Ethanol related disorders are one of the challenging current health problems with far reaching medical, social and economic consequences. High ethanol consumption causes critical problems in the body including alcoholic liver disease (ALD) (Pari and Karthikesan, 2007; Sivaraj et al., 2011). According to the World Health Organization report of 2005, approximately 2 billion people worldwide consume ethanol, and about 76 million of them have been estimated to be suffering from ethanol consumption related disorders. The most extensively investigated aspects of alcohol on health is ALD, which is one of the major causes of illness and death worldwide (Liu et al., 2010). Long term use of ethanol potentially results in serious illness, including alcoholic fatty liver, steatosis and cirrhosis (Ponnappa and Rubin, 2000).

The unabated alcoholism made researchers for the past two decades to develop remedies for alcoholism. However, developing low toxicity and high efficiency medicines, remains a challenging task (Xu et al., 2005). Use of some synthetic medicines/drugs against alcoholism and alcohol related damage have been reported to exert unwanted and undesirable side effects (Mann, 1996; Kiefer and Wiedmann, 2004). Now the globe is turned towards plant medicine seeking herbal remedies to treat alcoholic related disorders (Corns, 2003). A large number of studies are in progress aiming to identify natural substances that would be effective in reducing the severity of ALD. It has been found that some compounds in their natural formulations are more effective (Khopde et al., 2001). Hence, there has been a search for safer natural dietary supplementation of native plant extracts containing several principles for therapeutic purpose.

Flavonoids are ubiquitous in higher plants and are known to be beneficial to human health due to its diverse pharmacological properties. Morm is an important dietary constituent of food. It has been reported to have clinically relevant functions, including antitumour, antioxidant, antiinflammatory, antihypercholesterolaemic, antimitagene, antidiabetic, antihypertensive, neuroprotective, cardioprotective, and hepatoprotective effects.
This study intends to provide an update in terms of the current status of molecular therapy for hepatic injury and how far we are from clinical utilization of these new therapeutic modalities. We have discussed the mechanisms by which alcohol contributes to increased oxidative stress, the activation of Kupffer cells and hepatic stellate cells leading to inflammation and apoptosis cascade. Finally, we have made an attempt to evaluate the mechanisms of action of morin in ethanol-induced toxicity rats by analysing targeted gene expression at the molecular level approaches.

5.1 IN VITRO ANTIOXIDANT AND FREE RADICALS SCAVENGING ACTIVITIES OF MORIN

To know the underlying mechanism of action of morin, we have investigated the in vitro DPPH$^-$ and ABTS$^-$ radical scavenging effects of morin. For the quantitative investigation of hydrogen-radical donation, a stable radical has the advantage that their concentrations are readily and directly measurable. Among them, a stable free radical, DPPH$^-$ was investigated as a reactive hydrogen acceptor. Generation of the ABTS$^+$ radical cation forms the basis of one of the spectrophotometric methods that have been applied to the total antioxidant activities of solutions of pure substances. The present study revealed that morin scavenged DPPH$^-$ and ABTS$^+$ in a dose dependent manner. The highest percentage of scavenging effect of morin on DPPH$^-$ and ABTS$^+$ was found to be at the concentration of 200 μM. Thus, morin is a potent free radical scavenger and antioxidant.

In order to know the mechanism of action of morin, we also investigated in vitro effects of morin on scavenging O$_2^-\cdot$ and OH$^-$. Radical scavenging activities are very important due to the deleterious role of free radicals in biological systems. It has been reported that O$_2^-\cdot$ radicals directly initiate LOOH (Wickens, 2001). O$_2^-\cdot$ is a precursor of active free radicals that have the potential of reacting with biological macromolecules thereby inducing tissue damage. OH$^-\cdot$ radical is chiefly responsible for LOOH, which impairs the normal function of cell membranes. Any condition which disrupts redox homeostasis produces an
oxidative stress in cells where the redox steady state of the cell is altered in the direction of prooxidants that leads to the accumulation of ROS. In the present study, it was clear that morin scavenged $\text{O}_2^-$ and $\text{OH}^-$ in a dose dependent manner. The highest percentage of scavenging effect of morin on $\text{O}_2^-$ and $\text{OH}^-$ was found to be at the concentration of 200 μM. The present findings clearly demonstrated that morin is an effective free radical scavenger against $\text{O}_2^-$ and $\text{OH}^-$. 

Phenolic compounds from phytochemical are important low molecular mass antioxidants coming from the diet (Mullen et al., 2007). Many plant phenolics exhibited antiradical or antioxidative activity in vitro and in vivo (Prior, 2003; Lopez-Velez et al., 2003). The intensity of antiradical activity of phenols depends on many factors such as number of hydroxyl groups bound to the aromatic ring (Sroka and Cisowski, 2003) and the number and places of double bonds in the molecule (Burda and Oleszek, 2001). The chemical structure of morin and other bioflavonoids can be distinguished by the presence of two aromatic rings connected by c-pyrone ring where polar hydroxyl groups are bound in various positions. These hydroxyl groups are suggested to be responsible for the free radical scavenging properties shared by morin (Subash and Subramanian, 2009).

5.2 EFFECT OF MORIN ON ETHANOL INDUCED ALTERATIONS IN THE CIRCULATIONS

The ALD appears to be generated by the effects of ethanol metabolism and the toxic effects of the immune response to ethanol or acetaldehyde altered proteins (Jaya et al., 1993). Ethanol-induced hepatic hypoxia also has been invoked as a possible cause of the potentiation of hepatotoxicity (Ankoma-sey et al., 2000). This is leading to characteristic changes in the serum enzyme activities. When the liver cell membrane is damaged, a variety of enzymes such as AST, ALT, ALP and GGT are released into the blood stream. Elevated serum enzymes are indicative of cellular leakage and loss of functional integrity of the hepatocyte.

Early detection of significant liver disease allows therapeutic intervention and lifestyle changes, aiming at the regeneration of liver cells. So far,
determination of liver-enzymes has emerged as a tool for the detection of liver disease even in asymptomatic patients. AST can be found in the liver and other tissues such as cardiac muscle, skeletal muscle, kidney, brain, pancreas and erythrocytes etc. Whereas, ALT is a cytoplasmic enzyme present in highest concentration in the liver (Rej, 1978). AST and ALT are reliable markers of liver function. The levels of serum AST and ALT has been attributed to the damaged structural integrity of the hepatocyte in the liver (Darwish et al., 2012; Tahir et al., 2013). ALP is a membrane bound glycoprotein enzyme and has been shown to be present in high concentration in the sinusoids and the endothelium of the central and periportal veins (Aruna et al., 2007). It has been reported to be involved in the transport of metabolites across the cell membranes, protein synthesis, secretory activities and glycogen metabolism (Nemesanszky, 1996). Thus, the rise in serum ALP activity in ethanol induced rats may be due to a disturbance in the secretory activity or in the transport of metabolites or may be due to altered synthesis of certain enzymes as in the hepatotoxic conditions (Sharma et al., 1995). GGT, present in the cell surfaces of most cell types, is responsible for extracellular metabolism of glutathione, can be also considered as a marker of oxidative stress (Lim et al., 2004; Hou et al., 2010). We have observed the increased levels of serum enzymes such as AST, ALT, ALP and GGT in ethanol fed rats, which indicate the increased permeability, damage and/or necrosis of hepatocytes (Pushpakiran et al., 2005).

Morin supplementation to the ethanol fed rats showed a marked hepatoprotective effect which correlates with the previous researchers (Lee et al., 2008). They found that morin has protective effect against the hepatotoxin in experimental animals. The membrane stabilizing effect of morin is evidenced by a reduction in the ethanol induced rise in hepatic marker enzyme activities. Morin seems to maintain the structural integrity of liver cells against ethanol challenge. An extensive literature survey has shown that flavonoids are potent antioxidants and possess significant hepatoprotection. It is well documented that localization of flavonoids within the membranes may modify the plasma membrane fluidity and lipid peroxidation. Phenolic phytochemicals, due to their phenolic ring and
hydroxyl substituents, can function as effective antioxidants by virtue of their ability to quench free radicals (Wang et al., 2006). Quercetin, an isomer of morin seems to be a better antioxidant but it has lower bioavailability than morin (Hou et al., 2003). This was evident from the significant normalization of serum AST, ALT, ALP and GGT in the morin administration to ethanol fed rats.

Serum bilirubin is one of the most sensitive tests employed in the diagnosis of hepatic diseases. It provides useful information on how well the liver is functioning (Sharmila Banu et al., 2009). Bilirubin, a chemical breakdown product of hemoglobin, is conjugated with glucuronic acid in hepatocytes to increase its water solubility. Hyperbilirubinemia was observed in an ethanol-fed rat, which may be as a result of mass release of bilirubin from damaged and dead hepatocytes. Decrease in the serum bilirubin after treatment with morin indicates the effectiveness of morin in the maintenance of the normal functional status of the liver.

Several organs are involved in eliminating drugs from the body. The kidneys are the most important organs in this regard. These organs of homeostasis remove drugs and drug by-products from circulation by both passive action (filtration) and by active processes involving secretion and reabsorption of substances from the plasma. Chronic intake of ethanol leads to oxidative stress, altered kidney function and ultimately causes renal failure (Das and Vasudevan, 2008; Shanmugam et al., 2010). Ethanol abuse causes a variety of electrolyte and acid-base disorders, which include decreases in the levels of phosphate, magnesium, calcium and potassium. These abnormalities relate to disorders within the functioning of kidney tubules.

Creatinine and urea are increased under severe renal dysfunction and reduced renal blood flow (Rajendran, 2002). Uric acid is the end product of purine metabolism. Hyperuricemia is associated with impaired renal function. Lowering of elevated uric acid level in the blood could be achieved by xanthine oxidase inhibitors and inhibitors of renal urate reabsorption (Rott and Agudelo, 2003; Yu et al., 2006). Urea, uric acid and creatinine levels were increased significantly in the
serum of ethanol fed rats, which are considered as the significant markers of renal dysfunction.

Previous reports shown that morin administration to rats significantly reduced the kidney damage against oxidative stress induced by gentamycin (Hala and Khattab, 2012), mercuric chloride (Venkatesan et al., 2010), imipenem (Lim et al., 2008), ammonium chloride (Subash and Subramanian, 2011) deoxycorticosterone acetate induced hypertension (Prahalladhan et al., 2012a) and improve the kidney functions through the regulation of organic ions transports in kidney (Wang et al., 2010). Overall, the results of the present study support to the previous reports that morin decreases the levels of urea, uric acid and creatinine in ethanol fed rats. This could be due to the decreased disturbance of protein and nucleic acid metabolism. The protective role of morin might be due to the potent inhibitory action on urate uptake, xanthine oxidase, free radicals and ROS. (Yu et al., 2006; Venkatesan et al., 2010).

Hyperlipidemia has become an important factor for ALD (Onyesom and Osioma, 2001) It is mainly due to the decreased fatty acid oxidation, as a result of altered redox-changes and the enhancement of triglyceride synthesis (Aruna et al., 2002). Increased TG levels after ethanol ingestion may be due to the increased availability of FFA, glycerophosphates, decreased LPL activity, and decreased fatty acid oxidation. The observed increase in the levels of FFA may be directly due to lipid breakdown and indirectly due to the oxidation of ethanol to acetate, which in turn forms FFA. An increase in the FFA level can increase the synthesis of other major lipids and activate NADPH⁺ or NADH-dependent microsomal peroxidation. (Kaffarnik et al., 1978).

PLs are the basic components of cell membranes, mainly acting as regulators of membrane-bound enzymes in the membrane transport processes and also in determining the pathophysiology of alcoholism (Carrasco et al., 2001). It is possible that the effect of ethanol on membranes is mediated through alterations in the membrane PLs (Shenbagam and Nalini, 2011b). The high PL content in the plasma of ethanol fed rats may be due to increased concentration of FFA and TC.
The elevated levels of PL can result in the modification of composition, structure and stability of cell membranes, resulting in membrane dysfunction (Kehrer et al., 1990).

In chronic alcoholism changes in the metabolism of lipoproteins in the liver is observed (Lieber et al. 1994). The increased cholesterol during ethanol ingestion is attributed to the increased α-hydroxyl methyl glutaryl CoA (HMG CoA) reductase activity, which is the rate limiting step in cholesterol biosynthesis (Ashakumari and Vijyammal, 1993). Ethanol has multiple effects on plasma lipoproteins. During lipoprotein transport, LDL and HDL appear to be particularly important. LDL is a converted form of VLDL, rich in cholesterol and cholesterol esters, and is regarded as bad cholesterol, whereas, HDL contains relatively less cholesterol, as low levels are associated with hyperlipidemia.

We observed that ethanol-intoxicated groups showed increased levels of TC, TG, FFA, PL, VLDL, LDL-cholesterol and decreased HDL-cholesterol in the plasma. Whereas, ethanol fed rats treated with morin shows decreased levels TC, TG, FFA, PL, VLDL, LDL-cholesterol and increased level HDL-cholesterol in the plasma. It is suggested that morin may be attributed to enhanced peripheral utilization of cholesterol and modulates the lipid metabolizing enzymes.

Lipid peroxidation is an accumulated effect of ROS, which leads to deterioration of biological systems (Badmus et al., 2011). ROS such as superoxide anion (O2•−), hydroxyl radicals (OH•), nitric oxide (NO) and peroxy radical (ROO•) can attack biomolecules such as lipids, proteins and nucleic acids (Gul et al., 2013). The consequences of oxidation of these biomolecules have been linked to a variety of different human disorders, including ALD.

Ethanol metabolism and mitochondrial oxidative stress play a crucial role in the pathogenesis and progression of ALD. Metabolism of ethanol and induction of CYP2E1 leads to the formation of oxidative stress through the generation of ROS, which can start lipid peroxidation by exhausting antioxidants in the cells thereby changing the redox balance, which in turn favours increased peroxidation (Bansal et al., 2012).
ROS formation within the mitochondria can alter the membrane permeability transition, suppress mitochondrial functions, such as respiration, oxidative phosphorylation, affect the inner membrane barrier functions (Devi and Anuradha, 2010a) and causes the release of oxidants thus hepatocytes become more vulnerable by direct interaction with the protein and lipid moieties in the membrane. Moreover, lipid peroxidation has been implicated in a number of deleterious effects such as increased membrane rigidity, osmotic fragility, decreased cellular deformability and reduced lipid fluidity (Balasubramaniyan et al., 2003a).

Erythrocytes are much more vulnerable to oxidative damage because of their continuous exposure to high oxygen flux and their high concentration of polyunsaturated fatty acids (PUFA) (Setshedhi et al., 2010). The most common alteration of erythrocytes viability is membrane peroxidation and destabilizes the membrane, thereby compromising cell survivals. Acetaldehyde, produced due to the ethanol oxidation, bound to red blood cells can be distributed to various tissues and exert widespread toxic effects (Baraona et al., 1987).

Excessive lipid peroxidation as measured by the formation of TBARS, LOOII and CD has been found in most studies. (Nordmann, 1990). In agreement with these findings ethanol administered rats showed increased levels of lipid peroxidation markers such as TBARS and LOOH in the plasma and erythrocytes. In the present study, morin co-administered rats showed significantly decreased levels of these lipid peroxidative markers as compared to the ethanol fed rats. Morin exhibits strong antioxidant activity by decreasing lipid peroxidation (Subash and Subramanian, 2009).

Decrease lipid peroxidation on morin administration implies the decreased impact of ROS on the membrane, thus increased protection against ethanol induced liver injury. The inhibition of lipid peroxidation by morin may be one of the mechanism by which morin exerts its protection against ethanol mediated tissue injury. Some studies have shown that treatment with flavanoids protect the liver, probably through a decrease in lipid peroxidation (Pradhan and Girish, 2006;
Shenbagam and Nalini, 2011a). Similarly morin being a bioflavonoid may also have a similar mode of action.

ROS are generated from the leakage of electrons into oxygen from various systems. Endogenous enzymatic antioxidant defence is a very important source to neutralize the oxygen free radical-mediated tissue injury (Poliodoro et al., 1984). SOD, one of the first antioxidant enzymes in the line of defense against the deleterious effects of oxygen radicals in the cells, scavenges ROS by catalyzing the dismutation of superoxide to H₂O₂. The decrease SOD activity could be due to oxidative inactivation of enzyme proteins due to excessive ROS generation and production of α-hydroxyl ethyl radicals (Mallikarjuna et al., 2008; Shammugam et al., 2011). CAT acts as a preventive antioxidant and plays an important role in the protection against the deleterious effects of lipid hydroperoxide. Reports have shown that there is a significant decrease in the activities of CAT in alcoholic subjects (Husain and Somani, 1997). The present results also agree with the above observations. The decreased activity of CAT was due to exhaustion of the enzyme as a result of oxidative stress caused by ethanol. Presumably, a decrease in CAT activity could be attributed to cross-linking and inactivation of the enzyme protein in the lipid peroxides. The SOD and CAT activities were restored to normal after treatment with morin, which shows the antioxidant property of morin against oxygen free radicals.

GPx plays a pivotal role in H₂O₂ catabolism and the detoxification of endogenous metabolic peroxides and hydroperoxides. The decreased level of GPx in the ethanol-fed rats could be due to either free radical dependent inactivation of enzyme or depletion of its co-substrates, that is GSH and NADPH. Furthermore ethanol oxidation by CYP2E1 produces hydroxyl radicals, which has been shown to inactivate GPx. GPx may also be inactivated by peroxides and oxygen derived radicals, which may presumably bind to the active sites of the enzymes (Bailey et al., 2001). Morin co-administered rats showed significantly improved the GPx activity as compared to ethanol-fed rats. The observed results show that the maintenance of cellular antioxidants by morin is may be due to inactivation of ROS via its radical scavenging effects or its antioxidant spanning effects.
Fig 32. The toxic effects of ethanol and the possible mechanism of hepatoprotective action of morin (Anbu and Saravanam, 2013).

With respect to all the biochemical estimation carried out in phase I, those rats administered with morin at the doses 60 and 120 mg/kg BW showed more significant results with regard to hepatonephritic markers, oxidative markers and antioxidants in the plasma and erythrocytes as well as histological alterations, when compared to rats treated with low dose (15 and 30 mg/kg BW). As there is no significant difference in all the parameters was observed in 60 and 120 mg/kg BW, the low dose (60 mg/kg BW) of morin was chosen for the further studies.

5.3 EFFECT OF MORIN ON THE LEVELS OF SERUM PROTEIN

Albumin and globulin are the major components of serum proteins. Albumin, an important natural antioxidant, synthesized in the liver and it is used to monitor the liver function (Friedman et al., 1980; Saravanam et al., 2006). Decreased serum total protein and albumin are seen in the ethanol-administered rats as reported earlier (Ahmed et al., 2002). Albumin is known to have a set of
diverse beneficial functions, including oncotic pressure regulation, binding and transport capacities for a wide variety of metabolites, including those of therapeutic drugs (Scatchard et al., 1944). However, the most prominent property of albumin is its major antioxidant activity in a circulatory system that is constantly subjected to powerful oxidative stress (Halliwell, 1988; Kratz, 2008). It demonstrates the decreased functional ability of ethanol-administered rat liver. Decrease in serum total protein reflects the reduction in albumin because serum globulin level remains normal. Decrease in the A/G ratio is a sign of poor health and a predictor of a bad outcome. Acetaldehyde, the metabolic product of ethanol modify the structure of albumin thereby impair its antioxidant property. Significant increase in serum total protein, albumin and A/G ratio was observed in morin co-administration to the ethanol-fed rats. Stabilization of serum protein levels through the co-administration of morin is further a clear indication of the improvement of the functional status of the liver cells.

5.4 EFFECT OF MORIN ON THE TISSUE LIPIDS

Liver is a vital organ participates in the uptake, oxidation and metabolic conversion of FFA, synthesis of TC, PL (Vishnudutt et al., 2009). Previous reports have shown that ethanol increases lipid levels in liver, kidney and brain (Balasubramaniyan et al., 2003b). Ethanol is metabolized in the liver as a process of detoxification. The metabolism of ethanol occurs mainly via ADH, which requires the cofactor NAD⁺. The reduced form of NAD⁺ (NADH) is attenuated when the ethanol concentration is in excess, and this could cause hepatic NADH accumulation (Dancygier et al., 2010). The reducing equivalents impede tricarboxylic acid cycle (TCA) activity and fatty acid oxidation, decreased mitochondrial fatty acid β-oxidation and increased endogenous fatty acid synthesis or enhanced delivery of fatty acids to the liver. As a result, more FFA and TG would be accumulated in the liver and other tissues (Galli et al., 1999; Dancygier et al., 2010). Accumulation of ROS and polyunsaturated fatty acids (PUFA) would increase the oxidative stress and toxicity in the hepatic cells (Wu and Cederbaum, 2003).
Lowering of tissue lipid levels through dietary or drug therapy seems to be associated with a decrease in the risk of various diseases. The reduced tissue lipids (TC, TG, PL, and FFA) in morin co-treated rats might be due to increased in the mobilization and hydrolysis of certain lipoproteins for their selective uptake and metabolism by different tissues.

Phenolic compounds have the ability to normalize the levels of tissue lipids during diseased conditions. It was reported that the morin possesses both antioxidant effects and hypolipidemic effects against LDL oxidation in vivo (Sivaramakrishnan et al., 2008). Therefore, the possible mechanisms by which the morin modulates lipid peroxidation and lipid levels during ethanol fed condition could be attributed to the natural antioxidants. A decrease in fat accumulation in ethanol-fed rats was also observed in response to antioxidant therapy (Kaplowitz and Tsukamoto, 1996) Thus, in the present study, multiple mechanisms are probably involved in the reduction of the degree of fat accumulation observed in morin co-treated rats.

5.5 EFFECT OF MORIN ON LIPID METABOLISING ENZYMES

The concentration of cholesterol can be regulated by cholesterol biosynthesis, removal of cholesterol from blood, absorption of dietary cholesterol and excretion via bile and feces. HMG-CoA reductase plays a major role in the regulation of cholesterol metabolism and a rate limiting enzyme in the pathway of cholesterol biosynthesis. Increased HMG-CoA reductase activity leads to excessive production and accumulation of cholesterol (Delgado-Villa et al., 2009).

Flavonoids from various sources have been shown to cause significant reduction of hepatic HMG-CoA reductase in experimental animals and to inhibit the activity of this enzyme in vitro (Bok et al., 2002; Ohtsuki et al., 2003). Previous reports have shown that morin suppresses the expression of HMG-CoA reductase (Karthik Kumar et al., 2010; Prabhalathan et al., 2012c). We noted an increase in the activity of HMG-CoA reductase in the liver of ethanol fed rats, whereas treatment of morin to the ethanol-fed rats significantly lowered the activity of HMG-CoA reductase in the liver.
A significant decrease in the activity of LCAT was noted in the ethanol-fed rats. LCAT converts cholesterol into cholesterol esters on the surface of HDL. The lowered activity of LCAT observed in ethanol-fed rats might be due to increased lipid peroxidation. Decreased activity of LCAT inhibits the esterification of cholesterol in ethanol-fed rats. This leads to increased levels of lipids in the liver. After treatment with morin there was a significant increased in the HDL-cholesterol levels and decreased in lipid peroxidation which is further confirmed by its modulation of LCAT activity in ethanol-fed rats. One of the possible mechanisms for the hypocholesterolemic effect of morin (Bartosiková et al., 2003) may be due to the regulation of cholesterol biosynthesis by decreasing the activity of HMG-CoA reductase in ethanol-fed rats. In addition, the enhancement of turnover of HDL-cholesterol by increased LCAT activity may be another possible mechanism of morin in ethanol-fed rats. Thus, the observed effects clearly revealed that the morin has the property to decrease the lipid level.

5.6 EFFECT OF MORIN ON ETHANOL-INDUCED OXIDATIVE STRESS

Ethanol metabolism leads to the production of highly reactive molecules such as indirect metabolites malondialdehyde, 4-hydroxynonenal (Devi and Anuradha, 2010b), superoxide and hydroxyl radicals generated by the microsomal oxidizing system (MEOS) that can destroy vital cell components through a process called oxidation which may be involved in the pathogenesis of ethanol-related tissue injury (Faut et al., 2009).

Chronic ethanol administration induces oxidative stress in the central nervous system, mainly through increased lipid peroxidation of the cell membrane leading to increased membrane fluidity, disturbances of calcium homeostasis (increase in free intracellular calcium) and finally cell death (Montoliu et al., 1995). Increased LOOH (an index of oxidative stress) are a reflection of the liver’s susceptibility to ethanol-induced oxidative damage. Enzymatic antioxidants, the primary defense systems have been reported to decrease in liver (Faremi et al., 2008), kidney (Karthi Kesavan and Pari, 2007) and brain (Srivastava and Shivanandappa, 2010) of ethanol-fed rats.
All tissues have the capacity to neutralize oxygen radicals to some extent by using cellular antioxidants. Antioxidants constitute the foremost defense system that limits the toxicity associated with free radicals. The cells have enzymic and non-enzymic systems to neutralize free radicals.

Taken together, the findings obtained from this present study indicate that ethanol metabolites decrease the level of SOD, CAT GPx and GR activities may be due, in part, to an overwhelming oxidative modification of the enzymatic proteins by excessive ROS generation. Moreover, reduction in the activities of these enzymes may decrease in their rate of synthesis. Morin treatment to the ethanol-fed rat significantly enhanced the activities of SOD, CAT GPx and GR. Similarly oxidant damage in the liver have been reported by Huang et al., (2013) who recorded a significant decrease in reduced SOD, CAT GPx and GR in rats after administration of ethanol. Sultana et al., (2005) also reported that the mean value of SOD activity decreased significantly following the administration of ethanol. GST represents one of the major cellular defense mechanisms against electrophilic xenobiotics and their metabolites.

Enzymic antioxidants are inactivated by the excessive levels of free radicals and hence the presences of non-enzymic antioxidants are presumably essential for the removal of free radicals (Parthasarathy and Suresh, 2008). The non-enzymic antioxidant such as GSH, ascorbic acid and α-tocopherol, are the second lines of defense mechanism which scavenge residual free radicals escaping decomposition by the antioxidant enzymes.

GSH is a tripeptide (L-c-glutamyl cysteinyl glycine), an antioxidant and a powerful nucleophile, critical for cellular protection, such as detoxification of ROS, conjugation and excretion of toxic molecules and control of inflammatory cytokine cascade (Park et al., 2013). The levels of GSH were significantly decreased in ethanol-fed rats, as well as in earlier published reports, which shows that the GSH concentration decreases during ethanol ingestion (Mallikarjuna et al., 2008; Shanmugam et al., 2011). Alcohol-induced oxidative stress can be inhibited by antioxidants (Adaramoye et al., 2011). The GSH level was significantly reduced
in ethanol-fed rats; the reverse condition was observed after the treatment with morin. Restoration of GSH levels is known to inhibit ethanol toxicity (Li et al., 2012) therefore it can be presumed that the effect of morin might be related to the normalization mechanism by maintaining adequate levels of GSII for detoxification of xenobiotic.

Vitamin-C and vitamin-E are typical lipophilic antioxidants; scavenge the residual free radicals escaping the antioxidant enzymes. Vitamin-C present in aqueous environment has multiple antioxidant properties including the ability to regenerate α-tocopherol radicals (Senthilkumar et al., 2004). Vitamin E is the major lipophilic antioxidant in vivo. It scavenges lipid peroxyl radical, chain carrying species in the lipid peroxidation, to break chain propagation. The phytol side chain of vitamin E take part an important role in radical scavenging efficacy (Niki and Noguchi, 2004). The lowered concentrations of vitamins-C and-E observed in ethanol fed rats might be due to neutralizing the free radicals. While, treatment of morin to the ethanol fed rats enhanced the levels of these antioxidants. The increased concentration of these vitamins may protect the liver, kidney and brain tissue against ethanol mediated free radicals.

5.7 EFFECT OF MORIN ON NITRITE LEVEL IN THE LIVER

Nitric oxide (NO) is essentially involved in several important physiological functions including blood vessel relaxation, inhibition of platelet aggregation, and neuronal communication. NO is an unstable molecule that triggers formation of oxidative free radicals such as peroxynitrite. NO is induced by cytokines such as TNF-α (Alderton et al., 2001; Bogdan, 2001; Dawn and Bolli, 2002). Inhibition of iNOS may be beneficial for the treatment of inflammatory disease (Aktan et al., 2003). It is well documented that iNOS is required for the pathogenesis of early ethanol-induced hepatitis by production of nitric oxide-derived pro-oxidants (e.g., peroxynitrite). (McKim et al., 2003; Venkatraman et al., 2004).

Ethanol consumption increases NO level and may lead to toxicity by peroxynitrite, a potent oxidant that produces nitration of tyrosine and inactivation of many biologically important proteins and enzymes (Deng and Deitrich, 2007).
This has important ramifications for toxicity because NO and its metabolite peroxynitrite cross cell membranes through anion channels and reacts slowly enough to react more selective throughout the cell making the biological and pathological implications (Pacher et al., 1997).

In the present study, we have observed the increased level of NO in the liver of ethanol treated rats. This could be attributed by the excessive formation of peroxynitrite by its reaction with superoxide. Whereas the administration of morin to the ethanol fed rat shows significantly decreased level of NO. Phenolic antioxidants are a class of dietary compounds that possesses anti-inflammatory properties (Kagan and Tyurina, 1998) and prevent the ALD through the suppression of iNOS expression (Nanji et al., 2003). Recently, Chen et al., (2012) reported that, morin suppressed the production and expression of NO and other several inflammatory cytokines.

5.8 EFFECT OF MORIN ON HEPATIC IRON

Iron plays an important role in various essential cell functions. However, excess iron is toxic and causes lipid peroxidation and tissue damage. Its absorption and transport therefore needs to be tightly regulated. Approximately one third of total body iron is bound to storage proteins (ferritin or hemosiderin) and the other two thirds of total body iron is involved in metabolic or enzymatic functions (Swanson and Christine, 2003).

Iron is needed for essential cell functions such as DNA synthesis, transport of oxygen and electrons, and cell respiration (Pietrangelo and Antonello, 2003). Excess iron in the liver can induce oxidative stress by catalyzing the conversion of superoxide to hydrogen peroxide to more potent oxidants such as hydroxyl radicals, which can cause tissue injury by initiating lipid peroxidation. Increasing evidence supports that iron plays a significant role in secondary risk factor for progression and pathogenesis of ALD. Many studies have shown that ethanol elevates the hepatic iron and develops liver damage (Cederbaum, 2001: Ganne-Carrie et al., 2000). Understanding the underlying mechanisms by which iron participates in the initiation and development of ALD may help design strategies
for the treatment and prevention of the disease (Purohit et al., 2003). We observed that ethanol fed rat showed significant increase in the level of hepatic iron, whereas ethanol fed rats co-treated with morin significantly decrease the levels of hepatic iron. This may be due to the chelating properties of morin. Previous report has been shown that flavonoid with antioxidant and metal chelating activities can effectively suppress the iron induced oxidative stress (Zhao et al., 2005; Lee et al., 2009).

5.9 EFFECT OF MORIN ON ALCOHOL METabolizing Enzymes

Alcohol is initially oxidized into acetaldehyde in the cytosol, principally by ADH, and then into acetate by ALDH in the liver. These steps require NAD⁺-dependent cytosolic ADH and mitochondrial ALDH. Acetaldehyde, a highly toxic metabolite of ethanol, has already been implicated in the pathogenesis of ALD (Lieber, 1994). Theoretically, the accumulation of acetaldehyde in the liver after chronic alcohol ingestion is determined by its formation and removal rates as catalyzed by ADH and ALDH, respectively (Lee et al., 2001a,b). Due to the functional diversity of hepatocytes, alterations in the levels of these specific enzymes are used as an index of intoxication of cell populations (Jayaraman and Namasivayam, 2011; Ge et al., 2013). The prolonged ethanol administration could deplete NAD⁺ that could be responsible for reduced ALDH activity. Further acetaldehyde, might damage the mitochondrial ALDH which in turn impairs further metabolism of acetaldehyde to acetate (Matsuzaki and Lieber, 1977). In the present study, ethanol feeding resulted in increased activity of ADH and decreased activity of ALDH leading to the increased formation of acetaldehyde adducts which culminates in severe hepatic damage. Supplementation with morin to ethanol-fed rats showed decreased activities of ADH and increased activities of ALDH, emphasizing the fact that morin ameliorates hepatocellular damage.

5.10 EFFECT OF MORIN ON THE ACTIVITIES OF XEnOBIOtIC METAbOLISING EnZYMES

CYP450 is a group of enzymes which belong to the multigene superfamily of microsomal hemoproteins that play a key role in the biotransformation and
detoxification of a wide variety of xenobiotics (Pelkonen et al., 2008; Turpeinen et al., 2007). CYP2E1 plays an important role in the catalysis of lipid peroxidation and production of reactive oxygen intermediates such as \( \text{H}_2\text{O}_2 \) in higher amounts relative to other P450 isoforms by the regulation of NADPH oxidase activity (Song et al., 2003). CYP2E1 oxidizes ethanol to generate many toxic products, such as acetaldehyde, 1-hydroxyethyl radical, and other ROS, such as \( \text{O}_2^- \), \( \text{H}_2\text{O}_2 \) and \( \text{OH}^- \) as well as the lipid peroxidation-end product malondialdehyde (MDA) (Gill et al., 1996; Chen et al., 2013).

Our results also showed increased activities of CYP450, cytochrome b5, NADH-cytochrome b5 reductase, NADPH-cytochrome P450 reductase, cytochrome P4502E1 in the ethanol-fed rats. In this context, Jayachitra and Nalini (2011) have reported an increase in the activities of cytochrome b5, NADPH cytochrome P450 reductase and NADH-cytochrome b5 reductase activities in the liver of ethanol-fed rats. Porta (1997) have also shown that chronic ethanol consumption increases CYP2E1 activity in the liver. Co-treatment of morin to the ethanol-fed rats decreased the activities of cytochrome P450, cytochrome b5, NADPH-cytochrome P450 reductase, NADH-cytochrome b5 reductase and cytochrome P4502E1 in the liver, which may be because of the modulatory effect of morin on cytochrome P450 dependent monoxygenases, the primary enzyme involved in the metabolism of many xenobiotics (Yang et al., 2013). The action of morin on the phase I enzymes may be, at different time points, resulting in significant protection against ethanol induced toxicity, which may reflect the ability of morin to reduce the accumulation of free radicals generated during ethanol-induced lipid peroxidation.

The phase II detoxification enzymes, act by metabolizing xenobiotic and endobiotic compounds rendering them water soluble, thereby facilitating their removal from the body (Tukey and Strassburg, 2000). The GST enzymes are soluble proteins predominantly found in the cytosol of hepatocytes and catalyze the conjugation of a variety of compounds with the endogenous tripeptide, GSH. GST is subject to activation by endogenous metabolism of drugs (Mosialou and Morgenstern, 1989). Therefore, inhibition of GST activity and depletion of GSH
levels might potentiate the deleterious effects of many environmental toxicants and carcinogens. GST has the capacity to detoxify electrophilic xenobiotics by catalysing the formation of GSH conjugates.

DT-diaphorase, also called quinone reductase (QR), is an enzyme present in the hepatic cytosol that produces NAD\(^+\) from NADH. DT-diaphorase is generally induced concomitantly with other phase-II detoxifying enzymes. Morin supplementation enhanced the DT-diaphorase and GST activities in ethanol-fed rats. In this context, Devi et al., (2009) have shown that inducers of DT-diaphorase can enhance the regeneration of NAD\(^+\) and thereby enhance the \textit{in vivo} metabolism of ethanol and decrease hepatotoxicity. Recent studies have pointed out the importance of phase II detoxification enzymes in the elimination of toxic metabolites and thereby reducing the toxic burden of liver undergoing stressful situation (Tukey and Strassburg, 2000). Hence, a decrease in the activities of phase II enzymes in ethanol fed rats would lead to accumulation of toxic substances resulting oxidative damage. Ethanol fed rats treated with morin significantly restored the phase II enzymes.

5.11 Effect of Morin on Membrane Bound ATPase

Ethanol interacts with the cellular constituents causing profound changes in membrane structure, by altering the lipid components, ion-channels and its fluidity (Rubin and Rottenberg, 1982). Ethanol particularly affects the concentrations of intracellular cations to reduce ionic transfer through alterations in the monovalent cation pump (Guiet-Bara et al., 1995) and ATPase activities (Babich et al., 2000, Webb et al., 1996).

Assay of membrane bound enzyme activities like ATPases indicate alterations in membrane under pathological conditions. ATPases are intimately associated with the plasma membrane and participates in the energy requiring translocation of sodium, potassium, calcium and magnesium (Mourelle and Franco, 1991). Ca\(^{2+}\) dependent ATPase, responsible for active calcium transport, is known to be inhibited due to membrane lipid peroxidation in different types of muscles (Kukreja et al., 1988, Zeng et al., 1998).
Na\textsuperscript{+}/K\textsuperscript{+}ATPase are a lipid dependent and ‘SH’ containing enzyme. The inactivation of Na\textsuperscript{+}/K\textsuperscript{+}ATPase could be due to the free radicals formed during ethanol metabolism. Ca\textsuperscript{2+} overload in the liver cells activates the Ca\textsuperscript{2+} dependent ATPase of the membrane depleting high energy phosphate stores, thereby indirectly inhibiting the Na\textsuperscript{+} and K\textsuperscript{+} transport as well as the activity of Na\textsuperscript{+}/K\textsuperscript{+}-ATPase. Mg\textsuperscript{2+}-ATPase activity is involved in other energy requiring process in the cell and its activity is sensitive to lipid peroxidation. A previous study has shown that ethanol modulate the membrane bound ATPase in liver, kidney and brain (Pushpakiran et al., 2005; Balasubramaniyan and Nalini, 2006). In the present study, we observed a decreased in the activities of total ATPase, Na\textsuperscript{+}/K\textsuperscript{+}-ATPase, Ca\textsuperscript{2+}-ATPase and Mg\textsuperscript{2+}-ATPase in ethanol-fed rats. The decreased activities of these ATPase observed during ethanol administration may be due to depletion of GSH or increased lipid peroxidation, which inhibits thiol dependent enzymes (Kukreja et al., 1988).

In the present study, in accordance with the previous report, the ethanol fed rats treated with morin significantly modulate the activity of Na\textsuperscript{+}/K\textsuperscript{+}-ATPase, Ca\textsuperscript{2+}-ATPase and Mg\textsuperscript{2+}-ATPase. This could be due to the ability of morin to protect the ‘SH’ thiol groups from the oxidative damage through the inhibition of peroxidation of membrane lipids. This effect might be due to the membrane stabilizing and free radical scavenging activity. A Previous study has also shown that administration of morin to rats significantly modulates the activities of membrane bound ATPase (Al-Numair et al., 2012). Additionally, the localization of flavonoids within the membranes may modify membrane fluidity and lipid peroxidation.

5.12 EFFECT OF MORIN IN THE STRUCTURAL ALTERATIONS BY THE FT-IR SPECTRAL CHANGES IN THE LIVER

Infrared spectroscopy (IR) has proved to be a powerful tool for the study of biological molecules and the application of this technique to biological problems is continually expanding, particularly with the aid of sophisticated sampling techniques such as infrared imaging. Biological systems include
lipids, proteins, peptides, bio membranes, nucleic acids, animal tissues, microbial cells, plants and clinical samples, have been successfully studied by using infrared spectroscopy (Dole et al., 2011).

IR spectroscopy could be a convenient tool for monitoring metabolic changes in liver tissue after \textit{in vivo} exposure to ethanol and that it might be used in conjunction with biochemical and physiological data (Saravanan and Nalini, 2008). In the present study, we examined the effects of morin on ethanol induced liver tissue at the molecular level using FT-IR spectroscopy. The intensity, or more accurately the area of the absorption bands is directly related to the concentration of the molecules (Saravanakumar et al., 2012; Sivakumar et al., 2012).

ROS may attack any type of molecules, but their main target appears to be PUTA, the precursors of lipid peroxide formation. It is known that unsaturated lipids are more prone to lipid peroxidation (Halliwell and Gutteridge, 1989). Unsaturated lipids are highly vulnerable to oxidative attack because of their double bond content. FT-IR is useful in monitoring unsaturated lipid content by utilizing the olefinic (=CH) stretching mode at 3012 cm\(^{-1}\). The unsaturated olefinic (C=\text{C-H}) stretching vibration, which has a unique vibrational frequency of 3012 cm\(^{-1}\) and is well separated and distinguishable from the saturated aliphatic peaks (Sills et al., 1994; Severcan et al., 2005). The increase in the olefinic band is due to the lipid peroxidation end products (Toyran et al., 2004). In the present study, the olefinic (C=\text{C-H}) peak intensity increased in the ethanol intoxicated liver tissue. This may be due to a vulnerable attack of ethanol induced lipid peroxidation. In the present study, the olefinic (C=\text{C-H}) peak significantly decreased in the ethanol intoxicated rat co-administrated with morin with respect to the biochemical estimations of lipid peroxidation markers (TBARS, LOOH and CD) in plasma, hepatic and extra hepatic tissues.

5.13 EFFECT OF MORIN ON THE ETHANOL INDUCED CYTOKINE RESPONSE

Cytokines are extracellular proteins involved in the regulation of innate and immunologically inflammatory reactions. These proteins are involved in various
processes including cell differentiation, development, growth and repair processes that lead to the maintenance or recuperation of homeostasis (Gao, 2005). Many cytokines influence different cell types of binding to specific receptors that transduce signals into the target cell. Cytokines are generally active over a short space by binding to the cell of their origin or to a neighboring cell.

ALD is associated with imbalanced immune responses and increased production of pro-inflammatory cytokines/chemokines. Many of the processes related to alcohol-induced liver injury are mediated via cytokines (An et al., 2012). Cytokines are low-molecular weight polypeptide mediators of cellular communication that are produced and released by different cell types in the liver (McClain et al., 2004). In ALD, there is an increased pro-inflammatory cytokine production by ethanol (Kawarata et al., 2011). TNF-α is believed to be one of the major pro-inflammatory cytokines in alcohol-induced liver injuries, which is involved in inflammatory response, steatosis and cell death (Dou et al., 2012). Oku et al., (2002) suggested that TNF-α can be considered a key factor that contributes to the triggering of an inflammatory cascade after liver injury. TNF-α also induces the secretion of cytokines such as IL-1, IL-6 and IL-10 and activates T cells and other inflammatory cells (Vilcek and Lee, 1991). TNF-α induces mitochondrial formation of ROS and is considered to play a role in the onset and progression of ALD (Malhi et al., 2006). Indeed, oxidant stress as well as pro-inflammatory stimuli can result in the degradation of the cytoplasmic nuclear factor kappa B (NF-κB) inhibitor, allowing translocation of NF-κB to nuclei. Once activated, NF-κB resulted in increased expression of pro-inflammatory cytokines and chemokines (Kono et al., 2000; Taub, 2004). The present study gives the supporting evidence for the induction and activation of NF-κB in ethanol treated group. NF-κB is one of the major transcription factors involved in the activation of immediate early response genes in response to ethanol induced liver injury and inflammatory stimuli (Chen and Shi, 2002).

Several naturally occurring polyphenols, including flavonoids, are characterized by their anti-inflammatory and immunomodulatory properties. The effects of these polyphenols on inflammation has often been attributed to their
actions on the NF-κB pathway (Gonzalez et al., 2011). For instance, several flavonoids, such as morin (Manna et al., 2007) and fisetin (Goh et al., 2012) have been shown to interfere with the NF-κB pathway by inhibiting IkB kinase. These results suggested that morin could inhibit the initiation of inflammatory response, at least in part, by suppressing NF-κB activation.

NO is a reactive nitrogen species critical in the redox biology of hepatocytes. It is created by nitric oxide synthase (NOS), which is present in three forms: neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS), in the liver. iNOS was found to be critical in the development and propagation of inflammation (Chartrain et al., 1994; Taylor et al., 1998). Its expression is induced by TNF-α and NF-κB which is associated with both local and systemic inflammatory response could consistently release high levels of NO (Michel and Feron, 1997). Cytotoxicity of NO- may also be related to the genesis of oxidative intermediates such as peroxynitrite, a product of NO- and superoxide (Rockey and Shah, 2004).

Cyclooxygenase (COX) is another important mediator of inflammation, exist in two distinct isoforms. COX-1 is constitutively expressed in nearly all tissues, and provides prostaglandin (PG) to maintain normal physiological functions including cytoprotection and the regulation of renal blood flow (Giuliano and Warner, 2002). In contrast COX-2 is catalyzing the formation of prostaglandins and other eicosanoids from arachidonic acid at the site of inflammation. The production of prostanoids by COX-2 is often implicated in inflammatory diseases, characterized by edema and tissue injury (Muller-Decker et al., 2005; Tanaka et al., 1997). Some investigators suggested that the NO released from inflammatory cells, which synthesized by iNOS, could increase COX-2 activity (Salvemini et al., 1995). Morin prevents the expression of COX-2 (Sivaramakrishnan and Niranjali Devaraj, 2009). The present study showed that morin largely attenuated the upregulation of iNOS and COX-2 expression in the livers of ethanol fed rats. These results implicated that morin could attenuate inflammatory processes by suppressing the expression of iNOS and COX-2, which might in turn reduced the expression other cytokines. It was well-known that the
levels of expression of iNOS and COX-2 were tightly controlled by NF-κB. We suggested that morin might attenuate the upregulation of iNOS and COX-2 expression induced by ethanol due to its inhibition of NF-κB activity. These results suggested that morin could alleviate liver injury caused by ethanol through suppressing inflammatory response.

CD14 is a 55 kDa myeloid membrane glycoprotein, expressed mainly by monocytes and macrophages, and at low levels also on the surface of polymorphonuclear leukocytes (Antal-Szalmas et al., 1997; Kitchens, 2000). Alcohol increases the expression of CD14 in Kupffer cells (Lukkari et al., 1999) and triggers a signalling cascade, resulting in induction of cytokines that are known to participate in liver injury (Thurman, 1998). It has been shown previously that CD14-deficient mice do not produce significant levels of these cytokines involved in ALD (Yin et al., 2001). Pathologic fibrosis is mediated by TGF-β. Exogenous inhibitors of TGF-β receptor binding reduce or abolish fibrosis (Dai and Jiang, 2001). TGF-β is involved in many inflammatory processes. TGF-β was identified as the first defined mediator eliciting hepatocyte apoptosis. It seems to be involved in the normal growth regulation of the liver rather than in inflammatory or infectious hepatocyte apoptosis. TGF-β induced apoptosis is greatly enhanced in vivo and in vitro by conditions of liver size regression (Oberhammer et al., 1992). TGF-β was found upregulated in the liver after onset of alcohol dependent liver damage in animal models and human disease (Chen et al., 2002). Anti TGF-β strategies were successfully used to counteract this process the liver (Kaimori et al., 2007). Herrera, et al., (2004) found out details about its function and investigated TGF-β effects on primary cultured hepatocytes. TGF-β signalling itself increases the intracellular content of ROS and down-regulates antioxidant genes in different cell types, including hepatocytes (Ciucilan et al., 2008). We found, that ethanol treatment, TGF-β strongly promotes alcohol dependent oxidative stress and antioxidant depression leading to enhanced cellular toxicity. This “alcohol damage promoting” effect of TGF-β is strengthened by the finding that ethanol induced lipid peroxidation is significantly increased upon parallel activation of TGF-β signalling.
Morin was known to suppress the expression of proinflammatory cytokines, including TNF-α, IL-6 and COX-2 (Sivaramakrishnan and Niranjali, 2009) subsequently inhibits inflammation (Lee et al., 2008). In the present study, ethanol fed rats showed upregulation of proteins expression of TNF α, iNOS, TGF β, COX-2 and upregulation of mRNAs expression of CD14, NF-κB and IL-6, whereas treatment with morin significantly down regulated these mRNA and protein expressions when compared to the untreated ethanol fed rats.

5.14 EFFECT OF MORIN ON DNA DAMAGE

There are several methods to measure DNA damage including classical cytogenetic tests such as chromosome aberrations, micronuclei and sister chromatid exchanges. The single cell gel electrophoresis or comet assay is a state-of-the-art technique for quantitative DNA damage and repair in vivo and in vitro. This technique is rapid, non-invasive, sensitive, visual and inexpensive as compared to the conventional techniques. It measures, double strand breaks (DSBs), single strand breaks (SSBs), alkali labile sites (apurinic, apyrimidinic, deamination and phosphotriesters etc.). DNA-DNA/DNA-protein/DNA-drug crosslinking and oxidative DNA base damage (Fedeli et al., 2003).

The comet assay done with lymphocytes is an important biomarker for early biological effects to the measurement of genotoxicity (Grossi et al., 2008). It has also been used in various studies to investigate the effect of ROS on DNA and the protective effect of certain dietary antioxidants (Sierens et al., 2001). Ethanol oxidation gives rise to the generation of free radicals, both in vitro and in vivo, and such events are associated with the induction of DNA strand breaks, modification of DNA bases and DNA damage (Saravanan and Pugalendi, 2005; Navasumrit et al., 2000). The importance of ROS in inducing genetic toxicity is widely accepted and subsequently has been extensively studied in the past decade. Superoxide radicals can directly or indirectly damage DNA whereas hydrogen peroxide mediates DNA damage by the production of hydroxyl radical via events such as the Fenton reaction (Imlay et al., 1988).
The extent of DNA damage was quantified by measuring the displacement of the genetic material between the cell nucleus (comet head) and the resulting 'tail'. When the alkaline single-cell gel electrophoresis was introduced, the tail length was used as an index of DNA damage.

The introduction of computerized image analysis made it possible to calculate other features (such as tail moment) of the comet image as well, thereby providing better descriptions of the overall DNA damage. The significantly increased tail length and tail moment in ethanol-fed rats clearly showed the relation between ethanol administration and DNA damage. These results are in concordance with many human and animal studies (Grossi et al., 2008). Previous report states that morin was found to inhibit DNA tail length induced by γ-radiation, indicating protection of cellular DNA by morin treatment (Zhang et al., 2011). Thus, the antioxidant effect of morin could be responsible, at least partly, for its protective effect against ethanol-induced toxicity.

5.15 EFFECT OF MORIN ON THE LIVER COLLAGEN

Collagen is an important extracellular matrix protein in the body with several important functions and has marked changes in its structure and composition in a variety of pathological conditions. Collagen deposition is a complex process which depends on its synthesis in the HISC and degradation by collagenase. TGF-β and IL-6 upregulates the expression of type I collagen gene. Inflammatory responses are known to participate in collagen synthesis and accumulation. Oxidative stress plays an important role in fibrogenesis. A product of lipid peroxidation can activate HSC and promotes the transcription of collagen.

TNFα stimulates parenchymal cells to produce acute phase proteins that activate the fibrogenic process, and IL-6 has a direct mitogenic effect on HSCs (Zhuo et al., 2012). Both TNF-α and IL-6 are implicated in the hepatic fibrogenic process. During fibrosis, injured hepatocytes release ROS and fibrogenic mediators (cytokines and chemokines) and recruit mononuclear cells toward the site of injury (Huang et al., 2012). It is interesting to find an effective ways to inhibit liver fibrosis and preventing the developments of cirrhosis are of great significance. In
the present study, the levels of IL-6, and TNF-α in the ethanol-fed rats were significantly higher than those in the normal control group, which could be responsible for the deposition of collagen in the liver. However, up-regulation of these inflammatory factors was markedly inhibited after treatment with morin. These data suggest that the morin exerted a therapeutic effect for fibrogenesis by restricting the production and release of inflammatory mediators, which is evidenced by decrease level of collagen accumulation in the liver.

5.16 EFFECT OF MORIN ON APOPTOTIC MARKERS

Apoptosis is a protease cascade process. There are several pro-apoptotic and anti-apoptotic proteins interacting with each other for eliminating critically damaged cells without disturbing the tissue structure or function (Zhou et al., 2005). Chronic alcohol consumption is one of the mechanisms that is known to potentially activate the apoptotic process. leads to alcohol-induced organ damage (Hoeck and Pastimoro, 2002, Molina et al., 2003). There are number of factors involved in ethanol-induced toxicity, such as changes in redox status (NAD+/NADH ratio), the accumulation of acetaldehyde, the generation of ROS and depletion of antioxidants (Wu and Cederbaum, 2003; Hoeck and Pastorino, 2002). Furthermore, it has been shown that ethanol administration is linked to hepatocyte apoptosis and that the number of apoptotic cells detected in the liver correlates with the development of ethanol-induced pathological liver injury (Kaviarasas et al., 2006).

Studies have shown that apoptosis is regulated by the balance between protein Bcl-2 and Bax. The Bcl-2 and Bax exist as homodimer, though they can exist as heterodimers too. If the Bax exists as homodimer, apoptosis will occur; if the amount of Bcl-2 is more than Bax, when all the Bax are combined, the residual Bcl-2 will play the inhibitory effect, preventing apoptosis. On the contrary, if Bax is more than Bcl-2, Bax will lead to apoptosis (Kim et al., 2005). In other words, the ratio of Bax/Bcl-2 determines the trend of apoptosis (Lee et al., 2005). Ethanol has the ability to trigger both the pathways
Mitochondria play a key role in the development of apoptosis (Gradzka, 2006).

In accordance with the previous observations (Robin et al., 2005; Gyamfi et al., 2008), in the present study, we observed that ethanol administration drastically increases the expression of the pro-apoptotic protein-Bax and decreases the expression of the anti-apoptotic protein-Bcl-2 in the liver of ethanol fed rats, indicating that ethanol induces necrosis mediated cell death in the hepatocytes. On supplementation with morin to ethanol fed rats, the expression of the pro-apoptotic protein-Bax was reduced and the expression of the anti-apoptotic Bcl-2 was enhanced, which could explain the potentiating of liver cells survival in alcohol induced liver injury (Zhang et al., 2011).

5.17 EFFECT OF MORIN ON THE HISTOLOGY OF LIVER

Histological observations under light microscopy revealed the presence of hepatocytic necrosis and micro and macro fatty inflammatory cells in the ethanol-fed rats. This could be due to the enhancement of lipid peroxidation, as a result of hypertriglyceridemia and lipid accumulation in the liver of ethanol fed rats, which forms a basis of cellular damage. Our results are in accordance with the previous report stated that ethanol can cause oxidative stress and ultimately damage to the hepatocytes (Samuhasaneeto et al., 2009).

Flavonoids have been reported to protect against ALD (Pradhan and Girish, 2006; Shenbagam and Nalini, 2011a). In the present study, morin, a naturally occurring bioflavonoid, diminished the hepatic tissue injury as monitored by normal liver architecture except for mild inflammation. Consistent with the improved morphology, treatment with morin was associated with a corresponding reduction in levels of transaminases, ALP and GGT thereby indicate a protective role of morin against ethanol toxicity via regulation of mitochondrial oxidative stress.

Furthermore, antioxidant enzymes, namely, SOD, GPx and CAT were reverted by morin during ethanol administration. These actions along with
antiperoxidative property make morin a suitable candidate for the treatment of liver diseases. The above results demonstrate and support that morin preserves the structural integrity of the liver by virtue of its hepatoprotective, hypolipidemic, anti lipid peroxidative and antioxidant properties.

5.18 EFFECT OF MORIN ON THE HISTOLOGY OF KIDNEY

Ethanol induces kidney CYP450, enhances lipid peroxidation and which might be responsible for the kidney damage in ethanol fed rats. In the present study, histopathological analysis of kidney of control rat tissues revealed the normal section and healthy cells. However, histology of ethanol-fed rat kidney showed alteration in the glomeruli and tubules. Renal cells and renal parenchyma cells are also damaged. There was no evidence of pathological changes in the control rat treated with morin alone. The study revealed that co-administration of morin with ethanol fed rats showed less damage in the kidney. Morin may mop up free radical generation by ethanol and its metabolism and it may be responsible for the healthy state of renal cells. Major findings from the present study demonstrated that morin possesses nephroprotective activity. Our results corroborate to the previous studies that morin has been shown to nephroprotective. (Khattab, 2012).

5.19 EFFECT OF MORIN ON THE HISTOLOGY OF BRAIN

Oxidative stress is the major causes of ethanol induced neurotoxicity (Das et al., 2007). Ethanol readily crosses the blood–brain barrier, where it is oxidized by CAT, ADH or CYP450. This process produces ROS which include superoxide free radicals, hydrogen peroxide, and hydroxyl radicals (Halliwell, 2006). Disturbance of cellular normal redox state by excessive ROS leads to oxidative stress which causes cellular damage. Brain is particularly susceptible to oxidative stress due to its high oxygen consumption rate, elevated levels of PUFA, and relatively low content of antioxidative enzymes (Shirpoor et al., 2008). Evidence has been accumulated indicating that chronic ethanol consumption leads to direct or indirect changes in the viability of central nervous system cells via oxidative stress.
In the present study, there is a significant pathomorphological alteration in brain of ethanol fed rats. These changes can alter the properties of the cell. Spongiosis was demonstrated in the hypothalamic and thalamic regions of the brain in ethanol treated rats (Kumar Rajagopal et al., 2003). In this study, we observed that the brain of ethanol-fed rats showed marked edema and spongiosis. Morin treatment reversed the cerebral edema and spongiosis in the brain. This could be due to the neuroprotective action of morin (Zhang et al., 2010). Control rat treated with morin does not show any significant changes in the brain. These observations indicate that morin confers against ethanol induced neuronal toxicity.