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Research Article

COMPARATIVE STUDY OF MUTATIONS IN DIFFERENT GENES OF ALBINO MICE DUE TO SODIUM FLUORIDE TOXICITY

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ABSTRACT

Fluoride in drinking water is easily absorbed by the intestines and is quickly distributed throughout the body. Fluoride easily crosses membranes and enters tissues, thus affecting every phase of metabolism. Bones and teeth especially are the sink for fluoride, which accumulates in them and causes fluorosis. Only limited work has been done, however, on the toxicity of fluoride on soft tissues, *viz* liver, kidney, muscles and testes. Fluorosis caused by excess intake of fluoride is a slow, progressive degenerative disorder, known to affect predominantly the skeletal systems, teeth and also the structure and function of skeletal muscle, brain and spinal cord. Recent studies have shown accumulation of fluoride in the hippocampus of the brain causing degeneration of neurons and decreased aerobic metabolism and altered free-radical metabolism in the liver, kidney, and heart. However, the effect of fluoride on neuromuscular tissue is far from clear. This gene encodes an integral outer mitochondrial membrane protein that blocks the apoptotic death of some cells such as lymphocytes. Constitutive expression of BCL2, such as in the case of translocation of BCL2 to Ig heavy chain locus, is thought to be the cause of follicular lymphoma. Two transcript variants, produced by alternate splicing, differ in their C-terminal ends. The aim of the present study, therefore, was to examine the effects of Fluoride on genes coding for BCl2 proteins, Caspase 3 protein and Tumor Protein 53 in Swiss albino mice

Keywords: *Sodium Fluoride, Caspase 3, BCl2, Tumor Protein 53, Albino Mice*

INTRODUCTION

Fluorosis caused by excess intake of fluoride is a slow, progressive degenerative disorder, known to affect predominantly the skeletal systems, teeth and also the structure and function of skeletal muscle, brain and spinal cord. Recent studies have shown accumulation of fluoride in the hippocampus of the brain causing degeneration of neurons and decreased aerobic metabolism and altered free-radical metabolism in the liver, kidney, and heart. However, the effect of fluoride on neuromuscular tissue is far from clear. In high concentrations, soluble fluoride salts are toxic and skin or eye contact with high concentrations of many fluoride salts is dangerous. Referring to a common salt of fluoride, NaF, the lethal dose for most adult humans is estimated at 1–10 grams A lethal dose is approximately 28 mg per kilogram of body mass. Fluoride in drinking water is easily absorbed by the intestines and is quickly distributed throughout the body. Fluoride easily crosses membranes and enters tissues, thus affecting every phase of metabolism. Bones and teeth especially are the sink for fluoride (Anuradha, 2001) which accumulates in them and causes fluorosis. Only limited work has been done, however, on the toxicity of fluoride on soft tissues, viz liver, kidney, muscles and testes. Clinical manifestations in Fluorosis reveal severe involvement of dental and skeletal tissues (Allan, 1987). However, It has been reported that Fluorosis is not merely a disease of bone and tooth, but it also affects the non-ossues tissues Collage (Huang, 1992) n, one of the structural constituents of both osseus and non-osseus tissues, appears to be severely affected due to Fluoride intoxication (Dias and Hattori, 2000). The constitution of nascent collagen protein in fluoride toxicity has been reported to be defective. The major defect has been localized in the absence of low molecular weight peptides which normally are known to fabricate the collagen fiber.

The aim of the present study, therefore, was to examine the effects of Fluoride on genes coding for cysteine-aspartic proteases (CASP3), tumor protein 53 and Bcl-2–associated X protein, or Bax.

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MATERIALS AND METHODS

Sample Preparation

Twelve healthy, adult female albino mice, Mus musculus of Swiss strain, each weighing about 30 ± 2 g, were obtained from the Animal house. Body weight and organo-somatic index: The body weight of each animal was noted before treatment and also on day 15. The weight of liver of respective groups of animals was recorded.

• *Primary Objective*- To check the mutation in different genes at different doses of sodium fluoride in swiss albino mice.

• *Secondary Objective*- Protocol validation for DNA extraction, PCR setup and Electrophoresis of PCR products with DNA Ladder. Sequencing of the PCR products.

RESULTS AND DISCUSSION

Using the standardized protocol the quantity of DNA extracted was high; contamination was almost negligible as viewed under UV transilluminator and the consistency was very good (dense fibers of DNA and milky white in colour). Quantification of DNA was done using UV spectrophotometer. 260/280 ratio for standardized protocol was 1.8.The cycle conditions were standardized before starting with amplification of test samples. The amplification was studied at different annealing temperatures and suitable annealing temperature was selected for final reaction setup. The amplification was observed for the entire test DNA samples.

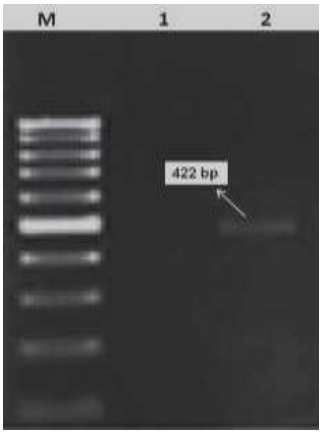
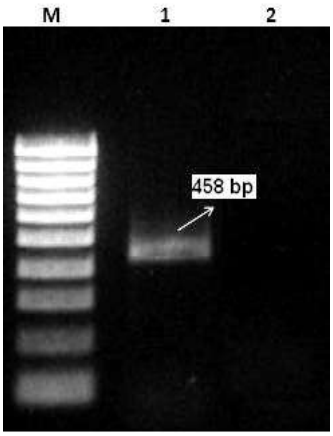


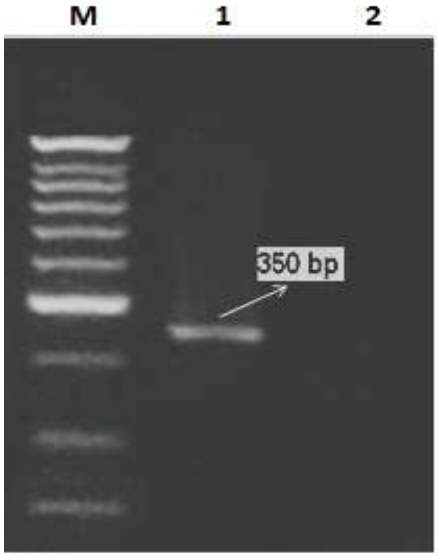
Figure 1: PCR product run on 2.0 % agarose gel and were stained with EtBr (10 mg/ml)
(Lane M: 100 bp DNA Ladder)



p53 or protein 53 or tumor protein 53p53 or protein 53 or tumor protein

Figure 2: PCR product run on 2.0 % agarose gel and were stained with EtBr (10 mg/ml)
(Lane M: 100 bp DNA Ladder : Lane 1: PCR product; Lane 2: PCR product)

Bcl-2–associated X protein, or Bax.



(Lane M: 100 bp DNA Ladder ; Lane 1: PCR product)

Figure 3: PCR product run on 2.0 % agarose gel and were stained with EtBr (10 mg/ml)

Discussion

The Albino mice have been subjected to fluoride intoxication for 14 days by administering the dose of aqueous NaF (20 mg/kg/body weight/day). At the end of the 14 day treatment, the animals were sacrificed by cervical dislocation, and the liver is dissected out, blotted free of blood, transferred to trays maintained at ice-cold conditions and used for isolation of DNA. Genomic DNA was isolated using standardized protocol and quantified on spectrophotometer to check its quality and then run on 8 % gel. Methods validated for the PCR Amplification and run for the four genes under study. The amplified PCR products were run on 2% Agarose gel with the 100 bp DNA ladder the check the amplified product size. The results obtained for the four genes under study did not vary for the normal and test mice, we get the same size of amplified products for both the cases. The PCR products obtained were further sequenced and matched but there was not any differences in their sequences.In conclusion, our findings revealed that we need to elaborate our study with more genes thats can be influenced and damaged by different forms of fluorine compounds. With our results and studies we assume may be substantially more evident effect was caused by other fluoride compounds compared to simple fluoride ion released by sodium fluoride.

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