SUMMARY & CONCLUSION
The last few years have seen a major increase in the use of herbal alternatives in the developed countries. They are primarily used in health care because of better acceptability, better compatibility with human body and because of lesser side effects.

Hawthorn extract is among the most popular herbal medicinal products in the United States as well as European countries such as Germany where it is marketed as a prescription medicine. Therefore, this over the counter product, was obtained and its antioxidant effects on carbon tetrachloride treated rats were evaluated.

Oxidative stress was induced by giving a single dose of CCl₄ (2.5ml/kg body weight) to rats. Its lipid-solubility allows it to cross cell membranes rapidly and it gets quickly distributed to all organs. Its main toxic effects are exhibited in the liver, although substantial injury has been documented in other organs. In rats, liver tissue, kidney issue and erythrocytes were taken up for study. Once the antioxidant potential was established in vivo in the rat model, its antioxidant potential was evaluated in vitro in normal human blood tissue. The following observations have been made in this study.

1. The drug extract proved to be a strong anti-lipidperoxidative agent in vitro against lipid peroxidation induced by ferrous sulphate in rat liver homogenate. The drug also prevented the oxidation of glutathione in vitro in rat liver homogenate.

2. Toxicological evaluation showed that the drug was practically non-toxic in nature. There was no change in the haematological and
biochemical parameters assayed in rats which were treated with the drug when compared with the control rats. Histology of liver and kidney tissue remained normal upon treatment with the extract.

3. *Crataegus* extract pretreatment decreased the CCl₄ induced changes. The drug proved to be strongly hepatoprotective.

a) It decreased the cytologic injury caused by CCl₄. It also reversed the depletion of liver glycogen which resulted from CCl₄ intoxication.

b) It decreased the levels of marker enzymes and bilirubin which were elevated upon intoxication with CCl₄.

c) The drug also reduced the plasma total cholesterol, triglyceride and lipoprotein levels which were elevated upon CCl₄ administration.

d) The drug could also ameliorate the lipid peroxidation induced by CCl₄. It also reversed the depletion of glutathione levels resulting from CCl₄ administration.

e) The drug increased the levels of primary antioxidant enzymes which became depleted after CCL₄ administration. The drug also increased the levels of glutathione-s-transferase whose levels became reduced after CCl₄ administration.

f) The drug also increased the levels of Kreb’s cycle enzymes studied. Carbon tetrachloride has a direct effect on mitochondria and it depleted the levels of NADH dehydrogenase and Cytochrome-c-
oxidase. The levels of these enzymes also increased upon drug pretreatment.

4. The kidney tissue also mirrored the changes observed in the liver tissue.

   a) The histology of the kidney tissue revealed gross alterations after CCl₄ administration. These changes were partially reversed with drug pretreatment.

   b) As seen in the case of liver, the drug decreased the lipid peroxidative effects of CCl₄. Glutathione levels also increased after drug administration.

   c) The drug could also increase the primary antioxidant enzymes which were decreased after CCl₄ administration.

   d) The levels of the detoxifying enzyme, glutathione-s-transferase also increased in the drug pretreated group.

5. The erythrocytes of rats also exhibited similar effects. The following aspects were observed.

   a) Lipid peroxidation levels increased in the haemolysate and the erythrocyte membrane after CCl₄ was administered. Glutathione levels were reduced after CCl₄ treatment and its levels increased with the administration of the drug. This brought about a decrease
in the levels of lipid peroxides both in the erythrocyte membrane and in the haemolysate.

b) There was a decrease in the levels of the primary antioxidant enzymes, SOD, CAT, and GPx in the carbon tetrachloride treated group. The enzyme levels increased significantly with the administration of drug.

c) Levels of membrane bound ATPases decreased with the administration of CCl₄. All the three ATPases namely, Na⁺K⁺-ATPase, Ca²⁺-ATPase and Mg²⁺-ATPase levels increased with pretreatment with the drug.

6. *In vitro* studies on human blood tissue was done using different concentrations of the drug. The erythrocyte fraction and the lymphocytes were taken for analysis. Here, hydrogen peroxide was used to produce oxidative stress.

a) There was an increase in the lipid peroxidation in the haemolysate of H₂O₂ group. This was effectively decreased in the drug pretreated group at a concentration of 10µl/ml of packed RBC. At this concentration, the levels of glutathione increased maximally.

b) The levels of the primary antioxidant enzymes, SOD, CAT, and GPx also increased to a maximum extent at that concentration.
c) The three membrane bound ATPases assayed, namely, Na⁺K⁺-ATPase, Ca²⁺-ATPase and Mg²⁺-ATPase decreased in the H₂O₂ treated group. The levels of this enzyme increased with the administration of the drug at a concentration of 10µl/ml of packed RBC.

7. Lymphocytes were incubated with the drug and the following changes were observed upon induction of oxidative stress.

a) Fluorescent staining of lymphocytes showed apoptotic changes upon treatment with H₂O₂ which was reversed after incubating with the drug.

b) Increased lipid peroxidation of the lymphocytes was decreased after treatment with the drug. The drug also increased the levels of glutathione, which was decreased after treatment with the drug. This was effectively done at a concentration of 10µl/10,000 cells.

c) The levels of primary antioxidant enzymes improved maximally at the above concentration.

d) DNA degradation induced by H₂O₂ was reversed to a greater extent at the same concentration.

All the above observations prove beyond doubt that *Crataegus* extract is definitely cytoprotective in nature. The extract could be a chain breaking antioxidant like Vitamin E. It could also have a direct action on Ca²⁺ metabolism as it prevented
endonuclease-dependent DNA degradation. It could also be an inducer of proteins which are antiapoptotic in nature such as Bcl2. Otherwise it could be an inhibitor of apoptosis inducing genes such as Bax and p53.