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The effects of thymoquinone and curcumin on the thyroid in rats with subacute fluoride poisoning

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Abstract

The purpose of this study was to determine the protective effect of thymoquinone (TQ), curcumin (CUR), and a TQ+CUR combination on thyroid hormones and histopathology in an experimentally-induced, subacute, sodium fluoride (NaF) toxicity rat model. Control, sham, and test groups (NaF, NaF+TQ, NaF+CUR, and NaF+TQ+CUR) were established in the study. At the end of the experiment, test groups had higher urine fluoride concentrations. Triiodothyronine (T3), thyroxin (T4), free triiodothyronine (FT3), and free thyroxin (FT4) levels decreased substantially, and thyroid-stimulating hormone (TSH) levels increased substantially when the control and test groups were compared. T3 and T4 decreased and TSH increased in the NaF group compared to the NaF+TQ, NaF+CUR, and NaF+TQ+CUR groups. Microscopic study of thyroid tissues revealed that the test groups showed statistically significant differences from the control group in terms of follicular degeneration (FD), decrease in colloidal fluid (DCF), fibrosis, atypical follicle epithelium (AFE), and mononuclear cell infiltration (MCI). When the NaF group and other test groups were compared, statistical significance was observed in the histopathological scoring of DCF, AFE, and MCI in the NaF+TQ group; FD, DCF, AFE, and MCI in the NaF+CUR group; and DCF, AFE, and MCI in the NaF+TQ+CUR group. Fluoride toxicity, therefore, caused problems with the thyroid and hormones. The study revealed that TQ and CUR combined with fluoride could not normalize the thyroid gland and thyroid hormones but could alleviate thyroid gland damage and provide a partial improvement in thyroid hormone levels.

Keyword: fluoride, rat, thyroid
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Introduction

Fluorine, a halogen, is an essential element for the body (Cerklewski, 1997; Çetin *et al.*, 2004). Almost all feed and water sources contain small amounts of fluoride, thus animals are regularly exposed to it throughout their lives. Although low levels of fluoride in the food have been shown to benefit bone and tooth development, elevated levels of fluoride in the body have been shown to harm teeth, bones, and other body systems (Shupe *et al.*,1964; Shupe & Olson 1971; Thompson, 2012). Excessive fluoride consumption and accumulation have been linked to thyroid dysfunction and histopathological changes in the thyroid gland (Patil & Dhurvey, 2015). It is reported that fluoride's harmful effects on the thyroid

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are caused by acute stress in the body's organs, which can cause serious illness and attack thyroid cells, replacing them with iodine ions (Foda & Shams, 2021).

Thymoquinone (TQ) is the primary active phenolic compound derived from the essential oil of *Nigella sativa* seed, and it has long been used to treat a variety of diseases due to its high antioxidant properties. TQ exhibits anti-inflammatory, antibacterial, and anticancer properties, along with many other beneficial effects (Guzelsoy *et al.*, 2018). *Nigella sativa* is reported to improve thyroiditis in patients with Hashimoto's thyroiditis (Farhangi *et al.*, 2016).

Curcumin is a component of turmeric used in traditional medicine (Nelson *et al*., 2017) that has antioxidant, anticarcinogenic, antimutagenic, antidiabetic, antibacterial, antiviral, and anti-inflammatory properties (Becit *et al*., 2017). CUR had an apoptotic effect in experimental hypothyroidism (Sourour, 2014). It inhibits cancer cell proliferation at various stages of the cell cycle and induces apoptosis in tumour cells (Su *et al.*, 2006). CUR inhibits cell viability in papillary thyroid cancer cells and promotes cell apoptosis in a dose-dependent manner (Song *et al.*, 2012). CUR has been shown to protect against hypothyroidism caused by potassium dichromate (Aboul-Fotouh *et al.*, 2018) and fluoride toxicity (Abdelaleem *et al.*, 2018).

Fluoride is present in high concentrations in some regions of the world in groundwater, vegetation, and soil (Johnston & Strobel, 2020). Fluorosis affects skeletal parts of the body, the brain, liver, kidney, thyroid, and spinal cord tissues in humans, animals, and birds that inhabit these regions (Shahab *et al*., 2017). The thyroid gland and its hormones play a critical role in regulating the development, differentiation, and metabolism of all mammalian tissues (Zhan *et al*., 2006). The lack of research on the effects of combined use of TQ and TQ+CUR on the thyroid gland and hormones in fluoride poisoning led us to conduct this study. The purpose of this study was to determine what kind of structural and histological changes occur in the thyroid glands of rats exposed to NaF, as well as how thyroid hormones are affected, and whether the combination of TQ, CUR, and a TQ+CUR has protective or therapeutic effects.

Materials and Methods

Tekirdağ Namık Kemal University Animal Experiments Local Ethics Committee granted permission for the study, numbered T2021-595, on 02.03.2021. The study included forty-four male, adult Wistar Albino rats aged 12–13 weeks, weighing 260–300 g for the experiment. The rats were kept in standard laboratory conditions at 23 ± 2 ℃, 40–50% relative humidity, and a 12 h light:12 h dark cycle. *Ad libitum* access to tap water (F concentration, 0.32 ppm) and a commercial pelleted diet (Optima Besin Maddeleri ve Ticaret A.Ş., Türkiye) was provided to them. The standard diet had 20% crude protein, 3.61% crude cellulose, 3.98% crude oil, and 7% ash. There was also 1.03% lysine, 0.62% methionine, 1.04% calcium, 0.5% phosphorus, and 0.05% sodium in the diet.

The rats were divided into six groups: control (n = 6), sham (n = 6), and four test groups (NaF (n = 8), NaF+TQ (n = 8), NaF+CUR (n = 8), and NaF+TQ+CUR (n = 8). The control group received no treatment. For 14 d, the sham group received 0.5 ml distilled water by oral gavage and 0.1 ml dimethyl-sulfoxide (DMSO) intraperitoneally (IP), while the test groups received NaF (30 mg/kg/day dose) by gavage. TQ (15 mg/kg/day IP dose), CUR (20 mg/kg/day IP dose), and TQ+CUR applications began one week before NaF administration and continued throughout the experiment.

TQ (Sigma–Aldrich, USA) and CUR (Sigma–Aldrich, USA) were dissolved in DMSO (Merck, Germany) and NaF (Merck, Germany) was dissolved in distilled water. All the chemicals used in the experiments were analytical grade.

On days 7 and 21 of the study, urine samples from all groups were collected. An equal volume of TISAB II (Total ionic strength adjustment buffer) (HANNA, USA) was added to the urine samples taken from the rats and mixed with a magnetic stirrer. Urine was measured with a specific fluoride electrode (HANNA, USA), calibrated with fresh, serially-diluted standard solutions using an ionometer (HANNA, USA).

At the end of the experiment, the rats were euthanized by decapitation under anaesthesia. Trunk blood was collected in sterile plastic tubes and allowed to clot. The blood samples were centrifuged at 3000 rpm for 20 min. Blood sera were separated and stored at -80 °C until analysis. Serum T3 (AFG Scientific, USA), T4 (AFG Scientific, USA), FT3 (BT Labs, China), FT4 (BT Labs, China), and TSH (BT Labs, China) levels were measured using commercial enzyme-linked immunosorbent assay (ELISA) kits, as directed by the manufacturer.

A routine tissue process was used to examine the histopathological findings of thyroid tissues. The tissues were immersed in a 10% buffered formaldehyde solution for one night. Paraffin-embedded tissues were cut at 3-µm thickness and stained with haematoxylin and eosin (Abdelaleem *et al.*, 2018). The preparations were examined and photographed using a light microscope (Olympus CX41, Zeiss Primo star). Histopathological scoring was used to grade tissue sections according to the follicular

degeneration (FD), decrease in colloidal fluid (DCF), fibrosis, atypical follicle epithelium (AFE), and mononuclear cell infiltration (MCI) (score 0, no structural damage; score 1, minor damage; score 2, moderate damage; score 3, severe damage) (Koyu *et al.,* 2014; Akkurt *et al.,* 2021).

Statistical analysis was conducted using the SPSS 24.0 package program. All values were expressed as mean ± standard error (X ± Sx). A one-way analysis of variance (ANOVA) was used to compare thyroid hormone levels between groups. After scoring the histopathological changes in the thyroid tissue, the Kruskal–Wallis H test was used to establish the difference between the groups. The Wilcoxon test was used to compare urine fluoride levels on the 7th and 21st days (Özdamar, 2019). Statistical significance was defined as a P value of 0.05 or less.

Results and Discussion

In the control and sham groups, no significant statistical difference could be detected in the urine fluorine concentration on days 7 and 21. When the urinary fluoride concentrations on the seventh day before NaF administration and the 21st day after NaF administration were compared in the test groups, a statistically significant increase in the urine fluoride concentration after NaF administration was found. Urinary fluoride concentrations did not differ between the test groups (Table 1).

Table 1 Urine fluoride concentrations on days 7 and 21

Groups	n	Day 7 (ppm)	Day 21 (ppm)
Control	6	1,63 ± 0,25^{a}	1,79 ± 0,22^{a}
Sham	6	1,80 ± 0,17^{a}	1,85 ± 0,16^{a}
Test Groups	Before NaF application		After NaF application
NaF	8	1,70 ± 0,11^{a}	9,64 ± 0,65^{b***}
NaF+TQ	8	1,81 ± 0,13^{a}	9,87 ± 0,49^{b***}
NaF+CUR	8	1,62 ± 0,16^{a}	11,35 ± 0,74^{b***}
NaF+TQ+CUR	8	1,4 ± 0,10^{a}	9,63 ± 0,99^{b***}

Superscript letters were used in the comparison of all groups. The difference between values with different letters on the same column is significant. Superscript asterisks were used for comparisons of the same group on the 7th and 21st days. ****P* <0.001 indicates significance. X ± Sx (Mean value ± Standard error). Rats were divided into six groups: control (n = 6), sham (n = 6), and four test groups [NaF (n = 8), NaF+ thymoquinone, TQ (n = 8), NaF+ curcumin, CUR (n = 8), and NaF+TQ+CUR (n = 8)]

Test groups showed a marked increase in TSH levels and a marked decrease in T3, T4, and FT4 levels compared to the control and sham. The serum FT3 level in the control group was higher than in the other groups. The NaF group showed a decrease in serum T3, T4, and an increase in serum TSH compared to the other test groups, however, there was no statistical significance in serum FT3 and FT4 levels (Table 2).

Based on a macroscopic examination, none of the thyroid tissues of the experimental groups had any pathological lesions or changes The histopathological scoring between the groups revealed different values (Table 3). There were differences in FD, DCF, fibrosis, AFE, and MCI in the test groups (*P* <0.01). The control and sham groups showed no histopathological changes (Figure 1). The NaF group showed flattening of the follicular epithelium, increased acidophilic staining of the cytoplasm, and karyopyknosis of the nuclei. The NaF group also had vacuoles in the follicles and vascular congestion in the thyroid tissues. The NaF+TQ, NaF +CUR, and NaF +TQ+CUR groups were found to be less affected than the NaF group in terms of FD, DCF, fibrosis, AFE, and MCI. There was a difference in FD (*P* <0.01), DCF (*P* <0.01), fibrosis (*P* <0.05), AFE (*P* <0.01), and MCI (*P* <0.01) between the NaF and NaF+CUR groups. A difference between the NaF and NaF+TQ groups was also seen in DCF (*P* <0.01), AFE (*P* <0.01), and MCI (*P* <0.05). There was a statistical difference in FD (*P* <0.01), DCF (*P* <0.01), AFE (*P* <0.01), and MCI (*P* <0.01) between the NaF and NaF+TQ+CUR groups.

Table 2 Serum thyroid hormone levels of rats divided into six treatment groups

Groups	n	T3 (pg/ml)	T4 (ng/ml)	FT3 (ng/L)	FT4 (pmol/L)	TSH (mIU/ml)
Control	6	2274,58 ± 62,9^{a}	332,00 ± 8,13^{a}	15,90 ± 1,28^{a}	22,39 ± 0,73^{a}	2,95 ± 0,05^{a}
Sham	6	2123,50 ± 59,9^{a}	334,13 ± 14,36^{a}	13,24 ± 0,49^{b*}	22,73 ± 1,14^{a}	2,91 ± 0,06^{a}
Test Groups						
NaF	8	1628,26 ± 81,3^{b***}	269,21 ± 5,33^{b***}	11,55 ± 0,57^{bc***}	18,16 ± 0,50^{b***}	3,63 ± 0,10^{b***}
NaF+TQ	8	1933,59 ± 54,3^{c**+++}	299,13 ± 4,50^{c**++}	12,60 ± 0,76^{bc**}	19,79 ± 0,39^{b*}	3,22 ± 0,04^{c*+++}
NaF+CUR	8	1837,40 ± 44,69^{c***+}	293,66 ± 6,03^{c**+}	11,57 ± 0,55^{bc***}	18,41 ± 0,60^{b***}	3,37 ± 0,06^{c***++}
NaF+TQ+CUR	8	1920,91 ± 59,86^{c***++}	296,32 ± 4,48^{c**++}	12,06 ± 0,74^{bc**}	19,06 ± 0,67^{b**}	3,34 ± 0,07^{c***++}

Superscript letters were used in the comparison of all groups. The difference between values in the same column with different letters is significant. (*): When comparing the control and other groups, *P <0.05, **P <0.01, and ***P <0.001 indicate significance. (+): When comparing NaF group to NaF+TQ, NaF+CUR, and NaF+TQ+CUR groups, ^{+}P <0.05, ^{++}P <0.01, and ^{+++}P <0.001 indicate significance. X ± Sx (Mean value ± Standard error). Rats were divided into six groups: control (n = 6), sham (n = 6), and four test groups [NaF (n = 8), NaF+ thymoquinone, TQ (n = 8), NaF+ curcumin, CUR (n = 8), and NaF+TQ+CUR (n = 8)]

Table 3 Grading thyroid tissue changes based on histopathological findings

Histopathological Findings	Control (n = 6)				Sham (n = 6)				Test Groups															
									NaF (n = 8)				NaF + TQ (n = 8)				NaF + CUR (n = 8)				NaF + TQ + CUR (n = 8)			
The score values	**0**	**1**	**2**	**3**	**0**	**1**	**2**	**3**	**0**	**1**	**2**	**3**	**0**	**1**	**2**	**3**	**0**	**1**	**2**	**3**	**0**	**1**	**2**	**3**
Follicular degeneration (FD)	6	0	0	0	6	0	0	0	0	0	6	2	0	4	4	0	0	5	3	0	0	6	2	0
Decrease in colloidal fluid (DCF)	6	0	0	0	6	0	0	0	0	0	4	4	0	5	3	0	0	5	3	0	0	6	2	0
Fibrosis	6	0	0	0	6	0	0	0	0	0	7	1	0	1	7	0	0	4	4	0	0	2	6	0
Atypical Follicle epithelium (AFE)	6	0	0	0	6	0	0	0	0	0	5	3	0	7	1	0	0	6	2	0	0	5	3	0
Mononuclear Cell Infiltration (MCI)	6	0	0	0	6	0	0	0	0	0	4	4	0	6	2	0	0	5	3	0	0	5	3	0

Score 0, no structural damage; score 1, minor damage; score 2, moderate damage; score 3, severe damage (Koyu *et al.,* 2014; Akkurt *et al.,* 2021)

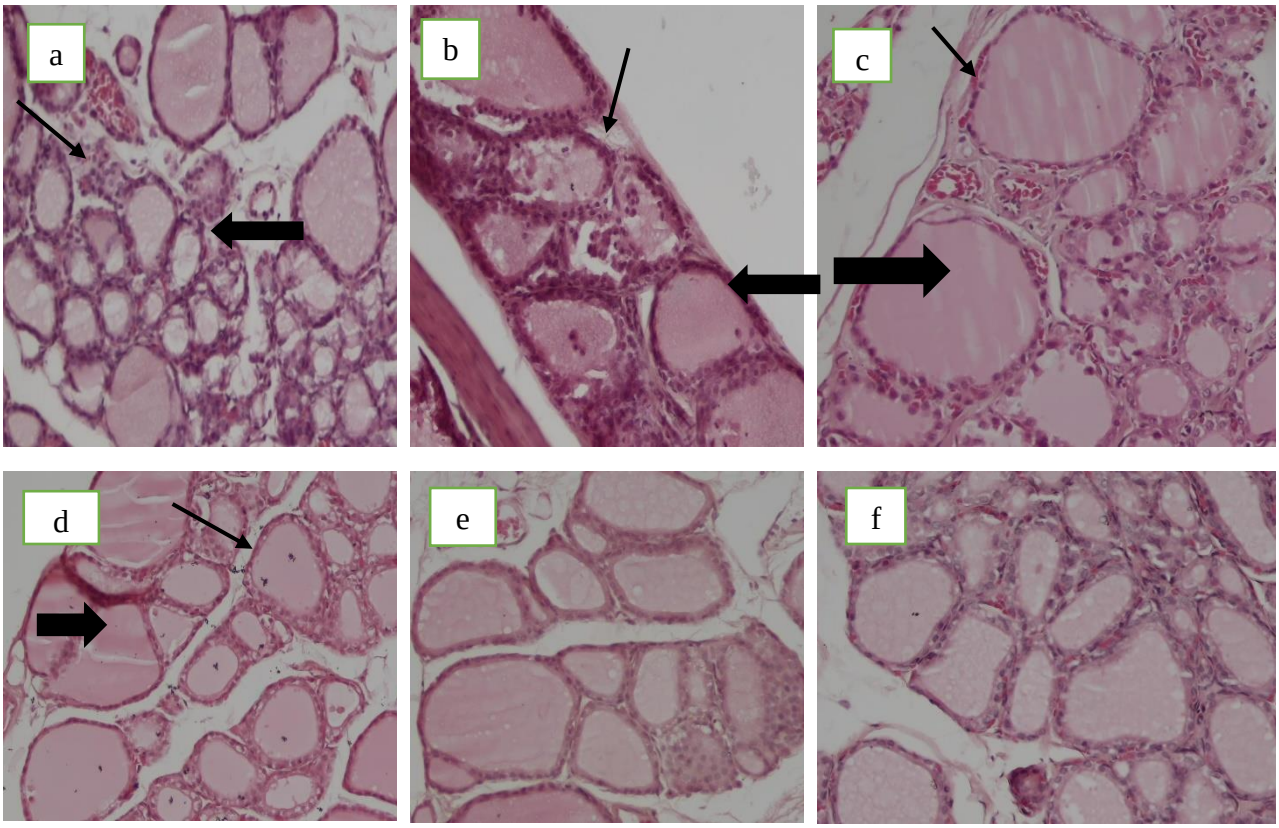

Figure 1 Microscopic appearance of thyroid tissues stained with haematoxylin–eosin (H&E) of NaF, NaF+TQ, NaF+CUR, NaF+TQ+CUR, Control, and Sham groups. (a) The DCF (thick arrow) with FD and MCI (thin arrow) in NaF group, H&E, 200× magnification. (b) FD (thin arrow) and DCF (thick arrow) in NaF+TQ+CUR group, H&E, 200× magnification. (c) AFE (thin arrow) with FD and DCF (thick arrow) in the NaF+CUR group, H&E, 200× magnification. (d) FD (thin arrow) and DCF (thick arrow) in NaF+TQ group, H&E, 200× magnification. (e, f) No histopathological findings were seen in the control and sham groups, normal thyroid tissue, H&E, 200× magnification. Rats were divided into six groups: control (n = 6), sham (n = 6), and four test groups [NaF (n = 8), NaF+ thymoquinone, TQ (n = 8), NaF+ curcumin, CUR (n = 8), and NaF+TQ+CUR (n = 8)]; follicular degeneration (FD), decrease in colloidal fluid (DCF), atypical follicle epithelium (AFE), and mononuclear cell infiltration (MCI)

Fluoride exposure is estimated using the fluoride concentrations in bone, teeth, nails, hair, urine, blood, or plasma (Vine, 1994; Whitford *et al.,*1994; ATSDR 2003). The digestive tract quickly absorbs fluoride, which is then integrated into calcified tissues and primarily accumulates in teeth and bone. The kidneys are the primary organs for the elimination of fluoride. Within 24 h of consumption, more than 50% of fluoride is excreted in urine (Spencer *et al.*, 1970; Torra *et al.*, 1998). Increased urinary fluorine concentrations indicate recent exposure or long-term release from bone (Cope, 2017). The urine fluorine concentration in the current study was within normal limits before NaF application, but it increased substantially after NaF application in the test groups. This indicates that fluorine poisoning occurred in the rats. There was no statistical difference in urinary fluorine concentration between the test groups, indicating that TQ and CUR have no effect on urinary fluorine concentration.

Some animal experiments (Zhan *et al.*, 2006; Wang *et al.*, 2009; Patil & Dhurvey 2015) have shown that high-dose fluorine concentrations cause substantial pathological effects on the thyroid gland and hormones. Fluorine actively accumulates in the thyroid tissue of rats (Hein, 1956), inhibiting protein and DNA synthesis (Shashi, 1993). Zhan *et al.* (2006) reported a decrease in serum T4 and FT4 levels in pigs treated with fluoride, as well as an increase in TSH, whereas Bouaziz *et al*. (2005) reported a decrease in serum FT3 and FT4 levels in mice. In different studies on rats treated with fluoride at various doses and periods, a decrease in T3, T4 (Wang *et al*., 2009; Nabavi *et al.*, 2011; Sarkar *et al.*, 2014; Dhurvey *et al.*, 2017; Abdelaleem *et al.*, 2018), FT3, and FT4 (Wang *et al.*, 2009) was found, and an increase in TSH (Dhurvey *et al.*, 2017; Abdelaleem *et al.*, 2018). An increase in TSH and a decrease in T3 and T4 levels suggest that NaF intake may cause hypothyroidism (Dhurvey *et al.,* 2017).

Fluoride may cause primary hypothyroidism, as shown in our study by the decrease in serum T3, T4, FT3, FT4 levels and the increase in serum TSH levels in the NaF-treated group compared to the control group. Our results agree with the abovementioned literature. The decrease in thyroid hormones (T3, T4, FT3, and FT4) in fluoride poisoning may be caused by a variety of factors. Iodine

and fluorine both belong to the halogen group and share a similar chemical structure. Iodine is less chemically active than fluoride (Dhurvey *et al.,* 2017). Fluoride damages the cell membrane internally, changes the permeability of the cell (Chinoy *et al*., 1994), attaches to the iodine receptor in the thyroid gland, inhibits Na/K-ATPase activity, and lowers the quantity of iodine in the gland (Dhurvey *et al.*, 2017). Fluoride disrupts thyroid physiology by interfering with the action of deiodinases, which catalyses the conversion of T4 to active T3 and lowers the amount of circulating thyroid hormones (Singh *et al.,* 2014). Fluoride is a TSH analogue. It not only acts like TSH in the absence of fluoride, but it can also enhance TSH's effects and change the expression of G proteins, affecting iodine uptake, transport, and conversion from T4 to T3 (Zhan *et al.,* 2006).

The thyroid gland is one of the most sensitive organs in histopathological and functional responses to excess fluoride (Zhan *et al.,* 2006). Patil and Dhurvey (2015) found a decrease in the amount of colloidal fluid and degeneration in the follicle epithelium after administering 5, 10, 15, and 20 mg/kg doses of NaF to rat drinking water for 15 d. It has been reported that the application of 500 ppm NaF to mice resulted in a decrease in colloidal fluid, vascularity, and proliferative changes in thyroid tissues (Bouaziz *et al.,* 2005). Similar histopathological findings were seen in the NaF group in our study.

Antioxidant-containing biological compounds help to protect cells and tissues from toxic damage. Antioxidant therapy protects cells from the lipid peroxidation caused by long-term fluoride exposure (Hassan & Yousef, 2009). Polyphenolic compounds have been shown in studies to reduce or prevent oxidative damage caused by toxic substances and oxidative materials (Altuntas *et al*., 2002). Thymoquinone (Guzelsoy *et al.,* 2018) and CUR (Becit *et al.,* 2017) are polyphenolic compounds with numerous health benefits, including anti-inflammatory, antioxidant, antimicrobial, and anticancer properties. Curcumin can help treat hypothyroidism induced by $NaClO_3$ (Sourour 2014). It was reported that administering 100 mg/kg/day curcumin along with 15 mg/kg/day NaF via gastric tube for two weeks protected both the function and structure of the thyroid gland from the toxic effects of NaF (Abdelaleem *et al.,* 2018). In another study, rats were given 600 mg/L NaF (in their drinking water) and 20 mg/kg curcumin (IP), and it was discovered that curcumin protected rats from thyroid dysfunction (Nabavi *et al.*, 2011). We found a statistical significance between the NaF+CUR group and the control group, but Abdelaleem *et al*. (2018) and Nabavi *et al*. (2011) observed no statistical significance between the NaF+CUR group and the control group. In our study, serum T3, T4 levels were lower in the NaF+CUR group than in the NaF group, indicating that CUR reduces the negative effect of fluoride on thyroid hormones. The NaF +TQ, NaF +CUR, and NaF +TQ+CUR groups were found to be less affected than the NaF group in histopathological examination of the thyroid glands. Although Abdelaleem *et al.* (2018) and Nabavi *et al.* (2011) reported that curcumin protects against fluoride-induced thyroid disorders, we found that CUR has a mitigating, rather than protective, effect. This could be due to the NaF and CUR doses, duration of administration, and the age of the animals.

To the best of our knowledge, no research has been conducted on the effect of thymoquinone on fluoride-induced thyroid disorders. However, in two separate studies on hypothyroidism induced by Propylthiouracil (PTU) in rats, Baghcheghi *et al.* (2018) reported that decreased T4 in rats with hypothyroidism increased with TQ administration while Ayuob *et al.* (2020) found that T3 and T4 increased and TSH decreased and reported that TQ had a protective effect against hypothyroidism and improved impaired thyroid function.

Our findings show that TQ treatment reduced the biochemical and histopathological damage caused by NaF. Thymoquinone inhibits nitric oxide production through macrophages during inflammation, contributing to the inflammatory process (El-Mahmoudy *et al.*, 2002). In our study, the mononuclear cell infiltration of the NaF+TQ and NaF+ TQ+CUR groups was statistically different to the NaF group. TQ has a curative role in the inflammatory process of the thyroid gland, according to the findings of this study.

Conclusion

As a result, it has been established that fluoride can cause hypothyroidism and that TQ and CUR, rather than normalizing serum thyroid hormone levels and thyroid gland histology, alleviate thyroid injury. Animals living in areas with high fluoride levels in drinking water are at risk and using TQ and CUR in conjunction with routine treatments may help alleviate the toxic effects of NaF. Controlled research should be conducted to investigate the preventive and therapeutic efficacy of TQ and CUR in an experimental fluorosis model developed in farm animals.

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Authors' Contributions

SA and SI collected data for this study, analysed the data, and drafted this article. SA and KY made the applications for the animals. SI performed the histopathological examination and evaluation. All authors approved the final version of the manuscript.

Conflict of Interest Declaration

The authors declare that they have no conflict of interest.

References

Abdelaleem, M.M. El–Tahawy, N.F.G., Abozaid, S.M.M. Abdel–Hakim, S.A., 2018. Possible protective effect of curcumin on the thyroid gland changes induced by sodium fluoride in albino rats: Light and electron microscopic study. Endocr. Regul. 52, 59–68. DOI: 10.2478/enr-2018-0007

Aboul-Fotouh, G.I., El-Nour, R.K.E., Farag, E.A., Boughdady, W.A.A., 2018. Histological study on the possible protective effect of curcumin on potassium dichromate induced hypothyroidism in adult male albino rats. EJH. 41, 220–235. DOI: 10.21608/EJH.2018.13844

Akkurt, G., Kartal, B., Alimoğulları, M., Çaylı, S., Alimoğulları, E., 2021. Effects of regular whey protein consumption on rat thyroid functions. Turk. J. Med. Sci., 30(51), 2213–2221. DOI: 10.3906/sag-20102-70

Altuntas, I., Delibas, N., Sutcu, R., 2002. The effects of organophosphate insecticide methidathion on lipid peroxidation and anti-oxidant enzymes in rat erythrocytes: role of vitamins E and C. Hum. Exp. Toxicol. 21, 681–685. DOI: 10.1191/0960327102ht304oa

ATSDR, 2003. Toxicological Profile for Fluorides, Hydrogen Fluoride, and Fluorine. U.S. Department of Health and Human Services, Public Health Service, Atlanta, GA., 11.

Ayuob, N., Balgoon, M.J., El-Mansy, A.A., Mubarak, W.A., Firgany, A.E.L., 2020. Thymoquinone upregulates catalase gene expression and preserves the structure of the renal cortex of propylthiouracil–induced hypothyroid rats. Oxid. Med. Cell. Longev. 2020, 1–15. https://doi.org/10.1155/2020/3295831

Baghcheghi, Y., Hosseini, M., Beheshti, F., Salmani, H., Anaeigoudar, A., 2018. Thymoquinone reverses learning and memory impairments and brain tissue oxidative damage in hypothyroid juvenile rats. Arq. Neuro–Psyquiatr. 76, 32–40. DOI: 10.1590/0004–282X20170182

Becit, M., Aydın, S., Başaran, N., 2017. Evaluation of therapeutic and toxic effects of curcumin: Review. Turkiye Klinikleri J. Pharm. Sci. 6(2), 126–42. DOI: 10.5336/pharmsci.2017–55938

Bouaziz, H., Soussia, L., Guermazi, F., Zeghal, N., 2005. Fluoride-induced thyroid proliferative changes and their reversal in female mice and their pups. Fluoride 38(3), 185–92.

Cerklewski, F.L., 1997. Fluoride bioavailability: Nutritional and clinical aspects. Nutr. Res. 17(5), 907–929. https://doi.org/10.1016/S0271–5317(97)00057–2

Chinoy, N.J., Walimbe, A.S., Vyas, H.A., Mangla, P., 1994. Transient and reversible fluoride toxicity in some soft tissues of female mice. Fluoride 27, 205–214.

Cope, R., 2017. Miscellaneous Inorganic Toxicants. Veterinary Toxicology for Australia and New Zealand. Ed. Dalefield, R. Elsevier. UK. Pp. 305–316.

Çetin, N., Bilgili, A., Eraslan, G., Koyu, A., 2004. Effect of fluoride application on some blood parameters in rabbits. E.Ü. J. Health Sci. 13(2), 46–50.

Dhurvey, V., Patil, V., Thakarea, M., 2017. Effect of sodium fluoride on the structure and function of the thyroid and ovary in albino rats (*Rattus norvegıcus*). Fluoride 50(2), 235–246.

El–Mahmoudy, A., Matsuyama, H., Borgan, M.A., Shimizu, Y., El–Sayed, M.G., Minamoto, N., Takewaki, T., 2002. Thymoquinone suppresses expression of inducible nitric oxide synthase in rat macrophages. Int Immunopharmacol. 2(11), 1603–1611. DOI: 10.1016/s1567-5769(02)00139-x

Farhangi, M.A., Dehghan, P., Tajmiri, S., Abbasi, M.M., 2016. The effects of *Nigella sativa* on thyroid function, serum Vascular Endothelial Growth Factor (VEGF)-1, Nesfatin-1, and anthropometric features in patients with Hashimoto's thyroiditis: A randomized controlled trial. BMC Complement Altern Med. 16, 471, 1–9. DOI: 10.1186/s12906–016–1432–2

Foda, D.S., Shams, S.G., 2021. A trial for improving thyroid gland dysfunction in rats by using a marine organism extract. Braz. J. Biol. 81(3), 592–600. https://doi.org/10.1590/1519-6984.226829

Ge, Y.M., Ning, H.M., Wang, S.L., Wang, J.D., 2005. DNA damage in thyroid gland cells of rats exposed to long–term intake of high fluoride and low iodine. Fluoride 38(4), 318–323.

Güzelsoy, P., Aydın, S., Başaran, N., 2018. Potential effects of thymoquinone the active constituent of black seed (*Nigella sativa L.*) on human health. J. Lit. Pharm. Sci. 7(2), 118–35. DOI: 10.5336/pharmsci.2018-59816

Hassan, H.A., Yousef, M.I., 2009. Mitigating effects of antioxidant properties of black berry juice on sodium fluoride induced hepato-toxicity and oxidative stress in rats. Food Chem. Toxicol. 47, 2332–2337. DOI: 10.1016/j.fct.2009.06.023

Hein, J.W., Bonner, J.F., Brudevold, F., Smith, F.A., Hodge, H.C., 1956. Distribution in the soft tissue of the rat of radioactive fluoride administered as sodium fluoride. Nature 178, 1295–1296. DOI: 10.1038/1781295a0

Johnston N.R., Strobel S.A., 2020. Principles of fluoride toxicity and the cellular response: A review. Arch. Toxicol 94(4), 1051–1069. DOI: 10.1007/s00204-020-02687-5.

Koyu, A., Gümral, N., Aşcı, H., Gökçimen, A., Özgöçmen, M., Özdamar, N., 2014. 2450 MHz electromagnetic field effect on the rat thyroid tissue: Protective role of selenium and L-Carnitine. Med. J. SDU. 21(4), 133–141.

Nabavi, S.F., Moghaddam, A.H., Nabavi, S.M., Eslami, S., 2011. Protective effect of curcumin and quercetin on thyroid function in NaF-intoxicated rats. Fluoride 44(3), 147–152.

Nelson, K.M., Dahlin, J.L., Bisson, J., Graham, J., Pauli, G.F., Walters, M.A., 2017. The essential medicinal chemistry of curcumin. J. Med. Chem. 9;60(5), 1620–1637. DOI: 10.1021/acs.jmedchem.6b00975

Özdamar, K., 2019. SPPS ile Bioistatistik. 11.Edition. Nisan Kitabevi Eskişehir.

Patil, V.V., Dhurvey, V.T., 2015. Exposure to sodium fluoride affects thyroid follicular cells in albino rats. International Journal of Plant, Animal, and Environmental Sciences (IJPAES). 5(1), 56–60.

Sarkar, C., Pal, S., 2014. Ameliorative effect of resveratrol against fluoride-induced alteration of thyroid function in male Wistar rats. Biol. Trace Elem. Res. 162(1–3), 278–87. DOI: 10.1007/s12011-014-0108-3.

Shahab, S., Mustafa, G., Khan, I., Zahid, M., Yasinzai, M., Ameer, N., Asghar, N., Ullah, I., Nadhman, A., Ahmed, A., Munir, I., Mujahid, A., Hussain, T., Ahmad, M.N., Ahmad, S.S., 2017. Effects of fluoride toxicity on animals, plants, and soil health: A review. Fluoride 50(4), 393–408.

Shashi, A.,1993. Nucleic acid levels in thyroid gland in acute and chronic fluoride intoxication. Fluoride 26(3), 191–196.

Shupe, J.L., Olson, A.E., 1971. Clinical aspects of fluorosis in horses. J. Am. Vet. Med. Assoc. 158, 167–174.

Shupe, J.L., Miner, M.L., Greenwood, D.A.,1964 Clinical and pathological aspects of fluoride toxicosis in cattle. Ann. N. Y. Acad. Sci. 24,111, 618–637. doi: 10.1111/j.1749–6632.1964.tb53130.x.

Singh, N., Verma, K.G., Verma, P., Sidhu, G.K., Sachdeva, S., 2014. A comparative study of fluoride ingestion levels, serum thyroid hormone & TSH level derangements, dental fluorosis status among school children from endemic and non-endemic fluorosis areas. Springerplus. 3:7, 1–5. DOI: 10.1186/2193-1801-3-7.

Song, F., Zhang, L., Yu, H.X., Lu, R.R., Bao, J.D., Tan, C., Sun, Z., 2012. The mechanism underlying proliferation, inhibitory, and apoptosis-inducing effects of curcumin on papillary thyroid cancer cells. Food Chem. 132(1), 43–50. DOI: 10.1016/j.foodchem.2011.10.024

Sourour, D.A., 2014. Curcumin induces apoptosis in thyroid cells in rats: Possible role of caspase 3. IJAR 2(1), 790–801.

Spencer, H., Lewin, Y., Wietrowski, E. 1970. Fluoride metabolism in man. Am. J. Med. 49, 807–813. DOI: 10.1016/s0002–9343(70)80062–6

Su, C.C., Lin, J.G., Li, T.M., Chung, J.G., Yang, J.S., Ip, S.W., Lin, W.C., Chen, G.W., 2006. Curcumin-induced apoptosis of human colon cancer Colo205 cells through the production of ROS, Ca^{2+}, and the activation of caspase-3. Anticancer Res., 26(6B), 4379–4389.

Thompson, L.J., 2012. Fluoride. Veterinary Toxicology. Basic and Clinical Principles. Second Edition Ed. Ramesh, CG., Elsevier, London, UK. Pp. 513–516.

Torra, M., Rodamilans, M., Corbella, J., 1998. Serum and urine fluoride concentration: Relationships to age, sex, and renal function in a non-fluoridated population. Sci. Total Environ. 220(1), 81–85. DOI: 10.1016/s0048-9697(98)00248-4.

Vine, M.F., 1994. Biological markers: Their use in quantitative assessments. Adv. Dent. Res. 8(1), 92–99. DOI: 10.1177/08959374940080011601

Wang, H., Yang, Z., Zhou, B., Gao, H., Yan, X., Wang, J., 2009. Fluoride-induced thyroid dysfunction in rats: Roles of dietary protein and calcium level. Toxicol. Ind. Health. 25(1), 49–57. DOI: 10.1177/0748233709102720.

Whitford, G.M., 1994. Intake and metabolism of fluoride. Adv. Dent. Res. 8(1), 5–14. DOI: 10.1177/08959374940080011001.

Xiang, Q., Chen, L., Liang, Y., Wu, M., Chen, B., 2009. Fluoride and thyroid function in children in two villages in China. J. Toxicol. Environ. Health Sci. 1, 54–59.

Zhan, X.A., Li, J.X., Xu, Z.R., 2006. Effect of fluoride on growth and thyroid function in young pigs. Fluoride 39(2), 95–100.