Summary & Conclusions
The present study was taken up in light of growing evidence of involvement of oxidative stress in pathogenesis of various diseases and the ability of plant derived antioxidants to alleviate the deleterious effects of the oxidative stress. Aqueous and ethanol extracts of the selected plants (*Ammannia baccifera* Linn, *Cocculus hirsutus* (Linn.) Diels and *Flacourtia indica* (Burn.f) Merr) were subjected to preliminary screening for their antioxidant potential by employing various electron transport based *in vitro* methods (DPPH, hydroxyl and superoxide anion radicals scavenging activities, reducing power and metal chelating activity). Ethanolic extract of *Ammannia baccifera* was found to possess highest free radical scavenging activity when compared to the other extracts. This may be due to the presence of flavonoids and phenols which are majorly responsible for the antioxidant activity. In addition to these constituents, sterols, triterpinoids, tannins, and alkaloids are also present which may contribute to the antioxidant activity of *A.baccifera*. Further TLC and HPLC analysis of EEAB revealed the presence of potent antioxidant compounds like rutin and quercetin. DNA protective efficacy of *A.baccifera* was proved by performing DNA nicking assay *in vitro*.

Acute toxicity study of EEAB revealed no behavioural changes and mortality and is nontoxic upto the dose level of 2000 mg/kg b. wt. In sub-acute toxicity studies EEAB did not produce any significant dose-related changes of hematological parameters, serum biochemistry and histopathological observations and so it was considered to be safe as per OECD-2000 guidelines.

Further to prove the antioxidant and DNA protective potential of EEAB *in vivo*, carbon tetrachloride was employed as oxidative stress inducer in albino rats. The treatment was in two phases, acute (10 days) and chronic (8 weeks/56 days). Hepatocellular damage caused by CCl₄ resulted in the increased levels of serum SGOT, SGPT, ALP, γGT, LDH and bilirubin. Administration of EEAB brought back the levels to the near normal in a dose and time dependent manner. The condition of renal tissue during CCl₄ intoxication was assessed by measuring serum urea, creatinine and uric acid levels. All the altered levels were reverted to near normal levels by the administration of EEAB.
Lipid (triglycerides, cholesterol and phospholipids) abnormalities play a vital role in development and progression of CCl₄ induced oxidative stress. The changes in the lipid profile such as increase in the levels of triglycerides and cholesterol and decrease in the levels of phospholipids in liver and kidney were observed in CCl₄ induced oxidative stress condition. On the other hand CCl₄ toxicity caused an increase in the levels of all the lipids in brain and heart tissues. All changes were reverted back to near normal levels by the treatment of EEAB.

Increased lipid peroxidation and their products lead to the damage of proteins and nucleic acids. CCl₄ toxicity results in the decrease of protein synthesis and some functional groups of proteins (sulfhydryls) in all tissues. The decreased levels of protein and total sulfhydryls in the tissues were reverted back to near normal values by the administration of EEAB during both acute and chronic treatments in a dose dependant manner, ensuring the antioxidative property of EEAB. Protein carbonyls, which are the biomarkers to denote the extent of protein damage and XOD, an oxidative enzyme were 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 EEAB suggesting the antioxidant potential of the extract.

Increased production of ROS and RNS during CCl₄ toxicity result in the increased level of LPO, which is due to decreased levels of both enzymic (SOD, CAT, GPx, GR and GST) and nonenzymic (GSH, Vitamin C and Vitamin E) antioxidants. This emphasizes the prooxidant/antioxidant imbalance in oxidative stress condition and this insufficiency in the antioxidant defense system were reverted back to near normal range by EEAB in tissues during both acute and chronic treatments via enhancing the activities of both enzymic and nonenzymic antioxidants and reducing the levels of LPO by scavenging the free radicals generated in CCl₄ induced oxidative stress condition. G-6-PDH, an ancillary antioxidant enzyme involved in glutathione redox cycle, decreased during CCl₄ toxicity. These decreased levels were dose dependently and time dependently brought back to near normal values by the treatment of EEAB.
Further, to prove the mechanism of the antioxidative property of EEAB, isoenzyme pattern and mRNA expression of antioxidant enzymes (SOD, CAT and GPx) were performed in the liver of control and experimental rats during chronic treatment as liver is the vital organ in metabolizing various chemicals. Down regulation of SOD, catalase and GPx gene expression was observed during CCl₄ induced oxidative stress. Administration of EEAB caused a remarkable up regulation in gene-expression levels of SOD, catalase and GPx. These results suggest that the beneficial effect of ethanol extract of *Ammannia baccifera* is related to its antioxidative property through the enhancement of mRNA expression of these antioxidant enzymes and thus could prevent the progression of oxidative damage caused by CCl₄.

The protective effect of EEAB against the DNA damage was assessed by single cell gel electrophoresis assay (comet assay). The results reveal that the EEAB has protective activity against the DNA damage caused by CCl₄ intoxication.

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 *A. baccifera*. 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 EEAB treated rats during acute and chronic treatments. This minimal disruption of cells in various tissues provides additional support to the study that EEAB has protective effect against oxidative stress.

In conclusion, ethanol extract of *Ammannia baccifera* is able to confer protection against oxidative damage induced by administration of CCl₄ in Wistar rats through its antioxidative and DNA protective property. In rats receiving ethanol extract of *A. baccifera* and CCl₄ near normal levels of direct and indirect oxidative stress markers (enzymic and non-enzymic antioxidants, LPO, XOD,
protein carbonyls, and total sulphydryls) and serum enzymes (SGOT, SGPT, ALP, GGT and LDH) were maintained, in contrast to alterations in all these parameters in rats receiving CCl₄ alone. Consequently, from these observations one can believe that EEAB hold the potential to become the antioxidative therapeutics of choice in future. The study also provides the scientific evidence to the folklore usage of *Ammannia baccifera*. However, further investigations are required in isolating and identifying the active principle(s) responsible for the antioxidant activity of ethanolic extract of *Ammannia baccifera* 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.