Drinking Water Toxicity of Sodium Fluoride in Steroid Producing Glands and Antioxidant Ascorbate Defense System in Albino Rats (Rattus norvegicus)

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Abstract

This investigation examined the reproductive toxic effects and oxidative stress of sodium fluoride (NaF) in male albino rats (Rattus norvegicus). The rats received acute doses of NaF as 0.5 ppm, 200 ppm and 500 ppm through drinking water for 15 days. Additionally 10 mg ascorbate was also supplemented after NaF treatment as an antidote. The dose 0.5 ppm was ineffective and 500 ppm was fatal after 4 days onwards. A significant decrease in ascorbate content of liver and increase in cholesterol content of liver and testes after 200 ppm NaF-15 days treatment was examined but their level from adrenals did not change significantly. Histological studies of testes in these animals indicated atrophy of steroidogenic cells. Almost similar results were obtained when ascorbate was supplemented as an antidote.

Keywords: Sodium fluoride toxicity; Steroid producing glands; Cholesterol; Ascorbate; Mammal

Introduction

Sodium fluoride (NaF), a well known water pollutant is important throughout the world, including India. The high percentage of fluoride in drinking water of contaminated areas exerts adverse effects on human beings and other vertebrate species [1,2]. The normal content of fluoride in drinking water should be under 5 ppm. The most obvious early toxic effects of fluoride in humans are dental and skeletal fluorosis, which are endemic in areas with elevated exposure to fluoride [3]. Sodium fluoride mainly exerts its adverse effects on soft tissues, including blood, brain, liver and eyes [3,4]. NaF is known to cross the cell membranes and to soft tissues and interfere with their normal metabolic activities [5,6]. It causes oxidative stress, enhances lipid per oxidation in the R.B.Cs, brain, liver and decreases activity of antioxidant enzymes i.e. glutathione (GSH), superoxide dismutase (SOD) in fluorosis patients [7,8]. Further, NaF- toxicity in drinking water in contaminated areas causes impairment of fertility in many vertebrate species including human beings [9,10]. NaF- exposure, at the dose available in drinking water in contaminated areas, led to inhibition of testicular gametogenesis and steroidogenesis in association with oxidative stress in male sex organs though they are protected significantly by dietary agents like Vitamin-E and calcium [11,12]. In some cases administration of ascorbate and calcium brought protection significantly by dietary agents like Vitamin-E and calcium [11,12]. In some cases administration of ascorbate and calcium brought protection [13] reported genotoxic effects of NaF in drinking water on mouse bone marrow cells. Moreover, according to Zhou et al. [14] NaF- acts as a specific inhibitor of protein synthesis in rabbits.

There are many reports on Fluoride -induced toxicity in organs like liver, kidney and bone marrow but very few on steroid producing tissues and also the role of ascorbate as an antioxidant, particularly in mammals. Thus, the present investigation was aimed to find out toxicity of sodium fluoride in adult male albino rats weighing 120-160gm after ingestion of certain doses of sodium fluoride in their drinking water for one or two weeks and to reflect its impact on antioxidant ascorbate status (from liver and adrenals) and on male fertility. The route chosen in this study for exposure to NaF via drinking water to mimic human exposure. An additional amount of ascorbate 1ml-10 mg daily for 15 days was also provided intramuscularly to find out whether ascorbate supplementation is sufficient to detoxify the Fluoride - toxicity or not.

Material and Methods

Total 50 adult male albino rats weighing 120-160 gms were used in the present investigation. All the animals were fed on a standard diet of soaked grams, green vegetables, wheat bread and drinking water was provided *ad libitum*. The animals were divided into five groups, subjected to the following treatments:

Group I: 10 animals, untreated control.
Group II: 10 animals, received 0.5 ppm NaF in drinking water for 15 days.
Group III: 20 animals, received 200 ppm NaF in drinking water for 15 days.
Group IV: 10 animals from this group III received 1ml-10mg ascorbate daily intramuscularly for another 15 days.
Group V: 10 animals, received 500 ppm NaF in drinking water for 15 days.

After 15 days of treatment, all the animals were sacrificed and the desired tissues (liver, adrenals and testes) were immediately taken out and kept in their respective media for biochemical and histological studies. Biochemical analysis of asorbate and cholesterol through UV-VIS spectrophotometer [15,16]. Histological studies include routine

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sectioning of adrenals and testes, preparation of permanent slides and their analysis under microscope.

**Results**

- Behavioural changes in animals
- Change in their body weights
- Change in bio concentration of ascorbate and cholesterol (mg/g) from respective tissues
- Histological changes in adrenals and testes of these animals

Visual observation of behaviour of animals of different groups was performed. There was no noticeable change in the behaviour in animals of group I and II. However, animals of group III and IV appeared tired, show unwillingness for food and water, blood spots appeared around their eyes and noses. Besides, the incisor teeth appeared relatively whiter as compared to animals of group I and II. The animals of group V died during experimentation. The body weight of animals of group III and IV were also significantly reduced as compared to animals of group I and II (Table 1).

There was no change in the level of cholesterol and ascorbate from liver, adrenals and testes after 0.5 ppm NaF-15 day’s treatment (group II). A significant decrease in ascorbate (liver) and increase in cholesterol (liver and testes) were noticed after 200 ppm NaF-15 day’s treatment (group III). But their level from adrenals did not change significantly. Additional supplementation of 10 mg ascorbate for 15 days was unable to recover any change over sodium fluoride toxicity i.e. there was no biochemical changes in respective tissues (Table-2,3).

No histological change in the structure of adrenals and testes after 0.5 ppm NaF treatment. A slight atrophy in adrenal cortex and in seminiferous tubules of testes was observed after 200 ppm NaF-15 day’s treatment. Further, ascorbate treatment in this group was unable to overcome these changes. (Figures 1 to 4).

**Discussion**

Generation of free radicals, lipid peroxidation and altered antioxidant defence system are considered to play an important role in the toxic effects of fluoride [4,17]. High doses of fluoride have repeatedly been found to interfere with the reproductive systems of animals. In another study, sodium fluoride was administered to the rats orally at a daily dose of 10 mg/kg body weight for 50 days did not cause significant change in the testicular cholesterol levels, indicating that metabolism was not altered and that there was no hypo/hyper cholesterolemic effect.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment and Doses</th>
<th>Initial body weight (gm)</th>
<th>Body weight after 15 days</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Untreated control</td>
<td>136.0 ± 4.78</td>
<td>135.5 ± 2.78</td>
<td>0.37</td>
</tr>
<tr>
<td>Group II</td>
<td>0.5 ppm NaF – 15 days</td>
<td>135.5 ± 4.63</td>
<td>136.5 ± 4.63</td>
<td>0.74</td>
</tr>
<tr>
<td>Group III</td>
<td>200 ppm NaF – 15 days</td>
<td>143.0 ± 3.29</td>
<td>131.1 ± 2.91</td>
<td>8.31</td>
</tr>
<tr>
<td>Group IV</td>
<td>200 ppm NaF – 15 days and 10 mg ascorbate 15 days</td>
<td>142.5 ± 3.95</td>
<td>132.5 ± 2.95</td>
<td>7.02</td>
</tr>
<tr>
<td>Group V</td>
<td>500 ppm NaF– 15 days and 10 mg ascorbate 15 days</td>
<td>139.8 ± 1.70</td>
<td>Mortality 0</td>
<td>0</td>
</tr>
</tbody>
</table>

Values are represented as mean ± SE

Table 1: Effect of sodium fluoride administration on Body weights (gm).
The degree of testosterone synthesis dependent on fluoride concentration. Testosterone concentration decreased in skeletal fluorosis patients [18]. According to Shivarajashankara [8] plasma ascorbic acid levels of children with skeletal fluorosis were increased. These findings suggest a definite role for ascorbate as an antioxidant and antistress factor in fluoride intoxication.

In the present investigation, 0.5, 200 and 500 ppm sodium fluoride was administered in adult male albino rats via drinking water for 15 days. Additional ascorbate was also supplemented after 200 ppm sodium fluoride treatment for 15 days. There were no significant biochemical and histological changes in group II. The ascorbate content from liver decreased and cholesterol content increased in liver and testes however there was no significant change in the cholesterol content of adrenals (group III and IV) as shown in Table 2 and 3. It indicates that sodium fluoride interferes with metabolic activities of liver. Not any biochemical or histological changes in adrenals indicating that adrenal steroids nothing to do with NaF toxicity. Increased level of testes cholesterol and atrophy of seminiferous tubules indicating the hypercholesterolimic effect of NaF on testes and male fertility. The decrease in the body weights of these animals was due to improper intake of food and water. The similar results were obtained when 1 ml-10 mg ascorbate supplemented after 200 ppm NaF- 15 day’s administration. It means that post ascorbate supplementation was unable to neutralize the effects of NaF toxicity in albino rats. All of this report is consistent with earlier reports of Chinoy and Sharma [7], Shivarajashankara [8]. No any observation was possible in group v i.e. 500 ppm NaF treated rats because they were die one by one with in four days of treatment. The cause of mortality was not established very strongly but it might be due to severe damage of liver cells and other vital organs.

**Conclusion**

On the basis of above foregoing accounts, it is clear that 0.5 ppm NaF – 15 days was not harmful, 200ppm NaF-15 days is complication creating dose and 500 ppm NaF is fatal for albino rats. The dose 200 ppm NaF- treatment creates metabolic imbalances in liver and is associated with impairment of male fertility. But adrenal glands appeared to have no significant response against NaF toxicity and ascorbate treatment did not bring about any alteration as an antidote.

**References**

9. Kaur K, Singh J (1980) Histological finding of mice testes following fluoride treatment for 15 days. Additional ascorbate was also supplemented after 200 ppm sodium fluoride-15 day’s administration.

