Chapter – 5
Summary & Conclusion
5. SUMMARY AND CONCLUSION

Mulberry (*Morus* sp.) has been domesticated over thousands of years and been adapted to a wide area of tropical, subtropical, and temperate zones of the world. In India, China, Japan etc. Morus species are cultivated as foliage to feed the silkworm. The extracts of *Morus indica* (MI), commonly known as Mulberry, have been reported to possess medicinal properties, including hypoglycemic, hypotensive, anti-inflammatory, antioxidant and diuretic activities. Many studies have demonstrated the hypoglycemic effect of *Morus indica* leaves or shoot culture extract using streptozotocin-induced diabetic animals. Our laboratory has also investigated and successfully proved the *Morus indica* antioxidant activity in food system and antidiabetic properties in animals and human subjects. During these studies, the cholesterol lowering property of *Morus indica* was observed along with antidiabetic properties in animals and human subjects. Various such scientific evidences on the medicinal properties of *Morus indica* intended a curiosity to carry an in depth research work and investigate its antioxidant properties at the biological level and its effect on lipid metabolism. Cultivation of MI throughout India and adaptability of the plant to different climatic conditions made a positive view for investigating the plant. This is the first report on the systematic study, wherein stepwise biological activity profiling is done resulting in successful elucidation of the mechanism of antihyperlipidemic, antioxidant activity and lipid oxidation inhibition by MI. It is also the first report on MI varieties (MI-M5, MI-V1, MI-S36). The summary and conclusion of the present study is presented here according to the work carried and results obtained.

➢ *Morus indica*-I - physicochemical properties, nutritional and phytochemical composition

The three samples of *Morus indica*- MI-M5, MI-V1 and MI-S36 investigated showed varied functional properties, nutritional and phytochemical composition. To utilize the sample as a functional ingredient in food formulations, the sample must provide vital nutrients such as vitamins, minerals and phytochemicals (phenolic, glutathione saponins etc.). The nutritional and phytochemical components help in illustrating the best performance as functional ingredient and fulfilling the purpose as
an antioxidant, glucose lowering and lipid lowering agent. These investigations also help in choosing the best sample. The results of this part of the study indicate that all the 3 samples of *Morus* can be used as functional ingredient as a single or in combination with other two varieties.

➢ **Antioxidant activity- *in vitro and ex vivo*.

The oxidation in food and biological system takes place in different stages, i.e., initiation, propagation and termination. An antioxidant should be potent enough to prevent or stop the oxidation reaction. The antioxidant activity of the samples was investigated at different concentrations by different methods. The antioxidant activity measured in different extracts i.e., Methanol (MIM), 80% methanol (MI8M), dechlorophyllised (MIDc), Aqueous cold (MIAQc) and aqueous hot (MIAQh), proved the capacity of the samples to fight against the different pro-oxidative conditions. The oxidation preventing, oxidants scavenging and de novo repair action of the antioxidants, remove the active species rapidly that attack biologically active molecules. Especially the correlation between the polyphenols and the free radical scavenging capacity of antioxidants express their second line defensive action at *in vivo*. A part from phenolics, various other antioxidants such as ascorbic acid, glutathione, tocopherols, etc, have acted as potent reducing agents or metal chelating agents. The antioxidant activity of the samples especially MI8M and MIDc extracts of MI-S36 and in preventing the oxidation by biological substrates, shows that the samples can exhibit their capacity at *in vivo* level against the oxidative stress biomarkers. Hence, understanding of mechanisms and dynamics of the antioxidant action is essential for designing appropriate experimental methods and proper interpretation of the data.

➢ **Hematological properties.**

The present experiment had shown the anticoagulant activity of aqueous extracts of MI- samples. The experiment provided the scientific evidence to promote the MI as anticoagulant agent. However, further in depth studies are needed to understand the mechanism of action.
➤ **Lipid lowering properties**

The *Morus indica* lipid lowering properties were exhibited by inhibiting HMG CoA reductase and binding bile acids. The major metabolic pathway for reducing cholesterol is via conversion to bile acids or preventing the cholesterol synthesis by inhibiting the HMG CoA reductase enzyme. Dechlorophyllised extract of all the three varieties, with high polyphenol content inhibited the enzyme better than the statins. Here the bile acid binding capacity was analyzed by different methods viz., hydration, diffusion and digestion and by combining these three methods (hydration, diffusion and digestion). The bile acid binding capacity expressed as Bile acid retardation index (BARI) of the samples was comparable with commercial drug cholesteramine, cereal bran and saponins. Combination of both the methods resulted in an improvement in the bile acid binding capacity of the samples and showed better bile acid binding capacity than the cereal bran. The optimization of bile acid binding method helped in understanding the gastrointestinal events involved in lipid metabolism. However, much more research is needed before prescribing the plant as ‘mainstream alternative therapy’.

➤ **Acute Toxicological studies**

The 80% methanol, dechlorophylis and aqueous extracts of Morus plant extracts at 2000mg/kg body weight, was investigated for14 days. The extracts did not cause any adverse effects on the animals, the biochemical parameters were within the normal range. Results of the liver histopathology suggests that, the extracts are safe and suitable for human consumption.

➤ **Hepatoprotective activity**

*Morus indica* extracts aqueous cold and dehlorophyllised extracts displayed a protective action against the CCl₄ hepatotoxicity. The phytochemicals of the plant have contributed towards the protection of the rat against toxicity of CCl₄. The protective nature of the Morus indicates that the plant can be used as a remedy for the diseases involving liver damage. Hence it can be inferred that the presence of antioxidant phytochemicals in the sample may have acted to reduce the CCl₄ induced toxicity. Since the plant demonstrated anti-hepatotoxic effect against CCl₄ induced oxidative damage, it can be utilized in any food formulation, as a nutraceutical or by individual administration to reduce or prevent the disorders involving oxidative stress.
Hypocholesterolemic / Antihypercholesterolemic properties

The cholesterol lowering capacity of the samples was investigated in hypercholesterolemic rats fed with high cholesterol diet, 80% methanol extract, saponin extract and treated with statins for 45 days. A significant reduction in total cholesterol and triglycerides was observed. Few parameters were found to be higher than healthy control group, however, the values were within the desirable range. Total cholesterol, TGL, LDL and HDL levels were maintained within the normal range in Morus treated groups. The 45 days treatment of animals with the Morus also resulted in reducing lipid peroxides and elevating glutathione levels. The lipid-lowering potential and antioxidant capacity of saponin extract appeared to be more pronounced than that of 80% methanol extract and Lovastatin. Hence, Morus may possibly be developed as an alternative cholesterol-lowering drug. Future studies must focus on identifying the bioactive compounds from Morus indica and their hypolipidemic under conditions of chronic hypercholesterolemia.

The following conclusions were drawn from the present investigation

- The use of different in vitro and ex vivo models and substrates have helped in confirming the antioxidant mechanism of Morus indica.
- The optimization of bile acid binding method helped in understanding the gastrointestinal events involved in lipid metabolism. However, much more research is needed before prescribing the plant as ‘mainstream alternative therapy’.
- The prime focus of the present investigation was on lipid metabolism. Morus indica was able to influence and modulate lipid or cholesterol metabolism by inhibiting the cholesterol synthesis and its absorption.
- The plant investigated was safe, inexpensive medicinal species with potential to be developed into alternatives or adjuncts to hypercholesterolemia.
- The chronic toxicity has to be studied before prescribing Morus indica as a therapeutic agent in long term prescription.