated lipid membrane, which leads to increases lipid peroxides along with pathological changes such as levels of serum marker enzymes like ALT, AST and ALP are elevated; decreased levels of Total Protein (TP), increased levels of Total Bilirubin (TBil) and Direct Bilirubin (DBil), Serum Triglycerides (STG) accumulation, depletion of GSH, increased Lipid Peroxidation and finally hepatocyte damage (Sies H, 1999). Therefore, CCl₄ / Ethanol induces liver injury by sharing a common property of free radical mechanism. The reduction of deleterious effects or preserve the normal hepatic physiological mechanisms which has been disturbed by hepatotoxin is the main ability of a hepatoprotective drug and the protection capability is the index of its protective effect (Suja SR et al, 2004).

The elevation of serum marker enzymes, which are released from liver into blood which leads to Hepatocellular necrosis (Shenoy KA et al, 2002). Hepatic cells play a part in variety of a metabolic activities and contain a host of enzymes. In tissues, AST and ALT in higher concentrations in cytoplasm and AST also exists in mitochondria. In liver injury, disturbances in transport function of hepatocytes, leakage of plasma membrane which leads to enzyme level are increased in serum (Das D et al, 2005). The activities of AST and ALT are elevated in serum which indicates cellular leakage and loss of functional integrity of the cell membranes in liver.
(Rajesh MG et al, 2004). In our results significantly increases the serum levels of enzymes like AST and ALT in rats after Administration of the CCl$_4$ and Ethanol. The activity of the above enzymes are decrease by oral administration of *Piper cubeba* extract at dose of 500 mg per kg body weight of rats, suggesting that the *Piper cubeba* showed the stabilization of the plasma membrane as well as repair of hepatic tissue damage caused by the CCl$_4$ and Ethanol.

The activity of Serum alkaline phosphatase (ALP) is related to functioning of hepatocytes, the levels of Serum alkaline phosphatase (ALP) increases in serum because of increased synthesis in presence of increased biliary pressure (Ghosh T et al, 2007). The elevation in serum bilirubin caused by the injury to hepatic parenchyma cells which is produced by toxicants like CCl$_4$ and Ethanol. Hyperbilirubinaemia is the very sensitive test for confirmation of the functional reliability of liver and severity of necrosis which increases conjugating, binding and an excretory capability of the hepatocytes that is proportional to erythrocyte degeneration rate (Rajesh MG et al, 2004). The reduction of higher levels of bilirubin along with suppression of ALP activity in the serum of Group III and Group IV rats, both groups are treated with *Piper cubeba* extract at dose of 250 mg per kg and 500 mg per kg respectively. Hence, suggests that during chronic injury with CCl$_4$ and Ethanol the ability of the *Piper cubeba* fruits extract to stabilize biliary dysfunction in rat liver.

In hepatotoxicity, due to defect in a protein biosynthesis depression in the Total Protein was observed and this is similar to our results. This is due to disruption and disassociation of polyribosomes from endoplasmic reticulum following CCl$_4$ and Ethanol administration (Das D et al, 2005, Hort MA et al, 2008). This change is prevented by administration of *Piper cubeba* extract at dose of 250 mg per kg and 500 mg per kg body weight. This indicates that the *Piper cubeba* possibly promote assembly of the ribosomes on endoplasmic reticulum to facilitate the uninterrupted protein biosynthesis.
In CCl\textsubscript{4} and Ethanol induced fatty liver the levels of Triglycerides (TG) are increase. Due to impairment in β-oxidation of fatty acids, increased synthesis of the fatty acids and decreased hepatic lipoproteins release in CCl\textsubscript{4} and Ethanol administered rats (Sun F et al, 2009). Due to the inhibition or destruction of TG secreting mechanism, the accumulation of TG in liver of CCl\textsubscript{4} and Ethanol treated rats (Kim SJ et al, 2008). This changes was prevented by administration of \textit{Piper cubeba} fruit extract at dose of 250 mg per kg and 500 mg per kg body weight. Hence, suggesting that \textit{Piper cubeba} probably improve β- oxidation of fatty acids.

The main reactive aldehyde is a Malondialdehyde (MDA) resulting from peroxidation of the biological membrane polyunsaturated fatty acid (PUFA). It is secondary product of the Lipid Peroxidation and used as indicator of the tissue damage. Malondialdehyde reacts with the thiobarbituric acid which produced red colored products (Das D et al, 2005). In pathogenesis of the increased membrane rigidity, reduced erythrocyte survival, osmotic fragility and perturbations in lipid fluidity assessment of Lipid Peroxidation is useful. (Shyamal S et al, 2003). The Lipid Peroxidation of the hepatocyte membranes by CCl\textsubscript{4} and Ethanol free radical derivatives causes CCl\textsubscript{4} and Ethanol induced hepatotoxicity (Shenoy KA et al, 2002). In present study the elevated levels of hepatic MDA observed in Group I rats (administered CCl\textsubscript{4} / Ethanol) is dependable on the above statement. Hence, the levels of hepatic MDA is maintained in Group IV rats (fruit extract, 500 mg per kg of body weight) is the most important. Therefore, due to antioxidant potential of \textit{Piper cubeba} it is suggest that \textit{Piper cubeba} provides hepatoprotective role.

The Glutathione (GSH) is naturally occurring tripeptide and is available in rich quantity, it is a type of non-enzymatic biological antioxidant existing in liver (Ghosh T et al, 2007). Glutathione (GSH) functions are concerned with the removal of free radicals such as superoxide radicals and H\textsubscript{2}O\textsubscript{2}, detoxification of foreign chemicals, maintenance of membrane protein and biotransformation of drugs (Jose JK et al, 2000). In the detoxification of reactive toxic metabolites of the CCl\textsubscript{4} and Ethanol the Reduced glutathione
(GSH) shows an important role, when the significantly depletion of reserves of GSH which leads to initiation liver necrosis (Hsiao G et al, 2001). Therefore, in the present investigation the levels of GSH are reduced than normal in group I rats (administered CCl₄ / Ethanol). Due to increasing efflux of GSH from the liver, binding of acetaldehyde and inhibiting the biosynthesis which leads to GSH levels are decreases (Kim SJ et al, 2008), the glutathione peroxidase which scavenge H₂O₂ leads to increase the utilization of GSH by antioxidant enzymes (Rajesh MG et al, 2004). In the present study, the GSH levels in Group IV rats (administered plant extract 500 mg per kg body weight) was significantly higher than Group I rats (administered CCl₄ / Ethanol) and nearby to the Group II (normal rats). the detoxifying enzymes can detoxify the reactive oxygen species (ROS) following administration of toxicants and *Piper cubeba* inducing the detoxifying enzymes. Therefore, it is suggest that *Piper cubeba* provides hepatoprotective role.

The liver injury caused by ROS and oxidative stress, the Serum activities of catalase (CAT) is the greatest sensitive enzymatic index (Sanmugapriya E et al, 2006). CAT is a type of haemoprotein, the accumulation of H₂O₂ CAT protects the cells by dismutating it to form H₂O and O₂ (Oh SI et al, 1997). The activity of CAT enzyme is reduced along with association of the accumulation of the highly reactive free radicals which leads to damaging effects such as loss of function of the cell membranes and integrity (Bhakta T et al, 1999). The generation of peroxy radical after administration of CCl₄ and Ethanol, O₂⁻ which is associated with inactivation of CAT enzyme. Hence, in present study the activities of the CAT are significantly reduced in rats challenged with the CCl₄ / Ethanol (Group I). The activity of CAT in rats receiving the CCl₄ / Ethanol and *Piper cubeba* extract (Group III, IV) are considerably higher than Group I rats, and very similar to normal rats (Group II). Therefore, suggests that *Piper cubeba* can reduce ROS which may causes decrease oxidative damage to hepatocytes and improve liver antioxidant enzymes activities, hence protecting the liver from CCl₄ / Ethanol.
Diabetes mellitus is one of the world’s major rising metabolic disease, and this disorder heterogeneity knowledge is progressive, and requirement for more appropriate therapy increases (Anjaneyulu AS et al, 2001). Throughout the world numerous traditional medicinal plants are used for diabetes. The study of traditional medicinal plants is a natural key for diabetologist’s pharmacy for the future (James AD, 1983).

The evaluation of biochemical parameter such as plasma glucose level, serum triglycerides, total cholesterol and HDL cholesterol in experimental rats having diabetes induced by alloxan. The alloxan has its own capacity for destroy the pancreatic β-cells (Laphookhieo S et al, 2004). By damaging insulin secreting β-cells of pancreas Alloxan and β-cytotoxin induces the “chemical diabetes” in the wide variety of animal species including rat. Alloxan produces time and the concentration dependent degenerative lesions of pancreatic β-cells (Lakshmi V et al, 1995). The dose of alloxan was 130 mg per kg required for induction of diabetes mellitus.

Insulin shows a significant role in metabolism of lipids. In lipolysis, Insulin is powerful inhibitor. The activity of the hormone sensitive lipase in adipose tissue was inhibited by Insulin and suppresses the release of the free fatty acids. The hyperlipidemia associated with increases in Lipid peroxidation.

In the diabetes the activity of the Lipid peroxidation was superior leads to increases lipolysis and release extra free fatty acids in to circulation. Increased fatty acids concentration leads to increases β-oxidation of the fatty acids, producing extra acetyl CoA and cholesterol during diabetes.

Due to lack of insulin, the levels of triglycerides were significantly increases in liver and the kidney in a diabetic control rats. Increased VLDL triglycerides hepatic secretion, probably a retarded lipolysis of VLDL triglycerides and increased serum free fatty acids level, all of these mechanisms which leads to high triglycerides in NIDDM. The low concentration of HDL cholesterol is another lipoprotein abnormality reported. Hence, low HDL cholesterol levels
leads to increased triglycerides. Enhancement of Triglyceride leads to increased production of slight dense form of LDL cholesterol and to depletion of the HDL cholesterol. However, in NIDDM the replacement of HDL cholesterol with triglycerides could not be the single justification for a reduced HDL cholesterol. The various causes for reduced HDL cholesterol such as reduced LPL, triglycerides levels elevated and increased hepatic triglyceride lipase, insulin resistance and glycosylation of HDL cholesterol.

The *Piper cubeba* produce significant alteration in levels of blood glucose or lipid profile in the diabetic rats (Baily CJ et al, 1986).

*The Piper cubeba* showed significant reduction in elevated levels of Total cholesterol in diabetic rats (Annapurna A et al, 2004).

*The Piper cubeba* showed significant increases levels of HDL cholesterol in diabetic rats (Bandole S et al, 2006).

*The Piper cubeba* showed the significant reduction in the elevated triglyceride levels in diabetic rats (Viana GSB et al, 2004).
5.2 CONCLUSION:

Ethanol extract of Piper cubeba fruits was assessed for hepatoprotective and antioxidant activities in rats. The ethanolic extract (250 and 500 mg/kg, p.o.) has demonstrated significant hepatoprotective and antioxidant activity against carbon tetrachloride and ethanol-induced hepatotoxicity as evaluated from the serum marker enzymes and antioxidant levels in blood and liver tissues. Ethanol and carbon tetrachloride induced significant increase in aspartate amino transferase, alanine amino transferase, alkaline phosphatase, total bilirubin, lipid peroxidase with a reduction of total protein, catalase, and glutathione peroxidase. Treatment of animals with different doses of ethanol extract of Piper cubeba fruits (250 and 500 mg/kg) significantly altered serum marker enzymes and antioxidant levels nearly same as normal against ethanol and carbon tetrachloride treated animals. The activity of the extract at dose of 500 mg/kg was comparable to the standard drug, silymarin (5 mg/kg, p.o.). A histopathological change of liver sample was compared with respective control. Results point out the hepatoprotective and antioxidant properties of Piper cubeba fruits against ethanol and carbon tetrachloride induced hepatotoxicity in rats.

The effect of ethanol extract of Piper cubeba fruits on several biochemical parameters in alloxan induced diabetic rats was carried out. The different doses of ethanol extract of Piper cubeba fruits was administered to alloxan-induced diabetic rats for a period of 21 days and its effect on some biochemical parameters on the blood serum of the rats were analyzed. The result indicates significant decreases in serum blood glucose levels at day 14 and 21 of the treated groups when compared to control group. In addition, ethanolic extract of Piper cubeba fruits significantly decreased total cholesterol, triglyceride levels, and elevated the level of HDL cholesterol that are the markers of liver enzymes and lipid profile. The result of this study indicates that ethanol extract of Piper cubeba has hypoglycemic effect in diabetic conditions.
From the observations of the present study, it can be concluded that,

- In vitro antioxidant studies using experimental models demonstrate that ethanolic extract of *Piper cubeba* fruits has potent antioxidant activity.

- Acute toxicity study of ethanolic extract of *Piper cubeba* fruits was carried out by oral administration. The tested doses did not show any mortality during observation period. Hence, on the basis of toxicity data, two doses were decided i.e. 250 mg/kg and 500 mg/kg.

- In both in vivo models i.e. Carbon tetrachloride induced hepatotoxicity and Ethanol induced hepatotoxicity, ethanolic extract of *Piper cubeba* fruits significantly decreased the levels of serum enzyme such as Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phophatase (ALP), Total bilirubin (Tbil) and Direct bilirubin (Dbil), whereas significantly increased Total protein (TP) content.

- Treatment of ethanolic extract of *Piper cubeba* fruits significantly decreased levels of liver tissue MDA, while increased the antioxidant enzymes glutathion and catalase that indicates ethanolic extract of *Piper cubeba* fruits has potent antioxidant activity which helps in preventing hepatic toxicity.

- *Piper cubeba* fruits offers the hepatoprotective activity against carbon tetrachloride induced hepatic injury and ethanol induced hepatic injury could be due to the cell wall membrane stabilization, hepatic cell regeneration and activation of antioxidant enzymes like catalase.

- Ethanolic extract of *Piper cubeba* fruits at dose of a 500 mg per kg had shown significant antidiabetic activity.

- Ethanolic extract of *Piper cubeba* fruits significantly decreased blood glucose level, total cholesterol, and triglyceride level, and increased HDL cholesterol.

- The present study concluded the ability of *Piper cubeba* fruits to reverse the chronic alloxan induced hyperglycemia in rats.

- The phytoconstituents estimated in the fruits such as saponins, glycoside, alkaloids and terpenoids appears to be responsible for significant antidiabetic activity.
Collectively, the ethanolic extract of Piper cubeba fruits possesses the significant antioxidant and hepatoprotective activity, and antidiabetic activity. Hence, the present study support the traditional believes of this plant and highlighted profound potential of Piper cubeba to be investigated for more appropriate individual bioactive compounds responsible for hepatoprotective and antioxidant effect, and antidiabetic activity.

Even though the significant development in treatment of the liver damage by an oral hepatoprotective agents, the synthetic drugs have a number of limitations due to now a days focused on plant derived drugs. In the present investigation, the ethanolic extract of the Piper cubeba fruits has been estimated for hepatoprotective activity against carbon tetrachloride induced hepatic injury and ethanol induced hepatic injury in rats.

As per present investigation following conclusions were seen:

- The Phytochemical screening of the ethanolic extract of Piper cubeba fruits showed the presence of alkaloids, glycoside, carbohydrates, saponin, flavonoides steroids and terpenoids.

- In vitro Antioxidant models demonstrate that ethanolic extract of Piper cubeba fruits have potent antioxidant activity.

- In acute oral toxicity study ethanolic extract of Piper cubeba fruits does not show any mortality during observation period, on the basis of toxicity study two doses were decided i.e. 250 mg per kg and 500 mg per kg.

- In both in vivo models i.e. Carbon tetrachloride induced hepatotoxicity and Ethanol induced hepatotoxicity, ethanolic extract of Piper cubeba fruits showed decrease in levels of serum enzyme such as Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phophatase (ALP), Total bilirubin (Tbil) and Direct bilirubin (Dbil) whereas increase in Total protein (TP).
Ethanolic extract of *Piper cubeba* fruits showed decrease in levels of liver tissue MDA and increases antioxidant enzymes glutathion and catalase which indicates ethanolic extract of *Piper cubeba* fruits has potent antioxidant activity which helps in preventing hepatic toxicity.

As per present investigation results, the mechanism of action of *Piper cubeba* fruits in affording the hepatoprotective activity against carbon tetrachloride induced hepatic injury and ethanol induced hepatic injury may be due to the cell wall membrane stabilization, hepatic cell regeneration and activation of antioxidant enzymes like catalase.

The present study confirmed the ability of *Piper cubeba* fruits to produce hepatoprotective activity against carbon tetrachloride induced hepatic injury and ethanol induced hepatic injury.

The phytoconstituents presence in the fruits such as saponins, glycoside, alkaloids and terpenoids responsible for significant antidiabetic activity.

Ethanolic extract of *Piper cubeba* fruits at dose of a 500 mg per kg shows significant antidiabetic activity.

It may be said that the Ethanolic extract of *Piper cubeba* fruits decreased blood glucose level, total cholesterol, and triglyceride level and increase in HDL cholesterol.

The present study confirmed the ability of *Piper cubeba* fruits to reverse the hyperglycemia of Alloxan hydrate treated rats in chronic study.
FUTURE PERSPECTIVES AND RECOMMENDATIONS

Future Perspectives:

The present study may confirm the ability of *Piper cubeba* fruits to produce hepatoprotective activity, decrease blood glucose level, total cholesterol, and triglyceride level and increase in HDL cholesterol due to phytoconstituents presence in the fruits such as saponins, glycoside, alkaloids and terpenoids responsible for significant antidiabetic activity and may have future scope to relace allopathic madications, hence reduces side effects, cost and enhance patient compliance.

Future Recommendations:

Need to be thorough Structural elucidation of *Piper cubeba* extract.

Need to be Structural level identifications to be discovered with suitable physio chemical methods in order to establish exact chemical structure.

The above research work need to be continued for the preparation and development of formulation.

The above research work need to be further continued for the confirmation of its appropriateness for usage in human being along with the developed explorative pharmacokinetic and pharmacodynamics studies.

The above research extract (formulation) need to be compare with marketed formulations.