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Fluoride induced oxidative stress, immune system and apoptosis in animals: a review

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Abstract: Halfway through the twentieth century, fluoride piqued the interest of toxicologists due to its deleterious effects due to high concentrations in animals as well as in human populations suffering from several types of disorders and in in-vivo experimental models. Until the 1990s, the toxicity of fluoride was largely ignored due to its "good reputation" for preventing caries via topical application and in dental toothpaste. However, in the last decade, interest in its undesirable effects has resurfaced due to the awareness that this element interacts with cellular systems even at low doses. In recent years, several investigations demonstrated that fluoride can induce oxidative stress and modulate intracellular redox homoeostasis; lipid peroxidation and protein carbonyl content, as well as alter gene expression and cause apoptosis. Genes modulated by fluoride include those related to the stress response, metabolic enzymes, the cell cycle, cell–cell communications and signal transduction. The primary purpose of this review is to examine recent findings on the effects of fluoride on oxidative stress, immune system and apoptosis in the animal as well as in human system.

Key words: Fluoride; Toxicity; Oxidative stress; Antioxidant enzymes; Immunity; Apoptosis

Introduction

Fluorine is a member of the halogen family, ubiquitously found in the environment as it is the 13th most abundant element in the earth crust (1, 2) (Fig. 1). Fluoride in drinking water is totally in an ionic form and hence, it rapidly, totally and passively pass through the intestinal mucosa and interferes with major metabolic pathways of the living system. About 96% of the fluoride in the body is found in bones and teeth (3). Fluorine is the most reactive element and it could not found in the free form. It behaves as a double-edged sword. Fluoride in small doses has remarkable prophylactic influence on the dental system by inhibiting the dental caries while in higher doses it causes dental and skeletal fluorosis in both human and animal (4-6). There are two patterns of fluoride toxicity in the world, endemic fluorosis and industrial fluorosis. Endemic fluorosis is related to the high concentration of fluoride present in the drinking water (7), while industrial fluorosis is mainly due to air pollution of fluorine. Fluoride toxicity caused several problems in the animal body. Due to its high concentration, it can be harmful to teeth, bones, heart, liver, kidneys, gastrointestinal tract, lungs, brain, blood, hormones, the immune system, various biochemical parameters, and reproductive system in both experimental animals and humans (8-18). All these hazards caused by fluorine are due to abnormality in the antioxidant defence system in the body, high level of oxidative stress, attenuated immune system, abnormality in cell signaling pathway, and elevation of apoptosis in the cell (19-22). Taking into account the studies cited above, the objective of the present review is to analyze and to update toxicity information due to F induced oxidative stress, immunological disorder, mortality of the cell due to apoptosis in animals; meanwhile human system also.

Fluoride as an inductor of Oxidative Stress

Oxidative stress is a state in which excess formation of highly reactive molecules such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) saturate the natural antioxidant defence mechanisms (23-25). Most widely studied examples of ROS are superoxide ($\cdot O_2^-$), hydroxyl ion ($\cdot OH$) and hydrogen peroxide (H_2O_2) (26). Common RNS include nitric oxide ($\cdot NO$) and per-oxynitrite ($ONOO^-$) (26). Oxidative stress induced by oxidant species occurs under conditions when antioxidant defence is depleted or when the rate constants of the radical reactions are greater than the antioxidant defence mechanisms (Table 1, Fig. 2) (27).

Fluoride exposure increased the generation of anion superoxide (O_2^-) (28); increased

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O_2^- concentration and its downstream consequences such as hydrogen peroxide, peroxynitrite, hydroxyl radicals seem particularly important in mediating fluoride's effects. Moreover, fluoride increased NO generation (29) and it can react with superoxide to form peroxynitrite, and with thiols and metal centres in proteins to form nitrosyl adducts. It has also been shown interference with the disulfide-bond formation and resulting in the accumulation of mis-folded proteins in the endoplasmic reticulum (ER) causing ER stress and ROS production.

Oxidative stress is a recognized mode of action of fluoride exposure that has been observed in vitro in several types of cells and also in vivo in soft tissues such as the liver, kidney, brain, lung, and testes in animals and in people living in areas of endemic fluorosis (28).

Reactive oxygen species (ROS) are implicated as important pathologic mediators in many disorders. Increased generation of ROS and enhanced lipid peroxidation are considered responsible for the toxicity of a wide range of compounds (30). Various authors have investigated relationships between fluoride and free radical reactions (31, 32). A close association between chronic fluoride toxicity and increased oxidative stress has been reported in humans (7) and experimental animals (33). Fluoride has been demonstrated in vivo and in vitro to cause increased lipid peroxidation in erythrocytes of humans and in blood and tissues of experimental animals (33). A marked increase in the concentrations of Thiobarbituric acid reactive substances (TBARS) and hydroperoxides are observed in liver and kidney of experimental rats. Increased lipid peroxidation in the liver can be due to the increasing oxidative stress in the cell as a result of depletion of antioxidant scavenger system. During the period of oxidative damage, the antioxidants of comprehensive antioxidant systems in organism play important roles (34).

In general, fluoride acts as an enzyme inhibitor due to its interactions with enzymes, involving its binding with the active sites on the enzymes (35). Fluoride is thought to inhibit the activity of antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase, and catalase (28, 36, 37). Moreover, fluoride can alter glutathione levels (38), often resulting in the excessive production of ROS at the mitochondrial level, lead to the damage of cellular components. Reduced glutathione (GSH) is known to protect the cellular system against the toxic effects of lipid peroxidation (39). GSH functions as a direct free radical scavenger as a co-substrate for glutathione peroxidase (GPx) activity and as a co-factor for many enzymes and forms conjugates in endo and xenobiotic reactions (40).

Enhanced lipid peroxidation and decreased activities of antioxidant enzymes have been recorded in soft tissues of fluoride-treated mice. The observed decrease in GSH level in fluoride-treated rats represents increased utilisation due to oxidative stress. GPx metabolises hydrogen peroxide to water by using GSH as a hydrogen donor (41). The depletion in the activity of GPx may result in the involvement of deleterious oxidative changes due to the accumulation of toxic products. Elevated concentration of ROS results in impaired cellular antioxidant defence system. GSH participate in the reaction that destroys hydrogen peroxide, or organic peroxide, free radicals and certain foreign compounds. Fluoride exposure restricts the antioxidant activity of GSH and inhibits various enzymes, which require GSH as a cofactor (42). Chinoy and Patel (2000) also reported decreased GSH level on fluoride exposure. The ratio between GSH/GSSG is a marker of oxidative stress (43). Chlubek *et al.,* (1999) reported that at fluoride at relatively low concentration creates a condition of oxidative stress while at higher concentration it acts as an inhibitor of free radical production (44).

Decreased SOD levels have been reported in the tissues of mice exposed to high fluoride intake. Reduced activities of super oxide dismutase (SOD) and catalase (CAT) in liver and kidney of experimental rats have been observed. SOD is an important defence enzyme, which converts superoxide radicals to hydrogen peroxide (45).

CAT is a heme protein, which decomposes hydrogen peroxide and protects the tissues from highly reactive hydroxyl radicals (46). The reduction in the activity of these enzymes may be due to oxidative stress exerted by fluoride intoxication. SOD is one of the key antioxidant enzymes in the body, which associating with GSH can convert the superoxide radical to less toxic hydrogen peroxide and water and protect the body from the toxicity of superoxide radical, maintain appropriate intracellular concentration of superoxide radical, and protect cells from the damage of active oxygen on the DNA and other biological macromolecules. Several studies showed that fluoride could inhibit the activities of antioxidants, such as SOD and GSH (28, 35). The decreased activity of SOD in the liver tissue may lead to the accumulation of reactive oxygen species. On the other hand, fluoride exposure could generate anion superoxide O_2^- (28).

All the above mentioned underlying mechanisms could explain the excessive production of ROS in the cells, which could initiate the peroxidation of membrane lipids and subsequently produced malondialdehyde (MDA), as evidenced in our study that a dose-dependent increase of MDA in the liver tissues after 90 days of exposure. Earlier

studies also indicated that exposure to fluoride could increase the content of lipid peroxidation (47, 48). Elevated MDA production has been considered as a reliable biomarker for oxidative stress in animals exposed to environmental contaminants (49).

Although a relationship in humans and animal fluorosis particularly between free radical generation, lipid peroxidation and antioxidant defence system has been investigated extensively, however, the results are mostly contradictory. Soni *et al.,* (1984) reported that sodium fluoride at a low concentration of 5 and 20 mg/kg body mass of rats caused an increase in lipid peroxidation in tissues while, the higher dose caused an inhibition in the liver, lungs and testes (50). Xiong *et al.,* (2007) also studied the dose-effect relationship between fluoride concentration in drinking water and damage to soft tissue. They reported that 2.0 mg/l fluoride can cause damage to the liver but not the kidney (51). In a report by Chlubek *et al.,* (1999) incubation of sodium fluoride increased concentration (2, 5, 50 and 500 µM) with the mitochondrial fraction from human placenta induced MDA formation, but higher concentration of sodium fluoride was less potent in raising levels of MDA (52). Even though fluoride toxicity is increasingly being considered to be important, very little information is available on the mechanism of action of fluoride.

In one study, it has been evaluated that the antioxidant defense system (both enzymatic and non-enzymatic) and lipid peroxidation both in human beings caused an endemic fluorosis area (5 ppm fluoride in drinking water). In rabbits, given water containing 150ppm of fluoride for 6 months, and found that there is no significant difference in lipid peroxidation, glutathione and vitamin C in blood of fluorosis patients and fluoride-intoxicated rabbits as compared to the controls. It has been also found that, there are not any changes in the activities of catalase, superoxide dismutase, glutathione peroxidase, or glutathione S-transferase in the blood due to fluoride intoxication (53). Some of the experiment which have been done earlier on fluoride exposure on the animal model, presented in table 2 and figure 2.

Immune system and Fluoride

Fluoride (F) and immunity has a well-known inverse relationship in animal body (54). F can be act as an adjuvant for mucosal and systemic immunity and is reported to affect oral immunity in chickens also (55). Studies with sodium fluoride (NaF) dosing has indicated that F can damage human lymphocyte chromosomes (56), induce adverse effects in the spleen (57), inhibit growth and general health in rabbits and increase their nonspecific immune-related acid phosphatase and lysozyme activities (58).

Dosing of high dietary F (800 to 1200 mg/kg) in chickens has the severe effects on immune system (59, 60). It is generally accepted that T-lymphocytes take part in cellular immunity in animals (61). Similar to those of mammalian species, T-cell populations in chickens can be represented into subsets based on their expression of cell-surface proteins like CD^{3+}, CD^{4+}, CD^{8+} etc. (62). CD^{3+} indicates the mature T-cell population in the animal body. T-cell subsets are further classified by their expression of molecules into CD^{4+} and CD^{8+} proteins. One study of Chen *et al.,* (2009a), in chicken, the CD^{4+} T-cells are considered as helper/inflammatory T-cells that respond to exogenous antigens in conjunction with the class major histocompatibility complex-II (MHC II) molecules. On the other hand, CD^{8+} T-cells are considered cytotoxic T-cells, and respond to endogenous antigens in conjunction with the class major histocompatibility complex-I (MHC I) antigens (59). High dietary F can induce the oxidative damage and subsequently decrease the T-cell subsets percentage in the chicken caecal tonsil (63, 64).

One of the most immune-regulatory proteins is cytokines which is the host mediators to respond against the stress, infection, and antigen invasion (65). However, Chabalgoity *et al.,* (2007) stated that, cytokine has the protective effects on immunity and act as a barrier to avoid immune-pathological changes (66). Lower level of T-cell subsets, serum interleukin-2 (IL-2) contents, and higher level of apoptotic splenocytes has been found after the high dietary F intake in the animal (59, 60).

On the other hand, Interleukin 8 (IL8 or chemokine (C-X-C motif) ligand 8, CXCL8) is a chemokine produced by macrophages and other cell types such as epithelial cells, airway smooth muscle cells and endothelial cells. In another report, it stated that IL-8 has a diametrical role in several pathological conditions, such as chronic inflammation, fibrosis and cancer (67). The inflammatory effect of fluoride exposure was evaluated in human lung epithelial cells. With 5-7 fold exposure increasing the dose to 3.75 and 5 (mM) NaF, has altered the human lung epithelial cell activity (68).

Effect of Fluoride on the programmed cell death (Apoptosis)

Programmed cell death is a complex phenomenon that includes death of a cell in any form through delicate regulation of signaling proteins via gene expression and/or protein activity (69). Reactive oxygen species (ROS) produced through the oxidative stress, induce the programmed cell death or apoptosis. Many researchers have found that Fluoride as the wide role on the apoptosis; caused after the severe oxidative stress which include lipid

peroxidation, thus causing mitochondrial dysfunction and the activation of downstream pathways of apoptosis (70-73). There has the controversy for the production of oxidative stress due to fluoride exposure (74). Intracellular calcium content has a great role on the induction of apoptosis. Many findings have showed that this intracellular calcium content gets altered directly or indirectly which caused apoptosis (75-77). Among all other components of apoptotic signaling pathways, pro- and anti-apoptotic genes are the most important. For example, B-cell lymphoma 2 (Bcl-2) has been demonstrated to be involved as a pro-apoptotic component in fluoride-induced apoptosis (Table 3, Fig. 2).

Meanwhile, among the executioners of apoptosis pathway, Caspases; class of apoptosis-related proteins, which can be divided into apoptotic initiators, plays the great role (78). One study showed that, F exposure can severely impair the immune function and induce thymus apoptosis (58, 79, 80). This may due to an F induced up-regulation in the thymus in the relative expression levels of caspase-3 and caspase-9 (79). Numerous studies have shown that F can induce apoptosis in different tissues. Matsui *et al.,* (2007) demonstrated that NaF may induce apoptosis-like necrosis in rat thymocytes with increases in the population of shrunken cells and cells positive to annexin V, both of which are associated with an early stage of apoptosis (17).

Miao *et al.,* (2013) and Cheng *et al.,* (2013) found that F-induced apoptosis was correlated with increased levels of Fas/FasL and the high expression of Bcl-2 and Bax (81, 82). In the study of Lee *et al.,* (2008) has showed that exposure of 20mM NaF, human gingival fibroblasts (HGF cells) showed a down-regulation of Bcl-2 followed by the activation of mitochondrial cell death pathway through the enhancement of cytochrome-C (Cyt C) release from the mitochondria into the cytosol as well as activation of the caspase cascade (with increased activities of caspase-3, -8 and -9), and the cleavage of Poly (ADP-ribose) polymerase (PARP) (74).

Some study also indicated that, there has the death receptor-dependent pathway in fluoride induced apoptosis. It was found that fluoride exposure resulted in the up-regulation of Fas ligand (Fas-L). The participation of the Fas-L pathway in fluoride-induced apoptotic cell death was also demonstrated. Exposure of 0.1–0.3mM NaF in osteoclast cells caused an increase of Fas and FasL and a decrease in NF-κβ expression (74, 83).

Fluoride exposure in human neuroblast cells at 2–4mM (sodium fluoride) NaF increased caspase-3 activity and caused the mRNA up-regulation of Fas, Fas-L, and caspases (3 and 8). NaF also affect the TNF-related apoptosis-inducing ligand (TRAIL). The exposure of 4-2 (2-aminoethyl) benzylsulfonyl fluoride (AEBSF), an organic fluoride compound, on adenocarcinoma cell line did not promote apoptosis signaling but has the morphological alterations associated with apoptosis (83).

One study reported that, there is no such above mentioned mechanism to inhibit the apoptosis but release of Cyt-C was not decreased. But, Cyt-c release is one of main cellular event in case of TRAIL mediated apoptotic pathway. From this incident, it could be emphasize that, this organic compound has the only capability of cell membrane blebbing (84).

One of the recent studies which was performed in a human population show that exposure to fluoride can modify the expression of apoptotic genes in peripheral blood mononuclear cells (PBMC). In PBMC of subjects with fluoride concentrations in a range of 2.16–7.3mg, demonstrated a significant down-regulation of CD40L, caspase-6, TNF receptor-associated factor 2 (TRAF-2) and TRAF-5 genes and an up-regulation of survivin when compared to the unexposed group (85).

Fluoride could enhance the lipid oxidative damage of cell membrane; consequently, affect the DNA which ultimately promotes the apoptosis (86). Cui *et al.,* (2005) demonstrated in his research paper that, fluoride could result in the apoptosis of rat spermatogenic cells at a certain dose and time (87). Sun *et al.,* (2001) showed that liver cell apoptosis of rabbit in the group treated with fluoride aggravated with the increase of the fluoride dose (88).

G-protein signaling pathways are also responsible for the apoptotic regulation in the cell after the fluoride exposure. It was found that, 5–7.5mM NaF doses can affects on the G-proteins with varying effects. Thus, NaF concentrations are capable to induce apoptosis with a sequential increase in protein kinase C (PKC), protein kinase A (PKA), tyrosine kinase and calcium-dependent protein activities (89).

Table 1: Summary of reactive oxygen and nitrogen species (90, 91).

Reactive Oxygen Species				Reactive Nitrogen Species			
Free Radicals		**Other Substances**		**Free Radicals**		**Other Substances**	
Superoxide anion radical	$O_2^{\bullet -}$	Hydrogen peroxide	H_2O_2	Nitric oxide radical	$NO^{\bullet}$	Peroxynitrite	$ONOO^-$
Hydroxyl radical	$HO^{\bullet}$	Hypochlorous acid	HOCl	Nitric dioxide radical	$NO_2^{\bullet}$	Nitrites	NO_2^-
Alkoxyl radical	$RO^{\bullet}$	Ozone	O_3			Nitrates	NO_3^-
Peroxyl radical	$ROO^{\bullet}$	Singlet oxygen	$^1O_2$			Nitrosyl	NO^+

Table 02: Oxidative stress an oxidative damage associated to fluoride exposure. Arrows refer to increases (↑), or decreases (↓) regulation.

Type of the study	Model & Dosage	End point	References
In-vitro (Animal cells)	Mouse pancreatic beta-cells (βTC-6) at 1.35 and 2.5mM for 12 h	↑Generation of O_2^-, ↓activity of SOD, ↓Δψ m	(28)
	Primary rat hippocampal neurons at 20, 40, and 80 mg/l, equivalent to 1.05, 2.1 and 4.2mM for 24 h	↑Generation of ROS, ↓level of GSH, ↓activities of GSH-Px, and SOD, ↑lipid peroxidation	(92)
	Murine hepatocytes at 100mM for 1 h	↑Generation of ROS, ↓level of GSH, ↓GSH:GSSG ratio, ↓activities of SOD, and catalase, ↑lipid peroxidation, and oxidation of proteins	(38)
In-vitro (Human Cells)	Hepatocellular carcinoma (HepG2) cells at 3mM for 6 and 24 h	↓GSH/GSSG ratio, ↑gene expression of Mn-SOD	(93)
	Neuroblastoma (SH-SY5Y) cells exposed at 0.05–5mM for 24 h	↑Lipid peroxidation, and ↑protein oxidation	(94)
	Male albino guinea pigs exposed at 250mg NaF/kg subcutaneously and sacrificed 8 h later	↑Generation of NO in blood	(95)
In vivo (Animals)	Male Wistar rats exposed at 5mg/kg body mass/day, orally for 8 weeks	↑Generation O_2^-, ↓activity of SOD, ↓Δψm, ↑lipid peroxidation in spermatozoa	(36)
	Male Swiss mice exposed at 50 mg/l in drinking water for 10 weeks	↑Generation of ROS, ↑lipid peroxidation, ↓activities of SOD, and catalase, ↑activities of GST, and GSH-Px, ↓ratio GSH:GSSG in brain ↑Level of ascorbic acid ↓ level of uric acid in plasma	(73)
	Albino rats exposed at 100 mg/l in drinking water for 4 months	↑Lipid peroxidation, ↑level of GSH, ↑activity of GSH–Px, ↓activity of SOD in erythrocytes	(20)
	Male albino Wistar rats exposed at 1, 10, 50, and 100 mg/l in drinking water for 12 weeks	↑Lipid peroxidation, ↑activities of GSH-Px, and GST, ↑GSH in brain and liver ↑Generation ROS, changes in levels of GSH in blood, ↑generation ROS in liver, kidney, and brain	(96)
	Second generation of Male Albino adult Wistar rats exposed at 10, 50, and 100 mg/l in drinking water for 180 days	↑Lipid peroxidation, ↓activities of SOD, catalase, and GSH-Px in lung	(97)
	Chicks exposed by diet to 100, 250, or 400mg F/kg for 50 days	↑Generation of NO, ↑lipid peroxidation, ↓activities of SOD, catalase, and GSH-Px in serum	(98)
	Male albino rats exposed at 10.3mg NaF/kg body weight/day, orally for 5 weeks	↑Lipid peroxidation, ↑generation NO, ↓activities of SOD, and catalase, ↓Total antioxidant capacity, and ↓level of GSH in liver	(29)
	Pig exposed to food supplemented with 250mg F/kg for 50 days	↓Expression of gen Cu/Zn SOD in liver	(99)
	Male rats exposed at 20 mg/kg/day for 29 days by oral gavage	↑Level of conjugated dienes in the testis, epididymis, and epididymal sperm pellet. ↓activities of GDH-Px, and catalase in the sperm	(100)

	Male Wistar rats exposed at 50 and 100 mg/l in drinking water during 4 months	↓Activity of CuZn-SOD in pancreas	(44)
	Male and female Wistar rats exposed at 50, 100, and 150 mg/l in drinking water during 3 months	↑Lipid peroxidation, ↓activities of SOD, and GSH-Px in liver ↑Generation of NO, ↑lipid peroxidation,	(101)
	Barrows exposed at 250 and 400 mg/kg (from NaF) in their diets for 50 days	↓activities of GSH–Px, and SOD in serum ↑Lipid peroxidation, ↓activities of GSH-Px, and SOD in thyroid, liver, and kidney	(102)
	Male Swiss mice exposed at 5mg/kg body mass/day, orally for 8 weeks	↑ROS in erythrocytes, ↓level of GSH in blood, ↓activities of SOD, catalase, and GSH-Px, ↑lipid peroxidation, in kidney and liver	(42)
	Female rats exposed at 100 mg/l in drinking water for 60 days	↑Lipid peroxidation, ↓activities of SOD, catalase, and GSH-Px in endometrium	(103)
	Swiss albino male mice exposed at 50 mg/l in drinking water for 3 weeks	↑Generation of ROS, ↓GSH level, ↓activity of SOD in blood, ↑activity of catalase in liver	(104)
	Male albino rats exposed at 10, 50 and 100 mg/l in drinking water for 10 weeks	↑Generation ROS in blood, liver, kidney, and brain ↓GSH/GSSG ratio in liver, kidney, and brain	(105)
	Female Albino mice exposed 5mg/kg body weight/day, orally for 30 days	↓Activities of SOD, catalase, and GSH–Px, ↓level of GSH, ↓total, dehydro and reduced ascorbic acid, ↑lipid peroxidation in ovary	(106)
	Male Balb/c mice exposed at 200 mg/l, in drinking water for 7 days	↓Activities of SOD, GSH-Px, and catalase, ↑lipid peroxidation, in erythrocytes, and liver	(107)
	Female Wistar rats exposed at 150 mg/l in drinking water for 28 days	↓Level of GSH, ↓activities of SOD, GPx, catalase and, glutathione reductase, ↑lipid peroxidation in brain	(108)
	Wistar albino pups placentally and lactationally exposed from mother rats at 50, and 150 mg/l in drinking water	↑Lipid peroxidation, ↑protein oxidation in developing central nervous system	(109)
	Residents from China-endemic area (mean urine concentration of 2mg F/l)	↓Activities of SOD, catalase, and GSH-Px ↑Lipid peroxidation, in serum	(110)
In-vivo (Human Cells)	Children with skeletal fluorosis from Indian-endemic area (mean water concentration of 5.53mg F/l)	↑Level of ascorbic acid, ↓level of uric acid in plasma ↑Lipid peroxidation, ↓GSH, ↓activities of SOD and GSH–Px in erythrocytes	(111)

Table 3: Regulation of apoptotic and cytokines related gene expression by fluoride exposure.

Type of the study	Model & Dosages	End point	References
In vitro (Human cells)	Neuroblastoma (SH-SY5Y) cells at 40, and 80 mg/l, equivalent to 2.1, and 4.2mM for 24 h	↑Apoptosis molecules Fas, Fas-L, and caspases 3 and 8.	(83)
In vivo (Humans)	Peripheral blood mononuclear cells from Mexican individuals drinking water with levels of 1.9–4.02mg F/l	↓Inflammatory Chemokines (CCL1, CCL18, CCL19), ↓cytokines (IL-11; IL-2), ↓pro- and anti-inflammatory molecules (LTA, TNF-a, TGF-a, TGF-b1,and TGF-b3), ↓Apoptosis molecules (TNF-a, FasL, CD30L, 4-IBBL, TANK, TRAIL, DR3, Casp-2, Casp6, CIDE-A and CIDE-B), ↑survivine	(85)

Arrows refer to increases (↑) or decreases (↓) genes regulation.

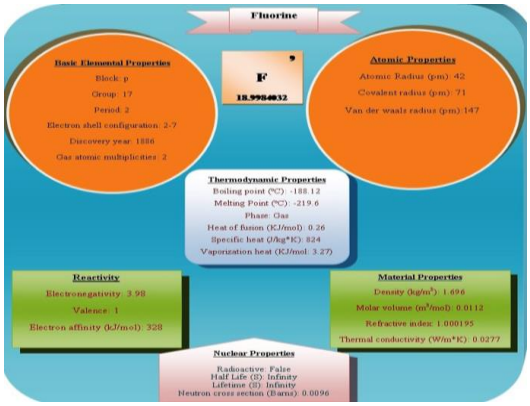

Figure 1: Basic properties of Fluoride

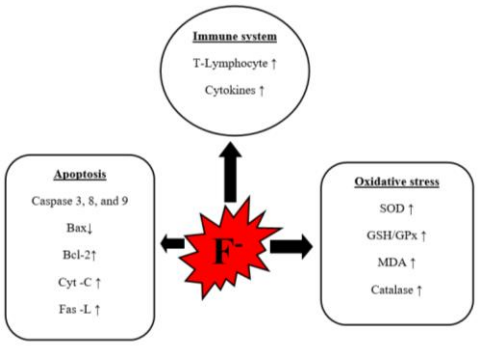

Figure 2: Diagrammatic representation of overall effects of fluoride on immune system, antioxidant enzymes, and apoptosis pathway

Conclusions

All the *in vivo* and *in-vitro* studies depict fluoride as an oxidative stress, immune system and apoptosis inducer that could render the host to effective on the antioxidant defence system, immune-compromised, and apoptotically sensitive in the animal body. All these effects can alter the whole physiology which could help explain increased risk of infections and several cancers observed in chronically exposed human populations. Fluoride-mediated alterations of cellular and humoral immunity reported in animal and in vitro models generally agree with immunological outcomes in humans. However, more work is needed to close the gap between experimental data and risk of human immunotoxicity. Moreover, inconsistencies in epidemiological findings, possibly due to differences in dose, sampling, genetic background, and environmental/ nutritional factors, indicate the need for larger participant numbers and diverse ethnic populations. Due to differential effects of exposures, populations having low, intermediate and high exposure should be evaluated to better understand dose-dependent relationships. Furthermore, strong evidence for an association between developmental fluoride exposure and elevated risk of human disease necessitates more investigations of early-life exposure outcomes. Finally, comprehensive genomic, proteomic and metabolomic profiling will be critical for identifying and validating potential molecular targets of fluoride to monitor the progression of fluoride-associated diseases and the mechanisms.

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