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Effect of experimentally induced acute fluoride poisoning on the thyroid gland and hormones in geriatric rats

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Summary

The aim of this study was to investigate the effects of fluoride on the thyroid gland and hormones in rats with acute poisoning by different doses of fluoride. For this purpose, a total of 5 groups were formed, including a control (C) group of 8 rats and 4 test groups consisting of 10 rats each. Toxicity was induced with NaF at doses of 45, 60, 70, and 90 mg/kg/bw in the test groups F45, F60, F70, and F90, respectively. During the study, 2 rats died in F45, 3 in both F60 and F70, and 4 in F90. Surviving rats were euthanized 24 hours after poisoning, and blood samples were collected. Urine samples were also taken before and after poisoning. Urinary fluoride levels increased after intoxication and were found to be statistically significant. There were no significant differences in the levels of thyroid hormones TT3, TT4, and FT4 between the test groups and the control group. However, a significant decrease in FT3 levels was observed in the test groups compared to the control group. Although there were no significant changes in TSH hormone levels in the F45 and F90 groups compared to the control group, a significant decrease was observed in the F60 and F70 groups. Histopathological examination revealed follicular degeneration, a decrease in the volume of colloidal fluid, and atypical follicular epithelium in the thyroid glands of the test groups. In conclusion, abnormal changes in the thyroid gland and hormones were observed in rats with acute poisoning by different doses of fluoride.

Keywords: acute, fluoride poisoning, thyroid, geriatric rat

Fluorine is a member of the halogen group in the periodic table. It is rarely found in elemental form in nature and is predominantly present in a compound (fluoride) form because of its high reactivity (26). Harmful levels of fluorides can originate from natural sources and industrial activities. For example, high fluoride levels in the environment may be due to presence of water from deep wells, volcanic ash, and industrial waste (27). Acute fluoride poisoning can occur as a result of accidents involving rodenticides used in agriculture or ingestion of excessive amounts of water, plants, and feed containing high levels of fluoride (6, 25). Currently, the most significant cause of acute fluoride poisoning are dental products (31). Sodium fluoroacetate and sodium fluoride are considered dangerous compounds in acute poisonings (25).

The follicular cells of the thyroid gland produce thyroid hormones, thyroxine (T4), and triiodothyronine (T3), which modulate various physiological processes, including normal growth and development (16, 17). Studies on the effects of fluoride on the thyroid gland have shown contradictory results (9). McLaren (18) reported that fluoride can accumulate in the thyroid, leading to structural and functional changes, while Demole (8) stated that fluoride does not have a specific toxic effect on the thyroid gland and does not accumulate in it.

Although studies exist on the effects of subacute and chronic fluoride poisoning on thyroid hormones, there is a lack of research on the effects of acute fluoride poisoning on these hormones. The aim of this study was to investigate the effects of different toxic doses

of fluoride on the thyroid gland and its hormones in geriatric rats.

Material and methods

Animals. Approval for this study was obtained from the Animal Experiments Local Ethics Committee of Tekirdağ Namık Kemal University (T2023-539). A total of 48 female Wistar Albino rats aged 18-24 months with a mean weight of 285 ± 21 g were included in the study. The animals were housed under a temperature of 25 ± 2°C, a relative humidity of 40-50%, and a 12-hour light-dark cycle in cages with daily cleaning. They were provided ad-libitum access to food and containers filled with city tap water.

Experimental design. One control group (C) and 4 test groups (F45, F60, F70, and F90) were formed. Eight rats in the control group were given distilled water via intragastric gavage. Four test groups of 10 rats each received a single intragastric dose of sodium fluoride (NaF; Merck M106449) dissolved in distilled water at doses of 45, 60, 70, and 90 mg/kg, respectively. After receiving the test doses, the animals were returned to their cages. The rats were given ad libitum access to water and a commercial pelleted diet (Optima Besin Maddeleri ve Ticaret A.Ş., Turkey). The standard diet composition included 20% crude protein, 3.61% crude cellulose, 3.98% crude oil, 7% ash, 1.03% lysine, 0.62% methionine, 1.04% calcium, 0.5% phosphorus, and 0.05% sodium. Euthanasia was performed 24 hours after intragastric gavage by taking blood from the heart under diethyl ether anaesthesia.

Biochemical analyses. Fluoride determination: Equal volumes of TISAB II (HANNA, USA) were added to urine samples collected from the rats and thoroughly mixed with a magnetic stirrer. Urinary fluoride levels were measured with a combined fluoride electrode (HANNA, USA).

Hormone determination. Blood samples were allowed to clot and were then centrifuged at 2000 rpm for 20 minutes. The resulting sera were stored at –80°C until analysis. Serum levels of TT3 (AFG scientific, USA), TT4 (AFG scientific, USA), FT3 (BT Labs, China), FT4 (BT Labs, China), and TSH (BT Labs, China) were determined using commercial ELISA kits according to the manufacturers' instructions.

Histopathological examination. Thyroid tissue samples were kept in 10% formalin fixation solution. The tissues were subjected to routine histopathological procedures and blocked in paraffin. Approximately 5 µm thick sections were taken and stained with hematoxylin and eosine.

The preparations were examined and photographed under a light microscope (Olympus CX41, Zeiss Primo star). The tissue sections were scored histopathologically according to follicular degeneration (FD), decrease in colloidal fluid (DCF), and atypical follicle epithelium (AFE) (score 0: no structural damage; score 1: minor damage; score 2: moderate damage; score 3: severe damage) (2, 15).

Statistical analyses. The statistical analysis of the data was conducted using the SPSS 24.0 package program. All values were presented as mean ± standard deviation. The normality of values measured in the study groups was assessed using the Shapiro-Wilk test. One-Way ANOVA was employed to compare thyroid hormone levels between the groups if the data exhibited normal (parametric) distribution, whereas the Kruskal-Wallis H test was used for non-parametric distribution. Following the scoring of histopathological changes in thyroid tissue, the Kruskal-Wallis H test was used to ascertain differences between groups. Additionally, the Wilcoxon test was used to compare pre- and post-application urinary fluoride levels within the same groups. A significance level of $p < 0.05$ was deemed statistically significant.

Results and discussion

During the study, 2 animals in the F45 group, 3 animals in the F60 and F70 groups, and 4 animals in the F90 group died within the first 2-4 hours.

There was no significant difference in urinary fluoride levels in the control group before and after the administration of distilled water, whereas a statistically significant increase was observed in the test groups after the administration of NaF (Tab. 1). While urinary fluoride levels before NaF application were not statistically significant in any of the groups, significant differences were detected after NaF application.

Tab. 1. Urine fluoride levels in the control group and the test groups

Group	Urine Fluoride Levels (ppm)			
	n	Before application	n	After application
Control	8	1.32 ± 0.35^{a}	8	1.39 ± 0.27^{a}
Test Groups		Before NaF application		After NaF application
F45	10	1.93 ± 0.42^{a}	8	25.80 ± 3.20***^{b}
F60	10	2.15 ± 0.62^{a}	7	35.50 ± 5.39***^{c}
F70	10	1.94 ± 0.48^{a}	7	54.00 ± 10.20***^{d}
F90	10	1.62 ± 0.76^{a}	6	70.16 ± 4.27***^{e}

Explanations: Letters were used in comparisons of urine fluoride levels between all groups before and after the application. Different letters in the same column indicate statistical significance. *** – sign was used to compare the urine fluoride levels of the groups in the same row before and after the application; *** $p < 0.001$ indicates significance

There were no statistically significant differences between the control group and the test groups in TT3, TT4 and FT4 hormone levels, but a significant decrease was detected in FT3. A statistically significant decrease in the TSH hormone level was detected in the F60 and F70 groups compared to the control (Tab. 2).

Macroscopically, no pathological changes were observed in the thyroid tissues across all groups. Histopathological examination, however, revealed statistically significant differences between the control group and the test groups concerning follicular degeneration (FD), decrease in colloidal fluid (DCF), and atypical follicle epithelium (AFE) (Tab. 3, 4). Additionally, the F45 group exhibited notable differences in DCF compared to the F70 and F90 groups. These statistical differences are presented in Table 4. No microscopic alterations were observed in the con-

Tab. 2. Serum thyroid hormone levels in the control group and the test groups

Parameters Groups	n	T3 (pg/ml) $\bar{x} \pm SD$	T4 (ng/ml) $\bar{x} \pm SD$	FT3 (ng/L) $\bar{x} \pm SD$	FT4 (pmol/L) $\bar{x} \pm SD$	TSH (mIU/ml) $\bar{x} \pm SD$
C	8	2366.01 ± 419.66	296.98 ± 33.49	16.11 ± 0.62^{a}	17.08 ± 1.55	3.73 ± 0.91^{a}
F45	8	2442.73 ± 218.56	239.84 ± 102.5	13.15 ± 1.72^{b**}	17.15 ± 1.50	3.15 ± 1.08^{a}
F60	7	2342.13 ± 516.81	297.79 ± 32.96	11.69 ± 3.49^{b*}	16.56 ± 1.86	0.76 ± 0.29^{b***}
F70	7	2100.41 ± 252.20	300.62 ± 37.33	11.15 ± 2.03^{b***}	16.29 ± 2.29	1.41 ± 1.15^{b**}
F90	6	2451.78 ± 656.60	252.88 ± 55.01	13.05 ± 2.12^{b**}	18.67 ± 2.38	3.80 ± 1.28^{a}

Explanations: Different letters in the same column indicate statistical significance between groups; * – $p < 0.05$, ** – $p < 0.01$, and *** – $p < 0.001$ indicate significance of differences between the control group with the test groups

Tab. 3. Scoring of thyroid tissue changes based on histopathological findings

Histopathological findings	Control (n = 8)				Test Groups															
					F45 (n = 8)				F60 (n = 7)				F70 (n = 7)				F90 (n = 6)			
The score values	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3
Follicular degeneration (FD)	8	0	0	0	0	8	0	0	0	7	0	0	0	7	1	0	0	6	0	0
Decrease in colloidal fluid (DCF)	8	0	0	0	0	4	4	0	0	5	2	0	0	6	0	0	0	6	0	0
Atypical follicle epithelium (AFE)	8	0	0	0	0	8	0	0	0	7	0	0	0	7	1	0	0	6	0	0

Explanations: Score 0 – no structural damage; Score 1 – minor damage; Score 2 – moderate damage; Score 3 – severe damage (Koyu et al. (15), Akkurt et al. (2))

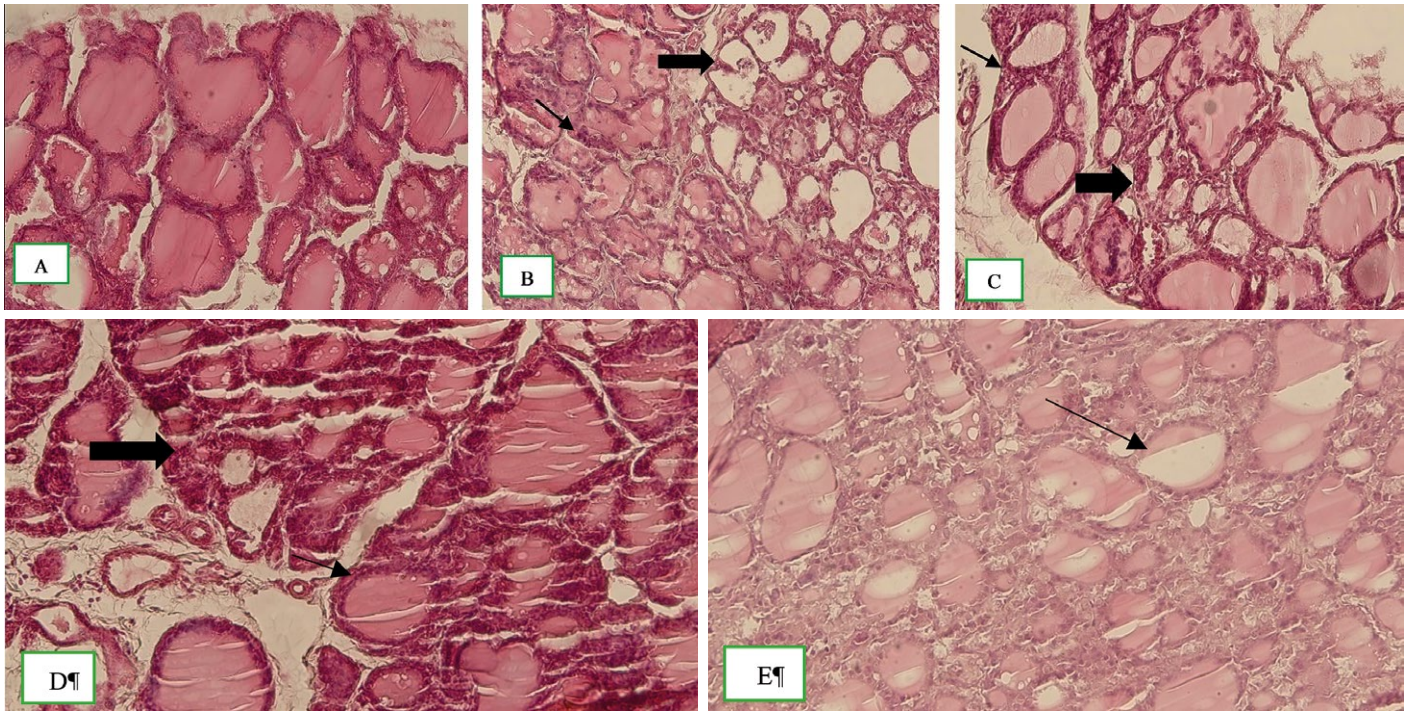

Fig. 1. Histopathological findings in thyroid tissue after acute floride exposure. A. No microscopic changes in the control group. B. Follicular degeneration (thin arrow) and a decrease in colloidal fluid (thick arrow) in the group F45 (H&E, × 200). C. Decrease in colloidal fluid (thick arrow) and atypical follicle epithelium (thin arrow) in the group F60 (H&E, × 200). D. Atypical follicle epithelium (thick arrow) and follicular degeneration (thin arrow) in the group F70 (H&E, × 200). E. A decrease in colloidal fluid (thin arrow) in the group F90 (H&E, × 200).

trol group (see Fig. 1). The test groups showed a noticeable flattening of the thyroid follicular epithelium, an increased acidophilic staining of cytoplasm, and the presence of vacuoles in the epithelium of some follicles.

In the rats subjected to fluoride intoxication, we observed clinical signs, such as hypersalivation, depression, lethargy, diarrhea, increased heart rate, and death. In geriatric rats in which acute fluoride poisoning was induced, the number of deaths increased with the dosage. In previous studies, Panneerselvam et al. (21) reported that no death was observed in rats aged 3 months and 2 weeks that had been intoxicated with NaF at doses of 45 and 90 mg/kg. Whitford et al. (33) found that in rats weighing between 152 and 202 g, rates of mortality due to NaF toxicity within the first

Tab. 4. Statistical significance of histopathological findings between groups

Histopathological findings Group comparisons	Follicular degeneration (FD)	Decrease in colloidal fluid (DCF)	Atypical follicle epithelium (AFE)
C-F45	P < 0.001	P < 0.01	P < 0.001
C-F60	P < 0.01	P < 0.01	P < 0.01
C-F70	P < 0.01	P < 0.01	P < 0.01
C-F90	P < 0.01	P < 0.01	P < 0.01
F45-F60	NS	NS	NS
F45-F70	NS	P < 0.05	NS
F45-F90	NS	P < 0.05	NS
F60-F70	NS	NS	NS
F60-F90	NS	NS	NS
F70-F90	NS	NS	NS

Explanation: NS – no significant

24 hours were 10% (1 out of 10) for the 67.8 mg/kg dose and 20% (2 out of 10) for the 88.1 mg/kg dose. Additionally, while no deaths occurred at doses of 50, 60.5, 66.6 and 73.2 mg/kg, it was reported that 37.5% of the rats (3 out of 8) died from a dose of 90.8 mg/kg. In our study, 2, 3, 3, and 4 (20%, 30%, 30%, 40%) deaths were observed at doses of 45, 60, 70, and 90 mg/kg, respectively. Contrary to the findings of Panneerselvam et al. (21) and Whitford et al. (33), the high mortality rate in our study may be due to the fact that old rats may be more sensitive to toxic fluoride doses compared to young rats.

Bone, tooth, nail, hair, urine, blood, and plasma fluoride concentrations are used to estimate fluoride exposure (29, 32). Fluoride is rapidly absorbed from the gastrointestinal tract and assimilates into calcified tissues, accumulating primarily in teeth and bones. The kidneys serve as the main route for fluoride elimination, with over 50% of fluoride excreted in the urine within 24 hours after ingestion (24, 28). Urinary fluoride concentration appears to have significantly increased in the test groups. Elevated urinary fluoride concentration indicates recent exposure or continual release from bone (7). In our study, prior to NaF application in the test groups, urinary fluoride concentration was within normal ranges. However, it rose to a notably high level after NaF application.

Animal experiments have shown that high fluoride concentrations have significant pathological effects on the thyroid gland and hormones (10, 30, 34). In various studies on rats treated with fluoride at different doses over different time periods, decreases in T3, T4 (1, 10, 20, 23, 30), FT3, and FT4 levels (30), along with increases in TSH (1, 10) were observed. An increase in TSH levels and a decrease in T3 and T4 levels are suggested to lead to hypothyroidism (10). In a study by Foda and Shams (11), no significant changes were observed in serum thyroid hormones with NaF administered at a dose of 40 mg/kg for 35 days. However, it has been noted that fluoride might have harmful effects on the thyroid gland and the hypothalamic-pituitary-thyroid (HPT) axis.

In our study, we found no significant differences in TT3, TT4, and FT4 levels between the test and control groups. However, FT3 levels showed a decrease. While TSH values in the F45 and F90 groups were not significantly different from those in the control group, we observed a decrease in the F60 and F70 groups. It has been reported that trauma, burns, sepsis, and acute intoxications have significant implications for the endocrine status, leading to „euthyroid sick syndrome" (5). Euthyroid sick syndrome (ESS), also known as nonthyroidal illness syndrome (NTIS), manifests with decreased levels of serum thyroid hormones (T3, free T3, and/or T4), while TSH levels remain normal or decrease (12, 14). In our study, we observed a decrease in FT3 levels across all test groups, while a decrease in TSH levels was noted in the F60 and F70 groups. However, TSH levels remained normal in the F45 and F90 groups. This suggests the possibility of euthyroid sick syndrome in the animals.

During illness, profound changes can occur in the hypothalamic-pituitary-thyroid (HPT) axis. The most consistent change is a decrease in serum triiodothyronine (T3) levels, but serum thyroxine (T4) can also decrease in severe illness. The persistence of normal or even decreased serum thyrotropin (TSH) levels combined with reduced serum thyroid hormone concentrations reveals a significant alteration in the regulation of the HPT axis set point. From the perspective of the hypothalamic-pituitary-thyroid-target organ axis, low fT3 and fT4 levels reflect thyroid and target organ changes. However, low TSH levels also reflect changes in the pituitary and hypothalamus, indicating a deterioration in the health status (19). The prevalence of euthyroid sick syndrome, especially in elderly patients with critical illness, has been reported to increase with age. Additionally, in elderly individuals, particularly geriatric patients with critical illness, euthyroid sick syndrome has been shown to be associated with poor prognosis, development of complications, aggravation of illness, and mortality (13).

The thyroid gland is one of the organs most sensitive to excessive fluoride (34). Subacute poisoning of rats with different fluoride doses (3, 22) has been shown to result in a decreased colloidal fluid volume and degeneration of follicular epithelium. A study on rats (4) also detected a decrease colloidal fluid in thyroid follicles, vascularity and proliferative changes in thyroid tissues. Similar histopathological changes were observed in our study groups.

In conclusion, we believe that NaF poisoning can lead to thyroid dysfunction, exerting particularly significant effects on FT3 and TSH levels. Further detailed research is needed to assess the potential risks of acute NaF toxicity for thyroid health.

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