CHAPTER 7

Chapter 5: Summary, Conclusion and Recommendations

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CHAPTER 7
SUMMARY, CONCLUSION AND RECOMMENDATIONS

7.1 Summary

Fluorine is one of the common, widely distributed and predominant environmental pollutant in the world. The major source of fluoride is ground drinking water. In India, almost every state either directly or indirectly has been suffering with fluoride toxicity due to lack of proper non economical techniques for purification and also due to rapid industrialization. On the whole, hydrofluorosis is more common than industrial emission induced fluorosis in India. Earlier research reports strongly suggested that when diets were fortified with nutrients with secondary metabolites resulted in reversal of fluoride induced alternations in the body.

The present work was an attempt to study alleviatory effects of *Brassica oleracea Var. Botrytis leaves* and *Arthrospira platensis* against sodium fluoride (NaF) induced toxicity. These plants were selected based on reported nutritional composition, phyto constituents and traditional uses in the treatment of various disorders. Therefore, the purpose of this work includes phyto chemical, *in vitro* antioxidant and pharmacological effects of *Brassica oleracea Var. Botrytis leaves* and *Arthrospira platensis* against NaF induced toxicity. Both materials were collected and authenticated by Dr. Sunita Garg, Chief Scientist, Raw Material Herbarium and Museum, Delhi (RHMD), CSIR-NISCAIR.

Based on percentage of yield obtained, 30: 70 (Water: Ethanol) ratio of both plant extracts were selected for phyto chemical studies and *in vitro* antioxidant assays. Preliminary phyto chemical studies revealed the presence of alkaloids, glycosides, steroids, flavonoids, phenols and tannins as major secondary phytoconstituents. Hydroalcoholic extracts of *Brassica oleracea Var. Botrytis leaves*(BOB) and *Arthrospira platensis*(ASP) were subjected to determine the *In vitro* antioxidant potential through DPPH radical assay, ABTS radical scavenging assay, metal chelation assay, total antioxidant assay, reducing power assay, total flavonoid content and total phenol content. Results of *in vitro* antioxidant assays revealed both plant extracts possessed good antioxidant potential and ASP was found to have more superior antioxidant potential than BOB.

Acute toxicity studies of BOB and ASP showed that both were well tolerated at 2000 mg/kg b. wt after oral administration. Hence, these extracts at the dose of 100, 200 and
400 mg/kg b. wt were used for screening the alleviatory effects of BOB and ASP against NaF induced toxicity.

In NaF control, decreased body weight and organ weights (heart, liver and kidney) were observed. Increased body weight and normalized organ weights were shown after treatment with BOB and ASP in a dose dependent manner. In the thyroid profile, NaF control has showed increased serum levels of T3 and T4. The treatment of BOB at doses of 100 and 200 mg/kg b. wt has showed significant reduction in the serum level of T4 and at doses of 200 and 400 mg/kg b. wt has showed significant increased serum level of T3. BOB control group showed significant increase in the serum level of T4 without significant variation in the serum level of T3. In the ASP treatment, 100, 200 and 400 mg/kg b. wt doses increased serum levels of T3 and T4 were observed which might be due to its relative richness in iodine content.

Fluoride exposure effectively alters the structural, functional and metabolic status of cardiac tissue. In this study, NaF control has showed significant increase in the levels of serum LDH, CK-MB and tissue lipidperoxidation and decreased tissue levels of reduced glutathione and catalase. Treatment of BOB and ASP were steadily decreasing the fluoride induced elevated levels of serum CK-MB and LDH levels and tissue lipidperoxidation and increased levels of tissue reduced glutathione and catalase in a dose dependent manner. Histopathological reports of cardiac tissue also further supported the alleviatory effects of plant extracts against NaF induced cardiotoxicity.

Liver is a major organ for detoxification of various endogenous and exogenous substances. Higher amount of fluoride disturbs the metabolic processes and detoxification capabilities of liver and induce necrosis, modifications of membrane lipids and apoptosis in hepatocytes. Toxic effects of fluoride were influenced by amount of intake, duration of exposure and dosage form. In this study, fluoride significantly increased the serum levels of glucose, total cholesterol, triglycerides, SGOT, SGPT, Total bilirubin and Direct bilirubin while decreasing the serum levels of total protein, albumin, magnesium and HDL-C. Treatment with BOB and ASP at 200, 400 mg/kg b. wt showed significant recovery from elevated levels of above specified serum levels and it indicates protective effect against NaF induced toxicity. Oxidative stress due to cardiac ischemia was observed by elevated levels of lipidperoxidation and decreased levels of reduced glutathione and catalase. BOB and ASP treatments at 200 and 400 mg/kg b. wt doses reduced lipidperoxidation and increased reduced
glutathione and catalase levels. These findings were further strengthened by the results of histopathological studies.

Kidney is the major excretory organ for excretion of fluoride from the body and there is a concern that continuous exposure of fluoride may directly contribute to its toxicity. In this study, NaF control showed increased serum levels of BUN, creatinine and tissue lipidperoxidation and decreased tissue levels of reduced glutathione and catalase. Treatment with BOB showed significant reduction in the serum levels of BUN, uric acid and creatinine in a dose dependent manner. Treatment with ASP showed dose dependent increased levels of BUN and Uric acid. This may be due to its relative richness in protein and nucleic acid contents. Significant reduction in the serum creatinine level was observed in a dose dependent manner after treatment with ASP, indicated its alleviatory effect against NaF induced nephrotoxicity. Treatment with BOB and ASP were shown significant reduction in the tissue lipidperoxidation and increased levels of reduced glutathione and catalase in a dose dependent manner.

Blood is a constantly circulating fluid, continuously supplying to the necessary substances such as nutrients, oxygen and also removes wastes from the body. Several studies have clearly indicated that fluoride effectively alters the blood profile in a dose and duration dependent exposure. In this study, NaF Control showed significant increase in leukocyte and MCHC levels and lowered MCV and platelet count. Treatment with BOB showed normalization of alerted leukocyte, MCHC, MCV and platelet counts. Treatment with ASP showed significant increase in the leukocyte count in a dose dependent manner indicating its potential immune stimulatory effect. No statistical differences were also observed in RBC, Hb, Hct and MCH levels in NaF control. In addition, increased serum iron levels also observed in a dose dependent manner after treatment with BOB and ASP when compared to the NaF control.

Bone is the major affected tissue in the body due to exposure with fluoride. Bone toxicity mainly depends on the dose and duration of exposure with fluoride. In the present study, decreased serum levels of Calcium and Phosphorus and bone weight, breaking strength and increase in the serum levels of ALP and bone fluoride were observed in NaF control. Treatment with BOB and ASP showed significant improvement in the serum levels of Calcium and Phosphorus and bone weight and breaking strength and reduction in the serum
ALP and bone fluoride levels in a dose dependent manner indicating their protective effect against fluoride induced bone toxicity.

7.2 Conclusion

- Based on the literature survey, *Brassica oleracea* Var. *Botrytis* leaves and *Arthrospira platensis* were selected as nutritionally rich with potential medicinal values for screening the alleviatory effects against sodium fluoride induced toxic effects.
- All extracts were subjected to preliminary phytochemical testing and revealed the presence of alkaloids, glycosides, steroids, flavonoids, phenols and tannins as major phytoconstituents.
- As per the findings of *In vitro* antioxidant ability assays, *Brassica oleracea* Var. *Botrytis* leaves and *Arthrospira platensis* were found to possess good free radical scavenging potential.
- Hydroalcoholic extracts of *Brassica oleracea* Var. *Botrytis* leaves and *Arthrospira platensis* were found to be safe and no mortality was found after acute toxicity studies.
- In the thyroid profile, hydroalcoholic extracts of *Brassica oleracea* Var. *Botrytis* leaves at higher dose and *Arthrospira platensis* at all treatment doses significantly increased T<sub>3</sub> and T<sub>4</sub> levels and it might be due their relatively rich source of iodine.
- The Plant extracts were effectively controlled elevated liver biomarkers and lipid parameters to normal but both have shown moderate effect over cardiac profile.
- In kidney biomarkers profile, *Arthrospira platensis* increased BUN and Uric acid levels in a dose dependent manner and it might be due its relatively rich source of proteins and nucleic acids.
- Further detailed molecular studies are required to study the exact mechanism involved in the various alleviatory effects of BOB and ASP.
- Results strongly support for further application of *Brassica oleracea* Var. *Botrytis* leaves and *Arthrospira platensis* in the herbal formulations and drug development for treatment of fluoride toxicity.

7.3 Recommendations

The present study clearly demonstrates the beneficial effects of the two plants i.e. *Brassica oleracea* Var. *Botrytis* leaves and whole plant of *Arthrospira platensis* as antioxidant and thyro protective, cardio protective, hepato protective, nephro protective and
bone protective action against sodium fluoride induced intoxication. The observed pharmacological potential of these plants may be due to their nutritional and phytochemical contents. In detailed molecular studies may provide the exact mechanism of action for observed pharmacological actions. However, the present results are very supportive for further development and discovery of various formulations acting against fluoride induced toxicity.