The common guava tree (*Psidium guajava*) is popular in an indigenous system of folk medicine. Traditionally *Psidium guajava* is used for the treatment of various ailments. Most scientific evidence examines the clinical efficacy of guava in treating gastrointestinal disorders. Other investigations examined antiamebic, antibiotic, antidiarrheic, antihyperglycemic, antimutagenic, antispasmodic, and sedative effects, as well as anticough and narcotic-like activities of the plant species. *Psidium guajava* contains a number of major pharmacologically active ingredients and so many other active principles. The important active constituents are essential oils, flavonoids, carotenoids, polyphenolic compounds, pentacyclic triterpenoids, esters, and aldehydes etc. In view of the immense medicinal importance of the plant, hence this study was carried out to evaluate its pharmacological activities.

### 5.1. Phytochemical analysis of the leaf sample of *Psidium guajava*

*Psidium guajava* leaves were collected in various areas in Coimbatore district and the crude leaves were subjected to extraction with hexane, chloroform and ethanol. From these three extractants, preliminary phytochemical investigation was carried out to identify 13 different bio active components with 26 different tests (Trease and Evans, 1983). The powdered leaves of *Psidium guajava* ethanolic extract showed the presence of a lot of secondary plant metabolites which are responsible for its numerous medicinal effects. Most phytochemical studies investigated the properties of *Psidium guajava* leaf products, revealing more than 20 isolated compounds, including anthocyanins, carotenoids, essential oils, fatty acids, lectins, phenols, saponins, tannins, triterpenes, and vitamin C (Akinpelu and Onakoya, 2006; Kamath *et al.*, 2008).

Followed by screening test the leaves also contain fixed oil. The leaves of *Psidium guajava* contain an essential oil rich in cineol, tannins, triterpenes, flavanoids, resin, tannin, eugenol, mallic acid, fat, cellulose, chlorophyll, mineral salts and a number of other fixed substances (Nadkarni and Nadkarni, 1999). The presence of these secondary
metabolites supports the claims made by the tradition healers about *Psidium guajava* as recalling the findings of the present study.

5.2. Isolation and Identification of Flavonoid "Quercetin" from *Psidium guajava* by Thin layer chromatography

Among the various methods used to identify the phytochemicals, the thin layer chromatographic procedure is the one of the most commonly used techniques (Kokate et al., 2006). TLC studies have provided new dimensions to the chemistry of flavonoids to such an extent that their presence have become important taxonomically (Smith, 1969). Presence of flavonoids has been reported from many plant species like *Lycium barbarum* (Harsh et al., 1983); *Passiflora planer* (Ulubelen et al., 1984); *Cassia angustifolia* (Goswami and Reddi, 2004); *Jatropha curcas* L. (Saxena et al., 2005)

In the present study, from the TLC pattern of isolated fraction of the leaf extract of *Psidium guajava* showed a spot with Rf 0.15 (Yellow) which exactly match with the colour and Rf of the standard quercetin. From the TLC analysis it is clearly depicted that the isolated fraction contains quercetin. Quercetin has been reported from many plant species like *Cicer arietinum* Linn. (Joshi, 1985) and *Acacia catechu* (Jain et al., 2007). Meena and Panti, (2008) have isolated and identified quercetin by TLC from *Citrullus colocynthis* (Linn.) Schrad.

5.3. Antimicrobial activity

The antimicrobial studies showed good activities for the ethanolic leaf extracts of *Psidium guajava* and the isolated compounds. In the present findings various phytoconstituents from the ethanolic leaf extract of *Psidium guajava* have been identified. These constituents may be responsible for the antimicrobial activity of *Psidium guajava*.

As antimicrobials, based on ethno-botanical data, considerable number of studies have been conducted on the antimicrobial activity of *Psidium guajava* and showed promising potency against multi-drug resistant microorganisms after the current antibiotics failed to eradicate them.
Coutino-Rodriguez et al. (2001) confirming the growth inhibition effect of *Psidium guajava*, particularly on *Staphylococcus aureus*, *Escherichia coli*, and other common enteropathogenic cultures. Sanches et al. (2005) have reported ethanol: water extract of *Psidium guajava* leaves inhibited the growth of *Staphylococcus aureus*. The *in vitro* antibacterial activity of *Psidium guajava* leaf extract on *Staphylococcus aureus* was possibly due to protein degrading activity of the extracts (Belemtougri et al., 2006).

The antibacterial activity and the anti-diarrhoeal effect was observed against *Salmonella* species, *Staphylococcus* species, *Escherichia coli*, *Shigella*, *Streptococcus mutans*, *Pseudomonas aeruginosa*, *Salmonella enteritidis*, *Proteus* species and *Bacillus* species (Adebolu et al., 2007; Perez et al., 2008). The aqueous extracts were more potent in inhibiting the growth of *Proteus mirabilis*, *Streptococcus pyogenes*, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeroginosa* than the organic extracts (Abubakar, 2009). Niaz Rahim et al., (2010) investigation showed strong antibacterial activity against by using multidrug-resistant *Vibrio cholerae*.

In the present study quercetin was isolated from the ethanolic extract of *Psidium guajava* leaf and the isolated fraction compound quercetin was confirmed by TLC. Interestingly the isolated fraction showed more potent antimicrobial activity than the plant extract. *Klebsiella pneumoniae*, *Bacillus* species and *Staphylococcus epidermidis* were recorded to be the most sensitive strains to isolated fraction of *Psidium guajava*. From these results, it is possible that isolated fraction may be used as natural microbial substance to replace antibiotics to control microbial infection.

Arima and Danno (2002) result indicates that two flavonoid glycosides; morin-3-O-α-L-lyxopyranoside and morin-3-O-α-L-arabopyranoside; and two known flavonoids; guaijavarin and quercetin; isolated from leaves of *Psidium guajava* presented the antimicrobial properties. Morin, quercetin and quercetin-3-O-arabinoside, bioactive compounds isolated in alcoholic extracts of *Psidium guajava* leaves also had antimicrobial activity (Lutterodt, 1989; Raucha et al., 2000).

Recently, Metwally et al., (2010) isolated five flavonoidal compounds from *Psidium guajava* leaves which are quercetin, quercetin-3-O-α-L-arabinofuranoside,
quercetin-3-O-β-D-arabinopyranoside, quercetin-3-O-β-D-glucoside and quercetin-3-O-β-D-galactoside and also had good antimicrobial activity for the extracts and the isolated compounds. This was true in this study also as quercetin showed antimicrobial activity.

5.4. Antidiabetic and antilipidemic activity

The experimental data shows increased plasma concentrations of glucose in alloxan-treated albino rats in the study. The most common pattern of atherogenic dyslipidemia, expressed as hypercholesterolemia, hypertriglyceridemia, and/or low-HDL cholesterolemia was also noted in alloxan-treated diabetic models.

Alloxan is the most prominent diabetogenic chemicals in diabetes research (Lenzen and Munday, 1991). In the present study alloxan at a concentration of 150mg/kg body weight successfully caused diabetes in albino rats. The diabetic animals showed the following signs of the condition: polydipsia (abnormal thirst), polyuria (increased urine volume) and weight loss.

Alloxan has two distinct pathological effects: it selectively inhibits glucose-induced insulin secretion through specific inhibition of glucokinase, the glucose sensor of the beta cell, and it causes a state of insulin-dependent diabetes through its ability to induce ROS formation, resulting in the selective necrosis of beta cells (Lenzen, 2008).

The present study revealed that the *Psidium guajava* leaf extract had marked hypoglycaemic as well as hypolipidemic effect in alloxan-induced diabetes. This extract, therefore, could be used for lowering glucose, TC, TG, LDL and VLDL levels and reducing thereby the risk of CVD by increasing HDL cholesterol level.

Mechanistically, in the current investigation the antidiabetic activity of ethanolic leaf extract of *Psidium guajava* may be due to the inhibitory activity of alpha-glucosidase. Deguchi et al., (1998) demonstrated that aqueous *Psidium guajava* leaf extract, inhibited the *in vitro* activities of maltase, sucrase, and alpha-amylase in a dose-dependent manner. Furthermore, Wang et al. (2007) also observed that the extract inhibited both sucrase and maltase activities.
The leaf extract of *Psidium guajava* stimulated glucose metabolic enzymes in liver tissues (Gutierrez et al., 2008; Shen et al., 2008). Treatment with freshly prepared leaf extracts of *Psidium guajava* significantly reduced blood glucose and lipid profile levels in diabetic albino rats and having similar effect in diabetic patients (Prasad et al., 2009; Wu et al., 2009; Rafiq et al., 2009; Rai et al., 2010).

In the current investigation not only plant extract but also its isolated fraction compound also has the ability to protect against alloxan-induced diabetes. Plant extract fraction compound treated alloxan-induced diabetic albino rats' significantly decreased glucose, TC, TG, LDL cholesterol and increased in HDL cholesterol level were observed. The experimental data revealed that the lower level of glucose and lipid profile in the plant was probably associated with high content of quercetin in *Psidium guajava* leaf extract and confirming thereby its administration for diabetic patients.

The findings of the present study were on par with the findings of others (Vessal et al., 2003; Kanter et al., 2006). Li et al. (2009) results indicated that quercetin, isoquercetin and rutin could bind alpha-glucosidase to form a new complex. Cheng et al. (2009) suggest that quercetin in the aqueous extract of *Psidium guajava* leaves promotes glucose uptake in liver cells, and contributes to the alleviation of hypoglycemia in diabetes as a consequence.

Oral administration of quercetin showed a decrease of plasma glucose and increase in insulin levels were observed along with the restoration of glycogen content and the activities of carbohydrate metabolic enzymes in quercetin-treated diabetic albino rats (Babujanarthanam et al., 2010; Wang et al., 2011). However, several studies have illustrated quercetin’s have the ability to reduce TC, TG, LDL cholesterol and augment of HDL cholesterol (Chopra et al., 2000; Torres-Piedra et al., 2010; Kim et al., 2011).

Ethanolic leaf extract of *Psidium guajava* had no effect on TC, TG, LDL cholesterol, VLDL cholesterol and HDL cholesterol concentration of normoglycemic animals. Furthermore, ethanolic leaf extract of *Psidium guajava* and its isolated fraction promotes similar effect in diabetic albino rats. In accordance with these reports, the present study elicited that *Psidium guajava* extract shows major constituent’s of
quercetin, which could regulate blood glucose and lipid profiles in alloxan-induced diabetic albino rats.

5.5. Antioxidant activity in diabetic induced animal models

In the present study oxidative stress status was assessed by measuring the TBARS formation, antioxidant enzymes such as, SOD, CAT, GPx and non-enzymatic antioxidant (GSH) activity in liver. The results showed that a significant increase in TBARS level and decrease in SOD, CAT, GPx and GSH activity in liver of the alloxan-induced diabetic albino rats.

In diabetes, hyperglycaemia generates ROS, which in turn cause lipid peroxidation and membrane damage and these free radicals play an important role in the production of secondary complications in diabetes (Hunt et al., 1988). Antioxidant has shown to prevent the destruction of β-cells (Murthy et al., 1992).

The result of the present study revealed that the administration of the *Psidium guajava* leaf extract showed a significant decrease not only in blood glucose level, conversely it also showed an improved antioxidant potential as evidenced by decreased lipid peroxidation and a significant increase in the activity of GSH and various antioxidant enzymes such as CAT, SOD and GPx in alloxan-induced diabetic albino rats.

In the current investigation, administration of fraction of *Psidium guajava* leaves significantly decreased blood glucose levels and improved the antioxidant status. Lipid peroxidation formation was also suppressed.

*Psidium guajava* markedly restored the activities of antioxidant enzymes, including SOD, CAT, and GPx in STZ induced diabetes (Huang et al., 2011). Oral administration of quercetin to diabetic albino rats resulted in a decrease in the levels of blood glucose, plasma TBARS and hydroperoxides. Quercetin also resulted in the activities of SOD, CAT coming to near normal, along with the levels of vitamin C and vitamin E (Mahesh and Menon, 2004; Coskun et al., 2005; Panda and Kar, 2007; Zhang et al., 2011). The present work clearly depict that the *Psidium guajava* leaf extract possess significant antioxidant against alloxan-induced diabetes. This may be due to the fraction
compound quercetin of the plants and as such make them potential natural chemoprophylactic agents.

5.6. *In vitro* antioxidant activity

Experiments were conducted to determine the free radical scavenging capacity of ethanolic leaf extract of *Psidium guajava* and its isolated fraction by *in vitro* assays (Nenadics *et al.*, 2007; Magalhaes *et al.*, 2008). It has been evaluated by different methods (DPPH radical scavenging activity, reducing power assay and NO assay) under different concentrations.

The experimental data of the present study of ethanolic leaf extract of *Psidium guajava* and its isolated fraction reveal that both are likely to have the effect of scavenging free radical in accordance with the standard, ascorbic acid. It was observed that a dose–response relationship is found in the DPPH radical scavenging activity, reducing power assay and NO assay; the activity increased as the concentration increased for ethanolic leaf extract of *Psidium guajava* and its isolated fraction.

In the current investigation the plant extracts showed maximum hydrogen-donating ability in the presence of DPPH stable radicals at high concentrations. DPPH reactivity is one popular method for screening the free radical-scavenging ability of compounds or the antioxidant activity of plant extracts, and has been used extensively as a free radical to evaluate reducing substances (Brand Williams *et al.*, 1995). DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule.

In the current study the relative reducing power of the extract of *Psidium guajava* leaf and its isolated fraction may be due to its strong reducing power potential and isolated compound in the extract which possess potent donating abilities. Substances, which have reduction potential, react with potassium ferricyanide (Fe$^{3+}$) to form potassium ferrocyanide (Fe$^{2+}$), which then reacts with ferric chloride to form ferric ferrous complex (Oyaizu, 1986). This may be true in the present study also.
NO is an important mediator generated by endothelial cells, macrophage, neurons etc., and involved in regulation of various physiological processes. The ethanolic extract of the plant drug under study showed the highest NO scavenging activity compared to standard. The reduction of the NO by the extract may be due the presence of isolated fraction which has the capacity to inhibit nitrite formation by directly competing with oxygen in the reaction with NO. Based on these results, it has been suggested that the scavenging free radical activity by the leaf extract is possibly due to the presence of ‘quercetin’ which is also confirmed by TLC pattern at molecular level.

Jimenez et al. (2001) reported the polyphenol compounds from *Psidium guajava* showed a remarkable antioxidant capacity. Qian and Nihorimbere (2004) found that the commercial *Psidium guajava* leaf extracts and ethanol *Psidium guajava* leaf extracts showed almost the same antioxidant power. The ethanol extract from the leaves of *Psidium guajava* showed the highest antioxidant capacity followed by the fruit peels of *Nephelium lappaceum* and *Garcinia mangostana* (Tachakittirungrod et al., 2007). This may be true in the present findings also.

The aquatic and the ethanol extracts from *Psidium guajava* possess the potential antioxidative activities and the flavonoids may be one of their antioxidative components (Wang et al., 2007). *Psidium guajava* contained the highest amount of total phenolics and total flavonoids. According to Akinmoladun et al. (2010), percentage DPPH radical scavenging activity was highest in *Psidium guajava* and compared with values obtained for ascorbic acid and gallic acid. This may be true in this study also.

The present findings and the findings of others indicate that useful bioactive substances exist in the *Psidium guajava* leaf extracts (You et al., 2011). In the present assessment, it was interesting to find that the isolated fraction from extract of *Psidium guajava* leaf showed the highest free radical scavenging activity than *Psidium guajava* leaf extracts.

A number of *in vitro* studies were performed with quercetin under several experimental conditions to monitor various indices of antioxidant/pro-oxidant activity.
Lee et al. (2003) incubated mouse thymocytes with quercetin (50 μM) and observed that, while quercetin alone did not induce any cytotoxicity, it did exhibit antioxidant activity by protecting cells against oxidative stress-mediated apoptosis. The results of the present findings and previous studies indicate that *Psidium guajava* and its component quercetin could be a suitable source of natural antioxidants.

5.7. Hepatoprotective activity

In the present study the activities of total protein, albumin, globulin, A/G ratio, total bilirubin, ALP, ALT and AST are commonly used to evaluate the status of liver function. Liver is the metabolic super achievers in the body and is the target organ for most toxicants which enters the body. It plays a central role in transforming and clearing chemicals (Norazmir et al., 2010).

CCl₄ is the most prominent hepatotoxic chemical (Junnila et al., 2000). Evidence suggests that various enzymatic and non-enzymatic systems have been developed by the cell to cope up with the ROS and free radicals. In many aspects it mirrors the pattern of human disease associated with toxic damage.

In the present study the significant decrease in albumin levels in CCl₄ treated albino rats could be attributed to suppressed protein synthesis in liver. Albumin is the most abundant circulatory protein and its synthesis is a typical function of normal liver cells. In the present study alteration of globulin content in CCl₄ induced animals appears to be compensatory, as the ratio of A/G ratio showed a significant fall in this group of animals.

In hepatic dysfunction a decrease levels in serum total protein and albumin and an increase in globulin fraction were also observed in previous studies (Premalatha and Sachidanandam, 1998). A healthy liver is so crucial for protein metabolism since liver disease is frequently associated with alterations in proteins and disturbances of protein metabolism (Marshall, 2000).

In the present investigation determination of serum bilirubin serves as an index for the assessment of hepatic function. Stabilization in the levels of serum bilirubin in
the *Psidium guajava* leaf extract, isolated fraction and silymarin treated groups as compared to CCl₄ alone fed group clearly indicate the improvement in the functional status of the liver.

Bilirubin is an endogenous organic anion that binds reversibly to albumin and is transported to the liver, where it is conjugated to glucuronic acid and excreted in bile (Friedman *et al.*, 1996). The increased levels of conjugated and unconjugated bilirubin in the present study could result from an impairment of uptake or conjugation, coupled with decreased excretion of the pigment. This is also showing the severity of hepatic dysfunction caused by the hepatotoxin.

In the present investigation, the study of serum enzymes (ALP, ALT and AST) activity has been found to be great importance in the assessment of liver damage in CCl₄ induced toxicity. In toxic liver injuries, variable changes in the activities of enzymes can be found in the serum and it extent of cellular damage. The rise in serum levels of ALP, ALT and AST have been attributed to the damage of structural integrity of the liver.

Increased activities of the enzymes in plasma may be due to leakage of all enzymes from the neoplastic cells into blood or may be due to the release of enzymes from normal tissue invaded by tumour or may be due to the possible effect of tumour on remote tissue leading to the loss of its enzyme and release into the blood (Plaa and Zimmermann, 1997; Devi and Devaki, 1998). This may be true in this study also.

The results of the present findings revealed that the alcoholic extract of *Psidium guajava* and its isolated fraction used the study seems to offer more significant protection and maintenance to the structural integrity of the hepatocellular membrane. This is evident from the fact that the treated albino rats with *Psidium guajava* extract and its isolated fraction significantly prevented the toxicity of CCl₄ on the liver as indicated by the decreased activities of the marker enzymes of hepatic function studies.

Treatment with alcoholic extract of *Psidium guajava* attenuated the CCl₄ induced to increase activities of these enzymes. A subsequent recovery towards normal level in the activities of these enzymes strongly suggests that the possibility of *Psidium guajava* leaf extract and its isolated fraction as a conditioner of the hepatocytes.
In the present study there were no significant change in the activities of total protein, albumin, bilirubin and liver enzymes in *Psidium guajava* ethanolic leaf extract and its isolated fraction alone treated albino rats. This proves the absence of any toxic effect of the plant on the mammalian system. This observation infers a protective effect by *Psidium guajava* leaf extract and its isolated fraction on impaired hepatic function caused by CCl$_4$. Administration of ethanolic leaf extract of *Psidium guajava* and its isolated fraction prevented the increase in the levels of bilirubin and these enzymes showed the pattern of recovery from the toxic effects.

5.8. Antioxidant Enzymes

The present investigation, reduced activities of SOD, CAT and GPx in the liver tissue were observed in CCl$_4$ induced toxicity. Treatment with *Psidium guajava* ethanolic leaf extract, its isolated fraction and silymarin showed a significant increase in SOD, CAT and GPx, which might be due to the antioxidant potential of these compounds. Results of the *in vitro* antioxidant studies of the present findings also supports the antioxidant and free radical scavenging effect of *Psidium guajava* ethanolic leaf extract, its isolated fraction and silymarin.

SOD is a metalloprotein and is the first enzyme involved in the antioxidant defense. CAT is a heme protein occurs abundantly in the body, with the highest activity in the liver, followed by erythrocytes, then the lungs. GPx is a seleno-enzyme two third of which is present in the cytosol and one third in the mitochondria (Govindarajan *et al.*, 2005). To cope with the oxidative stress, an increase in SOD must be accompanied by concurrent increase in CAT and/or GPx to prevent excessive buildup of H$_2$O$_2$ (Ratnam *et al.*, 2006).

In the present study *Psidium guajava* leaf extracts showed the strongest antioxidant activity, this result was on par with Chen *et al.* (2007). *Psidium guajava* has antioxidant properties attributed to polyphenols found in its leaves (Begum *et al.*, 2004). Wu *et al.* (2009) and Ling *et al.* (2010) have reported potent antioxidant activity in *Psidium guajava* leaf extracts and attributed it to the phenolic compounds.
Many antioxidant supplements and herb-containing medicaments contain high doses of flavonoids. In the current findings it was noted that the leaves of *Psidium guajava* are rich in flavonoids, particularly quercetin. Zhang et al. (2006) identified phytoconstituents and many flavonoids, quercetin was also one among them in *Psidium guajava* leaf. Recently, an extensive amount of in vitro and in vivo animal research has confirmed on the antioxidant potential of quercetin (Das et al., 2008; Hwang et al., 2009; Seufi et al., 2009; Park et al., 2010; Tota et al., 2010). Based on this the current study was also focused on in vitro and in vivo animal study.

5.9. Non-enzymatic Antioxidants

Lipid peroxidation was measured by formation of TBARS associated with CCl₄ induction, as an indicator of oxidative stress. Treatment with *Psidium guajava* leaf extracts, its isolated fraction and silymarin showed a significant reduction which might be due to the antioxidant ability of these compounds and the consequent reduction in lipid peroxidation which may lead to cancer.

Lipid peroxidation is one of the main manifestations of oxidative damage and has been found to play an important role in the toxicity and carcinogenicity of CCl₄. Peroxidation of membrane lipids initiated the loss of membrane integrity and membrane bound enzyme activities which in turn brought about a disturbance of cellular homeostatis (Tosulkao and Glinsukon, 1992).

Based upon the present experimental results for non-enzymatic antioxidants, it may be suggested that *Psidium guajava* leaf extracts and its isolated fraction have definitely antioxidative properties in CCl₄ induced toxicity. Among the non-enzymatic antioxidants GSH plays a critical role in important cellular functions, which includes maintenance of thiol status of proteins, the destruction of H₂O₂, lipid peroxides and free radicals, translocation of amino acids across cell membranes, the detoxification of foreign compounds and the biotransformation of drugs (James and Hrabison, 1982).

In the present study there was a significant decrease in Vitamin E levels in CCl₄ fed group, might be due to the excessive utilization of this antioxidants for quenching
enormous free radicals produced in these conditions. The level of vitamin E has increased in alcoholic leaf extract of *Psidium guajava*, its isolated fraction and silymarin treated albino rats which by its antioxidative nature results in the rejuvenation.

Vitamin E (α-tocophenol) is one of the most significant antioxidants in animal cells and is thought to act as a chain breaking antioxidant, by donating its labile hydrogen atom from phenolic hydroxyl groups to propagating lipid peroxyl and alkoxyl-radical intermediates of lipid peroxidation thus terminating the chain reaction (Wiseman, 1996). It has been found to have potent antioxidant activity due to its ability to penetrate to a precise site into the membrane which may be an important feature of protection against highly reactive radicals (Premalatha and Sachidanandam, 1999).

In the present findings the decreased level of vitamin C was found in CCl₄ fed group animals. The ascorbic molecule must be involved in the feed back inhibition of lysosoma L glycosidases responsible for malignant invasiveness (Cameron et al., 1979). In the present study the recoupment of vitamin C to near normal level in drugs treated albino rats was recorded. *Psidium guajava* by inducing the regulation of ascorbic acid has acted as a potent hepatoprotective drug to increase vitamin C level.

Vitamin C (ascorbic acid) is an excellent hydrophilic antioxidant (Frei and England, 1986). The availability of vitamin C is determining factor in controlling and potentiating host resistance. It can protect cell membranes and lipoprotein particles from oxidative damage by regenerating the antioxidant from vitamin E (Buettner, 1993). Thus vitamin C and E act synergistically in scavenging a wide variety of ROS (Beyer, 1994).

In the present study decreased levels of uric acid in the CCl₄ fed albino rats might be due to increased production of free radicals and subsequent decrease in lipid peroxidation. The marked increase in uric acid levels in the *Psidium guajava*, its isolated fraction and silymarin administered groups as compared to CCl₄ fed group would have resulted in free radical scavenging activity of the plant and its extract on lipid peroxidation chain reaction.
Uric acid, the end product of purine metabolism, has been proven to be a selective antioxidant, capable of reacting with free radicals and HOCl (Hasugawa and Kuroda, 1989). Urate protects ascorbate against oxidation by cupric ions and also against iron-induced oxidation. Urate possesses preventive antioxidant activity in addition to its chain-breaking action (Wayner et al., 1987).

In the present investigation, administration of the ethanolic leaf extract of *Psidium guajava* prior to CCl₄ intoxication could not only prevent the CCl₄ induced increased levels of serum marker enzymes, but also protect the antioxidant machineries of the liver as revealed from the enhanced levels of SOD, CAT, GPx, GSH and decreased level of lipid peroxidation. In the current study, the stimulation of this promising antioxidant defense provided a good natural antioxidant substance from *Psidium guajava* leaf and it has been an advanced remedy for hepatotoxic.

### 5.10. Histopathological studies of Liver

As observed in the present study, CCl₄ treated albino rats produced various histological changes in the hepatocytes. Pretreatment with *Psidium guajava*, its isolated fraction and silymarin prevented CCl₄ induced changes in the hepatic architecture and protect the liver tissue from necrotic, fatty and degenerative changes. In the liver injury induced by CCl₄, the higher dose (500 mg/kg, b.w.) of *Psidium guajava* leaf extract was found to be more effective than the lower dose (250 mg/kg, b.w.). This may be preventing the toxic chemical reactions which generate oxidative stress, lipid peroxidation and molecular changes.

In the present investigation, the severity of the toxicity is evidenced by the observation of pathological changes in the architecture of the liver viz., infiltration of inflammatory cells, Kupffer cell hyperplasia, neutrophil accumulation, focal necrosis and degenerative changes in the hepatocytes. This shows histological features of development of pure, well differentiated liver cell had abnormalities. Biliary proliferation is not seen in any of the rat livers. These lesions observed after CCl₄ treatment were on par with previous report on the administration of CCl₄ (Diao et al., 2011).
These above observations in the present study are in accordance with that of Chanchal et al., (2006), who have studied the impacts of the methonolic leaf extract of *Psidium guajava* in CCl₄ induced toxicity on rat liver cells. Sambo et al., (2009) study has shown that the aqueous extract of *Psidium guajava* leaf possesses hepatoprotective property.

In the present study both plant extract and its isolated fraction treatments were able to ameliorate CCl₄ induced hepatocellular damage as evidenced by prevention of any increase in serum transaminase (AST and ALT) levels subsequent to toxin exposure and the known anti-oxidant (Nardini et al., 1997), free radical scavenging (Kono et al., 1997) and anti-lipid peroxidation (Morton et al., 2000) properties of quercetin might be the contributing factor for these manifestations. Histopathological study of the liver tissue also supports hepatoprotective activity of *Psidium guajava* and its isolated fraction against the toxicity of CCl₄.

**5.11. Blood Glucose and Hepatic glycogen**

In the present study, it has been observed that CCl₄ induced a significant decrease in blood glucose and hepatic glycogen levels. The liver plays a central role in carbohydrate metabolism (Postic et al., 2004). Hepatic glucose metabolism is disrupted in the setting of cirrhosis (Haagsma et al., 1997; Petersen et al., 1999). Glycogen stores are markedly reduced in liver disease in addition to altered hepatic gluconeogenesis with the result that abnormalities of glucose homeostasis is a consistent finding in patients with chronic liver disease (Changani et al., 2001).

Studies have demonstrated decreased hepatic glycogen content after treatment with CCl₄, reflecting decreased gluconeogenesis by the liver (Krahenbuhl et al., 1991). In the impaired liver, diminished glycogen stores, resulting in failure of the liver to supply glucose in the post-absorptive state, may be the cause for further decrease in hepatocyte viability. In the present study the glycogen reduction indicates transformation of the preneoplastic to neoplastic cells as reported earlier (Changani et al., 2001).
In the present study replenishing glycogen stores is surely an indication of improved liver function and preservation of hepatic architecture integrity in the face of CCl₄ toxic insult. Administration of *Psidium guajava* leaf extract and its isolated fraction would have stimulated liver cells to convert more glucose to glycogen and thus glycogen content restored to normal. Based upon the above experimental results, it may be suggested that, ethanolic leaf extract of *Psidium guajava* and its isolated fraction has protective role on carbohydrate metabolism in CCl₄. This action is probably due to the synergistic effect of various compounds in the leaf extract.

5.12. Lipids

Liver plays a key role in lipid metabolism (Dessi *et al.*, 1982). In the present study elevation in serum cholesterol levels indicates high rate of cholesterol synthesis and it was observed in CCl₄ fed albino rats. Similar elevation in cholesterol levels was reported in hepatoma 27 cells (Diatlovitskia and Bergelson, 1982) and in N-nitrosodiethylamine induced hepato carcinoma (Tang *et al.*, 1992).

In the present study the increased TC level in serum of CCl₄ fed albino rats may be due to decreased uptake of cholesterol from blood. Blood supply to hepatomas also decreased and hence 80% decrease in uptake of blood born substances occurred in hepatoma conditions (Ericksun *et al.*, 1978).

The result of the present investigation showed decreased cholesterol content to near normal in the treated groups, ethanolic leaf extract of *Psidium guajava*, its isolated fraction and silymarin can be due to strong hypocholesteromic activity of *Psidium guajava* and its isolated fraction. This may inhibit cholesterol synthesis and accumulation. Lowering the elevated level of cholesterol not only retard progression but can even cause faster regression of the toxicity of CCl₄.

In the present study the hypertriglyceridemia observed in CCl₄ induced toxicity may be due to the clearance defects associated with deficient lipoprotein lipase activity. TG is probably metabolized by lipoprotein lipase and the reaction products, free fatty
acid and glycerol may be translocated into the liver readily crossing the liver cell membrane (Felt and Benny, 1971).

Hypertriglyceridemia which is frequently observed in various degrees in tumour bearing animals in combination with increased VLDL cholesterol and decreased HDL cholesterol is a defective catabolism rather than elevated hepatic synthesis of triglycerols rich lipoproteins (Damen et al., 1984).

In the present study the decreased content of TG in ethanolic leaf extract *Psidium guajava* and its isolated fraction administered animals. The hypolipidemic effect of *Psidium guajava* leaf extract can be attributed to the presence of flavanoids, in it. Because flavanoids have the ability to reduce serum TG level (Starvric and Matula, 1992). This may be due to the optimal activity of serum lipoprotein lipase and the antioxidant effect of the plant extract.

HDL cholesterol is considered to be a beneficial lipoprotein (Gordon, 1977). It helps in the scavenging of cholesterol from the extra hepatic tissues in the presence of lecithin cholesterol acyl transferase and brings it to the liver (Kavitha and Nalini, 2000). In this context Nikkila et al., (1987) have shown that the elevated activity of plasma lipoprotein lipase leads to an increase in HDL cholesterol production and reduction in LDL cholesterol constituents.

Therefore in the present study the increased HDL cholesterol levels might be due to the increased activities of lipoprotein lipase and lecithin cholesterol acyl transferase. In the present study the most active drug seems to be the ethanolic leaf extract of *Psidium guajava* for HDL cholesterol. Silymarin was the best drug for TC, TG, LDL cholesterol and VLDL cholesterol. Subsequently, ethanolic leaf extract of *Psidium guajava* and the isolated fraction quercetin of *Psidium guajava* are effective respectively.

5.13. Urea and Creatinine

Kidney is the second organ most frequently affected by any compound (Marshall 2000). Therefore, in the present investigation renal functions are assessed by measuring the concentration of creatinine and urea in plasma. Plasma urea and creatinine
concentrations are often used as an index of renal glomerular function and will be increased in renal injuries (Moshi et al., 2001; Hughes and Jefferson, 2008).

Urea is synthesized in the liver, primarily as by-product of the deamination of amino acids. In the present study the lowered urea level in CCl₄ treated conditions may be due to reduced hepatic urea synthesis which leads to reduction in blood urea (nitrogen) and this is an index of hepatocellular functional defect. Diminished urea synthesis results in decrease in the removal of ammonia, enhances the metabolic disturbances in cancer condition (Mc Intyre and Rosalki, 1992).

Creatinine is a nitrogenous waste product produced from creatinine in muscle and excreted by the kidneys. In the present study the slightly increased creatinine level during CCl₄ conditions may be due to impaired renal function which occurs as a secondary event to reduce hepatocellular function (Mc Intyre and Rosalki, 1992).

In the current investigation the reversal of these altered urea and creatinine levels to near normal state in ethanolic leaf extract of Psidium guajava and its isolated fraction treated animals could be attributed to strong inhibitory effect of Psidium guajava on CCl₄ conditions. Psidium guajava leaves prevent arsenic induced biochemical alterations (Roy and Roy, 2011).

Their study also reveals that the level of urea and creatinine found to be normal in Psidium guajava plus arsenic induced treated groups. In the present study nonsignificant increase in the creatinine level support that the ethanolic leaf extract of Psidium guajava and its isolated fraction does not causes renal damage.

5.14. Comet assay

In current investigation, an attempt was made to study the DNA protective potential of ethanolic leaf extract of Psidium guajava, its isolated fraction and silymarin on CCl₄ induced hepato toxicity using the comet assay. Since CCl₄ is a classic model compound for inducing free radical damage in the liver, CCl₄ poisoning was chosen as a primary rodent model of oxidative stress (Kadiiska et al., 2000).
It is known that CCl₄ is reductively bioactivated by cytochrome P450 to the trichloromethyl free radical, which, in the presence of oxygen, is subsequently converted into a peroxy radical. These free radical metabolites can abstract hydrogen from different molecules, thus initiating oxidation of lipids, proteins, and DNA (Recknagel, 1983; Recknagel et al., 1989; Reinke et al., 1992; Goeptar et al., 1995). In the current investigation this might be the main reason for increased severity of DNA damage in CCl₄-treated cells.

The present study findings are consistent with the other published reports, high concentrations of solvent extract from guava protected cells against DNA damage (Kong et al., 2010). Huang et al. (2011) noted that decreased oxidative stress in the plasma of *Psidium guajava* treated albino rats probably suggests that *Psidium guajava* exerts antioxidative activity that protects the tissues from the destructive effects of lipid peroxidation and DNA damage. The present investigation also confirmed the antioxidative properties of ethanolic leaf extract of *Psidium guajava* and its isolated fraction.

In this study DNA damage was significantly reduced in CCl₄ induction with the rat hepatocytes of quercetin fraction treated albino rats. In spite of its antioxidant property, quercetin is known to bind DNA strand at sites that would normally react with the active metabolites of carcinogen during carcinogen-DNA binding, a crucial step for initiation of carcinogenesis and toxicity (Bhattacharya and Firozi, 1988; Khanduja and Majid, 1993).

In the present study DNA protective property of plant extract may also be due to the synergistic effect of the various compounds in the *Psidium guajava* leaf extract. Alternatively, when the phenolics bind to DNA, its molecules could be positioned in such a way so as to effectively scavenge reactive intermediates that approach the critical sites on DNA, or phenolics may directly interact with the ultimate reactive metabolites of carcinogen by donating their electrons and rendering it inactive (Wood et al., 1982).

Dok-Go et al. (2003) demonstrated that quercetin rich plant extract and fraction acts in many cell-free experimental systems to scavenge reactive oxygen radicals and reduces CCl₄ generated free radical mediated DNA damage. With regard to other
genotoxicants, quercetin and rutin displayed antigenotoxic effects on DNA damage induced by mitomycin C, in a concentration-dependent manner (Anderson et al., 1997; 1998; Undeger et al., 2004; Yeh et al., 2005).

Recently, Orsolic et al. (2011) used the comet assay to assess the levels of DNA damage in the blood, liver and kidney cells in untreated and quercetin or naringenin treated with different experimental animals and the tested flavoinds have protective effects against alloxan-induced DNA-damage in peripheral lymphocytes may be true in this study also.

5.15. Cytotoxic effect against Ehrlich Ascites Carcinoma cell lines

Experimental tumors have great importance in modeling, and Ehrlich Ascites Carcinoma cell line is one of the commonest tumors. In the present study Ehrlich Ascites Carcinoma cell lines were used to screen the anticancer potential of the ethanolic leaf extract of Psidium guajava and its isolated fraction, using preliminary screening technique (Koul et al., 2003). The Ehrlich ascites tumor is a useful tool for testing the activity of chemicals and besides it provides an easy challenge to chemotherapeutic agents. This preliminary experiment was carried out using four different concentrations of the plant extracts and its isolated fraction.

Experimental data of the present study showed that the extracts found to be cytotoxic against Ehrlich Ascites Carcinoma. The cytotoxicity increased with increase in concentration of extract. 100 µg/ml concentrations showed 23.42% cell death where as, in high concentration (1000 µg/ml) 75.34% of cell death was noticed. The isolated fraction found to be cytotoxic against Ehrlich Ascites Carcinoma. At low concentration 5 µg/ml leaf extract showed 11.23% cytotoxicity whereas at high concentration 100 µg/ml showed 43.84% of cytotoxicity.

As a part of the apoptosis precede the loss of membrane integrity there by the cells were permeable to Trypan Blue. The extracts were found to have considerable cytotoxic effects and it may be found that it activates the apoptotic pathway inside the cancer cells. Further in depth cytotoxic activity of the plant extract and its isolated
fraction under study were evaluated against Ehrlich Ascites Carcinoma cell lines (MTT assay). 24hrs treatment with plant extracts showed growth inhibition of Ehrlich Ascites Carcinoma cells.

In the present investigation, leaf extract showed 72.99% of cytotoxicity with IC\textsubscript{50} Value 30 µg/ml and isolated fraction showed 97.70% of cytotoxicity with IC\textsubscript{50} Value 7.5 µg/ml. The death of the cells caused by the extract under study was due to the loss of mitochondria which is one of the hallmark of the apoptosis pathway. From the results it is clearly evident that at minimum concentration the extract activates the apoptotic pathways and results in death of Ehrlich Ascites Carcinoma cell lines.

In the current investigation, the electrophoerteic run of DNA of Ehrlich Ascites Carcinoma cells treated with two different concentrations of the ethanolic extract of the Psidium guajava exhibited extensive double strand breaks there by yielding a ladder appearance. The degradation of DNA may be due to activation of endonucleases. Fragmentation of DNA into nucleosomal units is caused by a specific enzyme known as CAD (Caspase activated DNase). Normally CAD exists as an inactive complex with ICAD also known as DNA fragmentation factor.

During the apoptosis ICAD cleaved by Caspase 3 to release CAD. Since CAD has DNase activity with high specific activity compared to DNase I and DNase II rapid fragmentation of DNA follows. The presence of DNA fragmentation has been extensively used as a marker for apoptotic cell death. In the present study DNA fragmentation caused by the plant extract and isolated fraction clearly indicated that the extract activates the apoptotic pathway of cancer cells.

The isolated fraction quercetin of this study has the ability to interfere with different targets identified as “hallmarks of cancer” makes this molecule, together with several other phytochemicals, a multi-target inhibitor with pleiotropic and synergistic effects in tumor cells (Lee et al., 2011). Quercetin inhibits the growth and proliferation of cancer cell lines of different origins (prostate, cervical, lung, breast, and colon) in vitro (Russo et al., 2012).