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Research Article

SODIUM FLUORIDE INDUCED TOXIC EFFECT ON BCL2 PROTEIN FORMING GENE IN SWISS ALBINO MICE

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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 in Swiss albino mice.

Key Words: *Fluoride, Toxicity, BCl2 protein, Gene, Albino Mice*

INTRODUCTION
Bcl-2 is the prototype for a family of mammalian genes and the proteins they produce. They govern mitochondrial outer membrane permeabilization (MOMP) and can be either pro-apoptotic (Bax, BAD, Bak and Bok among others) or anti-apoptotic (including Bcl-2 proper, Bcl-xL, and Bcl-w, among an assortment of others). There are a total of 25 genes in the Bcl-2 family known to date. Bcl-2 derives its name from *B-cell lymphoma 2*, as it is the second member of a range of proteins initially described as a reciprocal gene translocation in chromosomes 14 and 18 in follicular lymphomas.

Review of Literature
Fluoride is an essential trace element in humanbodies and is highly correlated with the metabolism of bone and tooth. But excessive exposure to fluoride for a long term leads to bone damage with complicated pathological changes such as osteoporosis and osteopetrosis. Arsenide is an environmental toxicant and a known carcinogen (National Research Council, 1999). Inorganic arsenide is highly accumulated *in vivo*. Arsenate deposits in skeleton because it takes the place of phosphate in the apatite crystal of bone. Fluoride and arsenide in groundwater, air, and food in some areas of China as a result of specific geographical and geological environment and living habits of local people have tremendous impact on health of local residents. Great attention has been paid to the possible combined effect of fluoride and arsenide (Huang *et al.*, 1992). There are different reports about the role of arsenide in the bone damage caused by fluoride (Li *et al.*, 1996). Both osteoblasts and osteoclasts are involved in bone damage. Studies indicate that the proliferation, differentiation, and maturity of osteoclasts are dependent on the existence of osteoblasts (Takahashi *et al.*, 1998). Osteoprotegerin (OPG) and osteoclast differentiation factor (ODF) are secreted by osteoblasts. The former is responsible for inhibiting osteoclastogenesis, while the latter plays an important role in stimulating the differentiation and maturity of osteoclasts, activating mature osteoclasts and inhibiting their apoptosis. So OPG and ODF may combine the functions

of osteoblasts and osteoclasts, which is of great help to the study of the influence of toxicants on bone remodeling.

Fluoride in drinking water is easily absorbed by the intestines (Carlson *et al.,* 1960) and is quickly distributed throughout the body. Fluoride easily crosses membranes and enters tissues, thus affecting every phase of metabolism (Zahvoronkov *et al.,* 1981) Bones and teeth especially are the sink for fluoride, which accumulates in them and causes fluorosis (Sing *et al.,* 1962) Only limited work has been done, however, on the toxicity of fluoride on soft tissues, *viz* liver, kidney, muscles and testes. Although artificial fluoridation of water supplies is practiced in many parts of the world in an effort to reduce the incidence of dental caries, there is growing evidence that the resulting increased exposure to fluoride (F) may cause serious toxic effects (Spittle *et al.,* 2009). Several clinical investigations and animal experiments suggest that F has adverse impacts on male reproductive function (Chinoy *et al.,* 1997; Luke *et al.,* 1997; Pushplata *et al.,* 2005) including structural and functional defects in spermatozoa (Chinoy 1992; Kumar *et al.,* 1994; Chinoy *et al.,* 1998) a decrease in sperm count (Chinoy *et al.,* 1995; Ghosh *et al.,* 2002; Narayana *et al.,* 1994), disturbances in the levels of reproductive hormones, alterations in the epididymis and accessory reproductive glands, and reduced fertility. Spermatogonia undergo various processes to ultimately fertilize an oocyte, including spermatogenesis, capacitation, and the acrosome reaction. F has been shown to impair all three of these processes. Like most soluble materials, fluoride compounds are readily absorbed by the stomach, intestines and excreted through the urine. Trace amounts are incorporated in bone. Urine tests have been used to ascertain rates of excretion in order to set upper limits in exposure to fluoride compounds and associated detrimental health effects. Ingested fluoride initially acts locally on the intestinal mucosa, where it forms hydrofluoric acid in the stomach (Chinoy and Sharma, 1998).Thereafter it binds calcium and interferes with various enzymes. A weakening of bones, leading to an increase in hip and wrist fracture. At the level used in fluoridated water, decreased fractures are expected, but the U.S. National Research Council found the overall evidence suggestive but inadequate for drawing firm conclusions about the risk or safety of exposures at [2 mg/L], but states that fractures do seem to increase as fluoride is increased from 1 mg/L to 4 mg/L, suggesting a continuous exposure-effect dose-response relationship at these levels adverse effects on the kidney. Within the recommended dose, no effects are expected, but chronic ingestion in excess of 12 mg/day are expected to cause adverse effects, and an intake that high is possible when fluoride levels are around 4 mg/L. Those with impaired kidney function are more susceptible to adverse effects. Little research has been done on possible liver damage, although some studies suggest negative effects at chronic ingestion of 23 mg/day. Chromosomal damage and interference with DNA repair. Overall, the literature from in vitro and rodent studies does not indicate genotoxicity, but the in vivo human studies are inconsistent.

Four epidemiological studies have noted a correlation between increased fluoride and low IQ. The most rigorous of these compared an area with mean water concentration of 0.36 ± 0.15 mg/L (range 0.18-0.76 mg/L) to an area with 2.47 ± 0.79 mg/L (range 0.57-4.50 milligrams per liter [mg/L]). Most of these studies did not publish important details, making them difficult to evaluate. If these correlations are caused by fluoride, the mechanism is not known, but the National Research Council speculates that effects on the thyroid could lead to poor test results. Two Chinese meta-analyses which included the previously mentioned studies have also noted this correlation. The high-fluoride areas studied had fluoride levels above those used in water fluoridation. The NRC report stated that many of the untoward effects of fluoride are due to the formation of AlFx [aluminum fluoride] complexes. This topic has been identified previously as cause for concern. The NRC noted that rats administered fluoride had twice as much aluminum in their brains. When water (1 ppm fluoride) is boiled in aluminum cookware more aluminum is leached and more aluminum fluoride complexes are formed. However, an epidemiological study found that a high-fluoride area had one-fifth the Alzheimer's that a low-fluoride area had, and a 2002 study found that fluoride increased the urinary excretion of aluminum. Fluoride's suppressive effect on the thyroid is more severe when iodine is deficient, and fluoride is associated with lower levels of iodine. Thyroid effects in humans were associated with fluoride levels 0.05-0.13 mg/kg/day when iodine

intake was adequate and 0.01-0.03 mg/kg/day when iodine intake was inadequate. Its mechanisms and effects on the endocrine system remain unclear. Bcl-2 is the prototype for a family of mammalian genes and the proteins they produce. They govern mitochondrial outer membrane permeabilization (MOMP) and can be either pro-apoptotic (Bax, BAD, Bak and Bok among others) or anti-apoptotic (including Bcl-2 proper, Bcl-xL, and Bcl-w, among an assortment of others). There are a total of 25 genes in the Bcl-2 family known to date. Bcl-2 derives its name from *B-cell lymphoma 2*, as it is the second member of a range of proteins initially described as a reciprocal gene translocation in chromosomes 14 and 18 in follicular lymphomas. There are a number of theories concerning how the Bcl-2 gene family exert their pro- or anti-apoptotic effect. An important one states that this is achieved by activation or inactivation of an inner mitochondrial permeability transition pore, which is involved in the regulation of matrix Ca^{2+}, pH, and voltage. It is also thought that some Bcl-2 family proteins can induce (pro-apoptotic members) or inhibit (anti-apoptotic members) the release of cytochrome c into the cytosol which, once there, activates caspase-9 and caspase-3, leading to apoptosis. Although Zamzami *et al.*, suggest that the release of cytochrome c is indirectly mediated by the PT pore on the inner mitochondrial membrane, strong evidence suggest an earlier implication of the MAC pore on the outer membrane. Another theory suggests that Rho proteins play a role in Bcl-2, Mcl-1 and Bid activation. Rho inhibition reduces the expression of anti-apoptotic Bcl-2 and Mcl-1 proteins and increases protein levels of pro-apoptotic Bid but had no effect on Bax or FLIP levels. Rho inhibition induces caspase-9 and caspase-3-dependent apoptosis of cultured human endothelial cells. The members of the Bcl-2 family share one or more of the four characteristic domains of homology entitled the Bcl-2 homology (BH) domains (named BH1, BH2, BH3 and BH4) (see the figure on the left). The BH domains are known to be crucial for function, as deletion of these domains via molecular cloning affects survival/apoptosis rates. The anti-apoptotic Bcl-2 proteins, such as Bcl-2 and Bcl-xL, conserve all four BH domains. The BH domains also serve to subdivide the pro-apoptotic Bcl-2 proteins into those with several BH domains (e.g. Bax and Bak) or those proteins that have only the BH3 domain (e.g. Bid, Bim and Bad). The Bcl-2 family has a general structure that consists of a hydrophobic helix surrounded by amphipathic helices. Many members of the family have transmembrane domains. The site of action for the Bcl-2 family is mostly on the outer mitochondrial membrane. Within the mitochondria are apoptogenic factors (cytochrome c, Smac/DIABLO, Omi) that if released activate the executioners of apoptosis, the caspases. Depending on their function, once activated, Bcl-2 proteins either promote the release of these factors, or keep them sequestered in the mitochondria. Whereas the activated pro-apoptotic Bak and/or Bax would form MAC and mediate the release of cytochrome c, the anti-apoptotic Bcl-2 would block it, possibly through inhibition of Bax and/or Bak.

The Bcl-2 gene has been implicated in a number of cancers, including melanoma, breast, prostate, and lung carcinomas, as well as schizophrenia and autoimmunity. It is also thought to be involved in resistance to conventional cancer treatment. This supports a role for decreased apoptosis in the pathogenesis of cancer.Cancer is one of the world's leading causes of death and occurs when the homeostatic balance between cell growth and death is disturbed. Research in cancer biology has discovered that a variety of aberrations in gene expression of anti-apoptotic, pro-apoptotic and BH3-only proteins can contribute to the many forms of the disease. An interesting example can be seen in lymphomas. The over-expression of the anti-apoptotic Bcl-2 protein in lymphocytes alone did not act in an oncogenic manner. But simultaneous overexpression of Bcl-2 and the protooncogene myc may produce aggressive B-cell malignancies including lymphoma. In follicular lymphoma, a chromosomal translocation commonly occurs between the fourteenth and the eighteenth chromosomest (National Research Council, 1999; Spittle, 2009) which places the Bcl-2 gene next to the immunoglobulin heavy chain locus. This fusion gene is deregulated, leading to the transcription of excessively high levels of bcl-2. This decreases the propensity of these cells for undergoing apoptosis.

Apoptosis also plays a very active role in regulating the immune system. When it is functional, it can cause immune unresponsiveness to self-antigens via both central and peripheral tolerance. In the case of defective apoptosis, it may contribute to etiological aspects of autoimmune diseases (Faccini *et al.*, 1969)

the autoimmune disease, type 1 diabetes can be caused by defective apoptosis, which leads to aberrant T cell AICD and defective peripheral tolerance. Due to the fact that dendritic cells (DCs) are of the most important antigen presenting cells of the immune system, their activity must be tightly regulated by such mechanisms as apoptosis. Researchers have found that mice containing DCs that are Bim -/-, thus unable to induce effective apoptosis, obtain autoimmune diseases more so than those that have normal DCs other (Faccini *et al.,* 1969) studies have shown that the lifespan of DCs may be controlled by factors such as a timer dependent on anti-apoptotic Bcl-2 (Faccini *et al.,* 1969).These investigations illuminate the importance of regulating antigen presentation as mis-regulation can lead to autoimmunity.Apoptosis plays a very important role in regulating a variety of diseases that have enormous social impacts. For example, schizophrenia is a neurodegenerative disease that may result from an abnormal ratio of pro- and anti-apoptotic factors. There is some evidence that this defective apoptosis may result from abnormal expression of Bcl-2 and increased expression of caspase-3. Further research into the family of Bcl-2 proteins will provide a more complete picture on how these proteins interact with each other to promote and inhibit apoptosis. An understanding of the mechanisms involved will help discover potential treatments such as inhibitors to target over-expressed proteins that may lead to new therapies in cancer, autoimmune conditions, and neurological diseases.

BH3-only family of proteins includes those of the Bcl-2 family proteins, which contain only a single BH-domain. The BH3-only family members play a key role in promoting apoptosis. The BH3-only family members are BAD, Bim and others. Various apoptotoc stimuli induce expression and/or activation of specific BH3-only family members, which translocate to the mitochondria and initiate Bax/Bak-dependent apoptosis.

Objectives

Female Swiss albino mice were has been used in the present study. They will be offered fluoride rich diet and drinking water for five days in two different concentrations of 10 mg/kg body weight to monitor their genetic effects. The objectives are as follows:

- **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.

1. Methodology

1.1. 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.

1.2. DNA Extraction

- After dissection tissue samples were stored at -20c freezer.
- Before DNA extraction samples from freezer were kept on ice.

Procedure:

1. Chopped small amount of tissue (as finely as you can) with a sterile scalpel blade.
2. Taken Approximately 200 µl chopped sample tissue in a 2 ml eppendorf tube.
3. Added 600 µl of TNES buffer and 35 µl of Proteinase-K (20 mg/ml) and Mixed the sample by inverting the tubes several times.
4. Tubes were incubated at 55°c overnight.
5. Remove tubes from oven and carefully add 800 µl PCI (phenol: chloroform: IAA).
6. Carefully removed aqueous layer for each sample into new, clean microfuge tube.
7. Add 45ul of 3M NaAC pH 5.3 to each tube. Closed tubes and Mixed properly and then added approx. 100 µl Ethanol (100%).
8. Invert to mix and precipitate DNA and kept in -20c freezer for 30 minutes.

9. Spin at 12,000rpm for 10 min at 4ºC.
10. Removed supernatent to a new, labeled 2 ml microcentrifuge tube.
11. Added an equal volume of cold 100 % ethanol and gently mix by inverting the tubes.
12. Centrifuged the samples at 12,000 rpm for 15 minutes at 4ºC.
13. Remove the supernatent and wash the DNA pellet in 500 µl of 100 % ethanol (add ethanol, close cap of tube and invert gently).
14. Pour (or pipette) off the ethanol and briefly spin the samples to keep the pellet at the bottom of the tube.
15. Washed DNA pellet with 70 % ethanol as above. After removing the 70 % ethanol, briefly
16. Centrifuge the samples to get the last of the ethanol to the bottom of the tube; pipette off the remaining ethanol.
17. The sample was to air dried for 1-3 hr depending upon the temperature.
18. Re-suspended the dried DNA sample into 100-200 µl of sterile distilled water or Tris-EDTA Buffer.

2. APPENDIX

2.1. TNES Buffer (Tris, NaCl, EDTA, SDS)

10 mM Tris, pH 7.5
400 mM NaCl
100 mM EDTA
0.6 % SDS
6 M NaCl is saturated salt solution stored at 37ºC: weigh out 6 M NaCl and heat the solution until it dissolves. Leave to cool at room temperature. Some crystals will form, this is normal.

2.2. TE Buffer (Tris-EDTA Buffer)

10 mM Tris-Cl, pH 7.5
1 mM EDTA
Make from 1M stock of Tris-Cl (pH 7.5) and 500 mM stock of EDTA (pH 8.0).

2.3. PCI mix (phenol: chloroform: Isoamyl Alcohol)

One part tris-saturated phenol to one part 24:1 Chloroform: Isoamyl alcohol, Shake thoroughly to make emulsion

2.4. Quantification and Quality check of DNA

After isolation of DNA, its quantification and analysis of quality are necessary to ascertain the approximate quantity of DNA obtained and the suitability of DNA sample for further analysis. This is important for many applications including digestion of DNA by restriction enzymes or PCR amplification of target DNA.

2.4.1. Quantification of DNA sample

1. The UV spectrophotometer was switched on and the deuterium lamp was allowed to warm-up.
2. A blank was set with TE buffer (or distilled water) in quartz cuvettes.
3. A 1:50 dilution was prepared with isolated genomic DNA sample in distilled water and then transferred in quartz cuvettes.
4. The reading at 260 nm gives the concentration of nucleic acid in the sample while reading at 280 nm specifies the concentration of aromatic amines.

2.4.2. Formula

OD 260nm/ OD 280nm = Ratio to determine the concentration of DNA in the sample.

DNA concentration in mg/ml = OD260 x 50 mg DNA/ml x Dilution Factor/1000

The 260/280 ratio should range from 1.6-1.9 for preparations of DNA that are to be used for PCR amplification. If there is contamination with protein or phenol, the ratio will be significantly less than 1.8 and if ratio is greater than 2.0, there can be possible contamination of RNA. Accurate quantitation of amount of nucleic acid will not be possible in such a sample and it cannot be used for further processes.

2.4.3. Agarose Gel Electrophoresis

Principle: Gel electrophoresis is a technique in which using an electric current applied to a gel matrix deoxyribonucleic acid, ribonucleic acid, or protein molecules are separated. Agarose gels are used for the electrophoresis of both proteins and nucleic acids. Agarose gel electrophoresis was used twice; once after PCR reaction to confirm the amplicon size.

Materials

Following is the list of materials typically required to carry out agarose gel electrophoresis of DNA:

1. Agarose

1) 0.8% for running genomic DNA samples
2) 2.0 % for PCR products.

2. Buffers and solutions

1) Electrophoresis buffer: - Tris Borate-EDTA (TBE) or Tris Acetate-EDTA (TAE) 1X, Tris phosphate-EDTA (TPE) pH 8.0
2) Gel loading buffer: - it contains Bromophenol Blue, a low molecular weight dye, to enable tracking the progress of the electrophoresis and glycerol, to make the DNA solution denser so it will sink into the wells of the gel.
3) Ethidium bromide: - it is an ultraviolet fluorescent dye.

3. Nucleic acid and oligonucleotides

1. Quantified DNA samples

4. Special Equipment

1. Equipment for agarose gel electrophoresis: The equipment should be clean and dry horizontal electrophoresis apparatus with chamber and comb, or clean dry glass plates with appropriate comb.
2. Disposable gloves.
3. Heating mantle.
4. Power supply device capable of up to 500 V and 200 mA.
5. UV lamp or UV light box or Gel Dock to visualize DNA in the gel

5. Preparation of Reagents

1) **50 X Tris acetate EDTA (TAE) Buffer** (2mM Tris acetate, 0.5 M EDTA (pH 8.0)

Table 1: Chemicals and their amount in preparation of 50X TAE buffer

Chemical	Amount
Tris base	24.2 grams
Glacial acetic acid	5.7 ml
0.5 M EDTA	10 ml

Add minimum amount of Distilled Water approximately 20 ml. allow it to dissolve and then adjust pH 8.0 by adding conc. HCl and make-up the volume upto 100 ml with distilled water.

2) Gel loading buffer

Table 2: Chemicals and their amount in preparation of gel loading buffer

Chemical	Amount
Glycerol	3 ml
Bromophenol blue	25 mg
D/W to make up the volume to 10 ml	

6. Protocol

6.1. Casting the Gel

- Weigh out the appropriate amount of agarose in weighing machine. For 0.8%/2.0% gel weigh 0.8/2.0 g and dissolve it in 100 ml of 1X TAE buffer by heating it in the heating mantle until the solution turns transparent.
- Allow the solution to cool a little and add approximately 1 – 2 drops of Ethidium bromide.
- Mix by swirling gently and pour the solution in the gel – casting tray such that no air bubbles are trapped. Place the comb such that the teeth dip into the gel solution and do not touch the casting tray.
- Allow the gel to solidify. Pour a little 1X TAE buffer over the gel and lift the comb out gently. The 1X TAE buffer will ensure that the wells do not dry up.
- Take the gel tray out of the holder and place it in the electrophoresis chamber.
- Pour 1X TAE (around 300-400 ml) buffer into the unit such that the gel is completely submerged under the buffer.

6.2. Preparation of Sample and Loading

- Aliquot the 3µl of GLB on a strip of parafilm.
- Add the 6µl of sample to the GLB. Mix well by titrating.
- Aspirate the entire contents of the mixture.
- Gently lower the tip into the well and release all the contents.

7. Running the Gel

- Connect the electrodes to the power pack.
- DNA is negatively charged so the gel should be placed in the electrophoresis unit such that the DNA is at the negative terminal (cathode) and would run towards the positive terminal (anode).
- The gel is run at constant voltage - 5V/cm distance between the electrodes. Therefore if the distance between the electrodes is 20cms, then the voltage is set to about 90-95V.
- The gel should be run till the gel loading buffer reaches the other end of the gel or the gel can be stopped when the gel front reaches mid-way to check if the samples loaded are present.

8. Viewing of the Gel

- The gel is viewed under a source of UV light (Trans-illuminator).

PCR Setup and Run

Materials

(A) DNA samples

- DNA extracted from test samples using Standardized protocol.

(B) Primers:

The details of Primers obtained from axygen:

Table 3: Primer sequences with product sizes

Sr. No.	Genes	Primers Sequence	Amplicon size
1.	BCL2	Forward primer: 5′-TAC CGT CGT GAC TTC GCA GAG -3′ Reverse primer: 5′-GGC AGG CTG AGC AGG GTC TT -3′	350 base pair (bp)

Reaction Set-up

Before starting with the reaction set-up, the sample DNA was diluted with HPLC water to make it 10ng/µl. Primers, both forward and reverse were diluted in the ratio 1:10.

Table 4: Reaction set-up in which DNA & primers was diluted 1:10 respectively using HPLC water as diluent

No.	Materials	Amount	Dilution
1.	AmpliTaq Gold PCR Master Mix	10µl	-
2.	HPLC water	3.0µl	-
3.	DNA (10 ng/ µl)	6.0µl	-
4.	Forward primer	0.5µl	1:10
5.	Reverse primer	0.5µl	1:10

Temperature Cycling

1. B-cell CLL/lymphoma 2 (BCL2)

Table 5: PCR cycling conditions for BCL2

Steps		Temperature	Time	No of Cycles
Step I	Initial Denaturation	94°C	5 min	1 cycle
	Denaturation			
		94°C	60 Sec	30 cycles
	Annealing			
Step II		59°C	30 Sec	
	Extension			
		72°C	30 Sec	
	Final Extension			1 cycle
Step III		72°C	5 min	
Hold at 4°C				

RESULTS

Results for DNA Extraction Protocol

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.

Results of PCR

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.

1. Bcl-2–associated X protein, or Bax.

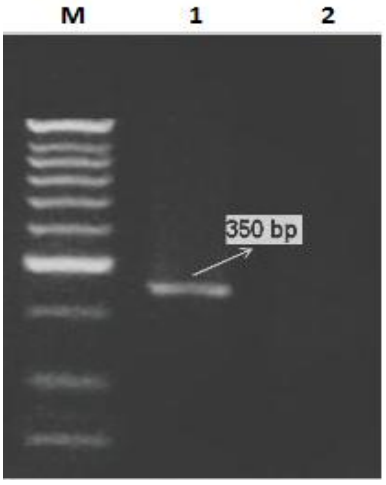

Figure 3: PCR product run on 2.0 % agarose gel and were stained with EtBr (10 mg/ml)
Lane M: 100 bp DNA Ladder
Lane 1: PCR product.

Sequencing

Most templates could be sequenced from a single round of amplification. Occasionally, templates provided too little product from a single amplification. In such cases, the first amplification product was gel-isolated and used as template for a re-amplification with a higher annealing temperature (50°C, all other parameters being held the same). In all instances, the PCR product for sequencing was obtainedby running the entire reaction volume on a 2% low-melting agarose gel, using wide-tooth combs. The reaction product was excised from the gel and subsequently purified. We used AmpliTaq Cycle-sequencing Kit, protocol according to the manufacturer of the double-stranded PCR products. Two electrophoretic analyses were required to sequence the complete fragment in each direction.

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 BCl2 gene 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 were not any differences in their sequences.

In conclusion, our findings revealed that we need to elaborate our study with more genes that's 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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