

## EFFECT OF SODIUM FLUORIDE ON SOME PHYSIOLOGICAL PARAMETERS AND HISTOPATHOLOGY IN ADRENAL AND THYROID GLANDS IN ADULT MALE RATS

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**Key words :** sodium fluoride, adrenal gland, thyroid gland

### ABSTRACT

This study was carried out to investigate the harmful effect of water fluoridation on both (thyroid and adrenal gland) in adult male rats its weight about (400-300gm) exposed to sodium fluoride (NaF) in the drinking water. We are used in this study (30) adult male rats (albino) were distributed randomly and divided into two equal groups (15 animals per group). The first group was given normal water and considered as a control group (control group), while the second group were given drinking water with 100 ppm of sodium fluoride (NaF) (treated group). this study carried out in the animal house in the college of Vet. Med. \ Tikrit University. blood was drawn through the eye pupil for periods (0,30,60 days) in order to measure the following parameters: Measurement of Glutathione (GSH) concentration, total cholesterol and blood glucose concentration. In addition taking tissue sections of the thyroid and adrenal glands. The results of this study showed that exposure of animals to sodium fluoride at a concentration of 100 ppm in drinking water for (60) days cause adrenal and thyroid gland dysfunction, represented by a significant decrease in the level of glutathione in blood serum on days 30 and 60 of the experiment and a significant increase in total cholesterol and blood glucose concentrations. The results of the histological examination of the thyroid gland of the treated group showed hyperplasia of the epithelial cell layer lining the acini

vesicles, and severe lipid changes were seen in the function of Reticularis in the histological sections of the thyroids gland of the same group animals. The results of this study confirmed the harmful effect of sodium fluoride (NaF) on thyroid and adrenal functions in addition to its effect on some biochemical parameters that are indicative of the occurrence of harmful effects of some chemical compounds .

## INTRODUCTION

The terms “fluorine” and “fluoride” are using interchangeably as a generic term. The term “fluorine gas” is often used as an emphasized reference to the elemental form of fluorine rather than a combined form (1). Fluoride is the light element of Group 17 (VIIA) of the periodic table. This group, also includes chloride, bromine, and iodine. As with the other halogens, fluorine happened as a diatomic molecule, F<sub>2</sub>, in its elemental form. It has one stable isotope and its valence in all compounds is -1. Fluorine is the most reactive of all the elements, which may be because of its large electronegativity (estimated standard potential +2.85 V). It reacts at normal room temperature or elevated temperatures with all elements other than nitrogen, oxygen, and the lighter gases. Fluorine is also notable for its small size; large numbers of fluorine atom fit around atom of other element. This, along with its electronegativity, allows the formation of many simple and complex fluorides in which the other elements is in its highest oxidation date (2). Sodium fluoride is an in-organic chemical compounds with the formula NaF. A colorless solid, it is a source of the fluoride ions in diverse applications. Sodium fluoride is less expensive and less hygroscopic than the related salt fluoride. Sodium fluoride is an ionic compound, dissolving to give separated Na<sup>+</sup> and F<sup>-</sup> ions (1). Sodium fluoride (NaF) is using as a flux for deoxidizing rimmed steel, as a component of laundry sours (removal of iron stains), in casein glues and heat-treating salts, and in the re-melting of aluminum, manufacture of vitreous enamels, pickling of stainless steel, and manufacture of coated papers (3). Sodium fluoride is also used in various pesticide formulations, including insecticides and woods preservations (4). Sources of exposure The major sources of fluoride in the world are food, drinking water (5), beverages, and fluoride-containing dental products (6). The atmosphere carries some fluoride, but it supplies a small fractions of the daily exposure except in heavily polluted areas (7).

## MATERIALS AND METHODS

1. **Animals:** We are used in this study thirty male albino rats (400-300 g) were used in this investigation. Animals were housed in iron cages in (22-25 °C) in the animal house at the College of Veterinary Medicine -University of Tikrit.

## **2. Preparation of NaF:**

Sodium fluoride (NaF) was used as described by (8).

## **3. Experimental Design:**

30 adult male were divided into 2 groups (15 rats/ group) and treated daily for (60) days as follows:

**1. Control Group:** all animals in this group were submitted to natural conditions.

**2. Treated Group:** all animals in this group were submitted to dose of 100 ppm of sodium fluoride with water (8).

## **4. Blood Sampling:**

Blood samples were collected from each animal groups after periods 30 days. blood was drawn from eye vein by a capillary tube containing heparin implanted in the inner corner of the eyeball. The blood was allowed to flow into a dry and clean test tube. The blood was then clotted and the tubes were placed in the centrifuge at 3000(rpm) for 15 minutes to separate the serum and kept it in refrigerator at - 22 C (9).

## **5. Studied Parameters:**

### **A. biochemical parameters include:**

1- Concentration of Glutathione (GSH).

The level of glutathione was estimated by using the modified method used by the researcher (10).

2- **Concentration Total Cholesterol (TC) (mg/dl):**

The level of cholesterol was estimated by using the method used by the researcher (11).

3- **Concentration of serum glucose:**

it was measured enzymatically by using glucose kit (Biomaghreb).

## **6. Histopathologic study:**

For histological examinations, rats were killed after completing period of study. Immediately, after killing, tissue pieces from thyroid and adrenal glands were preserved in 10% neutral buffer

formalin till the preparation of histological sections. Several tissue sections were prepared and stained according to (12).

**Statistical Analysis:**

Data were analyzed using two way analysis of variance (ANOVA) then followed with post hoc test (Least Square Deviation). The probability level ( $P \leq 0.05$ ).

**RESULTS AND DISCUSSION**

**1.Level of glutathione (GSH):**

The exposure to 100 ppm NaF (T1) on the mean value of serum (GSH) concentrations of adult male rats were clarified in table (1). A statistical analysis indicated that the mean values of serum (GSH) were non significantly different ( $P \leq 0.05$ ) in all experimental groups at the zero time when compared to each other. During treatment (After 30 days) a significant decreasing in (GSH) level with mean value of ( $16.8 \pm 0.15$ ) was detected in NaF Treated Group in comparing with control group ( $22.4 \pm 0.15$ ). and after 60 days there is significant increase in the level of glutathione in the infected group.

Table (1): level of glutathione concentration ( $\mu\text{mol/l}$ ) of adult male albino rats treated with (NaF) in drinking water for 30 and 60 days.

<b>Day \ Groups</b>	<b>Control G.</b>	<b>Treated G.</b>
Zero	$22.1 \pm 0.15$ Aa	$23.3 \pm 0.17$ Aa
30	$22.4 \pm 0.15$ Ba	$16.8 \pm 0.15$ Cb
60	$22.8 \pm 0.73$ Ba	$11.1 \pm 0.14$ Cc

- Values represent Mean  $\pm$  standard error.
- No. of animals in each group = 15.
- The different letters within the columns and rows mean a significant difference at a level of probability ( $p \leq 0.05$ ).

The mechanisms of fluoride effects have not been determined yet. However, oxidative stress with lipid peroxidation has been suggested as one of the important factor of toxic effects of fluoride (13). When an imbalance in the oxidant-antioxidant system occurs, the potentiality for tissue damage increases, with disturbances of the antioxidant system (14) including GSH concentration (15). it may be suggested that fluoride inhibits glucose-6-phosphates dehydrogenase (G6PD) due to an oxidative stress, and the decrease of Pentose phosphate pathway flow could make the cell unable to maintain the normal GSH/GSSG ratio, which is lowered due to fluoride. Increased consumption of Glutathione(GSH) and decreased capability to regenerate the reduced cofactor could, in turn, trigger a vicious circle, allowing a further oxidative damage of G-6-PD, lowering GSH concentration and cell injury (16).

## 2.Total serum cholesterol (TC) Concentration.

Table (2) showed the mean of TC concentration in the two groups along the experimental period. It can be seen that serum TC concentration was close in all groups ( $P \leq 0.05$ ) in the pretreated period. After 30 days of treatment, a significant increasing ( $P \leq 0.05$ ) in serum total cholesterol level was observed in NaF, ( $181.8 \pm 6.66$ ) as compared to control groups ( $109.8 \pm 3.15$ ). Further significant elevation in the (TC) parameter were observed at the end of experiment in NaF treated group comparing to control. The mean values of this parameter in treated group was ( $181.8 \pm 6.66$ ) and ( $256 \pm 3.14$ ), for the same previous treated periods (30 and 60 days respectively).

Table (2): level of Total serum cholesterol level (mg/dl) of adult male rats treated with (NaF) in drinking water for 30 and 60 days.

Day \ Groups	Control G.	Treated G.
Zero	$115.4 \pm 2.92$ A a	$118 \pm 3.22$ A a
30	$109.8 \pm 3.15$ C a	$181.8 \pm 6.66$ A b
60	$120.4 \pm 0.67$ C a	$256 \pm 3.14$ A c

- Values represent Mean  $\pm$  standard error.
- No. of animals in each group = 15.
- The different letters within the columns and rows mean a significant difference at a level of probability ( $p \leq 0.05$ ).
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### 3.Serum Glucose Concentration.

Table (3) illustrated the values of serum Glucose level in male rats after oral intubation of 100 ppm NaF for two months. Data pointed there is no significant differences ( $P \leq 0.05$ ) between groups at zero time. During treated period (after 30 days) a significant increasing in glucose level with mean value of ( $137.4 \pm 4.47$ ) was detected in NaF treated Group in-comparing with other group ( $92.20 \pm 2.59$ ). while at 60 days there is increase in the level of glucose in the treated group.

Table (3): level of glucose (mg/dl) of adult male rats treated with (NaF) in drinking water for 30 and 60 days.

Day \ Groups	Control G.	Treated G.
Zero	90.2 ± 12.13 A a	92.6 ± 11.53 A a
30	92.2 ± 12.59 C a	137.4 ± 14.47 A b
60	93.2 ± 14.61 C a	196.0 ± 17.92 A c

- Values represent Mean ± standard error.
- No. of animals in each group = 15.
- The different letters within the columns and rows mean a significant difference at a level of probability ( $p \leq 0.05$ ).

The effects of fluoride as mentioned by (17) who reported impaired glucose tolerance in 10 of 25 residents of an area with endemic fluorosis. A significant increase (17%) was observed in serum glucose in rats given  $F^-$  in drinking water at 100mg/L for 6 months (18). Additionally, (19) reported decreased insulin, increased plasma glucose and disturbance of the glucose tolerance test in rats after an oral dose of 40µM NaF per 100 g body weight. In *vitro* study showed that exposure of Langerhans islets isolated from rat to NaF, caused depression in insulin secretion in a dose dependent manner (20) with a subsequent elevation in glucose concentration.

Inhibition of enzymes involved in cellular metabolism is one of the mechanical toxic effects of fluoride on enzymes involved in the glycolytic pathway, such as hexokinase, enolase, and pyruvate kinase, these are all subject to  $F^-$  inhibition. Antioxidant enzymes such as superoxide dismutase (SOD).  $Na^+/K^+-ATPases$  are also inhibited by fluoride, leading not only to ATP depletion, but to

disturbances in the cell's membrane potential (21), with subsequent stimulation of glucagon dependent pathways like gluconeogenesis, glycogenolysis leading to an elevation of serum glucose.

Interestingly, mitochondria considers the main target of fluoride toxicity (22), because of increasing production of O<sub>2</sub>-(23). There is no evidence that explain linking between fluoride exposure and insulin expression. However, there was an evidence that fluoride exposure may be influence the transcription of many gens (24). Also its effects on expression of insulin mRNA production cells (25).

Apoptosis plays a rode role in fluoride toxicity in all cells (26), like pancreatic cells (27), possibly because its role in oxidative stress (28).which is greatly accompanied with a hyperglycemia (29).

### **Histological examination**

**Adrenal gland:** Figure (1) pointed to histological section of adrenal gland in animals treated with 100 ppm of NaF, where sever fatty changes which appeared as large clusters cystic structure in internal layer comparing to normal (Fig 2). There are some changes were observed in Thyroid and adrenal glands supported the (Functional) changes in all experimental groups.

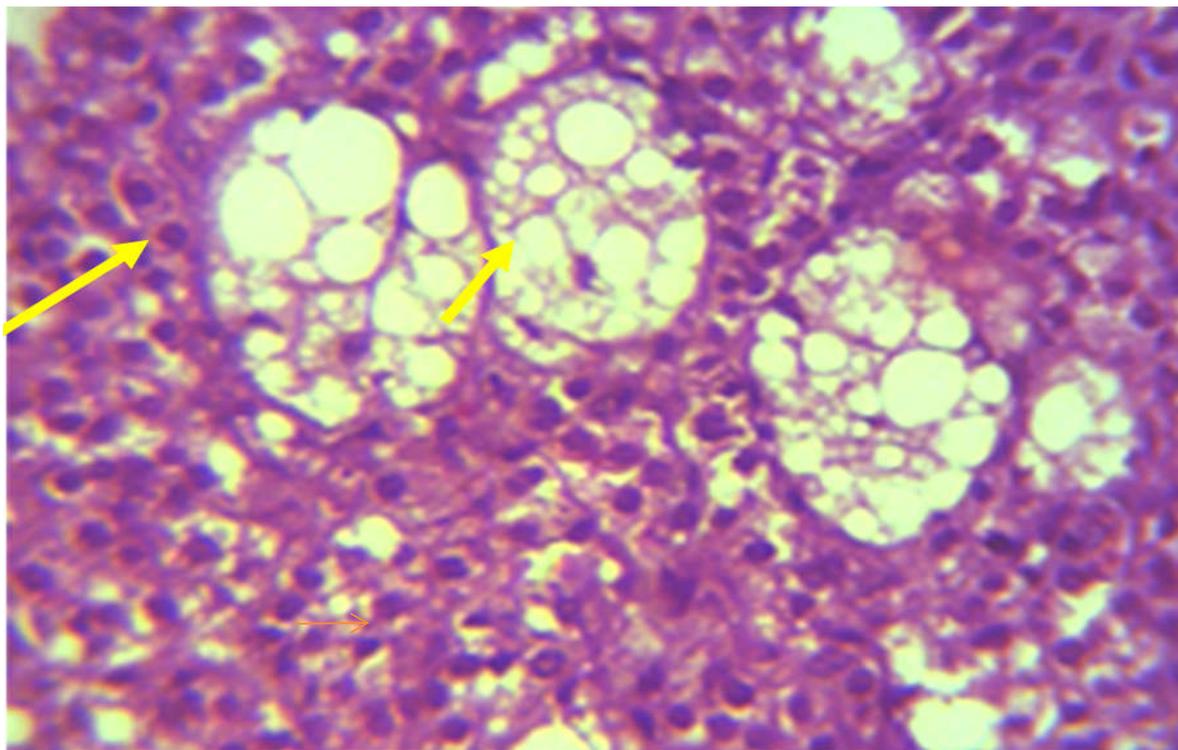


Figure (1) Histological section in adrenal of rats treated with 100 ppm of NaF for 60 days, reveals severe fatty change which appear large cluster cystic structure in internal layer (  ) (H and E 40X)

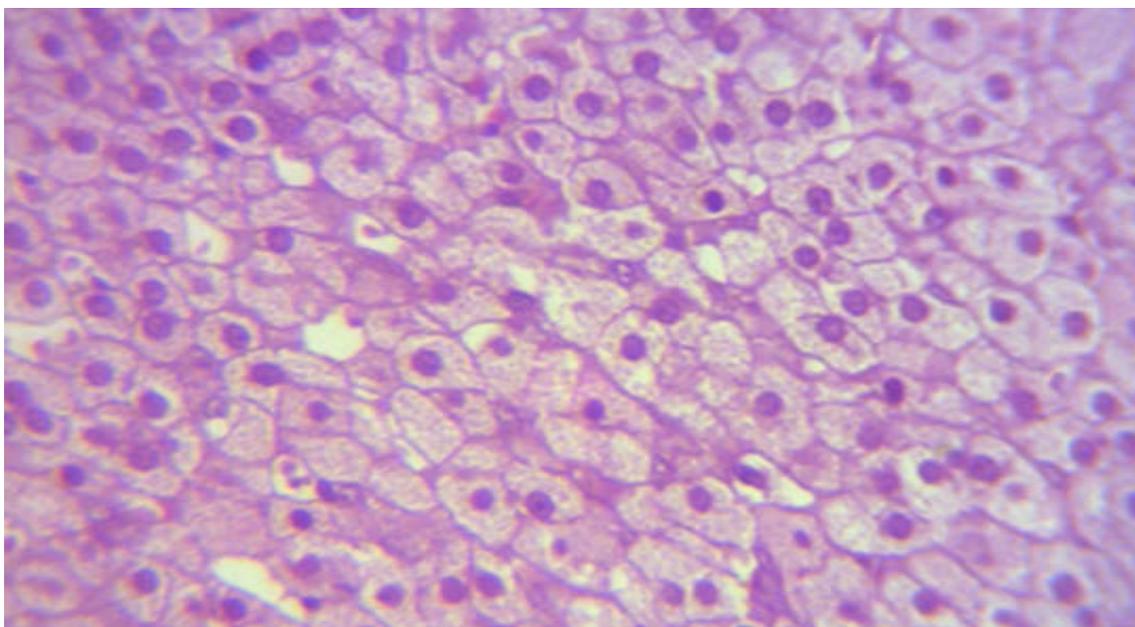


Figure (2) Histological section in control rats, showed normal structure of adrenal gland.(H and E 40X)

### Thyroid gland

Histological sections in the thyroid gland post 60 day of treatment with 100 ppm NaF showed irregular colloid substances with fluctuation, together with hyperplasia of epithelial lining cells of acinied (Fig 3) comparing to histological section of normal thyroid gland (Fig 4). Besides, uniform colloid substance .

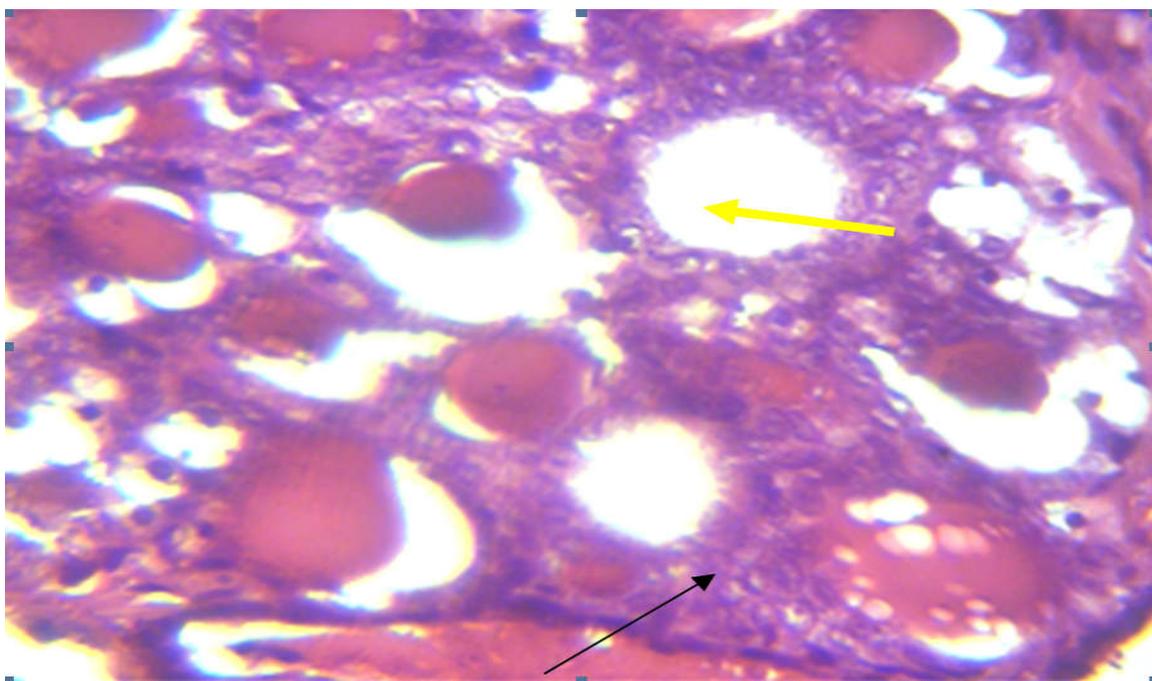


Figure (3) Histological section in the thyroid gland of rats treated with 100 ppm of NaF for 60 days, showed acini field with irregular colloid substance with vacuolation together with hyperplasia of epithelial lining cells of acinied ( —>).(H and E 40X).

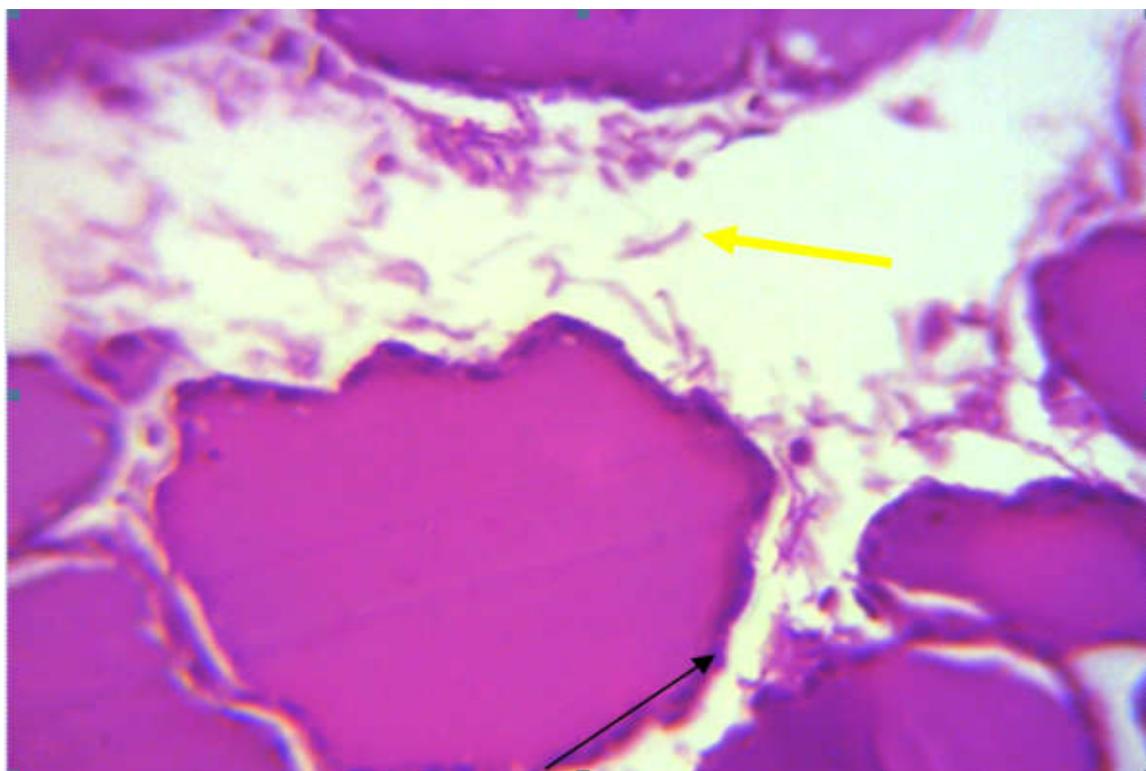


Figure (4) Histological section in control rats, showed normal structure of thyroid gland.(H and E 40X).

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