CHAPTER 7
SUMMARY AND CONCLUSION

7.1 SUMMARY OF WORK DONE

Medicinal herbs have been used from ancient times to cure various ailments. This tradition had been passed on across generations to promote health. India is known worldwide for its Ayurvedic treatments using medicinal plants that have natural substances which cure diseases. Due to the increase in trend to improve the quality of life, in the past few decades, there is a considerable attention and interest grown towards the use of herbal medicines.

Drug induced nephrotoxicity is common and responsible for a variety of pathological effects on the kidney. Many medications can lead to renal dysfunction in humans through various mechanisms, which can cause significant morbidity. Several research studies have revealed that the conventional drugs are not sufficient and hence found to be inadequate in treating the renal disorders. Search for nephroprotective agents has resulted in exploration of medicinal plants which were claimed to be useful in the treatment of renal disorders in folklore medicine.

This work has investigated the nephroprotective effects of two medicinal herbs, *Melia Azadirachta* and *Borrassus flabellifer*. This study has been applied in a rat model of nephrotoxicity, induced by acetaminophen and H$_2$O$_2$ in order to confirm that these two herbs posses therapeutic effects in kidney disease. The observations and inferences made in this dissertation are as follows.
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 *Melia Azadirachta* (MA) and *Borrassus flabellifer* (BF) 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 nephro protective effect of *Melia Azadirachta* (MA) extract against acetaminophen and H$_2$O$_2$ induced toxicity, were carried out in Vero cell line (*invitro*). The nephro protective effect of both MA and BF, were also been studied on albino rats (*invivo*).

The effects on cell viability by Trypan blue assay and MTT assay were determined. The cytotoxicity study was carried out from the plant extract of *Melia Azadirachta* (MA). Its extract was screened for its cytotoxicity against Vero cell lines at different concentrations to determine the CTC$_{50}$ (50% growth inhibition) by MTT assay. It is proved that 1000 $\mu$g/ml concentration of ethanolic extract of MA had the most efficient effect against H$_2$O$_2$ induced cytotoxicity. Dose response curve shows decreasing number of viable cells with increasing concentration of ethanolic extract of MA. As per MTT assay, Melia Azadirachta (MA) shows considerable activity on Vero cell.
plant extract of *Melia Azadirachta* has observed to offer good protection against the deleterious effects of acetaminophen and H$_2$O$_2$ indicating that it is a promising renal protectant than the *Borrassus fiabellifer*.

- 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 *Melia Azadirachta* extract brought down the elevated activities of marker enzymes and bilirubin to near normal.

- The levels of total protein were decreased significantly in acetaminophen intoxicated rats. These altered levels were brought back to near normal on treatment with *Melia Azadirachta* (MA) extract prior to acetaminophen challenge. The lowering of marker enzymes level is a definite indication of the hepatoprotective potential of the drug.

- 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 extracts of *Melia Azadirachta* (MA) and *Borrassus fiabellifer* (BF) have lowered the concentrations of serum urea and Creatinine 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 *Melia Azadirachta* (MA) and *Borrassus fiabellifer* (BF) 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 *Melia Azadirachta* (MA) and *Borrassus fiabellifer* (BF) extract have 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 extracts of *Melia Azadirachta* (MA) and *Borrassus fiabellifer* (BF), 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 *Melia Azadirachta* (MA) and *Borrassus fiabellifer* (BF) have 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 *Melia Azadirachta* (MA) and *Borrassus fiabellifer* (BF) extracts. Renal SOD activity was decreased significantly in the APAP-treated group. Treatment with *Melia Azadirachta* (MA) and *Borrassus fiabellifer* (BF) in APAP-treated rats.
significantly elevated the SOD activity levels compared to treatment with APAP, suggesting that MA and BF was exerting an antioxidant effect.

- Plant extracts alone treated group have shown similar activity in blood hematological parameters, biochemical markers and antioxidant studies.

- Histopathological profile of the normal animal showed normal nephrons with well preserved cytoplasm which has no sign of inflammation. The acetaminophen treated animals have shown severe centrilobular necrosis and fatty infiltration.

- Treatment with different doses of ethanolic extracts of MA and BF have produced mild degenerative changes and absence of centrilobular necrosis when compared with control. All these results indicate the nephroprotective potential of the ethanolic extracts of *Melia Azadirachta* (MA) and *Borrassus fiabellifer* (BF).

- Histopathological observations made on the kidney tissues obtained from these animals shown moderate cloudy swelling of proximal convoluted tubules and severe vacuolar degeneration of distal tubules. Treatment with ethanolic extracts of *Melia Azadirachta* (MA) and *Borrassus fiabellifer* (BF), 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 *Melia Azadirachta* (MA) and *Borrassus fiabellifer* (BF) extracts in APAP-administered rats.
The above experimental results obtained in terms biochemical changes, which were also confirmed by histopathological studies indicate the nephroprotective potential of *Melia Azadirachta* (MA) and *Borrassus fiabellifer* (BF) against acetaminophen-induced nephrosis. Theoretically, the nephroprotective action of *Melia Azadirachta* (MA) and *Borrassus fiabellifer* (BF) could be due to alterations in disposition and biotransformation of nephrotoxicants. And also due to the alterations in cellular detoxifying mechanisms as well as cellular response and regenerating processes. The possible mechanisms of nephroprotection may be stimulated by the synergistic presence of all phytoconstituents, which are reported to be present in the *Melia Azadirachta* (MA) and *Borrassus fiabellifer* (BF).

Based on the Biochemical analysis and histopathological observation, the results of the study indicate that the ethanolic extract of *Melia Azadirachta* (MA) shown more renal protectant than the ethanolic extract of *Borrassus fiabellifer* (BF).

Renal disorders are a worldwide problem. Conventional drugs used in the treatment of renal diseases are sometimes inadequate and can have serious adverse effects. It is therefore necessary to search for alternate drugs for the treatment of kidney diseases to replace currently used drugs of doubtful efficacy. Most of the effects of the ethanolic extracts of *Melia Azadirachta* (MA) and *Borrassus fiabellifer* (BF) 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 renal protective herbal medicine.