STUDY OF FLUORIDE-INDUCED HAEMATOLOGICAL ALTERATIONS AND LIVER OXIDATIVE STRESS IN RATS

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ABSTRACT
Fluorine is a necessary biological trace element for human health. However, fluoride accumulation leads to cascading effects resulting in altered physiological functions in human being. The current studies evaluated the effect of fluoride induced hematological alterations and oxidative stress in the liver of experimental rats. For this purpose, adult female Wistar albino rats divided into control and NaF treated group. Sodium Fluoride (400 mg/kg b.w) added in their drinking water for 70 days. Compared with the control, a significant decrease in the body weight gain (p<0.001) and increase in relative liver weight (p<0.001) were noticed in the NaF group. Our results showed that fluoride treatment caused an increase in Granulocyte (p < 0.001), White blood cell (p < 0.001), Lymphocyte (p < 0.01) and Platelets (p < 0.01) compared with the control. In addition, it led to an increases in triglyceride (p<0.001), cholesterol (p<0.01) level, GOT (p<0.01) and GPT (p<0.001) activities in serum animal compared to the control group. In contrast fluoride caused an oxidative stress by decrease in GSH level and GST activity and increase in MDA concentration compared with the control group. In Conclusion, Results demonstrated the toxic effect of high-dose of fluoride by causing oxidative stress and inflammation in hepatic tissue.

KEYWORDS: Sodium Fluoride; liver; hematological parameters; stress oxidative.

1. INTRODUCTION
Fluoride (F) is abundant in the environment and exists only in combination with other elements as fluoride compounds, which are constituents of minerals in rocks and soil. Sources of fluoride include natural fluoride in food stuffs and water (fluoridated water.
usually at 5.5 mg/L).\cite{1} The main source of fluoride for humans is the intake of ground water contaminated by geological sources. Although intake of low doses of fluoride is required to prevent dental caries, increased uptake for long time injured bone and soft tissues causes’ fluorosis.\cite{2} Endemic fluorosis is caused by excessive fluoride ingestion over a prolonged period. In addition to well-known effects on the skeleton and teeth, fluorosis can adversely affect many tissues and organs as exhibited by a broad array of symptoms and pathological changes.\cite{3} Earlier studies show that fluoride can produce abnormalities in the liver including degenerative and inflammatory changes, dilatations of sinusoids, hepatic hyperplasia, and accumulation of amorphous and crystalline bodies in the hepatocytes around the hepatic vein.\cite{4} The most commonly used medium for identifying fluoride exposure is urine.\cite{5} Acute exposure to high doses of fluoride damages renal tissue and causes renal dysfunction. The kidneys are the target organs for fluoride toxicity. On the other hand, pale, granular hepatocytes, characteristic of parenchymal degeneration were observed in mice treated with the compound (0.95 mg/kg). Liver congestion was observed in sheep given a single intragastric dose (9.5 mg/kg) of fluoride.\cite{6} One of the major mechanisms behind heavy metal toxicity has been attributed to oxidative stress.\cite{7} Toxic metals increase production of free radicals and decrease availability of antioxidant reserves to respond to the resultant damage stress.\cite{8} The aim of this study was to investigate the effect of sodium fluoride given orally on the haematological and oxidative stress parameters of rats.

2. MATERIALS AND METHODS

2.1. Animals and Handling: Adult females albino rats, weighing 180–230 g, were brought from the animal house of Pasteur institute, Algeria. They were placed in two groups of 4 rats in each and kept in animal’s house of Molecular and cellular biology Department, University of El oued, Algeria. Standard rat food and tap water were available ad libitum for the duration of the experiments unless otherwise noted.\cite{9} Animals were acclimated for two weeks under the same laboratory conditions of photoperiod (12h light/12 h dark) with a relative humidity 65.3 % and room temperature of 23 ± 2 C°. The experimental procedures were carried out according to the National Institute of Health Guide-lines for Animal Care and approved by the Ethics Committee of our institution.

2.2. Experimental design: The experiment was conducted over a period of 70 days. After a period of adaptation, the animals, at the age of 8 weeks, were divided into two experimental groups of 4 animals each: the control group was not treated with fluoride and the
experimental group received sodium fluoride (NaF). Fluoride (400 mg/kg b.w) as NaF added in drinking water for 70 days in animals. Evaluate the food intake, drinking water and body weight were monitored during the whole experiment.

2.3. Preparation of tissue samples: At the end of 10 weeks of sodium fluoride exposed, rats were fasted for 16 hrs, anaesthetized with chloroform by inhalation, rats were decapitated and blood was transferred into EDTA tubes for haematological studies and in non-heparinized tubes for serum biochemical analysis. Serum was obtained by centrifugation of the blood at 3000 rpm for 10 min and then quickly frozen at −20°C until used. The liver was rapidly excised, weighed and stored at -20°C for oxidative stress parameters analysis.

2.4. Measurement of biochemical parameters: The activities of GOT, GPT and ALP were determined using commercial kits from Spinreact (Girona, Spain) (refs: GOT-1001161, GPT-1001171 and ALP-1001131). Triglyceride (TG), Total cholesterol (TC), and Total protein concentrations were also measured using commercial kits (Spinreact) (refs: TG-1001311, TC-1001090 and total proteins-1001291).

2.5. Antioxidants measurement.

2.5.1. Preparation of homogenates: About 1 g of liver was homogenized in 9 ml of buffer solution of tris-NaCl buffer 1:9(w/v; 1g tissue 9ml TBS, pH=7.4). Homogenates were centrifuged at 9000xg for 15 min at 4°C, and the obtained supernatant was used for the determination of antioxidant activity.

2.5.2. Determination of malondialdehyde (MDA) level: tissue homogenates were prepared at 10% (w/v) in 0.1 mol/L Tris-HCl buffer, pH 7.4, and MDA steady-state level was determined. MDA was measured according to the method described by Sastre et al. (2000). Thiobarbituric acid 0.67% (w/v) was added to a liquots of the homogenate previously precipitated with 10% trichloroacetic acid (w/v). Then the mixture was centrifuged, and the supernatant was heated (100°C) for 15 min in a boiling water bath. After cooling, n-butanol was added to neutralize the mixture, and the absorbance was measured at 532 nm. The results were expressed as nmol of MDA/g tissue.

2.5.3. Determination of reduced glutathione (GSH) level: GSH concentration was performed with the method described by Ellman. Based on the development of a yellow color when DTNB is added to compounds containing sulfhydryl groups. In brief, 0.8 mL of
tissue homogenate was added to 0.2 mL of 0.25% sulphosalicylic acid and tubes were centrifuged at 2500 g for 15 min. Supernatant (0.5 mL) was mixed with 0.025 mL of 0.01 M DTNB and 1 mL TBS (pH 7.4). Finally, absorbance at 412 nm was recorded. Total GSH content was expressed as nmol GSH/mg prot.

2.5.4. Determination of Glutathione-S-transferase (GST) activity: Glutathione-S-transferase (GST) activity of tissues was measured spectrophotometrically by the method of Habig et al.\textsuperscript{[12]} using CDNB as electrophilic substrate that binds to GSH with the participation of the enzyme and forms a colored GSH-substrate complex, detected at 340 nm. The activity of GST was expressed in terms of μmol CDNBGSH conjugate formed/min/mg protein.

2.6. Statistical Analysis: carried out by using 1-way analysis of variance followed by the Student t test to compare means among the groups. Differences were considered statically significant at p<0.05.

3. RESULTS

3.1. Initial body weight, final body weight and relative liver weight
NaF treatment at a dose 400 ppm caused a decrease (p<0.001) in body weight and an increase in Relative liver weight (p<0.001) in the rats compared to the control rats. (Table 1).

<table>
<thead>
<tr>
<th>parameters</th>
<th>Control (n=4)</th>
<th>NaF (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (g)</td>
<td>199.20 ± 6.26</td>
<td>205.67±2.73</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>244.4 ±12.1</td>
<td>189.3 ±26.4***</td>
</tr>
<tr>
<td>Relative liver weight (g/g tissue)</td>
<td>0.023 ± 0.0006</td>
<td>0.031±0.0023***</td>
</tr>
</tbody>
</table>

* p<0.05, ** p<0.01, ***p<0.001: significantly different from control group.

Values are mean ± SEM, n=number of observations.

3.2. Blood biochemical values and transaminase Activities
As shown in Table 2, fluoride caused significant increases in serum triglyceride (p<0.001), serum cholesterol (p<0.01) and no significant effect of serum glucose, serum protein level and serum alkaline phosphatase activity compared to the control animals. In addition, the findings showed a significant elevation of Glutamate-oxaloacetate transaminase (GOT) (p<0.01) and glutamate-pyruvate transaminase (GPT) (p<0.001) activities in serum animal after NaF treatment for 70 consecutive days compared to the control group.
Table 2: Mean blood glucose, serum triglycerides, total cholesterol and protein concentrations and liver enzymes activities of control and NaF animals

<table>
<thead>
<tr>
<th>parameters</th>
<th>Control (n=4)</th>
<th>NaF (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood glucose (mg/dL)</td>
<td>107.60±7.59</td>
<td>98.50±2.5</td>
</tr>
<tr>
<td>Serum triglyceride (mg/dl)</td>
<td>125±4.6</td>
<td>447±1.4</td>
</tr>
<tr>
<td>Serum cholesterol (mg/dl)</td>
<td>80±0.6</td>
<td>105±1.3</td>
</tr>
<tr>
<td>Serum protein (g/l)</td>
<td>62.25±2.39</td>
<td>64±2.31</td>
</tr>
<tr>
<td>Serum ALP (U/l)</td>
<td>118.2±21.4</td>
<td>94±54</td>
</tr>
<tr>
<td>Serum GOT (U/l)</td>
<td>138.3±16.5</td>
<td>172.7±23.7</td>
</tr>
<tr>
<td>Serum GPT (U/l)</td>
<td>42.3±10.4</td>
<td>73.3±12.7</td>
</tr>
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</table>

** p<0.01, ***p<0.001: significantly different from control group.

Values are mean ± SEM, n=number of observations.

3.3. Haematological parameters

Seen from Table 3, NaF induced a significant increase in Granulocyte (GRN) (p < 0.001), White blood cell (WBC) (p < 0.001), Lymphocyte (LYM) (p < 0.01) and Platelets (PLT) (p < 0.01) and no significant effect in Red blood cell (RBC) and Haemoglobin (HGB) level compared to the control group.

Table 3: Haematological parameters in control and NaF animals

<table>
<thead>
<tr>
<th>parameters</th>
<th>Control (n=4)</th>
<th>NaF (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HGB (g/dL)</td>
<td>13.22±0.32</td>
<td>12.95±0.55</td>
</tr>
<tr>
<td>RBC (10^6/µL)</td>
<td>6.84±0.095</td>
<td>6.5±0.23</td>
</tr>
<tr>
<td>GRN (10^3/µL)</td>
<td>0.92±0.048</td>
<td>1.60±0</td>
</tr>
<tr>
<td>WBC (10^3/µL)</td>
<td>4.55±0.13</td>
<td>7.20±1.70</td>
</tr>
<tr>
<td>LYM (U/l)</td>
<td>3.05±0.12</td>
<td>4.55±1.55</td>
</tr>
<tr>
<td>PLT (10^3/µl)</td>
<td>596±16.4</td>
<td>770±56.5 **</td>
</tr>
</tbody>
</table>

RBC: Red blood cell count, HGB:Haemoglobin concentration, GRN: Granulocyt, WBC: White blood cell count LYM: Lymphocyte, PLT: Platelets

** p<0.01, ***p<0.001: significantly different from control group.

Values are mean ± SEM, n=number of observations.

3.4. Stress oxidative parameters

As showed in figure 1, there was a significant (p<0.001) increase in MDA concentrations in liver tissues after NaF treatment for 70 consecutive days compared to the control group. The GSH level and GST activity were significantly decreased (p<0.001) in Sodium fluoride (NaF) exposed rats as compared to control rats (figure 2 and 3).
Figure. 1 Level of MDA in liver tissues of control (C) and experimental group. Means± SE from 4 animals in each group. Significance from Control: ***p<0.001.

Figure. 2 Level of GSH in liver tissues of control (C) and experimental group. Means± SE from 4 animals in each group. Significance from Control: ***p<0.001.

Figure. 3 GST activity in liver tissues of control (C) and experimental group. Means± SE from 4 animals in each group. Significance from Control: ***p<0.001.

4. DISCUSSION
Through this study, we investigated to evaluation the toxic effect of orally exposed fluoride on haematological and oxidative stress parameters in liver rats. In this study we observed that
fluoride administration resulted in striking reduction in body weight of the albino rats. Similar types of findings were observed in a study conducted by Heindel et al.(1996). However, overall loss of body weight with continuous exposure to fluoride might be explained on the basis of anorexia which is induced by heavy metal ingestion. In this study, we observed a significant increase of the weight of liver which may be a direct effect of fluoride water on vital organs. increased in vital organs weight after NaF water treatment was reported in mammals. From our work it was observed that the values obtained for serum biochemical parameters like triglyceride and cholesterol showed significant elevation with higher fluoride contents as compared to control. The altered tissue biochemistry of liver may be due to toxic effect of fluoride. Liver is an important organ for metabolism and detoxification. SGPT and SGOT are markers of liver function. Drinking high-fluoride water over a long period can damage the liver. In the present study, elevated activities of SGPT and SGOT demonstrated liver damaged in the fluorotic rats, in agreement with results of previous investigations. The present study reported a significant increase in Hematological indices (GRN, WBC, LYM and PLT) in fluoride exposed rats. Similar results have been reported by Shanmugam et al. 2016. Who reported the elevated of WBC with 100 and 200 ppm of fluoride exposed. The total and differential WBC count is an important auxiliary diagnostic test as changes occur with systemic inflammation and other diseases. An increased granulocyte count has been associated with an increased risk of heart failure in apparently healthy men, while a decreased risk has been associated with an increased monocyte count. Since the mature immunocytes migrate from lymphoid organs to the peripheral blood and then exert their effects, a complete blood count is necessary to assess the immune status of the body. Chronic fluorosis can severely damage many systems of the animal body. ROS and lipid peroxidation have even been considered to play an important role in the pathogenesis of chronic fluoride toxicity and oxidative stress was as one of the important mechanisms of the toxic effects of fluoride. The liver is the main organ responsible for metabolism and detoxication. Fluoride exposure would induce both pathomorphological and metabolic changes in the liver. In this study, it is seen that administration of fluoride to rats caused a significant increase in the level of lipid peroxidation as indicated by the significant increase in MDA. Our results also corroborate well with that of Shivarajashankara et al. who demonstrated that 100 ppm of fluoride increases the rate of lipid peroxidation in liver. Oxidative stress is caused by a relative overload of oxidants, reactive oxygen species. Liu et al. suggested the mechanism of fluoride in injuring soft tissue as follows: fluoride causes excessive production of nitric oxide (NO), lipid peroxides (LPO), and oxygen free.
radicals, leading to the reduced capability of scavenging free radicals and antioxidating, which seriously damage the structure, especially the biological membrane structure, functions of cells, and biomacromolecules, such as proteins and nucleic acids, and even the entire soft tissues.\textsuperscript{[23]} The enhanced lipid peroxidation might result from the reduction in the liver activities of GST and GSH level observed in these animals. In agreement with previous studies, the level of GSH in liver was significantly decreased upon oral fluoride administration compared to the control group. Glutathione (GSH) participate in the cellular defense system against oxidative stress by scavenging free radicals and reactive oxygen intermediates\textsuperscript{[24]} as a co-substrate for glutathione peroxide (GPx), which explained decreased GSH concentration with increased oxidative stress\textsuperscript{[25]} it is a sulphhydryl peptide widely found in all biological systems. It forms the first line of defense against oxidative insult by acting as a nonenzymatic antioxidant. Its sulphhydryl (SH) group can directly interact with ROS or it can be involved in the enzymatic detoxification reaction of ROS as a cofactor or a coenzyme.\textsuperscript{[26]} This decrease in GSH levels may be due to its consumption in the scavenging free radicals generated by fluoride. Since oxidative stress is the first response to the environmental pollutants, liver cells may stimulate antioxidant and detoxification responses to counter heavy metal damages. The involvement of anti-oxidative enzymes such as GST play a considerable mission in protecting cells from oxidative stress.\textsuperscript{[27]} So, assessment of activities of this enzyme may supply important information about oxidative stress that cells exposed. We determined that fluoride used in this study were decreased enzyme activity.

5. CONCLUSION
The present study exhibits the toxic effects of sodium fluoride on haemotological and oxidative stress parameters in rats. From the study, it can be further concluded that excess fluoride water exposure caused altered blood cells and produce toxic effects on liver tissue.

CONFLICT OF INTEREST STATEMENT
We declare that we have no conflict of interest.

ACKNOWLEDGEMENTS
We thank members of Algiers Pasteur Institute for providing the rats. We thank also the biology laboratory members for help in the course of this work.
6. REFERENCE


