SUMMARY AND CONCLUSION
CHAPTER V
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In our present study, an attempt has been made to investigate the effect of sodium fluoride in cerebral hemisphere (CH), cerebellum (CB) of the brain as well as blood of adult Wistar rats (*Rattus norvegicus*) *in vivo* and haemolysis on human erythrocytes *in vitro*. The ameliorative actions of melatonin and amla *in vivo* and *in vitro* on fluoride induced toxicity were also investigated and compared withdrawal of the treatment for *in vivo* study only.

PART I: *IN VIVO STUDIES*

Fluoride toxic effects on Brain and Blood

This study was carried out to evaluate the fluoride induced toxicity at two dosage levels (5mg and 10mg/kg b.w.) for 60 days in brain and blood of male Wistar rats and to assess potential amelioration of antioxidants like melatonin (10mg/kg b.w.) and amla extract (20mg/kg b.w.) and their possible beneficial effects.

Gravimetric, biochemical and histological studies of cerebral hemisphere (CH) and cerebellum (CB) of the brain of control, treated, and antioxidant supplemented groups were evaluated. The results revealed significant alterations in body weights of fluoride treated groups. Total brain and individual CH and CB weights showed significant reduction by fluoride intoxication. The decline in body and organ weights could be attributed to low intake of food due to F⁻ exposure.

Sodium fluoride treatment also resulted in a significant reduction in the total protein levels and an increase in total lipids, cholesterol and altered enzymatic activities in the treated groups. This could be due to an inhibition of protein synthesis...
and increased oxidation resulted due to oxidative stress. An increase in total lipids could be a result of altered TCA cycle energetics. Reduction in the enzyme activity like adenosine triphosphatase (ATPase) and succinate dehydrogenase (SDH) indicated an altered energy metabolism and mitochondrial dysfunction by F poisoning. These are further correlated by histopathological observations in brain regions of treated groups.

On the other hand, elevation in lipid peroxidation was noted in brain of the fluoride treated animals indicating an increased oxidative stress within the regions due to fluoride intoxication. As a result of increased lipid peroxides, concomitant decline in superoxide dismutase (SOD) and catalase activities was observed. The altered enzymatic and non-enzymatic antioxidant system supported oxidative damage in the brain tissue. This is further evidenced by the fall of AChE in blood and brain regions indicating neurotoxicity. Increase of fluoride levels in neural tissues and blood also supported our findings.

Altered hematological parameters like hemoglobin (Hb), red blood corpuscles (RBC), white blood corpuscles (WBC), mean corpuscular hemoglobin concentration (MCHC), platelets (PLT), Haematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), erythrocyte sedimentation rates (ESR) and transminases in blood were observed due to fluoride intoxication, in addition to neurotoxic symptoms. Sodium fluoride, thus seemed to exert oxidative stress, followed by changes in histomorphology, metabolic functions in these brain regions and blood of treated rats.

Supplementation of melatonin and amla to treated groups exhibited considerable mitigation with respect to blood and tissue biochemical indices, defencing system, histoarchitecture and haematopoietic activity. Thus, these
antioxidants effectively mitigated fluoride induced toxicity by exercising antioxidant action on free radicals to reduce oxidative stress in this study. The defensive effects of melatonin, its metabolites and amla components may be responsible for their free radical scavenging features and antioxidant enzyme inductions to overcome F-toxicity. However, withdrawal studies for 60 days had no recovery.

**PART II: IN VITRO STUDIES**

This study was designed to evaluate the protective efficacy of melatonin and amola against in vitro hemolysis induced by fluoride (F-) in human red blood cells. Venous blood samples of healthy individuals of 2.0ml of the RBC suspensions were treated with different doses (50 – 500μg NaF/ml) revealed a notable dose dependent increase in RBC hemolysis in terms development of reddish colour in saline as compared to the control tubes. Melatonin and amla alone had essentially no effect and showed readings comparable to the control values. On the other hand, co-incubation of melatonin and amla with NaF added to RBCs demonstrated a significant decline in F-induced hemolysis compared to the respective pro-oxidant group. Thus both antioxidants have exhibited inhibitory effect on haimolysis due to possession of their strong antioxidant properties.
CONCLUSION

NaF exerted neurotoxicity in Wistar rats disturbing their structure, metabolism and function by induction of oxidative stress as evidenced by decreased activities of antioxidant enzymes, viz., superoxide dismutase and catalase with a concomitant increase in the lipid peroxidation. Similarly, haemopoietic system also affected by fluoride intoxication.

Melatonin and amla brought about amelioration of fluoride induced blood cell and neurotoxicity by virtue of their antioxidant and detoxifying properties in vivo.

Addition of sodium fluoride to RBCs suspension caused hemolysis which was curtailed by supplementation of melatonin and or/amlra to treated erythrocytes.

Thus, this study revealed that fluoride induced toxicity in brain and blood in vivo and human blood in vitro. But, addition of melatonin and amla in vivo as well as in vitro expressed mitigative effects. These results thus would be of immense help for use as antioxidants to population living in endemic areas of Gujarat, India and world over.
SUMMARY AND CONCLUSION

FUTURE LINE OF WORK:

1. Effects of the fluoride on endocrine and reproductive systems need to be further evaluated.

2. Genetic effects of fluoride need to be investigated at molecular level to understand long term exposure risk effects.

3. Teratological effects are necessary too in animals

4. Role of certain other antioxidants needs to be determined which is more active in ameliorating fluoride exerted toxicity than the known antioxidants in these studies.

5. In depth, behavioral effects need to be carried out.

6. Role of melatonin and amla as potent antioxidant needs to be studied combination.

7. Epidemiological studies are to be carried out in populations exposed to fluoride to identify pregnancy outcomes in terms of stillbirths, spontaneous abortions, premature births, birth deformities and abnormal offsprings

8. Supplementation of antioxidants is to be tried in population in endemic areas to minimize fluorotic effects.