Liver diseases have become one of the major causes of morbidity and mortality all over the world. Hepatotoxicity due to alcohol appears to be the most common contributing factor. Alcohol abuse, alcohol intolerance and other alcohol related disabilities are some of the most challenging public health problems. The biochemical, pharmacogenetic and pathological disturbances observed in human after acute and chronic intake of alcohol are complex. Ethanol permeates all tissues of the body, because it is a small molecule and soluble in both water and lipids. Ethanol by its property of generating free radicals causes severe damage to the membranes and thus affects almost all organs of the human body. Ethanol-induced acute liver injury in rats is a widely used experimental animal model to investigate the underlying mechanisms of clinical fulminate hepatic failure and to develop an effective therapeutic strategy. The use of natural remedies for the treatment of liver diseases has a long history and medicinal plants and their derivatives are still used all over the World in one form or the other for this purpose. Recent experience has shown that plant drugs are relatively non-toxic, safe and even free from serious side effects. In the present study, we have evaluated the protective role of morin, a naturally occurring bioflavonoid on ethanol-induced toxicity. The various biochemical parameters such as hepatic markers, renal markers, lipids, lipid peroxidation markers, enzymic and nonenzymic antioxidants, genotoxicity, mRNA and protein expressions of various cytokines responsible for inflammation and histopathological alterations in ethanol induced toxic rats were studied.

*In-vitro* antioxidant and free radical scavenging activities of morin

Morin scavenged DPPH* and ABTS* in a concentration-dependent manner (50, 100, 150, 200 and 250 μM). The percentage-scavenging activity of morin increased with increasing concentration. The maximum percentage scavenging effects of morin for DPPH* was 71% and 80% for ABTS* at 200μM/mL. Furthermore morin scavenged O₂* and OH* radicals. The highest percentage scavenging activity on O₂* was 77% and 68% on OH* radicals at 200 μM.
Effect of morin on the alteration in the circulations

Serum hepatic marker enzyme activities were increased in ethanol induced hepatotoxic rats. Treatment with morin at a dose of 15, 30, 60 and 120 mg/kg/body weight lowered the hepatic marker enzyme activities and decreased the levels of bilirubin. Kidney function markers (urca, uric acid and creatinine) were also elevated in the serum of hepatotoxic rats and treatment with morin significantly modulated all the above changes. Thus, the results showed that morin exerted hepatoprotective effect and consequently alleviated the functional abnormalities of liver and kidney associated with ethanol induced toxicity in rats.

Hepatotoxic rats had elevated levels of lipid peroxidation by products and decreased activities of enzymatic antioxidants in the plasma and erythrocytes. Treatment with morin protects the above changes and improved significantly towards normal, showing its antioxidant and antiperoxidative properties. Ethanol induced hepatotoxic rats showed elevated levels of TC, TG, FFA, PL LDL-C, VLDL-C and decreased level of HDL-C in the plasma. Treatment with morin protected the above changes in hepatotoxic rats and improved significantly. Among the four doses, the 60 mg dose was more effective.

Effect of morin on tissue lipids

In the present study, it was found that ethanol fed rats showed significant elevated levels of TGs, TC, FFA and PL in the tissues (liver, kidney and brain). Administration of morin to the ethanol fed rats significantly reduced the levels of lipids in the tissues. This might be due to the enhanced peripheral utilization and decreased synthesis of cholesterol or the antihyperlipidemic effect of morin. Control rats treated with morin did not show any significant changes.

Effect of morin on lipid metabolizing enzyme

Chronic administration of ethanol significantly decreased the activity of lipid metabolising enzymes such as LCAT, LPL and increases the HMG CoA reductase. Interestingly, these lipid metabolising enzymes activities were significantly modulated in the ethanol fed rats co-treated with morin.
Effect of morin on ethanol - induced oxidative stress

In the present study, the elevated levels of lipid peroxidation by products such as TBARS, LOOH and CD in the liver, kidney and brain of ethanol-fed rats were observed. However, morin administered to the ethanol-fed rats showed significant decrease in lipid peroxidation by products. The activities of the enzymatic antioxidants (SOD, CAT, GPx, GR and GST) and non-enzymatic antioxidants (vitamin-C, vitamin-E and GSH) were significantly decreased in the tissues (liver, kidney and brain) of ethanol-fed rats. Ethanol-fed rats co-treated with morin significantly increased the above antioxidants.

Effect of morin on the levels of nitrite and iron in the liver

In the present study, ethanol fed rats showed significant elevated levels of iron and nitrite. The increased body iron can cause oxidative stress and lipid peroxidation, resulting in cellular injuries. Nitrite production by ethanol triggers ALD, whereas ethanol fed rats treated with morin significantly showed reduced level of iron and nitrite production in the liver when compared to the untreated ethanol fed rats. Control rats treated with morin alone did not show any significant changes.

Effect of morin on alcohol metabolizing enzyme

Chronic administration of ethanol significantly decreased the activity of alcohol metabolising enzymes (ADH and ALDH). Interestingly, these enzymes activities were significantly increased in ethanol fed rats co-treated with morin.

Effect of morin on xenobiotic metabolizing enzyme

The activity of phase I enzymes significantly increased in the ethanol-fed rats as compared to the control rats. Morin supplementation resulted in a significantly decrease in the activities of phase I enzymes when compared to the untreated ethanol fed rats. Whereas, the activities of phase II enzymes (GST and DT-diaphorase) in the cytosolic fraction of the liver was significantly decreased in ethanol-fed rats, when compared to the control rats. Morin supplementation results
in a significant increased in the activities of GST and DT-diaphorase, when compared to the untreated ethanol-fed rats.

Effect of morin on membrane transporters

One among the various cellular mechanisms of ethanol toxicity is the alteration in membrane structure and functions as evidenced by several ATPase. Ethanol interacts with the cellular constituents causing profound changes in their structure, organization and functions. Alteration in lipid components and ion-channels by ethanol can cause changes in membrane function by altering its fluidity. In the present study, administration of morin significantly restored the activity of these enzymes, which might be due to the antilipidperoxidative and antihyperlipidemic effect of morin.

FT-IR Spectral changes in the liver

The olefinic band $3012\text{cm}^{-1}$ is assigned to monitor the lipid peroxidation. The FT-IR spectra revealed significant differences in the absorbance intensities between the control and ethanol treated groups indicating the lipid peroxidation in the ethanol fed group, while ethanol fed rats co-treated with morin significantly reduced the band intensity. It could be due to the decreased lipid peroxidation.

NF-κB, CD14 and IL-6 mRNA expression and TNF-α, TGF-β, COX-2 and iNOS protein expression

NF-κB, CD14 and IL-6 mRNA were significantly up-regulated in hepatotoxic rats whereas treatment with morin significantly down-regulated. In addition, a significant increase in hepatic TNF-α, TGF-β, COX-2 and iNOS protein expressions were found in the ethanol-fed rats. Whereas, ethanol fed rats co-treatment with morin suppressed these protein expressions, which may be due to the anti-inflammatory property of morin.

Effect of morin on ethanol mediated DNA damage

Ethanol induced DNA damage was observed in lymphocytes. DNA damage was observed by increased % tail DNA, tail length and tail moment of the comet
Morin administration to the ethanol-fed rats significantly reduced the DNA damage when compared to the untreated ethanol fed rats. This may be due to the DNA protective effect of morin.

**Effect of morin on collagen accumulation in the liver**

Control and control rats treated with morin show mild collagen deposition, the architecture of hepatic lobules is complete and there is no inflammatory cell infiltration. Ethanol fed rats showed fibrotic change; fibrosis is exuberant with bluish circular staining pattern around the portal triad. Marked reduction of collagen is seen in ethanol fed rats co-treated with morin.

**Effect of morin on apoptotic markers in control and experimental animals**

Bax protein expression was minimally detected in control rat liver. Control rats treated with morin showing normal expression of Bax. However, ethanol fed rats showed increased expression of Bax protein. Treatment with morin to the ethanol fed rats significantly reduced the expression of Bax. Bcl-2 protein expression was minimally detected in control and control rats treated with morin. However, ethanol fed rats showed decreased expression of Bcl-2. Treatment with morin to the ethanol fed rats significantly increases the expression of Bcl-2.

**Effect of morin on the histological changes**

Hematoxylin and eosin staining of tissues (liver, kidney and brain) of ethanol-fed rats showed significant structural alterations such as micro and macrovascular type of fatty change in the liver. Glomerulosclerosis and arteriolar thickening in the kidney as well as shrunken, pyknotic and neurons with darkly stained small nuclei in the brain. Treatment with morin significantly attenuated these changes to near normal when compared to untreated ethanol-fed rats.
Conclusion

The present study shows that morin possess hepatoprotective, antioxidant, antihypertensive properties in ethanol induced hepatotoxicity rats. It also possesses protective effect against liver, kidney and brain injuries associated with ethanol toxicity in rats. The mechanism of action may be due the regulation of oxidative stress pathways via down regulation of cytokines, apoptotic markers, alcohol metabolizing enzymes, iron metabolism and nitrosative stress. All the biochemical studies were supported by the histological studies. Morin protects the cell from the toxic effects of ethanol. Moreover, no toxic effects were observed on morin administration to control rats. Morin merits further development as a therapeutic agent and it may surpass other drugs in the future. Studies are warranted at the pharmacological levels.