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Protective effects of Vitamin C and E on fluorosis-induced gene expression in oxidative, apoptotic, and PI3K/AKT pathways in liver and kidney tissues

Efectos protectores de la vitamina C y E sobre la expresión génica inducida por la fluorosis en las vías oxidativas, apoptóticas y PI3K/AKT en los tejidos hepáticos y renales.

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ABSTRACT

This study aimed to investigate the modulatory effects of vitamin C and vitamin E on fluorosis-induced gene expression alterations in oxidative stress (Glutathione Peroxidase 1, Neutrophil Cytosolic Factor 1, Superoxide Dismutase 1), apoptosis (Caspase 3, Caspase 8, Caspase 9), and Phosphoinositide 3–Kinase/ Protein Kinase B/ Mechanistic Target of Rapamycin signaling pathways (Phosphoinositide 3–Kinase, Protein Kinase B, Receptor Tyrosine Kinase 2, mechanistic Target of Rapamycin, Tumor Protein p53) in rats. Forty-eight male Wistar Albino rats were randomLy allocated into six groups. Sodium Fluoride (150 mg·kg^{-1}·day^{-1}) was administered via drinking water for 16 weeks, followed by oral vitamin supplementation (C and E) for 4 weeks. mRNA expression levels were analyzed using RT-qPCR. Sodium Fluoride exposure increased Neutrophil Cytosolic Factor 1 expression in both kidney and liver tissues (P<0.001), while reducing Glutathione Peroxidase 1 and Glutathione Peroxidase 1 levels. Vitamin E, vitamin C, and their combination significantly suppressed Neutrophil Cytosolic Factor 1 expression (P<0.001), whereas the recovery of Glutathione Peroxidase 1 and Glutathione Peroxidase 1 was significant only in kidney tissues (P<0.05). Sodium Fluoride also markedly upregulated the apoptotic genes Caspase 3, Caspase 8, and Caspase 9 (P<0.05), and antioxidant treatments substantially attenuated these increases (P<0.0001). Furthermore, all Phosphoinositide 3–Kinase/Protein Kinase B pathway-related genes were strongly overexpressed following Sodium Fluoride administration (P<0.0001), and vitamin supplementation effectively reduced these elevations in both tissues (P<0.0001). Vitamin C and vitamin E, particularly in combination, exhibit substantial protective effects by restoring gene expression patterns toward homeostatic levels. This implies that a combination of antioxidant supplements may provide a promising therapeutic tool in combating fluorosis-induced cellular dysfunction.

Key words: Fluorosis; Antioxidants; Vitamin C; Vitamin E; Phosphoinositide 3–Kinase/Protein Kinase B pathway; Apoptosis; Oxidative stress; Gene expression

RESUMEN

Este estudio tuvo como objetivo investigar los efectos moduladores de la vitamina C y la vitamina E sobre las alteraciones de la expresión génica inducidas por fluorosis en el estrés oxidativo (Glutatión Peroxidasa 1, Factor Citosólico de Neutrófilos 1, Superóxido Dismutasa 1), la apoptosis (Caspasa 3, Caspasa 8, Caspasa 9) y las vías de señalización de la fosfoinositida 3–quinasa / proteína quinasa B / diana mecanicista de la rapamicina (fosfoinositida 3–quinasa, proteína quinasa B, receptor Erb-B2 tirosina quinasa 2, diana mecanicista de la rapamicina, proteína tumoral p53) en ratas. Se asignaron aleatoriamente cuarenta y ocho ratas Wistar Albino macho a seis grupos. Se administró fluoruro de sodio (150 mg·kg^{-1}·día^{-1}) a través del agua potable durante 16 semanas, seguido de un suplemento vitamínico (C y E) oral durante 4 semanas. Se analizaron los niveles de expresión del ARNm mediante RT-qPCR. La exposición al fluoruro de sodio aumentó la expresión de Factor Citosólico de Neutrófilos 1 tanto en los tejidos renales como en los hepáticos (P<0,001), al tiempo que redujo los niveles de Glutatión Peroxidasa 1 y Superóxido Dismutasa 1. La vitamina E, la vitamina C y su combinación suprimieron significativamente la expresión de Factor Citosólico de Neutrófilos 1 (P<0,001), mientras que la recuperación de Glutatión Peroxidasa 1 y Superóxido Dismutasa 1 solo fue significativa en los tejidos renales (P<0,05). El fluoruro de sodio también reguló al alza de forma notable los genes apoptóticos Caspasa 3, Caspasa 8 y Caspasa 9 (P<0,05), y los tratamientos antioxidantes atenuaron sustancialmente estos aumentos (P<0,0001). Además, todos los genes relacionados con la vía fosfoinositida 3–quinasa / proteína quinasa B se sobreexpresaron fuertemente tras la administración de fluoruro de sodio (P<0,0001), y la suplementación con vitaminas redujo eficazmente estos aumentos en ambos tejidos (P<0,0001). La vitamina C y la vitamina E, especialmente en combinación, exhiben efectos protectores sustanciales al restaurar los patrones de expresión génica hacia niveles homeostáticos. Esto sugiere que la suplementación combinada con antioxidantes puede servir como una estrategia terapéutica prometedora contra la disfunción celular inducida por la fluorosis.

Palabras clave: Fluorosis; antioxidantes; vitamina C; vitamina E; vía fosfoinositida 3–quinasa / proteína quinasa B; apoptosis; estrés oxidativo; expresión génica

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INTRODUCTION

Fluorine is seen as an important trace element that is needed in order to support the health of people as well as animals. A majority of the time, the consumption of fluorine occurs at normal physiological levels, however, if someone were to consume too much of this trace element, for long periods of time, they may experience systemic toxicity and/or pathological changes to their body. This type of toxicity has been demonstrated with persons suffering from an excess of fluoride which caused skeletal fluorosis. Evidence has also been gathered that an excess of fluoride will cause other structural, functional and metabolic abnormalities to occur within the soft tissues of the body such as the liver and kidneys [1].

Some studies have found that much fluoride can hurt the kidneys and the way they work, metabolic impairment and/or histopathological changes to the kidney. The toxicity that is induced by fluoride has multiple mechanisms. Some of these mechanisms include disruption of synapses, disruption of intracellular signalling, disruption of protein synthesis, disruption of transcription factors, increased levels of oxidative stress, and chronic inflammation [2].

The effects of fluorosis on gene expression are partly mediated by changes in oxidative stress and other pathways. Some of those pathways integrate multiple levels of cellular function (e.g., apoptosis), whereas others may only involve a single level of cellular function (e.g., inflammation). These pathways have been shown to contribute to cell injury following exposure to fluoride through disrupting normal cellular functioning, resulting in the persistence of apoptosis or an inability for cells to recover [3, 4, 5, 6, 7].

Researchers in this field recognize oxidative stress causes oxidative damage, which affects lipid peroxidation and oxidative phosphorylation to result in cell injury due to exposure to fluoride. The Mitogen-Activated Protein Kinase (MAPK) signal transduction pathway and the activation of apoptosis by fluoride are considered a metabolic regulatory failure at the cellular level. Therefore, when the normal homeostatic response to oxidative stress fails to maintain tissue homeostasis, then the normal homeostatic failure will ultimately end in tissue injury and apoptosis [8, 9].

The PI3K/Akt signaling cascade may contribute to the development of fluorosis and other diseases of the bone. The Phosphoinositide 3–Kinase / Protein Kinase B / mechanistic Target of Rapamycin (PI3K/Akt/mTOR) pathway plays an important role in regulating growth, survival, and stimulation of cell division and new blood vessel formation and is altered following chronic exposure to environmental toxins. Chronic administration of fluoride has been shown to cause a large increase in the expression of Phosphoinositide 3–Kinase (PI3K) and related genes in hepatocytes [10, 11].

Research has suggested that antioxidants can act as therapeutic agents in reducing the cellular and molecular effects of fluoride toxicity. Vitamin E is a lipid-soluble compound that protects membranes from oxidative damage caused by lipid peroxidation Furthermore, studies show that antioxidants have the potential to reduce oxidative damage in deoxyribonucleic acid (DNA) caused by fluoride toxicity. For example, the collaborative biological effects of vitamins E and C (i.e., vitamin E recycling) demonstrate how antioxidants act synergistically. [5, 12, 13, 14].

In addition to being an antioxidant, vitamin C also has a physiological role in collagen synthesis, regulating the immune system, absorbing iron and vitamin E, and metabolizing catecholamines [15].

The purpose of this experiment was to investigate the effects of both vitamin C and E, singularly or in combination, on fluoride-induced alterations in oxidative stress, apoptosis, and PI3K/Akt/mTOR signalling pathways through modulation of the expression of associated gene regulatory mechanisms within the respective tissues of the kidney and liver.

MATERIAL AND METHODS

Animals and Experimental Design

A total of 48 adult male Wistar rats (weighing 200–250 g) were obtained from the Experimental Research Center of Van Yüzüncü Yıl University (Van YYU). Throughout the study, the animals were housed in standard laboratory conditions and given unlimited access to standard rat food and water. The animals were randomly divided into six groups (n = 8 per group) as shown TABLE I. The Van Yüzüncü Yıl University Local Animal Experiment Ethics Committee has approved the project (decision no. 2017/11 dated November 30, 2017).

TABLE I
Experimental design and groups (*In vivo*)

Group		Treatment
Control	Control	No treatment
Corn oil	Corn	Corn oil (vehicle for vitamin E)
NaF	NaF	150 mg·kg^{-1}/day NaF in drinking water for 16 weeks
NaF+Vit E	NVE	NaF + Vitamin E (100 mg·kg^{-1}·day^{-1}, oral gavage) for 4 weeks
NaF+Vit C	NVC	NaF + Vitamin C (200 mg·kg^{-1}·day^{-1}, oral gavage) for 4 weeks
NaF+Vit C+Vit E	NVCE	NaF + Vitamin C (200 mg·kg^{-1}·day^{-1}, oral gavage) + Vitamin E (100 mg·kg^{-1}·day^{-1}, oral gavage) for 4 weeks

NaF: Sodium Fluoride

To investigate the potential role of vitamins in improving intoxication that developed between weeks 16 and 20, the study was continued until week 20.

Tissue collection and sample preparation

Following the experimental period, rats were sacrificed, liver and kidney tissues were excised under sterile conditions and immediately stored at –80°C (ILDAMLAB DF–360). Prior to molecular analyses, tissues were thawed at room temperature (20–25°C), and ~50 mg of each sample was transferred into sterile tubes. 0.2 mL of sterile phosphate buffered saline (PBS) was added to a tissue sample, homogenized, and centrifuged. Following homologation; the liquid phase of the sample was discarded, while the solid phase was extracted for RNA.

RNA extraction and quality assessment

Trizol reagents according to the protocol of Chomczynski and Mackey [16] were used to extract total ribonucleic acid (RNA) from the solid-phase tissue sample. RNA was verified as intact by running samples electrophoretically on a 0.7% agarose gel; and distinct bands at 28S, 18S, and 5S indicated that the RNA was intact. The concentration of RNA and the level of purity were measured on a NanoDrop spectrophotometer (BioDrop, UK).

Complementary deoxyribonucleic acid synthesis and quantitative real-time Polymerase Chain Reaction

WizScript™ cDNA Synthesis Kit (Wizbio, Korea) was used for the synthesis of complementary deoxyribonucleic acid (cDNA) from isolated RNA following the manufacturer's recommendations. Quantitative real-time Polymerase Chain Reaction (RT-qPCR) was performed using the Rotor-Gene Q system (Qiagen, USA) with the WizPure™ qPCR Mastermix (SYBR Green; Sentegen, Türkiye). The operating conditions for the RT-qPCR device were precisely defined to ensure optimal amplification. The thermal cycling protocol commenced with an initial denaturation phase at 95°C for 15 minutes (min). Subsequently, each cycle involved a denaturation step at 95°C for 15 seconds (s), followed by an annealing phase at 60°C for 30 s. The extension step was performed at 72°C for 30 s. A total of 40 cycles were executed for the complete amplification process.

Gene expression analysis was conducted for oxidative stress markers (Glutathione Peroxidase 1 (Gpx1), Neutrophil Cytosolic Factor 1 (Ncf1), Superoxide Dismutase 1 (Sod1)), apoptotic genes (Caspase 3 (Casp3), Caspase 8 (Casp8), Caspase 9 (Casp9)), Phosphoinositide 3–Kinase / Protein Kinase B (PI3K/Akt) signaling components (Pi3k, Akt1, Erb-B2 Receptor Tyrosine Kinase 2 (Erbb2), Mechanistic Target of Rapamycin (mTOR)), and Tumor Protein P53 (Tp53). The sequences of primers that were used for amplification are presented in TABLE II.

The control gene for normalizing the PCR results was beta-actin. Each reaction was performed in triplicate, and threshold cycle values were determined at the logarithmic phase of amplification. Relative gene expression was calculated using the $2^{-\Delta\Delta^{Ct}}$ method [17].

Statistical analysis

Data were processed as mean ± standard deviation (M±SD). Statistical differences and significance between groups were determined using one-way analysis of variance (ANOVA). Then, Duncan's multiple interval test was applied for post hoc comparisons. For all statistical analyses, a P value < 0.05 was considered statistically significant. Additionally, for certain analyses, a P value < 0.001 was used to denote a higher level of statistical significance. All statistical analyses were performed using SPSS software (version 22.0; IBM Corp., Armonk, NY, USA).

RESULTS AND DISCUSSION

Expression levels of oxidative stress-related genes

The mRNA expression levels of oxidative stress-related genes in kidney and liver tissues are presented in FIG. 1. The expression of Ncf1 was significantly upregulated in both kidney and liver tissues of rats exposed to NaF compared to the control group (P<0.001). However, co-administration of vitamin E, vitamin C, or their combination markedly reduced Ncf1 expression in both tissues (P<0.001).

Conversely, the expression levels of Gpx1 and Sod1 were significantly downregulated in the NaF-treated group compared to controls. Although treatment with vitamin E, vitamin C, or the combination improved the expression of these genes, the increase was statistically significant only in kidney tissues (P<0.05), with no significant change observed in liver tissues (P>0.05).

TABLE II
The base sequences of forward and reverse primers used in the Quantitative real-time Polymerase Chain Reaction

	Gen	Forward (5'–3')	Reverse (5'–3')
1	ACTB (Control Gene)	CTCCTCAAGGATGGCACC	GCTCATTGTAGAAAGTGTGGT
2	Gpx1	TCCACCGTGTATGCCTTCTC	TCTCTTCATTCTTGCCATTCTCC
3	Sod1	GCTTCTGTCGTCTCCTTGCT	CATGCTCGCCTTCAGTTAATCC
4	Ncf1	GTCGGAGAAGGTGGTCTACAG	CGATAGGTCTGAAGGATGATGG
5	Casp3	CGAAACTCTTCATCATTCAGG	GAGCATTGACACAATACACGG
6	Casp8	GATGTCCTGGTGCTATTTCAGAG	CCTCCTTGTCCATGTCTTCTG
7	Casp9	TCTCACACCAGAAACACCCA	GTCGTTCTTCACCTCCACCA
8	Erbb2	ATGCTCATCGCTCACAACCA	AACTCCTCCCTTCAGGATCTC
9	AKT1	CTCCTCAAGAATGATGGCACC	ACTCAAACTCGTTCATGGTC
10	PI3K	GGAGAACTATGAACAACCTGTG	CATCTTCCAGTAACGTAGGCAG
11	Tp53	CTGGACGACAGGCAGACTTT	GTCCCGTCCCAGAAGATTCC
12	mTOR	GCCATTGCCAGCCTCATTG	GAAAGTGTCCCCTGCCATTG

qRT-PCR: Quantitative Reverse Transcription Polymerase Chain Reaction; 5'–3': 5 prime to 3 prime (denotes the directionality of the nucleic acid strand); ACTB: Actin Beta; Gpx1: Glutathione Peroxidase 1; Sod1: Superoxide Dismutase 1; Ncf1: Neutrophil Cytosolic Factor 1; Casp3: Caspase 3; Casp8: Caspase 8; Casp9: Caspase 9; Erbb2: Erb-B2 Receptor Tyrosine Kinase 2; AKT1: AKT Serine/Threonine Kinase 1; PI3K: Phosphoinositide 3–Kinase; Tp53: Tumor Protein P53; mTOR: Mechanistic Target of Rapamycin

Expression levels of apoptotic genes

The expression patterns of apoptotic genes (Casp3, Casp8, Casp9) are shown in FIG. 2. NaF exposure significantly increased the expression of all three apoptotic genes in both kidney and liver tissues compared to the control group (P<0.05). Supplementation with vitamin E, vitamin C, or their combination significantly attenuated the expression of these apoptotic markers (P<0.0001).

Fluorine is widely used in various industrial and medical applications, including dental care products, cholesterol-lowering drugs, antibacterial and antidepressant agents, and insecticides. However, it is known to interfere with bone mineral metabolism, leading to structural and functional disruption of bone tissue [18].

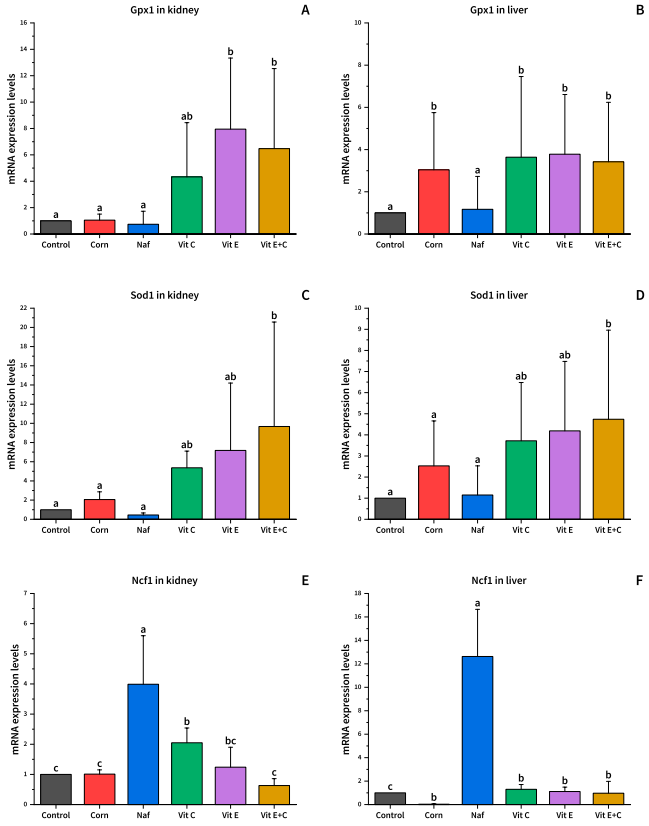

FIGURE 1. Expression levels of oxidative stress-related genes (Gpx1, Ncf1, Sod1) in kidney and liver tissues across experimental groups. (A) Gpx1 mRNA expression in kidney tissue. (B) Gpx1 mRNA expression in liver tissue. (C) Sod1 mRNA expression in kidney tissue. (D) Sod1 mRNA expression in liver tissue. (E) Ncf1 mRNA expression in kidney tissue. (F) Ncf1 mRNA expression in liver tissue

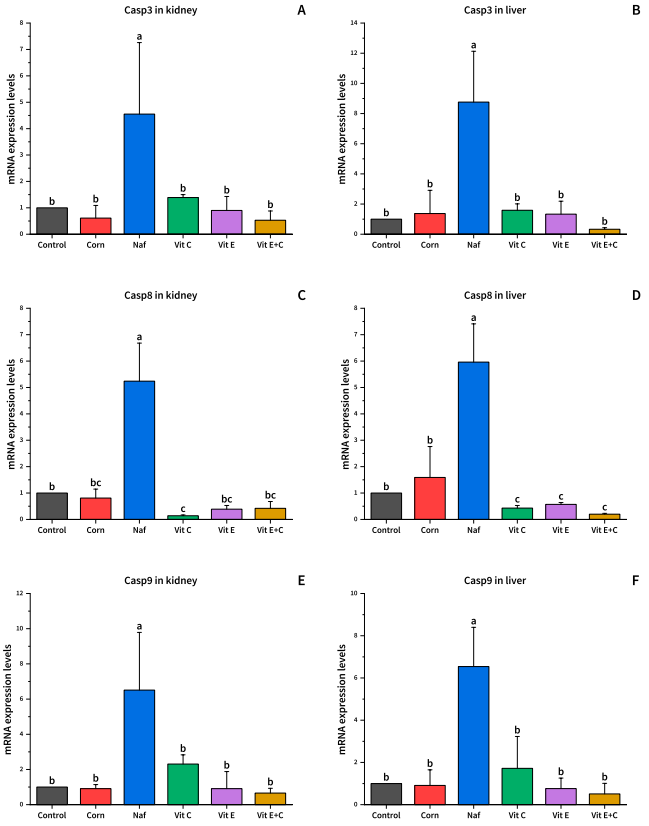

FIGURE 2. Expression levels of apoptotic genes (Casp3, Casp8, Casp9) in kidney and liver tissues of experimental groups. (A) Casp3 mRNA expression in kidney tissue. (B) Casp3 mRNA expression in liver tissue. (C) Casp8 mRNA expression in kidney tissue. (D) Casp8 mRNA expression in liver tissue. (E) Casp9 mRNA expression in kidney tissue. (F) Casp9 mRNA expression in liver tissue

Following hepatic metabolism, fluorine is primarily excreted via the kidneys. Prolonged and excessive exposure results in fluorosis, which is associated with cellular damage in liver and kidney tissues [19]. Excess fluoride induces soft tissue damage through oxidative stress mechanisms, including lipid peroxidation and mitochondriopathy [7]. Moreover, fluorine has been reported to alter energy metabolism and promote metabolic disorders [20, 21].

Antioxidants have shown promising effects in mitigating fluoride-induced toxicity [5, 13]. Fat-soluble α–tocopherol (vitamin E) protects cellular membranes by interrupting the lipid peroxidation cascade. It also reduces fluoride accumulation in hard tissues and exerts protective effects against fluorosis. Vitamin E has demonstrated the ability to ameliorate fluoride-induced reproductive toxicity in animal models [22]. Furthermore, its administration has been associated with enhanced antioxidant defense and reduced fluoride-induced damage [9]. Consistent with these findings, decreased activities of antioxidant enzymes Catalase and GPx, alongside increased Malondialdehyde levels, have been reported in liver tissues exposed to NaF [23]. In NRK–52E kidney epithelial cells, vitamin E has been shown to counteract NaF-induced oxidative DNA damage [24].

This study found that NaF administration significantly reduced the expression of the primary antioxidant defense genes Gpx1 and Sod1 in both liver and kidney tissues, while simultaneously upregulating the pro-oxidant gene Ncf1. Collectively, these observations are important evidence to show that fluoride exposure produces a high degree of oxidative stress [25]. Vitamin supplementation was able to significantly suppress the fluoride-induced increase in Ncf1 expression in both kidney and liver tissue. In particular, only the increase of Gpx1 and Sod1 in kidney tissue after vitamin supplementation was statistically significant ($P<0.05$) while in the liver tissue the increase was not statistically significant ($P>0.05$).

This difference in response between kidney and liver may suggest that the kidney, being the organ primarily responsible for the elimination of fluoride, has a unique (or much stronger) compensatory antioxidant response to vitamin supplementation compared to the liver, and this may be due to differing rates of fluoride accumulation in the respective tissues or the existence of tissue-specific metabolic pathways for fluoride excretion [26]. Studies have shown that antioxidant defense restoration is tissue-specific and that both vitamins C and E are effective in protecting tissues from fluoride-induced oxidative damage; this is confirmed by the beneficial results of using these vitamins together in clinical studies [27].

Chronic exposure to fluoride has been associated with inflammation-mediated apoptosis of several types of tissues, including cardiomyocytes, through extracellular matrix remodeling and altered calcium signalling [23]. In cardiomyocytes, cytotoxicity induced by NaF (production of mitochondrial ROS and programmed cell death) has been previously demonstrated in F9 embryonic carcinoma cells. Use of vitamin C was able to help lessen the cytotoxic damage caused by fluoride through upregulation of Sirt1 suggesting that it may be involved in regulating mitochondrial oxidative stress. [3].

Fluoride has been shown to activate L-type calcium channels in kidney cells and to upregulate apoptotic processes by causing an increase in the BcL–2 levels relative to bAX. In addition, vitamin – C pre-treatment may promote Sirt1 levels and reduce apoptosis in NaF-induced cells by promoting SOD2 function. Also, a 2020 study specifically examined the protective role of vitamins E and selenium as a combination treatment for DNA damage caused by fluoride to NRK–52E, which emphasizes how important supplementation with many antioxidants is for reducing genetic toxicity due to environmental contaminants [24].

Expression levels of PI3K/Akt signaling pathway genes

Gene expressions for signalling pathway includes PI3k, AKt1, TP53, Erbb2, and mtor which are shown the expression of these genes in FIG. 3 (L-intersection). All five genes (PI3k, Akt1, TP53, Erbb2, and mTOR) were strongly overexpressed when comparing NaF treatment with Control Subjects (P<0.0001). Combined (additive) treatment of vitamin E and vitamin C demonstrated significant suppression of gene expressions in both liver and kidney tissues (P<0.0001).

Phosphoinositide 3–Kinase / Protein Kinase B / mechanistic target of rapamycin signaling pathway modulation

The PI3K/Akt pathway functions as an important anti-apoptotic and pro-survival receptor-mediated signalling pathway [10, 11]. In this research, the primary result is the increased expression of all five genes in the PI3K/Akt/mTOR pathway (PI3k, Akt1, TP53, Erbb2 and mTOR) due to NaF treatment. With the addition of Vitamins C and E, the over-expressed genes were suppressed significantly (P<0.0001).

The significant upregulation of the PI3K/Akt pathway following exposure to NaF, along with the increased expression of pro-apoptotic markers suggests that there is a complicated cellular response to fluoride toxicity where the PI3K/Akt pathway is activated as a compensatory mechanism to counteract the severe oxidative stress and apoptotic signalling NaF produces. This hypothesis is in accordance with current literature describing the disruption of the SIRT1/PI3K/AKT axis in fluoride-induced cardiotoxicity and studies demonstrating that fluoride regulates chondrocyte proliferation and autophagy through the activation of the PI3K/AKT/mTOR pathway [28]. Therefore, the current research supports the idea that the PI3K/Akt pathway is a major target for organ damage caused by fluoride and a key target for protective agents.

The data suggests that there is a significant presence of Tp53 overexpression in our study. Tp53 functions as a tumor suppressor and pro-apoptotic component, but it also plays a critical role in the regulation of the cellular stress response. Elevated Tp53 levels evident in the present study was accompanied by also elevated levels of activation of the PI3K/Akt pathway.

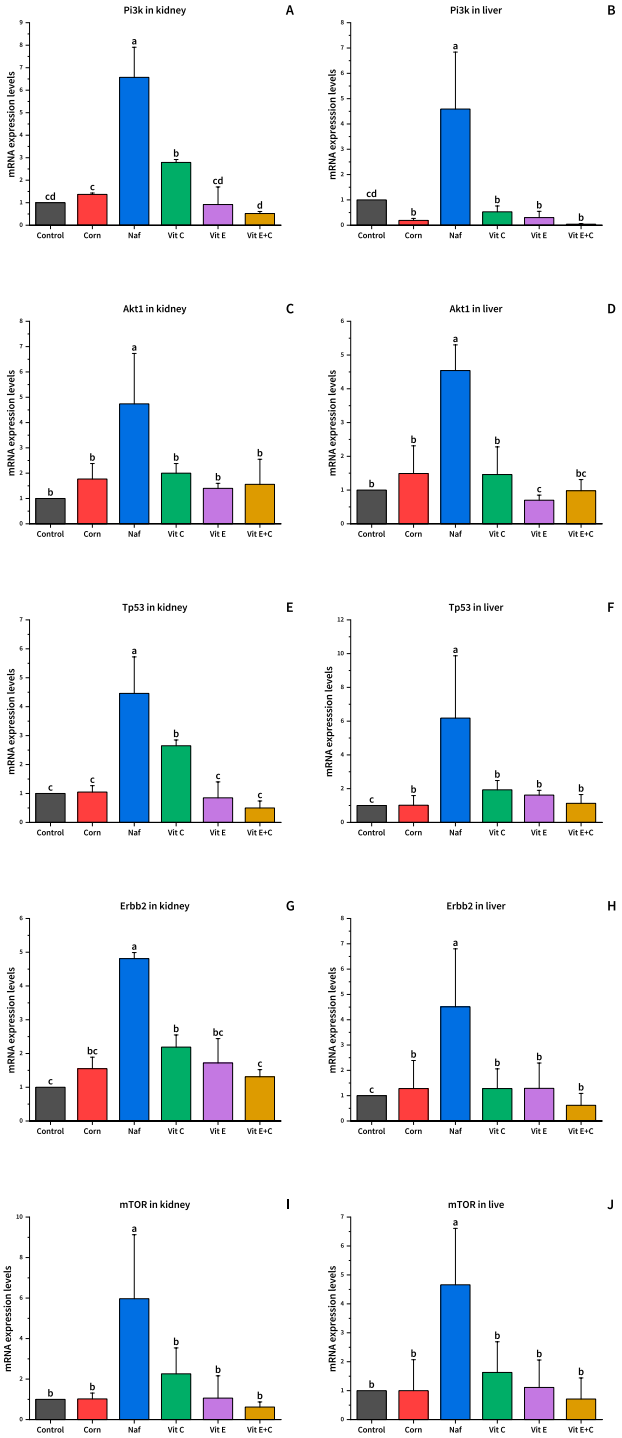

FIGURE 3. Expression levels of phosphatidylinositol 3–kinase (PI3K)/Akt pathway genes (Pi3k, Akt1, Tp53, Erbb2, mTOR) in kidney and liver tissues. (A) Pi3k mRNA expression in kidney tissue. (B) Pi3k mRNA expression in liver tissue. (C) Alt1 mRNA expression in kidney tissue. (D) Akt1 mRNA expression in liver tissue. (E) Tp53 mRNA expression in kidney tissue. (F) Tp53 mRNA expression in liver tissue. (G) Erbb2 mRNA expression in kidney tissue. (H) Erbb2 mRNA expression in liver tissue. (I) mTOR mRNA expression in kidney tissue. (J) mTOR mRNA expression in liver tissue

This may potentially indicate cellular crisis, where both the cell is receiving strong signals for survival and strong signals for apoptosis. The fact that vitamin C and vitamin E significantly downregulated all of the observed gene products suggests that the antioxidant compounds are not simply scavenging free radicals but are also actively returning the cellular signaling environment to a state of homeostasis by inhibiting the initial stressor, which in turn, reduces the need for the cell to produce conflicting high-level signals. The mechanistic data we present regarding the role of the PI3K/Akt pathway in fluorosis and its regulation by vitamins adds additional information to the body of literature.

CONCLUSIONS

Results of this work provide evidence that chronic exposure to NaF has substantial effects on oxidative stress, apoptosis and the PI3K/Akt signaling pathway in both the liver and kidney. Chronic exposure to NaF transformed the expression level of important antioxidant genes (Gpx1, Sod1) to lower levels and elevated Ncf1 (pro-oxidant) gene than normal control groups. Also, chronic exposure to NaF significantly increased the expression of Casp 3, Casp 8, Casp 9 (apoptotic markers) and several major genes associated with the PI3K/Akt/mTOR signaling pathway (Pi3k, Akt1, Erbb2, mTOR, Tp53).

Supplementation with vitamin C or vitamin E alone was able to reverse the negative effects of chronic NaF exposure on the expression of oxidative stress-related genes, reduce the level of apoptosis and negate the overstimulation of the PI3K/Akt/mTOR signaling pathway. Vitamin C and vitamin E worked together with a greater effectiveness than either supplement alone in restoring gene expression and in reversing detrimental cellular effects from NaF exposure. These data suggest that vitamin C and vitamin E may prevent fluoride-induced multi-organ toxicity and thus represent a therapeutic strategy to redirect the cellular damage resulting from chronic exposure to fluoride (fluorosis).

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Conflict of interest

The authors declare no conflict of interest.

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