Ref: Ro J Med Pract. 2024;19(4)
DOI: 10.37897/RJMP.2024.4.10

# The association between the serum levels of Molybdenum and Cadmium with the development of urolithiasis

Qayssar Shaheed JASIM[1], Basim Jasim HAMEED[1], Zainab Haroon AHMED[2]

[1]Clinical Laboratory Sciences Department, College of Pharmacy, University of Basrah, Basrah, Iraq
[2]Pharmacology and Toxicology Department, College of Pharmacy, University of Basrah, Basrah, Iraq

## ABSTRACT

***Background.*** *Kidney stones have increased globally and become a public health problem. The association between kidney stone risk and environmental heavy metal exposure was examined in some observational studies. Environmental and industrial metal contaminants commonly found include excess molybdenum (Mo) and cadmium (Cd). The objective of this study is to examine the correlation between the presence of kidney stones in a group of adults and the levels of these two metals in their blood serum.*

***Methods.*** *In this study, 44 patients with renal stones were taken against 44 healthy subjects from Basra Governate in Iraq. We employ the Agilent Inductively Coupled Plasma Mass Spectrometry (ICP-MS Agilent 7500/USA) technique to quantify the concentrations of molybdenum (Mo) and Cadmium (Cd).*

***Results.*** *The results indicate a significant difference in patients' molybdenum (Mo) and cadmium (Cd) levels. Molybdenum (Mo) and cadmium (Cd) levels in the patient group were (179 ppb, 2.86 ppb) and in the healthy group (156 ppb, 1.22 ppb), respectively. The study also includes the antioxidant enzymes (catalase, glutathione peroxidase, malondialdehyde, xanthine oxidase, and superoxidase dismutase), which may be affected by increased heavy metal levels and significant differences between the patient and healthy groups. We use the enzyme-linked immune sorbent assay (ELISA) technique to assay antioxidant enzymes. The study also assesses the function of the liver and kidneys (serum uric acid, urea, serum creatinine, total serum bilirubin, direct and indirect bilirubin, GOT, GPT, and ALP). The patient and the healthy group significantly differed in liver and renal function parameters.*

***Conclusion.*** *The study determined that both molybdenum (Mo) and cadmium (Cd) are identified as risk factors for the formation of kidney stones. Elevated blood Mo and Cd exposure has been linked to increased urolithiasis in adults, and urolithiasis may be predisposed to high serum Mo and Cd levels.*

**Keywords:** heavy metals, Cadmium, Molybdenum, kidney stones, antioxidant enzymes

## INTRODUCTION

Heavy metals are a significant class of environmental contaminants that have a profound influence on the functioning of several parts of the body. Arsenic, iron, mercury, bismuth, chromium, uranium, copper, and lead are the primary nephrotoxic heavy metals that can cause damage to the glomeruli and tubules [1]. Humans are becoming exposed to low amounts of toxic metals in the natural environment due to increasing variables, including contaminated food, potable water, and air. Over 40 million individuals in Bangladesh are potentially vulnerable to consuming water that has been poisoned [2]. Various disorders linked to drinking water polluted by heavy metals have been documented in other publications [3,4].

Molybdenum is a biologically essential trace element in substantial quantities in humans, plants, and

*Corresponding author:*
Qayssar Shaheed Jasim
*E-mail:* basim.hameed@uobasrah.edu.iq

*Article History:*
Received: 4 June 2024
Accepted: 23 December 2024

animals [5]. It is widely recognized that it is found in the active areas of more than fifty enzymes that serve a crucial function in their molecular structure. Additionally, it engages in the redox process and facilitates oxygen atom transfer activities, promoting enzyme activity and maintaining the viability of cells [6]. Mo also has significant industrial applications, particularly in producing fertilizer, cast iron, mining, and industrial stainless steel. Consequently, due to its widespread industrial use, the amount of Mo content in the environment continuously rises [7]. Prior studies have demonstrated that elevated levels of Mo can harm several organs, such as the liver, kidneys, bones, spleen, and reproductive systems [8]. One of the primary organs that is exposed to Mo is the kidney. The kidney eliminates Mo, and it takes several weeks for total elimination. A diet high in Mo may lower the function of the antioxidant enzymes, exacerbate the accumulation of harmful free radicals, modify the expression of genes associated with programmed cell death [apoptosis), and ultimately result in different levels of oxidative harm and cellular death [8,9]. According to the findings, exposure of Duck kidney tubular epithelial cells to Mo and/or Cd via the mitochondrial pathway may induce oxidative damage and death. Additionally, the two metals may have a synergistic impact [10].

Cadmium (Cd) through their diet, nearly every member of the population becomes subjected to Cadmium [11]. Cadmium, a natural element, is frequently released into the environment by various human activities, including extracting it, melting it, manufacturing nickel/Cd batteries, plating metal with colors, stabilizing material, disposing of wastewater, and applying phosphate fertilizers and manure [11,12]. The biological half-life of Cadmium in humans is around 10-33 years. Prolonged intake of Cadmium in food leads to the buildup of this metal in the renal system, which can eventually cause malfunction of the tubules and impairment of the kidney. The skeletal system is also harmed by Cadmium [12,13]. Kidney necrosis, autophagy, and apoptosis have all been linked to Cd exposure, according to an investigation by Choudhury et al. and Gu et al. [14,15].

Furthermore, it was shown that Cd reduced ATPase activity, exacerbated calcium efflux in cells, and eventually resulted in irreversible kidney injury [15,16]. According to research, Cd may upset the Ca 2+ (calcium ion) equilibrium and cause autophagy [15,16]. An imbalance in intracellular Ca 2+ homeostasis was shown to be responsible for Cd-induced nephrotoxicity [17]. Results from earlier in vitro studies demonstrated autophagy's critical role in renal impairment that Cd and Mo co-induced in ducks [18]. Prior research has concluded that prolonged exposure causes cadmium accumulation in the proximal tubule epithelial cells, resulting in the death of these cells and functional impairment [19,20]. Tobacco plants acquire Cd at significant levels (650 to 3630 ng/g tobacco), mainly if cultivated in polluted soil [21]. From a pathophysiological perspective, investigations have identified three potential initial reaction strategies in the proximal tubule thus far. The mechanisms include (i) altering intracellular signaling cascades, (ii) upregulating cadherin-mediated cell adhesion, and (iii) oxidative stress induction [19]. According to recent studies, up to 50% of Cd deposits may accumulate in the kidney, where their mean half-life might cause renal toxicity [22].

## METHODS

**Participant's criteria**

Forty-four participants, ages between 23 and 75, who were diagnosed by a specialist urologist with a kidney stone(s), participated in this study. The serum samples were obtained from patients in Al-Mwanei hospital in Basra governorate after diagnosis of kidney stones. The social history of each subject was taken according to a questionnaire (age, gender, weight, height, protein food, UTI recurrence, renal stone recurrence, smoking, chronic disease, and chronic drug intake).

**Control group**

Forty-four healthy people, ranging in age from 24 to 69, were selected as the control group from members of the public seeking medical checkups and from individuals accompanying patients in the hospital.

**Ethical committee approval**

The Ethics Committee approved the study's methods after each participant gave verbal and written notification permission to take part in the study and have their blood collected for research purposes.

**Methods for collecting, preparing, and analyzing samples**

Each person (patient and healthy control) had their veins punctured with a 5mL disposable syringe to collect their venous blood. To get the serum, a blood sample was centrifuged at 2450xg for 10 minutes after being left to clot on the bench for 20 minutes. Subsequently, it was divided into three Eppendorf tubes. The initial Eppendorf tube containing serum was utilized to examine biochemical tests, such as serum (uric acid, urea, and creatinine), liver enzymes, and function (including alanine aminotransferase, aspartate aminotransferase, total serum bilirubin, direct bilirubin, and alkaline phosphatase) will be analyzed using the clinical chemistry analyzer Architect Chemistry System (Abbott, USA). The following Eppendorf tube was kept at a temperature of -20°C until they were used for metal assay (using Agilent Inductively Coupled Plasma Mass Spectrometry (ICP-MS Agilent 7500/USA). The final Eppendorf tubes group was kept at a temperature of -20°C

until used for assays of antioxidant enzymes by the ELISA technique (enzyme-linked immune sorbent assay).

**Body mass index measurement**

The body mass index (BMI) is presently used to define adult anthropometric height/weight characteristics. It was computed using the square-meter formula BMI=Weight (kg)/height.

**Statistical Analysis**

In this work, the results of all experiments are offered as Mean ± SD. For statistical analysis, one-way ANOVA was used, followed by Dunnett's t-test. Probability (P) values just under 0.05 were considered statistically significant.

## RESULTS

Research subjects' essential traits. This study included 88 individuals who completed health checkups, performed tests for several biochemical markers, and provided information through questionnaires. Out of the total, 61 individuals (69.31%) were males, while 27 individuals (30.68%) were females. The age range of patients with renal stones was from 23 to 75 years, with a mean age of 42.6 years. In contrast, healthy control participants ranged in age from 24 to 69 years, with a mean age of 40.7 years. The investigation revealed a significant correlation between the group of patients with kidney stones and the control group in terms of blood creatinine levels, blood urea and uric acid, serum cadmium and molybdenum, total serum bilirubin, direct and indirect bilirubin, liver enzymes (GOT, GPT, ALP), oxidative enzymes (catalase, glutathione peroxidase, malondialdehyde, xanthine oxidase, and superoxidase dismutase) and these correlations were statistically significant (P <0.05). No statistical correlation was seen between age, weight, height, or body mass index, as shown in Table 1.

***TABLE 1.*** *Demography for patient and control group*

| Parameter | Patients | Healthy | P value |
|---|---|---|---|
| Age | 42.6±12.6 | 40.7±10.7 | 0.074 |
| Weight (Kg) | 81.7±13.6 | 80.5±18.3 | 0.225 |
| Height (cm) | 168.1±8.8 | 172.5±9.6 | 0.02 |
| BMI (Kg/m$^2$) | 28.9±4.6 | 27.1±6.1 | 0.22 |

Data expressed as Mean ± SD. * p <0.05, ** p <0.01, *** p <0.001 compared to negative control

Table 2 shows the levels of heavy metals (Mo and Cd) in the serum samples of participants. The cadmium levels in the patient group (2.86 ppb) were elevated in high significance (P=0.00001) in comparison to the control group (1.22 ppb). The molybdenum levels in the patient group (179 ppb) were elevated in high significance (P = 0.0006) compared to the healthy group (156 ppb).

***TABLE 2.*** *Heavy metals levels (ppb) in the serum of the patient and control group*

| Parameter | Patients | Healthy | P value |
|---|---|---|---|
| Mo (ppb) | 179±31.6 | 156±26.4 | 0.0006 |
| Cd (ppb) | 2.86±1.9 | 1.22±0.8 | 0.00001 |

Data expressed as Mean±SD. * p <0.05, ** p <0.01, *** p <0.001 compared to negative control

The glutamic oxaloacetate transaminase (GOT) levels in the patient group (27.81 U/L) were elevated in high significance (P = 0.0019) compared to the healthy group (14.84 U/L). The alanine aminotransferase (ALT or GPT) levels in the patient group (23.31 U/L) were elevated in high significance (P = 0.0067) compared to the healthy group (16.15 U/L). Alkaline phosphatase (ALP) levels in patients (58.95 U/L) were elevated in high significance (P = 0.0003) compared to the healthy group (26.47 U/L), as mentioned in Table 3.

In Table 3, biochemical parameters show differences in significance as follows: Blood urea levels in the patient group (37.97 mg/dl) were elevated in high significance (P = 0.002) compared to the healthy group (26.52 mg/dl). Serum uric acid levels in the patient group (6.23 mg/dl) were elevated in high significance (0.0001) compared to the healthy group (4.55 mg/dl). Serum creatinine levels in the patient group (0.85 mg/dl) were elevated in high significance (P = 0.0003) compared to the healthy group (0.55 mg/dl). The total serum bilirubin levels in the patient group (0.82 mg/dl) were elevated in high significance (P = 0.0009) compared to the healthy group (0.63 mg/dl). Direct bilirubin levels in the patient group (0.36 mg/dl) were elevated in high significance (P = 0.0017) compared to the healthy group (0.22 mg/dl). Indirect bilirubin levels in the patient group (0.46 mg/dl) were elevated in significance (P = 0.013) compared to the healthy group (0.41 mg/dl).

***TABLE 3.*** *Biochemical parameters and liver enzyme levels in serum of patient and control group*

| Parameter | Patients | Healthy | P value |
|---|---|---|---|
| GOT (U/L) | 27.81±8.1 | 14.84±7.9 | 0.0019 |
| GPT (U/L) | 23.31±9.4 | 16.15±7.7 | 0.0067 |
| ALP (U/L) | 58.95±16.2 | 26.47±13.5 | 0.0003 |
| B. Urea (mg/dL) | 37.97±13.5 | 26.52±7.5 | 0.002 |
| S. Uric acid (mg/dL) | 6.23±1.52 | 4.55±1.12 | 0.0001 |
| S. Cr(mg/dL) | 0.85±0.27 | 0.55±0.14 | 0.0003 |
| TSB (mg/dL) | 0.82±0.22 | 0.63±0.15 | 0.0009 |
| Dir. Bilirubin (mg/dL) | 0.36±0.17 | 0.22±0.09 | 0.0017 |
| Ind. Bilirubin (mg/dL) | 0.46±0.1 | 0.41±0.12 | 0.013 |

Data expressed as Mean ± SD. * p <0.05, ** p <0.01, *** p <0.001 compared to negative control

Table 4 shows the results of antioxidant enzymes. Catalase serum concentration levels in the patient group (129.45 KU/L) were elevated in high significance (P = 0.0019) compared to the healthy group (116.62 KU/L). Glutathione serum concentration levels in the patient group (5.95 ng/ml) were elevated in significance (P = 0.049) compared to the healthy group (3.41 ng/ml). Malondialdehyde serum concentration levels in the patient group (11.83 nmol/ml) were elevated in significance (P = 0.039) compared to the healthy group (9.02 nmol/ml). Xanthine oxidase serum concentration levels in the patient group (19.54 ng/ml) were elevated in significance (P = 0.032) compared to the healthy group (16.42 ng/ml). Superoxidase dismutase serum concentration levels in the patient group (21.35 ng/ml) were elevated in high significance (P = 0.0063) compared to the healthy group (14.11 ng/ml).

***TABLE 4.*** *Antioxidant enzyme levels in serum and blood of patient and control group*

| Parameter | Patients | Healthy | P value |
|---|---|---|---|
| Catalase KU/L | 129.45 ± 44.5 | 116.62 ± 26.7 | 0.0019 |
| Glutathione peroxidase ng/ml | 5.95 ± 2.1 | 3.41 ± 1.6 | 0.049 |
| Malondialdehyde nmol/ml | 11.83 ± 8.6 | 9.02 ± 7.6 | 0.039 |
| Xanthine oxidase ng/ml | 19.54 ± 9.2 | 16.42 ± 8.4 | 0.032 |
| Superoxidase dismutase ng/ml | 21.35 ± 8.9 | 14.11 ± 4.6 | 0.0063 |

Data expressed as Mean±SD. * p <0.05, ** p <0.01, *** p <0.001 compared to negative control

## DISCUSSION

We collected physical questionnaire information and serum samples from people in Basra Governorate in Iraq to examine the correlation between blood levels of Mo and Cd and the development of kidney stones compared to a healthy control group. The study also examined the biochemical parameters and liver enzyme levels in the serum of the patient and control group (i.e., GOT, GPT, ALP, B. urea, S. uric acid, S. Cr, TSB, Dir. Bilirubin, and Ind. bilirubin). It also evaluated blood antioxidant enzyme levels for the same groups (catalase, glutathione peroxidase, malondialdehyde, xanthine oxidase, and superoxidase dismutase). The demographic variables were collected through the reported questionnaire and studied as possible risk factors associated with kidney stones in this study; there was no statistical correlation of kidney stones with healthy control for BMI, age, weight, and height.

The heavy metal (Table 2) shows higher average levels of Mo (179 ppb) and Cd (2.86 ppb) in the urolithiasis group against the healthy group (156 ppb, 1.22 ppb, respectively), leading to a significantly reduced difference in comparison to the healthy control (P=0.0006, P=0.00001, respectively). The kidney stone group had greater levels of urea (37.97mg/dl), creatinine (0.85mg/dl), and uric acid (6.23mg/dl) than the control group (26.52mg/dl, 0.55mg/dl, 4.55mg/dl, respectively), and the difference was highly significant (Table 3).

Mo and Cd have been demonstrated to cause dose-dependent nephrotoxicity, and it is now well-acknowledged that large dosages of these substances are nephrotoxic toxicants [23]. One of the primary organs that are exposed to Mo is the kidney [24]. Mo is eliminated through the kidneys, which take many weeks to do so thoroughly. Excessive consumption of Mo can decrease the activities of antioxidant enzymes, increase the formation of free radicals, alter the expression of genes associated with apoptosis, and eventually result in various levels of cell oxidative harm and cell death in the human body [8]. There are several possible explanations for the correlation between the occurrence of kidney stones and Cd exposure. First off, one of the main pathophysiological factors for calcium nephrolithiasis is hypercalciuria, which can be brought on by exposure to Cd [25,26]. Hypercalciuria, kidney stones, and obstructive nephropathy can result from abnormalities in the management of calcium in the kidneys [27]. Cd can compete and replace calcium ions at different target locations since it is a divalent cation [28,29]. Second, kidney stones can occur as a result of tubular dysfunction, another degenerative condition brought on by Cd exposure. Nephrolithiasis was more common in those living in Thailand's cadmium-contaminated villages and those exposed to Cadmium at work [30]. An additional contributing factor in chronic tubulointerstitial nephritis is occupational contamination with Cadmium [31].

The traditional markers UREA, CR, and UA are frequently employed in medical work to assess kidney function. Glomerular filtration function can be more precisely represented by the blood creatinine level [32]. Growing epidemiological data suggests a connection between serum uric acid levels and urolithiasis and the possibility that it may harm tubular and glomerular function [33,34].

Liver function tests (GOT, GPT, ALP, and Bilirubin) show a highly significant difference between the urolithiasis and control group. Experimental liver harm caused by molybdenum (Mo) exposure was demonstrated by hepatocyte apoptosis using a mitochondrial route. Nitric oxide synthase, nitric oxide, and malondialdehyde were upregulated in the hepatocytes and correlated with decreased levels of catalase and superoxide dismutase [35]. Finally, after consuming excessive Mo, a localized Cu deficit may result from Mo's replacement of intracellular Cu, potentially aggravating liver damage. The experimental liver damage caused by cadmium (Cd) injection was accompanied by an increase in

the activity of liver antioxidant enzymes, including glutathione S-transferase, glutathione peroxidase, and glutathione reductase [35].

Our study also includes the antioxidant enzymes (Table 3); catalase was elevated in a significant difference (P=0.0019) in the urolithiasis patient group (129.45 KU/L) than that in the control group (116.62 KU/L), glutathione was elevated in significant difference (P= 0.049) in urolithiasis patient group (5.95 ng/ml) than that in the control group (3.41 ng/ml), malondialdehyde was elevated in significant difference (P= 0.039) in urolithiasis patient group (11.83 nmol/ml) than that in control group (9.02 nmol/ml), xanthine oxidase was elevated in significant difference (P= 0.032) in urolithiasis patient group (19.54 ng/ml) than that in control group (16.42 ng/ml), and superoxidase dismutase was also elevated in significant difference (P =0.0063) in urolithiasis patient group (21.35 ng/ml) than that in control group (14.11 ng/ml).

The elevation of antioxidant enzymes may compensate for the oxidative stress caused by high amounts of reactive nitrogen and oxygen species resulting from heavy metal toxication. Exposed to heavy metals (Mo, Cd) might lead to excessive ROS-induced oxidative stress and trigger death in tubular epithelial cells duck kidney via the mitochondria-mediated route. Additionally, the two metals had a synergistic impact [10]. The equilibrium between free radicals and antioxidants is disrupted by oxidative stress, which produces reactive nitrogen and oxygen species. Oxidation of proteins, DNA, and lipids is brought on by mitochondrial dysregulation, which also causes an increase in ROS generation and lower antioxidant levels. Inducing pro-inflammatory mediators worsens cellular damage and accelerates the course of CKD [36]. This research added to a series of research conducted in Basra that has previously quantified heavy metal pollution levels in several biological systems, including people, plants, food, soil, and water, as well as the severity of these levels' effects on human health [37-40].

## CONCLUSION

The study in the Basra Governorate, Iraq, looked at the relationship between cadmium (Cd) blood levels, molybdenum (Mo), and kidney stones. The findings showed that, compared to healthy individuals, patients with renal stones had much higher blood levels of Mo and Cd. Additionally, greater exposure to heavy metals had an impact on oxidative enzymes. The study's conclusion, which highlights the significance of monitoring environmental heavy metal exposure to address this public health concern, indicated that increased blood levels of Mo and Cd are risk factors for adult kidney stones.

*Conflict of interest:* The authors declare no conflict of interest.

*Financial support:* The authors declare they received no financial support from any organization for the submitted paper.

*Authors' contribution:* all authors contributed equally to the manuscript.

## REFERENCES

1. García JD, Arceo E. Renal damage associated with heavy metals: review work. *Revista Colombiana de Nefrología.* 2018 Jun;5(1):45-53. https://doi.org/10.22265/acnef.5.2.254
2. Uddin R, Huda NH. Arsenic poisoning in Bangladesh. *Oman Med J.* 2011; May;100(323):1-3. https://doi: 10.5001/omj.2011.51
3. Colak EH, Yomralioglu T, Nisanci R, Yildirim V, Duran C. Geostatistical analysis of the relationship between heavy metals in drinking water and cancer incidence in residential areas in the Black Sea region of Turkey. *J Environ Health.* 2015 Jan 1;77(6):86-93. https://www.jstor.org/stable/26330189
4. Zhang T, Ruan J, Zhang B, Lu S, Gao C, Huang L, et al. Heavy metals in human urine, foods and drinking water from an e-waste dismantling area: Identification of exposure sources and metal-induced health risk. *Ecotoxicol Environ Saf.* 2019;169:707-13. https://doi.org/10.1016/j.ecoenv.2018.10.039
5. Helaly AM, Mokhtar N, Firgany AE, Hazem NM, El Morsi E, Ghorab D. Molybdenum bupropion combined neurotoxicity in rats. *Regul Toxicol Pharmacol.* 2018;98:224-30. https://doi.org/10.1016/j.yrtph.2018.08.001
6. Hille R, Rétey J, Bartlewski-Hof U, Reichenbecher W, Schink B. Mechanistic aspects of molybdenum-containing enzymes. *FEMS Microbiol Rev.* 1998;22(5):489-501. https://doi.org/10.1111/j.1574-6976.1998.tb00383.x
7. Ema M, Kobayashi N, Naya M, Hanai S, Nakanishi J. Reproductive and developmental toxicity studies of manufactured nanomaterials. *Reprod Toxicol.* 2010;30(3):343-52. https://doi.org/10.1016/j.reprotox.2010.06.002
8. Zhuang Y, Liu P, Wang L, Luo J, Zhang C, Guo X, et al. Mitochondrial oxidative stress-induced hepatocyte apoptosis reflects increased molybdenum intake in caprine. *Biol Trace Elem Res.* 2016;170:106-14. https://doi.org/10.1007/s12011-015-0450-0
9. Bersényi A, Berta E, Kádár I, Glávits R, Szilágyi M, Fekete S. Effects of high dietary molybdenum in rabbits. *Acta Vet Hung.* 2008;56(1):41-55. https://doi.org/10.1556/avet.56.2008.1.5
10. Wang C, Nie G, Yang F, Chen J, Zhuang Y, Dai X, et al. Molybdenum and cadmium co-induce oxidative stress and apoptosis through mitochondria-mediated pathway in duck renal tubular epithelial cells. *J Hazard Mater.* 2020 Feb 5; 383:121157. https://doi.org/10.1016/j.jhazmat.2019.121157
11. Schaefer HR, Dennis S, Fitzpatrick S. Cadmium: Mitigation strategies to reduce dietary exposure. *J Food Sci.* 2020 Feb;85(2):260-7. https://doi: 10.1111/1750-3841.14997
12. Nordberg GF, Bernard A, Diamond GL, Duffus JH, Illing P, Nordberg M, et al. Risk assessment of effects of cadmium on human health (IUPAC Technical Report). *Pure and Applied Chemistry.* 2018;90(4):755-808. https://doi.org/10.1515/pac- 2016-0910

13. Ougier E, Fiore K, Rousselle C, Assunção R, Martins C, Buekers J. Burden of osteoporosis and costs associated with human biomonitored cadmium exposure in three European countries: France, Spain and Belgium. *Int J Hyg Environ Health.* 2021;234:113747. https://doi.org/10.1016/j.ijheh.2021.113747
14. Choudhury C, Mazumder R, Kumar R, Dhar B, Sengupta M. Cadmium-induced oxystress alters Nrf2-Keap1 signaling and triggers apoptosis in piscine head kidney macrophages. *Aquat Toxicol.* 2021;231:105739. https://doi.org/10.1016/j.aquatox.2020.105739
15. Gu J, Ren Z, Zhao J, Peprah FA, Xie Y, Cheng D, et al. Calcimimetic compound NPS R-467 protects against chronic cadmium-induced mouse kidney injury by restoring autophagy process. *Ecotoxicol Environ Saf.* 2020;189:110052. https://doi.org/10.1016/j.ecoenv.2019.110052
16. Zhang F, Xing S, Li Z. Antagonistic effects of lycopene on cadmium-induced hippocampal dysfunctions in autophagy, calcium homeostasis, and redox. *Oncotarget.* 2017;8(27):44720. https://doi: 10.18632/oncotarget.18249
17. Li S, Jiang X, Luo Y, Zhou B, Shi M, Liu F, Sha A. Sodium/calcium overload and Sirt1/Nrf2/OH-1 pathway are critical events in mercuric chloride-induced nephrotoxicity. *Chemosphere.* 2019;234:579-88. https://doi.org/10.1016/j.chemosphere.2019.06.095
18. Zhang C, Wang X, Pi S, Wei Z, Wang C, Yang F, et al. Cadmium and molybdenum co-exposure triggers autophagy via CYP450s/ROS pathway in duck renal tubular epithelial cells. *Sci Total Environ.* 2021;759:143570. https://doi.org/10.1016/j.scitotenv.2020.143570
19. Prozialeck WC, Edwards JR. Mechanisms of cadmium-induced proximal tubule injury: new insights with implications for biomonitoring and therapeutic interventions. *J Pharmacol Exp Ther.* 2012;343(1):2-12. https://doi.org/10.1124/jpet.110.166769
20. Thijssen S, Maringwa J, Faes C, Lambrichts I, Van Kerkhove E. Chronic exposure of mice to environmentally relevant, low doses of cadmium and lead to early renal damage, not predicted by blood or urine cadmium levels. *Toxicology.* 2007;229(1-2):145-56. https://doi.org/10.1016/j.tox.2006.10.011
21. Piadé JJ, Jaccard G, Dolka C, Belushkin M, Wajrock S. Differences in cadmium transfer from tobacco to cigarette smoke, compared to arsenic or lead. *Toxicol Rep.* 2015;2:12-26. https://doi.org/10.1016/j.toxrep.2014.11.005
22. Yan LJ, Allen DC. Cadmium-induced kidney injury: Oxidative damage as a unifying mechanism. *Biomolecules.* 2021;11(11):1575. https://doi.org/10.3390/biom11111575
23. Shi L, Cao H, Luo J, Liu P, Wang T, Hu G, Zhang C. Effects of molybdenum and cadmium on the oxidative damage and kidney apoptosis in Duck. *Ecotoxicol Environ Saf.* 2017;145:24-31. https://doi.org/10.1016/j.ecoenv.2017.07.006
24. Abramovich M, Miller A, Yang H, Friel JK. Molybdenum content of Canadian and US infant formulas. *Biol Trace Elem Res.* 2011;143:844-53. https://doi.org/10.1007/S12011-010-8950-4
25. Hara A, Yang WY, Petit T, Zhang ZY, Gu YM, Wei FF, et al. Incidence of nephrolithiasis in relation to environmental exposure to lead and cadmium in a population study. *Environ Res.* 2016;145:1-8. https://doi.org/10.1016/j.envres.2015.11.013
26. Moe OW. Kidney stones: pathophysiology and medical management. *Lancet.* 2006;367(9507):333-44. https://doi.org/10.1016/S0140-6736(06)68071-9
27. Renkema KY, Bindels RJ, Hoenderop JG. Role of the calcium-sensing receptor in reducing the risk for calcium stones. *Clin J Am Soc Nephrol.* 2011;6(8):2076-82. https://doi.org/10.2215/CJN.00480111
28. Handlogten ME, Shiraishi N, Awata H, Huang C, Miller RT. Extracellular Ca2+-sensing receptor is a promiscuous divalent cation sensor that responds to lead. *Am J Physiol Renal Physiol.* 2000;279(6)): F083-91. https://doi.org/10.1152/ajprenal.2000.279.6.F1083
29. Rosen JF. The metabolism of lead in isolated bone cell populations: interactions between lead and calcium. *Toxicol Appl Pharmacol*. 1983 Oct 1;71(1):101-12. https://doi.org/10.1016/0041-008x(83)90049-2
30. Kaewnate Y, Niyomtam S, Tangvarasittichai O, Meemark S, Pingmuangkaew P, Tangvarasittichai S. Association of elevated urinary cadmium with urinary stone, hypercalciuria and renal tubular dysfunction in the population of cadmium-contaminated area. *Bull Environ Contam Toxicol.* 2012;89:1120-4. https://doi.org/10.1007/s00128-012-0856-8
31. Järup L, Elinder CG. Incidence of renal stones among cadmium-exposed battery workers. *Br J Ind Med.* 1993;50(7):598-602. https://doi.org/10.1136/oem.50.7.598
32. Nah H, Lee SG, Lee KS, Won JH, Kim HO, Kim JH. Evaluation of bilirubin interference and accuracy of six creatinine assays compared with isotope dilution–liquid chromatography-mass spectrometry. *Clin Biochem.* 2016;49(3):274-81. https://doi.org/10.1016/j.clinbiochem.2015.10.015
33. 33. Hsieh YP, Chang CC, Yang Y, Wen YK, Chiu PF, Lin CC. The role of uric acid in chronic kidney disease patients. Nephrology. 2017 Jun;22(6):441-8. https://doi.org/10.1111/nep.12679.
34. Braga TT, Foresto-Neto O, Camara NO. The role of uric acid in inflammasome-mediated kidney injury. *Curr Opin Nephrol Hypertens.* 2020 Jul 1;29(4):423-31. https://doi.org/10.1097/MNH.0000000000000619
35. Teschke R. Aluminum, arsenic, beryllium, cadmium, chromium, cobalt, copper, iron, lead, mercury, molybdenum, nickel, platinum, thallium, titanium, vanadium, and zinc: molecular aspects in experimental liver injury. *Int J Mol Sci.* 2022 Oct 13;23(20):12213. https://doi.org/10.3390/ijms232012213
36. Mishra M, Nichols L, Dave AA, Pittman EH, Cheek JP, Caroland AJ. Molecular Mechanisms of Cellular Injury and Role of Toxic Heavy Metals in Chronic Kidney Disease. *Int J Mol Sci.* 2022;23(19):11105. https://doi.org/10.3390/ijms231911105
37. Khalaf EA, Abduljaleel SA, Al-Jassani HM. Appraisal of trace elements and heavy metals levels in breast cancer patients of Basrah Province. Toxicology International. 2021;28(2):8-14. https://doi.org/10.18311/ti/2021/v28i2/26484
38. Nashmi AD, Hassan AF, Hammady MM. Estimation the Level of Metals (Lead, Cadmium, Copper and Zinc) In Multiple Sclerosis Patients in Basra\Iraq. Indian Journal of Forensic Medicine & Toxicology. 2020 Jul 1;14(3). https://DOI:10.37506/ijfmt.v14i3.10508
39. Talal A, Alkinani SA, Almansoory AF, Abbas KF. Some of Heavy Elements in Water, Fish and Plant Species from Shatt Al-Arab at Basrah Governorate–Iraq. *Bas J Sci.* 2020;38(1):60-74. https://doi.org/10.29072/basjs.20200107
40. Al-Garory NH, Al-Kaabi WJ. Determination of some heavy metals concentrations in some dairy products from three different regions of Basrah, Iraq. Basrah J Agric Sci. 2020;33(2):1-3. https://doi.org/10.37077/25200860.2020.33.2.01