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EFFECTS OF ACUTE AND CHRONIC FLUORIDE ADMINISTRATION ON SOME KIDNEY PARAMETERS OF RATS (CYS-c, KIM-1, AND NGAL)

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ABSTRACT: Fluorosis is an important disease both in modern societies as well as in the previous periods. Paleopathological studies reveal that people in ancient periods suffered from fluorosis which causes adverse health effects, especially on the musculoskeletal system as well as soft tissues such as kidneys. In this study, we aimed to investigate the effects of acute and chronic fluoride administration on some kidney markers in rats. Fifty-six Wistar albino rats were divided into 7 groups, 8 in each group. Acute fluoride intoxication was established by administering, in drinking water, 5 ppm (group 2), 15 ppm (group 3), and 50 ppm (group 4) for 7 days. Chronic fluoride intoxication was established by administering 5 ppm (group 5), 15 ppm (group 6), and 50 ppm for 90 days (group 7). Control group (group 1) was given tap water. At the end of the study, the rats were sacrificed under anesthesia and blood samples were taken. The blood was centrifuged and their serums were separated. CYS-c, KIM-1, and NGAL levels were measured by ELISA method, and urea, creatinine, total protein, and albumin levels were measured spectrophotometrically. CYS-c levels were increased in all groups administered fluoride ($p>0.05$). Similarly all groups had higher levels of NGAL due to fluoride exposure and chronic fluoride 5 mg/L group showed significant increase compared to control ($p<0.05$). In KIM-1 values, a significant increase occurred in acute fluoride 15 and 50 mg/L ($p<0.05$). Significant alterations were also observed in creatinine and urea values due to fluoride exposure. Consequently, exposure to fluoride may cause an increase in serum inflammation markers (NGAL, KIM-1) due to differences in dosage and exposure period. Further long term studies, including molecular and histopathological assessments, are needed to elucidate the impact of long term exposure to fluoride on the renal system.

Keywords: Fluoride; Fluorosis; CYS-c; Kidney; KIM-1; NGAL.

INTRODUCTION

Pollutants in the environment are constantly interacting with us and affect our health. Drinking water can transmit many diseases and in addition it is a route for exposure to different chemical pollutants. One of the most common chemicals that can cause health problems in drinking water is fluoride. Fluorine (F) is a toxic agent that causes adverse health effects on the reproductive and neurological systems, and may cause endocrine diseases as well as dental and skeletal fluorosis.[1-3] Fluoride is one of the most abundant elements in the earth's crust. It combines with other elements to produce compounds known as fluorides. Therefore, fluoride is a naturally occurring substance that can be found everywhere in the environment.[4] Fluoride in drinking water is often the main source of F intake.[5] Fluoride containing waters occur especially in areas where volcanic activities are intense. A study of the victims of the eruption of Mount Vesuvius in 79 AD in the nearby cities of Herculaneum, Pompeii, and Nocerq Inferiorie, found that dental fluorosis has occurred in this area since Roman times.[6] In the study, Petrone et al. analysed the changes in shape and

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color in the teeth from samples taken from individuals settled near Vesuvius and found dental fluorosis was present.[6] In another study carried out by Yoshimura et al., the cases showing pathological lesions and ones not showing such pathological lesions in the dental samples, taken from skeletal remains from 2nd and 3rd century underground tombs in Palmyra, Syria, were evaluated in terms of fluorosis.[7] As a result, it was determined that the teeth with lesions had a high fluoride content. For this reason, it has been reported that necessary measures should be taken to reduce the fluorine concentration in drinking water in order to prevent fluorosis.[8]

Animal modeling of diseases is an important data source for medical research. For example, in a study conducted on rats exposed to chronic lead accumulation, water containing 1,000 ppm lead acetate was administered to for 90 days to monitor the morphological changes on the femoral bone.[9] They are also important sources for understanding the diseases of people who lived in historical as well as prehistorical times. One of these studies is about the disease fluorosis. When fluoride is ingested in excess, it causes damage to bones and teeth and even soft tissues such as kidneys.

The main excretion route of fluoride from the body is the kidneys, so the soft tissue with the highest fluoride content is the kidney. Due to renal dysfunction, the half-life of fluoride in plasma is prolonged and clinical toxicity may occur even when taken at low concentrations.[10] The kidney is among the organs most sensitive in response to excessive amounts of fluoride.[11] The kidneys are the main organs in the excretion and retention of fluoride and therefore participate in chronic fluoride poisoning.[11] Nephrotoxic effects of fluoride exposure have been shown in experimental studies.[12] Moreover, ecological studies have shown that the fluoride content in drinking water is high in regions with a high prevalence of chronic kidney damage, and it has been suggested that there is a possible relationship between kidney damage and the amount of fluoride in drinking water.[13,14] In the kidney injury, some pathophysiological processes and molecules can be used as markers of early kidney damage. It has been stated that these markers may be more sensitive and specific than traditional markers such as creatinine and urea.[12] In previous studies, it has been reported that markers such as kidney injury molecule-1 (KIM-1), Cystatin-C (CYS-c) and NGAL increase in kidney damage.[15] Early detection of renal effects of fluoride exposure is important. Several biomarkers are currently available for early detection of kidney damage in correlation with histopathological changes. Therefore, we proposed that the evaluation of serum CYS-c, KIM-1, NGAL levels may be important in evaluating early kidney damage due to fluoride exposure. This study was carried out to examine the effects of acute and chronic fluoride exposure on the CYS-c, KIM-1, and NGAL markers.

MATERIALS AND METHODS

Ethical permission: Animal Experiments Local Ethics Committee permission was obtained for this study with the decision number 2018/03, dated 29.03.2018 and both institutional and national guidelines for the care and use of laboratory animals were followed during experimental procedures.

Study design: The material of the study was composed of 56 adult male Wistar albino rats. Rats were divided into 7 groups with 8 rats in each group.

Group 1: Control group. No administration was performed on rats.

In all the acute fluoride groups NaF (Sigma) was dissolved in drinking water, available *ad libitum*, for 7 days.

Group 2: Acute fluoride group (5 mg/L): administered daily 5 mg/L NaF.

Group 3: Acute fluoride group (15 mg/L): administered daily 15 mg/L NaF.

Group 4: Acute fluoride group (50 mg/L): administered daily 50 mg/L NaF.

In all the chronic fluoride groups NaF was dissolved in drinking water, available *ad libitum*, for 90 days.

Group 5: Chronic fluoride group (5 mg/L): administered daily 5 mg/L NaF.

Group 6: Chronic fluoride group (15 mg/L): administered daily 15 mg/L NaF.

Group 7: Chronic fluoride group (50 mg/L): administered daily 50 mg/L NaF.

At the end of the experimental protocol, animals were sacrificed under anesthesia with xylazine 10 mg/kg IP (2% Rompun,® Bayer) and Ketamine (HCl) (10% Alfamine,® Atafen) 75 mg/kg IP injectable anesthetics. Serum was obtained from the obtained blood. NGAL (catalog number; SG-20801), KIM-1 (catalog number; SG-20751) and CYS-c (catalog number; SG-20197) levels were also determined by ELISA method with commercial kits. Urine and serum urea and creatinine levels were measured in an Architect ci16200 model auto analyzer using Abbott branded commercial kits.

Statistical analysis: All the values were expressed as mean±SD of eight rats in each group. The data were analyzed with one-way analysis of variance (ANOVA) followed by Turkey's multiple comparison test. $P<0.05$ was considered to indicate statistical significance.

RESULTS

No significant difference was found in the CYS-c level in all the groups. However all the values were higher in all the fluoride groups compared to the control group. The KIM-1 level was found to be highest in the acute fluoride 15 and 50 mg/L groups and this increase was significant compared to the chronic fluoride 50 mg/L group ($p<0.05$). The differences between the other groups showed no statistical significance. All the values for NGAL in the fluoride groups were higher compared to the control group. However the only significant difference was found between the control group and the 5 mg/L chronic fluoride group. The serum creatinine level was found to be significantly higher than the control group in the acute fluoride group administered 5 and 15 ppm fluoride and also in the chronic fluoride 15 mg/L group. Serum urea level was lower in acute fluoride 50 mg/L and chronic fluoride 5 mg/L group compared to the chronic fluoride 15 mg/L group ($p<0.05$). The serum total protein and albumin levels were similar between the groups with no statistically significant differences. The NGAL, CYS-c, KIM-1, total protein, albumin, urea, and creatinine levels of the groups are given in Table 1.

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Table 1. Biochemical parameters of control group and acute as well as chronic fluoride administered groups. KIM-1 = kidney injury molecule-1, CYS-c = Cystatin-C; Data are presented as mean±SD.

Parameter	Group						
	Control Mean ±SD*	Acute fluoride 5 mg/L Mean ±SD*	Acute fluoride 15 mg/L Mean ±SD*	Acute fluoride 50 mg/L Mean ±SD*	Chronic fluoride 5 mg/L Mean ±SD*	Chronic fluoride 15 mg/L Mean ±SD*	Chronic fluoride 50mg/L Mean ±SD*
CYS-c (ng/mL)	37.9 ±6.1^{a}	39.0 ±6.4^{a}	38.5 ±14,5^{a}	39.6 ±4.6^{a}	50.6 ±6.9^{a}	46.8 ±7.06^{a}	41.1 ±5.37^{a}
KIM-1 (pg/mL)	77.1 ±5.6^{ab}	76.6 ±5.6^{ab}	83.3 ±6.3^{b}	82.8 ±6.8^{b}	77.9 ±7.5^{ab}	75.4 ±3.0^{ab}	72.1 ±2.4^{a}
NGAL (ng/mL)	0.57 ±0.0^{a}	0.66 ±0.1^{a}	0.66 ±0.1^{a}	0.65 ±0.1^{a}	0.81 ±0.2^{b}	0.69 ±0.1^{ab}	0.66 ±0.1^{a}
Creatinine (mg/dL)	0.53 ±0.02^{a}	0.68 ±0.04^{b}	0.66 ±0.17^{b}	0.63 ±0.01^{ab}	0.60 ±0.01^{ab}	0.64 ±0.04^{b}	0.61 ±0.07^{ab}
Urea (mg/dL)	49.3 ±2.4^{ab}	53.7 ±2.5^{bc}	49.0 ±8.3^{ab}	45.0 ±7.0^{a}	44.7 ±3.1^{a}	56.7 ± 5.3^{c}	53.7 ±5.3^{bc}
Total protein (g/L)	64.0 ± 2.0^{a}	71.3 ±2.5^{a}	70.3 ±12.8^{a}	67.0 ±1.7^{a}	66.3 ±3.7^{a}	72.3 ± 5.1^{a}	63.3 ±3.6^{a}
Albumin (g/L)	31.9 ±2.0^{a}	32.7 ±1.2^{a}	32.5 ±5.9^{a}	32.0 ±1.0^{a}	29.3 ±1.5^{a}	32.3 ± 2.0^{a}	29.3 ± 1.9^{a}

*Different letters on the same line indicate statistical significance ($p < 0.05$).

DISCUSSION

An excessive fluoride intake may impair health. The fluoride ion can pass through the intestinal barrier and be distributed throughout the body and stored in various tissues.[16] Fluorine has an effect on the function of the thyroid gland and this effect causes thyroid complications in pregnant women.[17] It has been reported that the serum fluoride concentration increases after oral administration of sodium fluoride.[16,18] It has been suggested that there is a constant absorption rate of fluoride from the gastrointestinal tract after sodium fluoride is administered via drinking water, food or the oral route.[18] Since fluoride-induced renal dysfunction alters fluoride concentration, urinary excretion of fluoride decreases, which may contribute to the increase of fluoride serum concentration.[18] Intestinal fluoride absorption is

dependent on gastric acidity because fluoride is absorbed from the gastrointestinal system after soluble hydrogen-fluoride formation under the normal acidic pH in the stomach.[18] Chronically, 500 ppm NaF application has been shown to decrease the total protein level in the skeletal muscle of rabbits and in the liver and serum of rats.[19] The decrease in protein level was explained by altered food intake.[19] Prolonged exposure to fluoride may result in inhibition of protein synthesis as it suppresses Na-K ATPase activity, which is important for amino acid uptake.[4] The progression of kidney damage largely overlaps with the kidney's ability to repair itself. Kidney damage and tissue repair are dynamic events with progression and regression of kidney damage.[15]

Since the Food and Drug Administration (FDA) and the European administrative authority responsible for similar issues (European Medicines Agency) have accepted the use of CYS-c and KIM-1 in preclinical studies as urine markers of kidney injury in 2008[20] and as NGAL is also accepted as an early biomarker along with CYS-c and KIM-1,[21] we wanted to measure CYS-c, KIM-1, and NGAL in serum samples for a comparison of acute and chronic fluoride exposure in an acute and a chronic fashion.

CYS-c is a low molecular weight cysteine protease inhibitor. CYS-c is produced from all cells containing a nucleus. CYS-c is filtered freely from the glomeruli and reabsorbed by proximal tubule cells and catabolized.[12] The serum CYS-c level is closely related to the glomerular filtration rate.[22] CYS-c is used as a biomarker of kidney injury in different models such as intoxication with fluoride, exposure to biological molecules such as gentamicin,[23] and experimental models such as diabetes mellitus.[24] A positive relationship was found between urinary CYS-c and fluoride exposure in a study conducted on people living in areas with 0.1–5 mg/L fluoride in drinking water.[12] Environmental fluoride exposure also caused an augmentation in CYS-c in adult human urine.[12] In the present study, the administration of fluoride caused an non-significant increase in the serum CYS-c levels compared to the control group ($p>0.05$). Although statistically insignificant, the chronic groups had higher CYS-c levels compared to the acute groups. This finding suggests an augmentation of the impact of fluoride during chronic administration. In future studies, with a greater sample size, significant results might be found. In a study by Cárdenas-González et al. conducted on rats, it was shown that the urine CYS-c level was increased after subchronic fluoride exposure.[15] The results of the present study are in accordance the findings of Cárdenas-González et al. In a study by Ge et al., renal ischemia reperfusion injury caused a peak increase in serum CYS-c 24 hours post reperfusion but the level was still high on the 7th day.[25] Since in our experiment, the reason for the injury (uninterrupted intake of fluoride containing water) was continued for 90 days it was expected that we would see augmented levels of CYS-c in the chronic groups. Although the observation of high values in the chronic groups indicates that an injury due to fluoride exposure has occurred, no significant value was found. Biological unpredictable variations may also interfere with expected results. More research is required to elucidate relations between fluoride exposure and its effects on some kidney markers both in human and rat experimental models.

KIM-1 is a recently discovered transmembrane protein. KIM-1 is expressed by differentiated proximal renal epithelial cells at damage sites. It can participate in the progression of kidney damage and repair. In many studies, different functions of

KIM-1 have been shown in acute and chronic kidney damage.[26] KIM-1 is usually found in damaged tubule cells that differentiate and replicate. However, it is difficult to detect KIM-1 in damaged tubule cells, such as completely flattened and atrophic cells.[27] Tubular KIM-1 expression is also associated with tubular interstitial damage and inflammation.[28] Fluoride (25 mg/kg body weight) given to rats for 4 weeks was reported to increase KIM-1 in the inner proximal tubules of kidney.[29] It has been suggested that KIM-1 is expressed as part of the tissue repair process after the damaged cells leave the tubule and the surviving cells migrate to the area at the base of the membrane to repair the epithelial barrier.[30] Transition to these cells differentiated from normal epithelial cells is associated with a dramatic upregulation of KIM-1 expression.[31] It has been shown that the KIM-1 mRNA level is upregulated in the renal cortex of rats exposed to fluoride in drinking water.[11] In the study by the Cárdenas-González et al., KIM-1 levels increased in the 15 mg/L ($p>0.05$) and the 50 mg/L ($p<0.05$) fluoride groups at 40 days of exposure.[15] Three hundred mg/L NaF exposure to Wistar-albino rats, given via drinking water for 7 days, resulted in a significant increase in KIM-1 expression, as shown by immunohistochemistry.[32,33] In our study, both the 15 and 50 mg/L acute fluoride groups caused a significant increase in serum KIM-1 levels compared to the control with 7 days of administration ($p<0.05$). Our results are consistent with this study. KIM-1 is stated to be an early indicator of kidney damage and, in a study by Bontemps et al., it was shown that its gene expression was induced in 3 days when NaF is administered i.p. at 2 mg/kg dose.[34] Although expression of KIM-1 in the early stage of damage is considered as an adaptive response, its chronic expression is concluded to be a maladaptive response and associated with the development of fibrosis.[35] Our results showed higher levels in acute exposure (15 and 50 mg/L). It has been reported that KIM-1 and CYS-c showed a positive correlation due to exposure to aflatoxin-B1 in Mexican indigenous women.[21]

NGAL is a small protein of the lipocalin family, weighing 25 kDa. In recent studies, it has been reported that NGAL starts to increase in the early period of renal damage[36] and can be used as an early predictor of acute kidney injury.[37] In a study comparing NGAL and serum creatinine in determining early stage renal damage, Wagener et al. reported that serum creatinine was delayed 1–3 days in determining renal damage compared to NGAL.[38] Moreover, NGAL plays a protective role in early acute kidney injury due to its antiapoptotic properties.[39] NGAL is expressed in other tissues and it is induced in inflammation and other types of damage.[40] In mouse models, it has been observed that the NGAL mRNA level in the kidney increases after a short period of cisplatin and renal ischemia.[41,42] Plasma NGAL levels have been shown to be lower in dogs with chronic kidney disease than dogs with acute kidney disease. It is known that acute kidney disease upregulates a variety of inflammatory genes, including the lipoqualin2 genes encoding NGAL. In acute kidney injury, the decrease in the filtration capacity of the kidney leads to a decrease in NGAL clearance and therefore it accumulates systemically.[43] Plasma NGAL level does not only increase in renal diseases but it also increases in other diseases such as acute infection.[43] Increasing serum NGAL level may reflect inflammatory damage rather than acute injury. It has also been emphasized that NGAL is not organ-specific.[44] NGAL level has been shown to stabilize in 24–48 hours after the injury. In our study, similar to CYS-c, NGAL levels were also high in all the fluoride

administered groups which reached significance in the chronic fluoride 5 mg/L group. It can be stated that fluoride administration triggers kidney inflammation even in the acute period and also in low dose. It is known that NGAL increases 2 hours after kidney injury. However, from the literature, it was also observed that an increase in NGAL still persists at a high level after 60 days in rats with conditions such as hypertension and hyperglycemia.[45] In our study, high NGAL levels were still observed in the chronic fluoride groups and reached statistical significance in the 5 mg/L chronic fluoride group. A decrease of NGAL due to the administration of a chemical to rats such as curcumin is accepted as an alleviation of this damage.[46] Our animals still had higher values in the chronic period compared to the control which reached significance in the 5 mg/L chronic group and suggested persistence of a chronic kidney injury. A study by Severin et al., found that excretion of NGAL in urine varies in a time course; it makes a dramatic decrease in first 4 days and then increases.[47] They considered this situation as a reflection of molecular changes such as down-regulation due to methotrexate-induced kidney injury.

The increase in creatinine (significant in the acute fluoride groups administered 5 and 15 ppm fluoride and also in the chronic fluoride 15 mg/L group) in all the fluoride groups indicates that the fluoride exposure to rats had an adverse effect in our study. The findings of our study are in accordance with Cárdenas-González.[15] Although the BUN and creatinine are accepted as gold standards for the assessment of renal function, CYS-c, NGAL[48] and KIM-1[49] are emerging as sensitive biomarkers which enable a more specific detection of damage.

The serum urea level showed a significant decrease in the acute fluoride 50 mg/L and chronic fluoride 5 mg/L groups compared to the chronic fluoride 15 mg/L group. This finding suggests a complex interplay of different physiological events with results which do not have a simple relationship with the dosage given or the duration of exposure.

The total protein and albumin levels showed no significant difference among the groups and this finding indicates that the impact of fluoride on the kidney does not cause a great change in these parameter. Decreased albumin and total protein values have been reported due to renal injury with agents such as doxorubicin.[50]

CONCLUSION

In this current study, Wistar albino rats fluoride were exposed fluoride in different doses (5, 15, and 50 mg/L) and periods (7 days acute and 90 days chronic). Our results suggest a potential harmful renal effect of fluoride which was made visible by alterations of some kidney markers such as KIM-1, NGAL and CYS-c, which are emerging biomarkers for renal tubular diseases. Fluoride may therefore, at an appropriate dosage and duration of exposure, have a proinflammatory impact on kidneys.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

REFERENCES

1 Jarquín-Yañez L, de Jesús Mejía-Saavedra J, Molina-Frechero N, Gaona E, Rocha-Amador DO, López-Guzmán OD, at al. Association between urine fluoride and dental fluorosis as a toxicity factor in a rural community in the state of San Luis Potosi. The Scientific World Journal 2015:2015;647184. doi: 10.1155/2015/647184.

2 Biglari H, Chavoshani A, Javan N, Mahvi AH. Geochemical study of groundwater conditions with special emphasis on fluoride concentration, Iran. Desalination and Water Treat 2016: 57(47);22392-9.

3 Mohammadi AA, Yousefi M, Yaseri M, Jalilzadeh M, Mahvi AH. Skeletal fluorosis in relation to drinking water in rural areas of West Azerbaijan, Iran. Sci Rep 2017:7(1);17300.

4 Perumal E, Paul V, Govindarajan V, Panneerselvam L. A brief review on experimental fluorosis. Toxicol Lett 2013;223(2):236-51.

5 Dobaradaran S, Mahvi AH, Dehdashti S, Dobaradaran S, Shoara R. Correlation of fluoride with some inorganic constituents in groundwater of Dashtestan, Iran. Fluoride 2009:42(1);50-3.

6 Petrone P, Graziano V, Sastri C, Sauvage T, Mezzasalma M, Paternoster M, et al. Dental fluorosis in the Vesuvius towns in AD 79: a multidisciplinary approach. Ann Hum Biol 2019;46:(5):388-392.

7 Yoshimura K, Nakahashi T, Saito K. Why Did the Ancient Inhabitants of Palmyra Suffer Fluorosis. J Archaeol Sci 2006;33(10):1411-8.

8 Yousefi M, Ghoochani M, Mahvi Ah. Health risk assessment to fluoride in drinking water of rural residents living in the Poldasht city, Northwest of Iran. Ecotoxicol Environ Saf 2018;148:426-40.

9 Alvarez-Lloret P, Ming Lee C, Inés Conti M, Romina Terrizzi A, González-López S, Pilar Martínez M. Effects of chronic lead exposure on bone mineral properties in femurs of growing rats. Toxicology 2017;(377):64-72.

10 Karabulut E, Otlu O, Pakdemirli A, Yarum M, Salt A, Cenesiz S. The effects of quercetin on the fluorosis toxicity in kidney of mice. Journal of Tumour Research and Reports 2019;4:126.

11 Shashi A, Singh J, Thapar SJF. Toxic effects of fluoride on rabbit kidney. Fluoride 2002;35(1):38-50.

12 Jiménez-Córdova MI, Cárdenas-González M, Aguilar-Madrid G, Sanchez-Peña LC, Barrera-Hernández Á, Domínguez-Guerrero IA, et al. Evaluation of kidney injury biomarkers in an adult Mexican population environmentally exposed to fluoride and low arsenic levels. Toxicol Appl Pharmacol 2018;352:97-106.

13 Chandrajith R, Nanayakkara S, Itai K, Aturaliya T, Dissanayake C, Abeysekera T, et al. Chronic kidney diseases of uncertain etiology (CKDUE) in Sri Lanka: geographic distribution and environmental implications. Environ Geochem Health 2011;33(3):267-78.

14 Dharmaratne RW. Fluoride in drinking water and diet: the causative factor of chronic kidney diseases in the North Central Province of Sri Lanka. Environ Health Prev Med 2015; 20(4):237-42.

15 Cárdenas-González MC, Del Razo LM, Barrera-Chimal J, Jacobo-Estrada T, López-Bayghen E, Bobadilla NA, et al. Proximal renal tubular injury in rats sub-chronically exposed to low fluoride concentrations. Toxicol Appl Pharmacol 2013;272(3):888-94.

16 Aydin G, Cicek E, Akdogan M, Gokalp O. Histopathological and biochemical changes in lung tissues of rats following administration of fluoride over several generations. J Appl Toxicol 2003;23(6):437-46.

17 Kheradpisheh Z, Mirzaei M, Mahvi AH, Mokhtari M, Azizi R, Fallahzadeh H, et al. Impact of drinking water fluoride on human thyroid hormones: A case-control study. Sci Rep 2018;8(1):2674.

18 Ekambaram P, Paul V. Calcium preventing locomotor behavioral and dental toxicities of fluoride by decreasing serum fluoride level in rats. Environ Toxicol Pharmacol 2001;9(4):141-6.

19 Shashi AJF. Histopathological effects of sodium fluoride on the duodenum of rabbits. Fluoride 2002;35(1):28-37.

20 Dieterle F, Sistare F, Goodsaid F, Papaluca M, Ozer JS, Webb CP, et al. Renal biomarker qualification submission: a dialog between the FDA-EMEA and Predictive Safety Testing Consortium Nat Biotechnol 2010;28(5):455-62.

21 de León-Martínez LD, Díaz-Barriga F, Barbier O, Ortíz DLG, Ortega-Romero M, Pérez-Vázquez F, et al. Evaluation of emerging biomarkers of renal damage and exposure to aflatoxin-B1 in Mexican indigenous women: a pilot study. Environ Sci Pollut Res Int 2019;26(12):12205–16.

22 Zaffanello M, Franchini M, Fanos V. Is serum Cystatin-C a suitable marker of renal function in children? Ann Clin Lab Sci 2007;37(3):233-40.

23 Cárdenas-González M, Estrada TJ, Rodríguez-Muñoz R, Barrera-Chimal J, Bobadilla NA, Barbier OC, et al. Sub-chronic exposure to fluoride impacts the response to a subsequent nephrotoxic treatment with gentamicin. J Appl Toxicol 2016;36(2):309-19.

24 Yuan D, Liu XM, Fang Z, Du LL, Chang J, Lin SH. Protective effect of resveratrol on kidney in rats with diabetic nephropathy and its effect on endoplasmic reticulum stress. Eur Rev Med Pharmacol Sci 2018;22(5):1485-93.

25 Ge YZ, Wu R, Xin H, Liu H, Lu TZ, Zhao YC, et al. Effects of ischemic preconditioning on the systemic and renal hemodynamic changes in renal ischemia reperfusion injury. Int J Clin Exp Pathol 2015;8(2):1128–40.

26 Huo W, Zhang K, Nie Z, Li Q, Jin F. Kidney injury molecule-1 (KIM-1): a novel kidney-specific injury molecule playing potential double-edged functions in kidney injury. Transplant Rev (Orlando) 2010;24(3):143-6.

27 van Timmeren MM, van den Heuvel MC, Bailly V, Bakker SJ, van Goor H, Stegeman CA. Tubular kidney injury molecule-1 (KIM-1) in human renal disease. J Pathol 2007;212(2):209-17.

28 Waanders F, van Timmeren MM, Stegeman CA, Bakker SJ, van Goor H. Kidney injury molecule-1 in renal disease. J Pathol 2010;220(1):7-16.

29 Thangapandiyan S, Miltonprabu S. Epigallocatechin gallate supplementation protects against renal injury induced by fluoride intoxication in rats: Role of Nrf2/HO-1 signaling. Toxicol Rep 2014;27(1):12-30.

30 Prozialeck WC, Vaidya VS, Liu J, Waalkes MP, Edwards JR, Lamar PC, et al. Kidney injury molecule-1 is an early biomarker of cadmium nephrotoxicity. Kidney Int 2007; 72(8):985-93.

31 Ichimura T, Bonventre JV, Bailly V, Wei H, Hession CA, Cate RL, et al. Kidney injury molecule-1 (KIM-1), a putative epithelial cell adhesion molecule containing a novel immunoglobulin domain, is up-regulated in renal cells after injury. J Biol Chem 1998;273(7):4135-42.

32 Oyagbemi AA, Omobowale TO, Ola-Davies OE, Asenuga ER, Ajibade TO, Adejumobi OA, et al. Ameliorative effect of Rutin on sodium fluoride-induced hypertension through modulation of Kim-1/NF-κB/Nrf2 signaling pathway in rats. Environ Toxicol 2018;33(12):1284-97.

33 Oyagbemi AA, Omobowale TO, Ola-Davies OE, Asenuga ER, Ajibade TO, Adejumobi OA, et al. Luteolin-mediated Kim-1/NF-kB/Nrf2 signaling pathways protects sodium fluoride-induced hypertension and cardiovascular complications. Biofactors 2018;44(6):518-31.

34 Bontemps A, Conquet L, Elie C, Magneron V, Gloaguen C, Kereselidze D, et al. *In vivo* comparison of the phenotypic aspects and molecular mechanisms of two nephrotoxic agents, sodium fluoride and uranyl nitrate. Int J Environ Res Public Health 2019;16(7),1136.

35 Bonventre JV. Kidney injury molecule-1: A translational journey. Trans Am Clin Climatol Assoc 2014;125:293-9.

36 Hirsch R, Dent C, Pfriem H, Allen J, Beekman RH, Ma Q, et al. NGAL is an early predictive biomarker of contrast-induced nephropathy in children. Pediatr Nephrol 2007;22(12):2089-95.

37 Kong HY, Zhu SM, Wang LQ, He Y, Xie HY, Zheng SS. Sevoflurane protects against acute kidney injury in a small-size liver transplantation model. Am J Nephrol 2010;32:347-55.

38 Wagener G, Jan M, Kim M, Mori K, Barasch JM, Sladen RN, et al. Association between increases in urinary neutrophil gelatinase-associated lipocalin and acute renal dysfunction after adult cardiac surgery. Anesthesiology 2006;105(3):485-91.

39 Haase M, Bellomo R, Haase-Fielitz A. Neutrophil gelatinase-associated lipocalin. Curr Opin Crit Care 2010;16(6):526-32.

40 Cowland JB, Borregaard N. Molecular characterization and pattern of tissue expression of the gene for neutrophil gelatinase-associated lipocalin from humans. Genomics 1997;45(1):17-23.

41 Mishra J, Ma Q, Prada A, Mitsnefes M, Zahedi K, Yang J, et al. Identification of neutrophil gelatinase-associated lipocalin as a novel early urinary biomarker for ischemic renal injury. J Am Soc Nephrol 2003;14(10):2534-43.

42 Mishra J, Mori K, Ma Q, Kelly C, Barasch J, Devarajan P. Neutrophil gelatinase-associated lipocalin: a novel early urinary biomarker for cisplatin nephrotoxicity. Am J Nephrol 2004;24(3):307-15.

43 Steinbach S, Weis J, Schweighauser A, Francey T, Neiger R. Plasma and urine neutrophil gelatinase-associated lipocalin (NGAL) in dogs with acute kidney injury or chronic kidney disease. J Vet Intern Med 2014;28(2):264-9.

44 Smertka M, Chudek J. Using NGAL as an early diagnostic test of acute kidney injury. Ren Fail 2012;34(1):130-3.

45 Caires A, Convento MB, Castino B, Leme AM, de Andrade Pessoa E, Aragão A, et al. Antioxidant effect of endothelin-1 receptor antagonist protects the rat kidney against chronic injury induced by hypertension and hyperglycemia. J Bras Nefrol 2019;41(4):451-61.

46 Zhao YH, Shen CF, Wang GJ, Kang Y, Song YH, Liu JW. Curcumin alleviates acute kidney injury in a dry-heat environment by reducing oxidative stress and inflammation in a rat model. J Biochem Mol Toxicol 2021;35:e22630.

47 Severin MJ, Campagno RV, Brandoni A, Torres AM. Time evolution of methotrexate-induced kidney injury: A comparative study between different biomarkers of renal damage in rats. Clin Exp Pharmacol Physiol 2019;46(9):828-36.

48 de León-Martínez LD, Ortega-Romero M, Grimaldo-Galeana JM, Barbier O, Vargas-Berrones K, García-Arreola ME, et al. Assessment of kidney health and exposure to mixture pollutants in the Mexican indigenous population. Environ Sci Pollut Res Int 2020;27:34557–66.

49 Oyagbemi AA, Akinrinde AS, Adebiyi OE, Jarikre TA, Omobowale TO, Ola-Davies OE, et al. Luteolin supplementation ameliorates cobalt-induced oxidative stress and inflammation by suppressing NF-κB/Kim-1 signaling in the heart and kidney of rats. Environ Toxicol Pharmacol 2020;80:103488.

50 Khames A, Gad AM, Abd El-Raouf OM, Kandeil MA, Khalaf MM. Sodium thiosulphate shows promising anti-inflammatory role against doxorubicin-induced renal injury depending on tlr4 pathway inhibition. Plant Arch 2020;20(2):2948-58.