4. SUMMARY AND CONCLUSION

Liver & Renal diseases are a worldwide problem. Conventional drugs used in the treatment of hepatorenal diseases are sometimes inadequate and can have serious adverse effects. It is therefore necessary to search for alternate drugs for the treatment of hepatorenal diseases to replace currently used drugs of doubtful efficacy. Hence the present study was undertaken to evaluate the hepato and nephro protective efficacy of Caesalpinia sappanand Clitoria ternatea L. (CT) in a rat model of hepatorenal toxicity evoked by acetaminophen in order to confirm that this plant does indeed have a therapeutic benefit in liver and kidney disease. The findings of the present study have been summarized below.

- Acetaminophen (APAP) is generally accepted as a safe analgesic and an antipyretic when administered within the therapeutic range; however, after overdose absorption or in specific conditions, APAP is known to be hepatotoxic and nephrotoxic in humans and experimental animals. Excessive use of APAP can cause multiple organ damages, especially of the liver and kidney.

- Phytochemical analysis of ethanolic extract of Caesalpinia sappanand Clitoria ternatea L. (CT) leaves by GC-MS, revealed that the presence of most of the biologically active compounds such as alkaloids, flavonoids, glycosides, saponins, tannins, phytosterols, triterpenoids.

- The hepato and nephro protective effect of Caesalpinia sappanand Clitoria ternatea L. (CT) extract against acetaminophen-induced toxicity was carried out in HepG2 and
Vero cell line respectively (*in vitro*), as well as in *albino* rats (*in vivo*). The effects on cell viability by Trypan blue assay and MTT assay were determined. Both the plant extracts offered good protection against the deleterious effects of acetaminophen indicating that it is a promising hepatorenal protectant.

- The activities of serum marker enzymes such as AST, ALT and ALP were found to be increased along with elevated levels of serum bilirubin in rats challenged with acetaminophen. Prior oral treatment with Caesalpinia sappan and Clitoria ternatea L. (CT) extract brought down the elevated activities of marker enzymes and bilirubin to near normal.

- The standard drug, silymarin treated animals (Group 5) also showed a significant increase in antioxidant enzymes levels and decrease in liver marker enzymes when compared to APAP induced group.

- The levels of total protein were decreased significantly in acetaminophen intoxicated rats. These altered levels were brought back to near normal on treatment with Caesalpinia sappan and Clitoria ternatea L. (CT) extract prior to acetaminophen challenge. The lowering of marker enzymes level is a definite indication of the hepatoprotective potential of the drug.

- Experimental evidence suggests that free radicals and reactive oxygen species play an important role in acetaminophen induced...
liver injury. The increased levels of lipid peroxides and decreased antioxidant (SOD, CAT, GSH, GPx and GST) status brought about by acetaminophen reverted to near normal on Caesalpinia sappan and Clitoria ternatea L. (CT) extract pretreatment. This indicates that the anti-peroxidative nature and the antioxidant property of the extracts of CS and CT.

- Mitochondria are the most important intracellular source and target of reactive oxygen species. A decrease in antioxidant status was noticed in liver mitochondria of rats that received acetaminophen. Treatment with Caesalpinia sappan and Clitoria ternatea L. extract prior to acetaminophen induction enhanced the antioxidant status by its antioxidant potential.

- Studies demonstrated that the acetaminophen-induced liver injury resulted in dysfunctions in oxidative energy metabolism of rat liver mitochondria. A decline in the activities of TCA cycle enzymes (ICDH, α-KGDH, MDH and SDH) and respiratory chain enzymes (NADH dehydrogenase and cytochrome c oxidase) were found in Caesalpinia sappan and Clitoria ternatea L. (CT) extract treated animals. This effect of the extract may be attributed to its effect of improving the electron transport and subsequent energy production.

- Impairment in the activities of microsomal mixed function oxidase was observed in acetaminophen intoxicated rats. The potentiality of detoxification system was found to be elevated upon prior oral administration of Caesalpinia sappan and Clitoria
ternatea L. (CT) extract by optimizing the activities of heme oxygenase.

- The activities of lysosomal enzymes namely β-glucuronidase and β-galactosidase were found to be significantly increased in acetaminophen intoxicated rats. Oral administration with Caesalpinia sappan and Clitoria ternatea L. (CT) extract prior to the toxic insult of acetaminophen decreased the enzyme activities, which could be attributed to the membrane stabilizing property of the extract.

- The standard drug, silymarin treated animals also showed a significant increase in ICDH, α-KGDH, SDH, NADH, CYT C Oxidase levels and decrease in Heme oxygenase, β-D glucuronidase and β-D galactosidase when compared to APAP induced group.

- The kidney is an important target of the toxicity of drugs, xenobiotics, and oxidative stress, which plays an essential role in health, disease and overall development and growth. The main function of the kidney is to maintain total body fluid volume, its composition and acid base balance. A number of environmental variables including certain drugs influence these functions.

- Serum urea and creatinine concentrations were significantly increased in APAP-treated animals compared with controls, indicating the induction of severe nephrotoxicity. Treatment with extract of Caesalpinia sappan and Clitoria ternatea L.
(CT) lowered concentrations of serum urea and CR compared with treatment with APAP alone. However, the levels of uric acid were significantly decreased in the APAP-treated groups compared with control. Treatment with Caesalpinia sappan and Clitoria ternatea L. (CT) in APAP-treated rats significantly increased uric acid levels compared with treatment with APAP alone.

- Screening of various haematological parameters depicts the significance in diagnosis to estimate the degree of renal function. Studies on hematological parameters indicated that administration of an overdose of acetaminophen induced remarkable decrease in MCH, MCHC, Grans, PLC and PCV along with increased level of MCV. Prior oral administration with Caesalpinia sappan and Clitoria ternatea L. (CT) extract reverted the above changes to near normal, which indicates its prophylactic action in maintaining haemostasis.

- APAP overdose increases lipid peroxidation and suppresses the antioxidant defense mechanisms in renal tissue. In APAP-treated animals the TBARS levels increased significantly, while administration of ethanolic extract of Caesalpinia sappan and Clitoria leschenaultia takeda, significantly lowered TBARS levels compared with APAP induced rats.

- The activity of catalase in the APAP-treated group was significantly decreased compared with control. Treatment with
Caesalpinia sappan and Clitoria ternatea L. (CT) significantly prevented the decrease in CAT activity induced by APAP.

- Similarly, the decreased GPx activity as a result of the treatment with APAP was also restored by Caesalpinia sappan and Clitoria ternatea L. (CT) extract. Renal SOD activity was decreased significantly in the APAP-treated group. Treatment with Caesalpinia sappan and Clitoria ternatea L. (CT) in APAP-treated rats significantly elevated the SOD activity levels compared to treatment with APAP, suggesting that CT was exerting an antioxidant effect.

- Plant extract alone treated group did not show any significant changes in blood hematological parameters, biochemical markers and antioxidant studies.

- Histopathological profile of the normal animal showed normal hepatocytes with well preserved cytoplasm with no sign of inflammation. The acetaminophen treated animals showed severe centrilobular necrosis and fatty infiltration. Treatment with different doses of ethanolic extract of CS and CT and silymarin produced mild degenerative changes and absence of centrilobular necrosis when compared with control. All these results indicate the hepatoprotective potential of the ethanolic extract of Caesalpinia sappan and Clitoria leschenaultia takeda.

- Histopathological observations made on liver sections of rats treated with APAP showed a marked necrosis in the proximal
tubules. The kidney tissues obtained from these animals showed moderate cloudy swelling of proximal convoluted tubules and severe vacuolar degeneration of distal tubules. Treatment with ethanolic extract of Caesalpinia sappan and *Clitoria leschenaultia takeda*, in APAP-treated animals showed a mild degree of necrosis and degeneration. The normal tubular pattern with a mild degree of swelling was also obtained on treatment with Caesalpinia sappan and *Clitoria ternatea* L. (CT) extracts in APAP-administered rats.

The above experimental results obtained in terms biochemical changes, which were also confirmed by histopathological studies indicate the hepatoprotective potential of Caesalpinia sappan and *Clitoria ternatea* L. (CT) against acetaminophen-induced hepatic injury. Theoretically, the hepatoprotective action of Caesalpinia sappan and *Clitoria ternatea* L. (CT) could be due to alterations in disposition and biotransformation of hepatotoxicant, alterations in cellular detoxifying mechanisms as well as cellular response and regenerating processes. The possible mechanisms of hepatoprotection may be stimulated by the presence of alkaloids, which are reported to be present in the leaves of Caesalpinia sappan and *Clitoria leschenaultia takeda*.

Based on the Biochemical analysis and histopathological observation, the results of the study indicate that the ethanolic extract of Clitoria ternatea L. (CT) shown more hepatorenal protectant than the ethanolic extract of *Monochoria vaginalis*.
Hepatorenal disorders are a worldwide problem. Conventional drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effects. It is therefore necessary to search for alternate drugs for the treatment of liver and kidney diseases to replace currently used drugs of doubtful efficacy. Most of the effects of the ethanolic extract of Caesalpinia sappan and Clitoria ternatea L. (CT) are due to the antioxidant activities and synergistic effect of the chemical compounds present in them making them good sources for the production of a hepato-renal protective herbal medicine.