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Effects of Thymoquinone and Curcumin on Certain Biochemical Parameters in Rats with Subacute Fluoride Toxicity

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ABSTRACT

Purpose: Fluoride is a common environmental contaminant that, when consumed in high amounts, can cause oxidative damage and inflammation, leading to serious dysfunction in vital organs such as the liver, kidneys, and heart. This study was conducted to evaluate the protective and restorative effects of thymoquinone (TQ) and curcumin (CUR) in rats with subacute fluoride toxicity.

Methods: The experiment involved a control group, a sham group, and four test groups (NaF, NaF+TQ, NaF+CUR, and NaF+TQ+CUR), each consisting of six rats. While no treatment was given to the control group, the sham group received 0.5 mL of distilled water by oral gavage and 0.1 mL of dimethyl sulfoxide (DMSO) intraperitoneally (i.p.). TQ (15 mg/kg/day, i.p.), CUR (20 mg/kg/day, i.p.), and the combination (15 mg/kg/day + 20 mg/kg/day, i.p.) were administered starting one week before NaF exposure. The test groups then received 30 mg/kg/day of NaF by oral gavage for 14 days. Serum samples were analyzed for alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), albumin (ALB), glucose (GLU), direct bilirubin (D.BIL), total bilirubin (T.BIL), blood urea nitrogen (BUN), urea (URE), creatinine (CRE), C-reactive protein (CRP), calcium (Ca), chloride (Cl), potassium (K), sodium (Na), phosphorus (P), procalcitonin (PCT), and high-sensitivity troponin T (TnT-HS).

Results: ALB and GLU levels decreased in the test groups compared with the control and sham groups. TnT-HS levels were higher in the NaF group compared with both the control and sham groups, while they were lower in the NaF+TQ, NaF+CUR, and NaF+TQ+CUR groups relative to the NaF group.

Conclusions: NaF administration elevated TnT-HS levels; however, TQ and CUR reduced this increase, indicating a cardioprotective effect. These findings suggest that TQ and CUR may be important protective agents against fluoride toxicity.

Keywords: *Subacute Fluoride toxicity; Nigella sativa oil; Curcuma longa Cardiotoxicity; Hepatotoxicity*

INTRODUCTION

Fluorine is the most electronegative element. Fluoride gradually accumulates in the environment through volcanic emissions, mineral dissolution, and industrial by-products.1 In regions where soil and water contain high fluoride concentrations, the likelihood of encountering chronic endemic fluorosis is significantly higher.2 Chronic excessive fluoride exposure has adverse effects on teeth, bones, and various other body systems.3 As a result of fluorosis, degenerative changes have been reported in the bone marrow, kidneys, liver, adrenal glands, heart muscle, and central nervous system.4

Fluoride poisoning leads to alterations in various biochemical parameters due to dysfunctions in the liver, kidneys, and metabolic processes. The liver is one of the primary target organs of fluoride toxicity,5 and increased serum ALT, AST, and LDH levels have been reported in rats treated with NaF.6-7

Damage to the kidneys, the main organs responsible for fluoride excretion, causes elevated BUN and CRE levels in serum, indicating reduced renal clearance.8-10

Because of their anti-inflammatory and antioxidant properties, thymoquinone, the primary component of Nigella sativa extracts and its volatile oil,11-12 and curcumin, a polyphenol found in turmeric,13-14 are reported to have protective effects on the liver, kidneys, and cardiovascular system.11-16

It is well established that chronic or subacute fluoride exposure triggers oxidative stress and causes damage to hepatic, renal, and cardiac tissues; however, the search continues for safe and readily accessible agents that can mitigate these harmful effects. Although previous studies have examined the effects of thymoquinone and curcumin on fluoride toxicity individually, no study to date has investigated their combined or simultaneous administration in this context.

The aim of this study is to investigate the potential changes in liver function tests (ALT, AST, LDH), metabolic and hepatic indicators (albumin, glucose, direct and total bilirubin), kidney function markers (BUN, urea, creatinine), inflammatory markers (CRP, PCT), electrolyte balance (Ca, Cl, K, Na, P), and the cardiac biomarker TnT-HS caused by NaF exposure, and to evaluate the protective or reparative effects of TQ and curcumin on these changes.

MATERIAL AND METHODS

Ethics

This study was approved by the Tekirdağ Namık Kemal University Animal Experiments Local Ethics Committee (approval no. T2023-1444).

Animals

A total of 36 adult male Wistar Albino rats, weighing 260–300 g and aged 12–13 weeks, were used. The rats were housed under standard laboratory conditions at 23±2 °C, 40–50% relative humidity, with a 12-hour light/dark cycle, and had ad libitum access to tap water and commercial pelleted food.

Experimental Design

The animals were divided into six groups: a control group (n=6), a sham group (n=6), and four test groups (NaF, NaF+TQ, NaF+CUR, and NaF+TQ+CUR; each n=6). The control group received no treatment. The sham group was given 0.5 mL of distilled water by oral gavage and 0.1 mL of dimethyl sulfoxide (DMSO, Merck M116743) intraperitoneally. In the test groups, sodium fluoride (NaF, Merck M106449) was administered by oral gavage at a dose of 30 mg/kg/day for 14 days, with NaF dissolved in distilled water. Thymoquinone (TQ, Sigma 274666) at 15 mg/kg/day, curcumin (CUR, Sigma C1386) at 20 mg/kg/day, and their combination (TQ+CUR, 15 mg/kg/day and 20 mg/kg/day, respectively) were dissolved in DMSO and administered intraperitoneally. These treatments were initiated one week before NaF administration to allow for a protective effect and were continued concurrently with NaF for the subsequent two weeks.

Biochemical Analysis

At the end of the experiment, the animals were anesthetized and decapitated, and blood samples were collected. The blood was centrifuged at 3000 rpm for 20 minutes to separate the serum, which was then stored at –80 °C until analysis. Serum levels of ALT, AST, LDH, ALB, GLU, D.BIL, T.BIL, BUN, URE, CRE, CRP, Ca, Cl, K, Na, and P were measured using a Cobas® 6000 analyzer (Roche Diagnostics- Switzerland), while PCT and TnT-HS levels were determined using a Cobas® e411 analyzer (Roche Diagnostics, Switzerland).

Statistical Analysis

The statistical analysis was performed using the SPSS 24.0 software package. All values are expressed as mean ± standard error (X ± Sx). The normality of the data was assessed using the Shapiro-Wilk test. Biochemical parameter comparisons between groups were conducted using one-way ANOVA for normally distributed data and the Kruskal-Wallis test for non-normally distributed data. A p-value <0.05 was considered statistically significant.

RESULTS

This study aimed to evaluate the changes in certain biochemical parameters induced by NaF toxicity and to assess the effects of TQ, CUR, and their combined use on these changes. To this end, biochemical data obtained from Wistar albino rats—divided into a control group, a sham group, and four test groups with six animals in each—were compared, and the results are presented in detail below.

No statistically significant differences were detected among the groups in terms of biochemical parameters including D.BIL, T.BIL, CRP, PCT, BUN, URE, CRE, Ca, Cl, K, Na, and P ($p > 0.05$; Table 1).

ALT, AST, and LDH levels were elevated in the NaF group compared to the control and sham groups; however, these differences were not statistically significant ($p > 0.05$). In the NaF+TQ, NaF+CUR, and NaF+TQ+CUR groups, ALT and AST levels exhibited a non-significant decreasing trend relative to the NaF group. Furthermore, no statistically significant differences were observed among the NaF+TQ, NaF+CUR, and NaF+TQ+CUR groups.

Table 1. Biochemical parameters in adult male Wistar albino rats (age: 12–13 weeks, weight: 260–300 g) from control, sham, NaF, NaF+TQ, NaF+CUR, and NaF+TQ+CUR groups

Parameters	Control	Sham	NaF	NaF+TQ	NaF+CUR	NaF+TQ+CUR
ALT (U/L)	53.00±10.43[a]	48.00±13.08[a]	100.33±48.78[a]	24.67±2.96[a]	43.00±2.98[a]	45.67±6.39[a]
AST (U/L)	157.25±19.39[a]	146.75±18.35[a]	314.50±122.73[a]	146.67±16.76[a]	147.17±8,84[a]	143.33±28.47[a]
LDH (U/L)	2392.40±124.84[a]	2078.67±253.51[a]	3254.20±484.21[a]	1967.33±66.06[a]	2374.00±75.60[a]	2524.67±526.69[a]
D.BIL (mg/dl)	0.020±0.004[a]	0.17±0.15[a]	0.030±0.008[a]	0.030±0.007[a]	0.010±0.003[a]	0.040±0.012[a]
T.BIL (mg/dl)	0.040±0.013[a]	0.07±0.02[a]	0.050±0.009[a]	0.080±0.003[a]	0.040±0.008[a]	0.060±0.006[a]
ALB (g/dl)	4.24±0.03[a]	4.11±0.11[a]	3.51±0.12[b]	3.18±0.19[b]	3.36±0.10[b]	3.08±0.09[b]
TNT-HS (pg/ml)	4134.25±1170.98[b]	4276.00±230.38[b]	8543.20±473.24[a]	2560.00±301.26[b]	4148.40±938.04[b]	3570.00±1346.64[b]
CRP (mg/L)	0.05±0.01[a]	0.04±0.01[a]	0.06±0.01[a]	0.08±0.02[a]	0.07±0.01[a]	0.05±0.02[a]
PCT (ng/ml)	<0.02[a]	<0.02[a]	<0.02[a]	<0.02[a]	<0.02[a]	<0.02[a]
BUN (mg/dl)	23.40±0.68[a]	17.60±1.66[a]	17.50±0.85[a]	22.67±11.68[a]	17.67±1.36[a]	16.00±2.00[a]
URE (mg/dl)	50.44±1.56[a]	37.52±3.57[a]	37.33±1.81[a]	48.73±24.99[a]	38.10±2.84[a]	34.10±4.26[a]
CRE (mg/dl)	0.42±0.05[a]	0.38±0.04[a]	0.32±0.03[a]	0.37±0.12[a]	0.28±0.03[a]	0.27±0.03[a]
Ca (mg/dl)	11.26±0.31[a]	10.37±0.24[a]	10.14±0.36[a]	10.27±0.34[a]	10.12±0.29[a]	10.43±0.07[a]
Cl (mmol/L)	96.68±0.58[a]	99.50±2.54[a]	96.27±1.26[a]	97.53±0.30[a]	96.27±3.05[a]	100.53±1.62[a]
K (mmol/L)	6.42±0.62[a]	7.17±1.12[a]	6.08±0.38[a]	5.44±0.21[a]	5.06±0.27[a]	6.91±0.71[a]
Na (mmol/L)	144.80±0.80[a]	141.20±3.12[a]	142.00±1.39[a]	146.00±1.00[a]	144.00±4.41[a]	145.67±0.67[a]
P (mg/dl)	8.34±0.82[a]	8.90±0.70[a]	7.41±0.45[a]	9.12±1.47[a]	6.59±1.24[a]	9.57±0.53[a]
GLU (mg/dl)	148.25±5.14[a]	144.80±5.40[a]	77.67±5.33[b]	100.67±16.41[b]	112.00±5.14[b]	98.33±15.30[b]

Superscript letters were used for the comparison among all groups. Differences between values in the same row with different letters are considered significant. A p-value <0.05 was regarded as statistically significant.

Albumin levels were significantly decreased in the NaF group compared to both the control ($p < 0.01$) and sham ($p < 0.05$) groups. Similar reductions were observed in the NaF+TQ, NaF+CUR, and NaF+TQ+CUR groups, with albumin levels significantly lower than those in the control ($p < 0.05$, $p < 0.01$, and $p < 0.05$, respectively) and sham ($p < 0.05$, $p < 0.01$, and $p < 0.05$, respectively) groups. However, no statistically significant differences in albumin levels were detected among the NaF, NaF+TQ, NaF+CUR, and NaF+TQ+CUR groups (Table 1).

Glucose levels were significantly lower in the NaF group compared to both the control and sham groups ($p < 0.001$). Similarly, the NaF+TQ, NaF+CUR, and NaF+TQ+CUR groups also showed significantly reduced glucose levels relative to the control and sham groups ($p < 0.05$ for all comparisons). However, no statistically significant differences in glucose levels were observed among the NaF, NaF+TQ, NaF+CUR, and NaF+TQ+CUR groups (Table 1).

High-sensitivity troponin T levels were significantly elevated in the NaF group compared to the control and sham groups ($p < 0.05$). Importantly, TnT-HS levels were significantly reduced in the NaF+TQ, NaF+CUR, and NaF+TQ+CUR groups compared to the NaF group, with p-values of < 0.05, < 0.01, and < 0.05, respectively (Table 1). However, no statistically significant differences were observed among the NaF+TQ, NaF+CUR, and NaF+TQ+CUR groups.

DISCUSSION

Various studies conducted in endemic fluorosis regions and in experimental animal models have reported that fluoride toxicity causes significant alterations in several biochemical parameters. 7,10,17-20 In our study, although the NaF group exhibited higher ALT, AST, and LDH levels compared to the control and sham groups, these increases did not reach statistical significance. This finding differs from several previous studies that reported significant elevations in these enzymes following NaF administration.[7,10,17–20] One possible explanation for this discrepancy is the relatively short exposure duration (14 days) and the moderate NaF dose (30 mg/kg/day) used in our

experimental model. In contrast, studies reporting significant increases in these enzymes often used higher doses or prolonged exposure periods, which may have led to more pronounced hepatocellular damage.

Thymoquinone and curcumin, known for their antioxidant, anti-inflammatory, immunomodulatory, antihistaminic, antimicrobial, and antitumor properties, have also demonstrated hepatoprotective, cardioprotective, gastroprotective, nephroprotective, and antidiabetic effects.21 It is well established that oxidative stress is a key factor in fluoride toxicity.22 Fluoride increases free radical production and depletes antioxidant enzymes, thereby triggering lipid peroxidation, protein oxidation, and cell death.

In the NaF+TQ, NaF+CUR, and NaF+TQ+CUR groups, ALT and AST levels demonstrated a downward trend compared to the NaF group; however, the differences were not statistically significant. These non-significant decreases may suggest a partial hepatoprotective effect of TQ and CUR, potentially linked to their antioxidant and anti-inflammatory properties. Nevertheless, the absence of statistical significance indicates that the protective effect may have been limited under the specific dose and duration conditions of this study.

Regarding LDH, while some studies in rats have documented significant increases in LDH activity following NaF exposure,[7,19] others such as Guan et al.[23] found no statistically significant change, despite a numerical increase. Interestingly, a study conducted on sheep even reported a reduction in LDH levels.24 Our findings are consistent with those of Guan et al.,[23] suggesting that LDH response to fluoride may vary depending on species, experimental design, and exposure intensity. The modest elevation in LDH levels observed in our study, although not significant, may reflect mild cellular stress insufficient to cause overt cytolysis.

Regarding bilirubin parameters, various studies in rats have yielded differing results. Some researchers have reported increases in direct, indirect, and total bilirubin,18,20 whereas others have observed a decrease in total bilirubin levels7 or no significant changes in bilirubin levels in mice.25 In our study, no significant changes were detected in total or direct bilirubin levels. The absence of significant bilirubin alterations in our study suggests that the subacute fluoride dose used may not have induced substantial hepatobiliary dysfunction.

In the evaluation of CRP levels, studies on chronic fluorosis models in rabbits and in humans living in endemic fluorosis regions have reported increased CRP levels.26-29 Çenesiz et al.27 noted that CRP levels significantly increased as the duration of exposure progressed. In contrast, we observed no significant changes in CRP levels, which may be due to the shorter exposure period or insufficient inflammatory activation at the selected NaF dose. These findings suggest that CRP may be more responsive to chronic rather than subacute fluoride toxicity.

Although procalcitonin (PCT) is widely used as a biomarker for sepsis, it has also been reported to increase in various toxic conditions such as acetaminophen poisoning.30 In our study, PCT levels remained below the measurement limits of the autoanalyzer following NaF exposure and did not show any significant change. As there is currently no direct evidence linking fluoride exposure to altered PCT levels, our results suggest that PCT may not be a sensitive biomarker for subacute fluoride toxicity. It is also possible that the degree of systemic inflammation induced by NaF in our model was too mild to stimulate procalcitonin production.

While some studies have reported a decrease in urea and an increase in creatinine levels following fluoride exposure,[19] others have observed elevations in BUN, uric acid, and creatinine.[10] For instance, Siddiqi et al.31 noted a reduction in urea after a single intraperitoneal dose of 30 mg/kg NaF, without significant changes in BUN, creatinine, or uric acid, whereas Guan et al.[23] found no significant alterations in BUN and creatinine levels. Our findings are in agreement with those of Guan et al.,[23] as we also observed no significant differences in renal biochemical parameters (BUN, urea, creatinine) among the groups. This suggests that NaF at 30 mg/kg/day for 14 days may have a relatively low nephrotoxic potential under subacute exposure conditions. The nephrotoxic effects of fluoride vary with dose and exposure duration, with chronic high-dose exposure posing a greater risk[8]. Supporting this, Nabavi et al.32 demonstrated that in rats given high-dose fluoride (600 ppm), creatinine, urea, and BUN levels rose significantly—but pretreatment with curcumin at 10 and 20 mg/kg effectively preserved renal tissue and normalized these serum markers. However, in our study, the absence of significant renal damage in the NaF group limited our ability to evaluate the nephroprotective effects of thymoquinone and curcumin. These antioxidant therapies may only demonstrate measurable renal benefits under more severe toxic conditions. Future studies employing higher fluoride doses or longer exposure periods may be necessary to induce more pronounced renal impairment and thus allow a clearer assessment of the protective efficacy of TQ and CUR.

In terms of electrolyte changes, previous studies have reported decreases in potassium and sodium,19,33 as well as in calcium, phosphorus,33 and chloride19 levels following NaF administration. Siddiqi et al.31 observed a decrease in calcium and phosphorus with a single NaF dose, but no significant changes in sodium and potassium, while Chinoy et al.17 reported increases in sodium and potassium levels. In our NaF group, no significant differences in sodium, potassium, chloride, calcium, or phosphorus were found compared to the control. These results suggest that under

subacute exposure conditions, the systemic electrolyte homeostasis remained largely intact. The absence of alterations may be due to adaptive renal and hormonal mechanisms that buffer electrolyte changes in moderate fluoride exposure.

Regarding albumin and glucose levels, our study observed a decrease in the NaF group relative to the control. Some studies have reported no significant change in albumin levels,18,31 while others have reported decreases.7,23 Our results are in line with the findings of Bouasla et al.7 and Guan et al..23 For glucose levels, although some studies report no significant changes,23 others have observed either increases10,20 or decreases.25 Our findings are consistent with those of Pillai et al.,25 suggesting that the decrease in glucose may be related to reduced food intake or disturbances in hepatic carbohydrate and protein metabolism. Interestingly, although TQ, CUR, and their combination were expected to exert hepatoprotective effects, albumin levels remained significantly lower in all treatment groups compared to control and sham. Furthermore, there were no statistically significant differences among the NaF+TQ, NaF+CUR, and NaF+TQ+CUR groups. This suggests that the hepatoprotective effects of TQ and CUR, while present, may have been modest under the conditions tested. One likely explanation is the use of fixed single doses (15 mg/kg for TQ and 20 mg/kg for CUR) and a relatively short treatment duration. The liver's synthetic function may require longer recovery time and possibly higher or repeated doses of antioxidants to restore protein levels such as albumin. Moreover, the similar outcomes across all treatment groups imply that co-administration of TQ and CUR did not result in additive or synergistic effects at the doses employed. A similar pattern was observed with glucose levels. Treatment with TQ, CUR, or their combination led to partial recovery in glucose levels; however, these levels remained significantly lower than in the control and sham groups, and no significant differences were observed among the treatment groups themselves. This suggests that the applied therapies may have had a limited effect on restoring carbohydrate metabolism within the relatively short duration of exposure.

Acute or chronic high-dose fluoride exposure has been reported to cause toxic effects on cardiac tissue and an increase in cardiac injury markers.19,34-35 Previous studies have shown increases in troponin I19,34 and troponin T levels.35 In our study, a significant increase in high-sensitivity troponin T levels was observed in the NaF group compared to the control, supporting the cardiotoxic effects of NaF. This finding is consistent with similar studies in the literature and provides important evidence of fluoride's potential harmful effects on the cardiovascular system. This protective effect is likely mediated through their potent antioxidant and membrane-stabilizing properties, which may help prevent oxidative damage to myocardial cells and reduce cardiac enzyme leakage. Although the combination of TQ and CUR did not show superior efficacy compared to their individual use, all treatment groups demonstrated significant attenuation of fluoride-induced cardiac injury, highlighting their therapeutic potential.

The combined administration of thymoquinone and curcumin did not produce statistically significant improvements in biochemical parameters compared to their individual use. This suggests that the combination therapy was not more effective than monotherapies. Such an outcome may be attributed to overlapping mechanisms of action or to suboptimal dosing ratios of the compounds.

CONCLUSIONS

This study demonstrated that subacute exposure to sodium fluoride (NaF) at a dose of 30 mg/kg/day for 14 days led to significant reductions in serum albumin and glucose levels, along with a marked increase in high-sensitivity troponin T (TnT-HS), indicating hepatic metabolic disruption and cardiotoxicity. Although ALT, AST, and LDH levels showed upward trends, these changes were not statistically significant, but may suggest early signs of liver injury. No significant alterations were observed in bilirubin, CRP, PCT, renal markers (BUN, urea, creatinine), or electrolyte levels, indicating that the administered NaF dose had limited effects on renal and systemic parameters under the tested conditions.

Thymoquinone and curcumin, when administered individually or in combination, significantly attenuated the elevation in TnT-HS levels, supporting their cardioprotective effects. However, they did not restore albumin or glucose levels to control values, and no statistically significant differences were found among the NaF+TQ, NaF+CUR, and NaF+TQ+CUR groups. These findings suggest that while TQ and CUR provide partial protection against fluoride-induced toxicity, their effectiveness may be influenced by dose and duration of administration.

A limitation of this study is that the efficacy of thymoquinone, curcumin, and their combination was evaluated using single fixed doses. Therefore, further studies using multiple dose regimens and longer exposure periods are warranted to determine the optimal therapeutic strategy and assess potential synergistic effects.

CONFLICT OF INTERESTS

None.

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