Summary & Conclusions
The present study focuses in evaluating the hepatoprotective and antioxidant efficacy of Polygonum glabrum in treating liver disorders in the traditional folklore medicine.

Three medicinal plants (Polygonum glabrum, Ecbolium viride and Kyllinga triceps) with antioxidant activities were screened based on ethnobotanical approach. The aqueous and ethanolic extracts of the plants were prepared by soxhlation and screened for in vitro antioxidant activity through DPPH, hydroxyl, superoxide radical scavenging, metal chelating, anti-lipid peroxidation and reducing power assays. Ethanolic extract of P. glabrum showed highest antioxidant activity in all the in vitro models when compared with the other extracts. The phytochemical analysis of the ethanolic extract of P.glabrum showed the presence of phenols, flavonoids, glycosides and tannins which may be responsible for the antioxidant activity. The HPLC analysis of the extract confirmed the presence of flavonoids like quercetin, genistein, rutin and diadzein. A fair correlation between phytochemical contents and in vitro antioxidant activity was observed in EEPG. The DNA protective nature of the P.glabrum extract was confirmed by the DNA strand scission assay with FX174 RF1 supercoiled DNA in vitro. The strong antioxidant properties of ethanolic extract of P.glabrum make it a good candidate for further animal intervention studies.

Safety evaluation (Acute and Subacute toxicity) studies of the ethanolic extract of Polygonum glabrum were done using male Wistar rats. No mortality and behavioural changes were noted in acute toxicity studies upto the dose level of 2000 mg/kg b.wt after the administration of the extract. In sub-acute toxicity studies, EEPG did not produce any significant dose-related changes of SGOT, SGPT, ALP, total protein, bilirubin, cholesterol, triglycerides and haematological parameters (ESR, PCV, hemoglobin) and histopathological observations and so it was considered to be safe as per OECD-2000 guidelines.
A rat model of CCl₄ induced acute (10 days) and chronic (8 weeks) oxidative stress was used to assess the antioxidant and hepatoprotective activities of *P. glabrum*. CCl₄ intoxication caused free radical generation in many tissues such as liver, kidney, brain and heart. The hepatorenal damage caused by CCl₄ was protected by EEPG dose dependently, by stabilization of the levels of liver markers (SGOT, SGPT, ALP, GGT, LDH and bilirubin) and non-protein nitrogenous substances (urea, uric acid and creatinine) in serum.

Lipids are more easily attacked by the activated metabolites of CCl₄ resulting in damage to intracellular membranes and the plasma membrane. CCl₄ toxicity caused increase in cholesterol and triglycerides and decrease in phospholipids in liver and kidney while all the lipid profiles increased in brain and heart tissues. EEPG effectively reduced the lipid levels altered by CCl₄ metabolism in all the tissues.

Oxidation products of lipids (lipid peroxidation), proteins (protein carbonyls and total sulfhydryls) are the biomarkers of oxidative stress in CCl₄ poisoning. Lipid peroxidation, protein carbonyls were significantly elevated whereas the total sulfhydryl groups were decreased in CCl₄ treated animals. In EEPG treated rats these values were altered significantly and brought back to near normal values. XOD, an oxidative enzyme was also observed in high levels during CCl₄ induced oxidative damage to the tissues. These increased levels were reverted to near normal values by the administration of EEPG in both acute and chronic studies indicating the antioxidative activity of EEPG.

Biological systems protect themselves against the damaging effects of reactive oxygen species by several innate defense systems. These include enzymic (SOD, CAT, GPx, GR, GST and G-6-PDH) and non enzymic (GSH, vitamin C & E) antioxidants. Administration of EEPG appeared to protect the liver, kidney, brain and heart of Wistar rats against CCl₄ induced acute and chronic oxidative
stress by reducing the intensity of lipid peroxidation and by enhancing the activities of enzymic (SOD, CAT, GPx, GR, GST and G-6-PDH) and non enzymic antioxidants (GSH, Vitamin C & E).

To resolve whether the hepatoprotective effect of EEPG was mediated through its antioxidant activity; spectral analysis, isozyme analysis and mRNA expression of antioxidant enzymes (SOD, CAT and GPx) in liver was performed. The administration of the EEPG up-regulated the antioxidant enzymes which were down-regulated by CCl₄ toxicity and thus could prevent the progression of liver injury.

Direct breakage of the DNA strands occurs when reactive oxygen species interact with DNA. The DNA damage due to CCl₄ intoxication was protected by EEPG treatment as assessed in blood by comet assay. The comets showed shorter tail length of the cells in the EEPG treated groups when compared to the CCl₄ treated group.

Histopathological studies were performed to provide direct evidence of the toxicity of CCl₄ on the tissues, and of the protective effect of the ethanol extract of P.glabrum. Marked disruption of the structure of cells was observed in liver, kidney, brain and heart tissues of CCl₄ treated rats. Only minimal disruption of the structure of cells was noted in liver, kidney, brain and heart tissues of EEPG treated rats during acute and chronic treatments. This minimal disruption of cells in various tissues provides additional support to the study that EEPG has protective effect against oxidative stress.

In conclusion, the present investigation reveals the potential of ethanolic extract of P.glabrum as an antioxidant and hepatoprotective agent, which is effective in countering the various biochemical and histological changes characteristic of CCl₄ toxicity and this study rationalizes its folklore use in the
liver disorders. The spectrum of these properties is attributed to the presence of phytochemicals like saponins, tannins, glycosides flavonoids and polyphenols which acts synergistically and brings out the antioxidant and hepatoprotective activity. However, further investigations are required in isolating and identifying the active principle(s) responsible for the antioxidant activity of ethanolic extract of *P. glabrum* to ascertain the role of individual constituents in the efficacy of the above described properties of the plant in order to ascribe potential pharmacological applications to this plant.