



Pretreatment of ellagic acid protects ifosfamide-induced acute nephrotoxicity in rat kidneys: A mitochondrial, histopathological and oxidative stress approaches

Mohammad Shabani^{a,b,c}, Deniz Bayrami^a, Amin Ashena Moghadam^a, Zhaleh Jamali^{d,e}, Ahmad Salimi^{b,c,*}

^a Students Research Committee, Faculty of Pharmacy, Ardabil University of Medical Sciences, Ardabil, Iran

^b Traditional Medicine and Hydrotherapy Research Center, Ardabil University of Medical Sciences, Iran

^c Department of Pharmacology and Toxicology, School of Pharmacy, Ardabil University of Medical Sciences, Ardabil, Iran

^d Department of Addiction Studies, School of Medicine, Shahrood University of Medical Sciences, Shahrood, Iran

^e Student Research Committee, School of Medicine, Shahrood University of Medical Sciences, Shahrood, Iran

ARTICLE INFO

Handling Editor: Prof. L.H. Lash

Keywords:

Natural compounds
Kidney damages
Antineoplastic drugs
Nephroprotective agent
Polyphenols

ABSTRACT

Ifosfamide (IFO) kidney damage is an important organ toxicity in children and adults undergoing chemotherapy. Previous evidence has shown that IFO toxic metabolites such as acrolein and are associated with mitochondrial dysfunction, depletion of antioxidants, oxidative stress and may predispose the kidney to IFO toxicity. Bioactive food compounds such as ellagic acid (EA) found in fruits has been described as antioxidant and mitochondrial protective agents against toxicity-related mitochondrial damage and oxidative stress. In current study, the protective effects of EA on IFO-induced nephrotoxicity in male Wistar rats were investigated with histopathological, biochemical, and mitochondrial methods. The rats were randomly divided into four groups, control, IFO, IFO + EA, and EA groups. EA (25 mg/kg, i.p. daily) were administered to animals for 2 consecutive days and IFO (500 mg/kg, i.p.) was administered on third day. The results showed that pretreatment EA significantly increased mitochondrial succinate dehydrogenases (SDH) activity, and protected mitochondrial swelling, mitochondrial membrane potential (MMP), reactive oxygen species (ROS) formation, lipid peroxidation (LPO) and depletion glutathione (GSH). Histopathological findings demonstrated that EA had protective effects and reduced histopathological abnormalities caused by IFO. These results showed that EA administration protects the kidneys against mitochondrial dysfunction, oxidative stress and histopathological abnormality induced by IFO. Taken together, our results demonstrated that EA played a protective role against IFO-induced nephrotoxicity through mitochondrial protection and antioxidant properties.

1. Introduction

Ifosfamide (IFO) is a commonly used alkylating agent in cancer therapy and is belong to the nitrogen mustard analog [1]. This drug almost in combination with other chemotherapeutic agents is used for treatment of some refractory lymphomas, sarcomas and testicular tumors both in children and in adult individuals [1]. Despite the good effectiveness of IFO in the treatment of above cancers, unfortunately, various toxicities have been reported in both children and adults after its administration [2]. Due to structural similarity of IFO to cyclophosphamide and production of active metabolites such as acrolein and chloroacetaldehyde, it is probable to induce central nervous system

(CNS) toxicity, cardiotoxicity, hepatotoxicity, nephrotoxicity [3]. One of the most common toxicities after treatment with IFO is nephrotoxicity. It has been reported acute as well as chronic kidney dysfunction with range of moderate to severe in 18–28% of patients treated with IFO [4]. IFO-induced nephrotoxicity is associated with mainly injury to the renal tubules, hemorrhagic cystitis (due to injury to urinary epithelium), glycosuria at normal serum glucose levels, Fanconi syndrome (proximal tubular damage) with urinary potassium and phosphorus wasting, non-anion gap metabolic acidosis (proximal renal tubular acidosis) and aminoaciduria [5]. The mechanism of IFO-related neurotoxicity has not been fully elucidated, but it seems that its activation to toxic metabolites be important in induction kidney damage. It has been reported that this

* Correspondence to: Toxicology and Pharmacology School of Pharmacy, Ardabil University of Medical Sciences, P.O. Box: 56189-53141, Ardabil, Iran.

E-mail addresses: a.salimi@arums.ac.ir, salimikd@yahoo.com (A. Salimi).

<https://doi.org/10.1016/j.toxrep.2023.04.005>

Received 17 February 2023; Received in revised form 5 April 2023; Accepted 12 April 2023

2214-7500/© 2023 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

prodrug is metabolized to its active metabolites by cytochrome P450 3A (CYP3A) family, mainly CYP3A4 and CYP3A5 [6]. This process leads to hydroxylation of IFO and generation therapeutic and toxic metabolites phosphoramidate mustard and acrolein [6]. Despite above pathway in IFO metabolism, this drug also is detoxified to N-dechloroethylation, and creates 2-dechloroethylifosfamide (2-DCE) and 3-dechloroethylifosfamide (3-DCE). The production of 2-DCE and 3-DCE yields equal amounts of chloroacetaldehyde [6]. The metabolites responsible for toxicity of IFO are acrolein, chloroacetaldehyde and itself therapeutic metabolite, phosphoramidate mustard. The produced phosphoramidate mustard, after metabolic activation alkylate or bind with many intracellular molecular structures, in cancerous and normal cells [7]. Moreover, acrolein and chloroacetaldehyde as main toxic metabolites of IFO can induce mitochondrial dysfunction and oxidative stress [8,9]. In the kidneys mitochondrial damage and oxidative stress are linked to inflammation, cell death via necrosis/apoptosis, and tissue injury [10]. Therefore, it seems that a promising strategy to reduce the toxicity of IFO, especially nephrotoxicity be the use of antioxidant and mitochondrial protective agents without interfering with therapeutic mechanism of the drug.

Studies have reported that dietary polyphenolic compounds could modulate kidney damages [11]. These studies have suggested that the diet rich in grain, fruits, and vegetables is related to a lower incidence of kidney diseases [11]. In recent years, several polyphenolic compounds such as ellagic acid (EA) have attracted attention as nephroprotective agents, particularly due to antioxidant potential and mitochondrial protective effects [12]. EA is found in vegetables and fruits, such as blackcurrants, walnuts, grapes, raspberries and strawberries [13]. The antioxidant, anti-inflammatory, mitochondrial protection effects of EA have reported in various studies [14–18]. Beneficial effects of EA have reported on nephrotoxicity, nephropathy and kidney injury in different models. It has reported that EA can increase superoxide dismutase (SOD) levels and reduce serum malondialdehyde (MDA) levels in streptozocin induced diabetic nephropathy [14]. A recent study showed that EA is able restore lead-induced nephrotoxicity via anti-inflammatory, anti-apoptotic and free radical scavenging activities [19]. Further, EA reversed the swelling of mitochondrial kidney, reduced the mitochondrial ROS content, and prevented loss of MMP [20]. It was suggested that EA treatment triggers SIRT1 overexpression and enhances renal tolerance to oxidative stress in renal tissues [21]. Moreover, EA via SIRT1 expression, suppresses p53 and promotes cell survival via expression of antioxidant enzymes [21]. Overall, above studies suggest that EA deserves to be further studied as a nephroprotective agent. For this purpose, in the current study, we investigated a preventive strategy to reduce IFO-induced nephrotoxicity by EA with mitochondrial, histopathological and biochemical approaches in the adult Wistar rats.

2. Materials and methods

2.1. Chemicals

2',7'-Dichlor-fluorescein (DCF), Rhodamine123, Ellagic Acid, N-(2-hydroxyethyl) piperazine-N'-(2-ethanesulfonic acid) (HEPES), Bovine Serum Albumin (BSA), Hank's Balanced Salt Solution (HBSS), Dimethyl sulfoxide (DMSO), Rotenone, D-mannitol, Sucrose, Ellman's reagent (5,5'-dithiobis-(2-nitrobenzoic acid, 3-morpholinopropane-1-sulfonic acid (MOPS), Monopotassium phosphate, 2-Amino-2-hydroxymethylpropane-1,3-diol (TRIS), 4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Sodium succinate, Potassium chloride and Magnesium chloride were purchased from Sigma (St. Louis, MO USA). Ifosfamide (IFO) with purity 99% and CAS number 3778-73-2 was obtained from 22 Bahman Training Pharmacy (Ardabil, Iran).

2.2. Animals

A total of 36 male Wistar rats with 8 weeks of age and 180–220 g

weight, were obtained from the Pasteur Institute of Iran (Tehran, Iran). Rats were acclimatized in the Animal House of the Faculty of Pharmacy, Ardabil University of Medical Sciences for two weeks and housed in cages under a 12 h light/dark cycle, at 25 ± 2 °C with $50 \pm 20\%$ humidity. Animals had free access to standard diet for rats and water. The protocols were approved by the Animal Ethics Committee of the Ardabil University of Medical Sciences (No. IR.ARUMS.AEC.1400.027).

2.3. Study design

The administration doses of EA and IFO were chosen according to the previous studies [22,23]. Male Wistar rats were weighed and randomly divided into 4 groups (n = 6) as follows: Group I (Control): Animals received intraperitoneal normal saline for 4 days. Group II (IFO): Animals received IFO (500 mg/kg BW, i.p.) on the third day. Group III (IFO + EA): Animals received EA (25 mg/kg/day; i.p.) for 2 consecutive days, with IFO (500 mg/kg BW, i.p.) single dose on the third day. Group IV (EA): Animals received EA (25 mg/kg/day; i.p.) for 2 consecutive days. On the fourth day, 24 h after last administration, the rats were anaesthetized (50 mg/kg ketamine+10 mg/kg xylazine). Blood samples and kidneys were collected to examine of biochemical, mitochondrial and histopathological analyses.

2.4. Assessment of histopathological abnormality in kidney tissue

24 h after the last administration, the kidneys from all groups were simultaneously harvested and immediately fixed in 10% formalin. Briefly, using ascending concentrations of alcohol, the tissue samples were dehydrated and cleaned by xylene to remove alcohol. The tissue samples were placed in paraffin and allowed to harden. Subsequently, the paraffin blocks were cut to 5- μ m sections by microtome and floated in the water bath. Then, the sections were mounted onto microscopical slides and dried in an oven at 60 °C. Afterward, the section were stained with hematoxylin and eosin (H&E) and histological examinations were carried out using light microscopy. The severity of pathological lesions was assigned as follows: score - = normal; score \uparrow = mild; score $\uparrow\uparrow$ = moderate; score $\uparrow\uparrow\uparrow$ = severe.

2.5. Assessment of lipid peroxidation in tissue samples

MDA levels as indicator of lipid peroxidation were using thiobarbituric acid-reactive substances (TBARS) test [24]. Briefly, 100 mg kidney tissue was homogenized by a glass homogenizer in 1 ml 0.1% of trichloroacetic acid (TCA). The homogenate was centrifugated at 10,000 x g for 10 min and the obtained supernatants were placed in 4 ml of 0.5% thiobarbituric acid (TBA) and 20% TCA. The samples were left in boiling water for 20 min, and after centrifugation at 10,000 x g for 10 min, the absorbance was measured at 535 nm. The kidney tissue MDA amount presented as nmol MDA/mg protein.

2.6. Assessment of GSH and GSSG in tissue samples

The concentrations of the reduced glutathione (GSH) and oxidized disulfide (GSSG) were measured in kidney tissue as oxidative markers [25]. Briefly, 100 mg kidney tissue was homogenized by a glass homogenizer in 1 ml phosphate buffer, and centrifuged at 10,000 x g for 10 min at 4 °C. The obtained supernatants were collected and used for detection of GSH and GSSG. For measurement of GSH and GSSG, 100 μ L supernatant were mixed with 3 ml of reaction solution containing (GSH reductase, 1 mM EDTA, 150 μ M NADPH, 3 mM $MgCl_2$, 500 mM Tris-HCl and 10 mM DTNB) and (500 mM Tris-HCl and 10 mM DTNB), respectively incubated for 15 min at 25 °C. The absorbance of the assay mixture was measured at 412 nm.

2.7. Isolation of renal mitochondria

Briefly, blood, lipid and connective tissues were removed from the kidneys and were washed with normal saline solution (4 °C). The whole kidney tissue was cut, minced and homogenized with a glass homogenizer in an isolation buffer (75 mM sucrose, 225 mM D-mannitol and 0.2 mM EDTA, pH 7.4). The homogenate was centrifuged at 1000 g for 10 min, and the obtained supernatant was centrifuged at 10,000 g for 10 min. All procedures were performed on the ice at 4 °C. The mitochondrial pellet was kept on ice and used within 4 h and used for measurement of mitochondrial toxicity parameters [26]. Mitochondrial protein concentration was measured by Bradford method with bovine serum albumin (BSA) as a standard.

2.8. Assessment of mitochondrial succinate dehydrogenase activity in isolated mitochondria

The MTT assay was used to measurement SDH as mitochondrial function test. Renal isolated mitochondria were suspended in mitochondrial assay buffer (20 mmol/L HEPES, 0.5 mmol/L KH_2PO_4 , 10 mmol/L NaCl, 0.5 mmol/L EGTA, 2 mmol/L MgCl_2 , 140 mmol/L KC; supplemented with 10 mmol/L succinate and 1 mg/ml rotenone). Then, 100 μL of isolated mitochondria (100 μg /well) 25 plus μL of MTT day (0.5 mg/ml) were added to 96-well plates and incubated at 37 °C for 30 min. After incubation, 100 μL of DMSO was added to each well to dissolve the formazan crystals. The absorbance was measured using an ELISA reader at 570 nm [27].

2.9. Assessment of mitochondrial swelling in isolated mitochondria

Mitochondrial swelling is widely performed in suspensions of isolated mitochondria by measuring light scatter at 540 nm. Decrease the absorbance at 450 indicates mitochondrial swelling and mitochondrial permeability transition (MPT) pore opening [25]. Renal isolated mitochondria were suspended in mitochondrial swelling buffer (20 mmol/L HEPES, 0.5 mmol/L KH_2PO_4 , 10 mmol/L NaCl, 0.5 mmol/L EGTA, 2 mmol/L MgCl_2 , 140 mmol/L KC; supplemented with 10 mmol/L succinate and 1 mg/ml rotenone). Then, 100 μL of isolated mitochondria (100 μg /well) was added to 96-well plates and the absorbance was read at 540, during 10 min

2.10. Assessment of mitochondrial membrane potential collapse in isolated mitochondria

The MMP collapse was assessed by monitoring the mitochondrial uptake of a fluorescence dye, rhodamine 123, using flow cytometry on FL-1 channel. One milligram/ml of mitochondrial protein was suspended in MMP buffer (5 mM KH_2PO_4 , 68 mM D-mannitol, 220 mM sucrose, 10 mM HEPES, 2 mM MgCl_2 , 2 μM rotenone, 50 μM EGTA, 5 mM sodium succinate, 10 mM KCl and 5 μM rhodamine 123 at 37 °C for 15 min. Then the above MMP buffer was replaced with fresh MMP buffer without rhodamine 123 and incubated again for 15 min. The fluorescence intensity of rhodamine 123 for 2×10^4 particles, was measured on the FL1 channel using flow cytometry (Cyflow Space-Partec, Germany).

2.11. Assessment of mitochondrial membrane potential collapse in isolated mitochondria

The MMP collapse was assessed by monitoring the mitochondrial uptake of a fluorescence dye, rhodamine 123, using flow cytometry on FL-1 channel [25]. One milligram/ml of mitochondrial protein was suspended in MMP buffer (5 mM KH_2PO_4 , 68 mM D-mannitol, 220 mM sucrose, 10 mM HEPES, 2 mM MgCl_2 , 2 μM rotenone, 50 μM EGTA, 5 mM sodium succinate, 10 mM KCl and 5 μM rhodamine 123) at 37 °C for 15 min. Then the above buffer was replaced with fresh MMP buffer without rhodamine 123 and incubated again for 15 min in dark. The

fluorescence intensity of rhodamine 123 for 2×10^4 particles, was measured on the FL1 channel using flow cytometry (Cyflow Space-Partec, Germany).

2.12. Assessment of mitochondrial ROS formation in isolated mitochondria

The mitochondrial ROS formation was assessed by H_2DCF , using flow cytometry on FL-1 channel [25]. One milligram/ml of mitochondrial protein was suspended in respiration buffer (50 mM EGTA, 0.5 mM MgCl_2 , 10 mM Tris, 0.32 mM sucrose, 0.1 mM KH_2PO_4 , 5 mM sodium succinate, 20 mM MOPS and 10 μM H_2DCF) at 37 °C for 15 min. Then the above buffer was replaced with fresh respiration buffer without H_2DCF and incubated again for 15 min in dark. The fluorescence intensity of H_2DCF for 2×10^4 particles, was measured on the FL1 channel using flow cytometry (Cyflow Space-Partec, Germany).

2.13. Statistical analysis

All results were presented as mean \pm standard error of the mean (SEM). Statistical analyses were conducted by the one-way analysis of variance, followed by hoc Tukey's test and were performed using the GraphPad Prism Software, version 9.0 (GraphPad Software, San Diego, CA, USA) for Windows. $P < 0.05$ was chosen as the level of significance,

3. Results

3.1. The effect of EA on IFO-induced histopathological abnormalities

All H&E-stained sections from various experimental groups were histologically evaluated. Kidney sections from the control group showed apparently normal morphology regarding tubules, glomeruli and interstitial tissue. Also, the kidney sections of the EA group did not show any significant histopathological abnormalities. However, the histopathological evaluation of the kidneys of IFO-treated animals showed severe renal damage such as vacuolar degeneration of tubules, cloudy swelling, interstitial hemorrhage, tubular cast and moderate tubular necrosis. In the EA+IFO group the severity of the IFO-induced histopathological abnormalities, mentioned above was decreased compared to the IFO group (Fig. 1 and Table 1).

3.2. The effect of EA on IFO-induced oxidative stress markers

IFO treatment led to a significant increase in oxidative stress markers such as GSH, GSSG and MAD levels in kidney tissue, compared with the control group. However, pretreatment of EA, in the EA + IFO group reduced oxidative stress markers and normalized these markers, compared with the IFO group. Treatment of animal with EA produced non-significant changes in oxidative stress markers, compared to the control group (normal saline) (Table 2).

3.3. The effect of EA on IFO-induced mitochondrial dysfunction

As shown in Fig. 2A, mitochondrial SDH activity as indicator of function test of mitochondria were significantly ($p < 0.01$) down-regulated in animals exposed to IFO, compared with the control group, in isolated mitochondria obtained from rat kidney tissue. Pretreatment of EA for two consecutive days significantly increased of SDH mitochondrial activity. There is not a significant difference between mitochondrial SDH activity of the EA group and the control group.

3.4. The effect of EA on IFO-induced mitochondrial dysfunction

Information on mitochondrial swelling status in isolated mitochondria obtained from rat kidney tissue is shown in Fig. 2B. Mitochondrial swelling in rats exposed to IFO were increased to 1.5-fold compared with

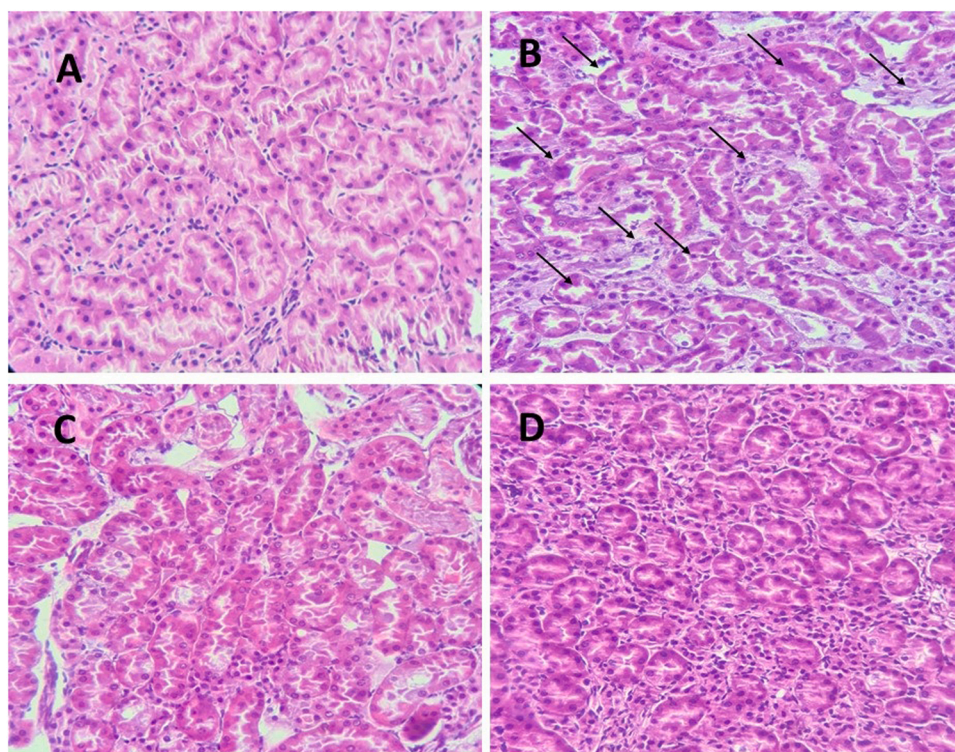


Fig. 1. Histopathologic section of kidneys from different experimental groups. thick black arrows = vacuolar degeneration of tubules; thin arrows = interstitial hemorrhage; white arrows = tubular necrosis. (A) Normal view of the control group. (B) Ifosfamide induced vacuolar degeneration of tubules, cloudy swelling, interstitial hemorrhage, Bowman's space with proteinaceous filtrate, tubular cast and moderate tubular necrosis in the kidney tissue. (C) Microscopic appearance of EA+IFO group in kidney tissue. (D) Microscopic appearance of the ellagic acid group in the kidney tissue. H&E 400×.

Table 1

Kidney tissue microscopic scoring between groups treated with normal saline, IFO, EA + IFO and EA.

Histopathological changes	Control	IFO	IFO + EA	EA
Vacuolar degeneration of tubules	-	+++	+	-
Cloudy swelling	-	+++	+	-
Interstitial hemorrhage	-	+++	+	-
Tubular cast	-	++	+	-
Moderate tubular necrosis	-	+++	+	-

Scores represent values obtained from tissue sections of six animals of each group. score - = normal; score + = mild; score ++ = moderate; score +++ = severe. IFO, Ifosfamide; EA, Ellagic Acid.

Table 2

Statistical significance of oxidative stress markers (GSH, GSSG and LPO) in rats treated with normal saline, IFO, EA + IFO and EA.

Groups	GSH (nmol/mg)	GSSG (nmol/mg)	MDA (nmol/mg)
Control	64 ± 3.18	12 ± 2.35	35 ± 2.7
IFO (500 mg/kg)	38 ± 2.58 ^a	39 ± 2.47 ^a	57 ± 1.9 ^a
IFO + EA (25 mg/kg)	48 ± 4.20 ^b	28 ± 3.11 ^b	33 ± 2.4 ^b
EA (25 mg/kg)	65 ± 1.45 ^b	11.4 ± 2.15 ^b	29 ± 3.1

^a Significant difference ($P < 0.01$) with control group.

^b Significant difference ($P < 0.01$) with IFO-treated group. IFO, Ifosfamide; EA, Ellagic Acid.

that of the control group. While, in the EA + IFO group, EA significantly ($p < 0.01$) decreased IFO-induced mitochondrial swelling. The rats that received EA alone for two consecutive days did not show significant alteration in mitochondrial swelling status of isolated mitochondria obtained from rat kidney tissue, compared with the control group.

3.5. The effect of EA on IFO-induced mitochondrial ROS formation

The effect of EA on IFO-induced mitochondrial ROS formation in isolated mitochondria obtained from rat kidney tissue is shown in Fig. 3.

A significant increase in the mean of fluorescence intensity of DCF, as reflection of ROS formation was observed in IFO group, when compared with the control group. While, treatment with EA in the EA + IFO group significantly decreased the mean of fluorescence intensity of DCF. The rats that received EA alone for two consecutive days did not show significant alteration in the mean of fluorescence intensity of DCF, compared with the control group.

3.6. The effect of EA on IFO-induced MMP collapse

Exposure to IFO in the IFO group resulted in a significant increase in the mean of fluorescence intensity of rhodamine 123 and translocation of the histogram from left to right (Fig. 4). Treatment with EA in the EA + IFO group significantly decreased the mean of fluorescence intensity of rhodamine 123 as reflection of MMP collapse. The rats that received EA alone for two consecutive days did not show significant alteration in the mean of fluorescence intensity of rhodamine 123, compared with the control group.

4. Discussion

It has been reported that mitochondria play an important role in the pathogenesis of different forms of renal disease and nephrotoxicity induced by chemicals and drugs [28]. Due to functions such as filtration and reabsorption, the renal cells are one of the most energy-demanding cells and require their mitochondria to produce energy via oxidative phosphorylation [29]. In kidneys, mitochondria also perform vital activities for survival of the renal cells such as the catabolism of branched-chain amino acids, glutaminolysis, the management of metabolic by-products, nucleotide biosynthesis, fatty acid beta-oxidation, redox balance, heme metabolism, calcium homeostasis, cellular death regulation, etc. [30]. In the renal cell especially in tubular cells, to maintain the activity of the transmembrane electrochemical gradients and the ATPase pumps, there are the high number mitochondria. In the tubule, the proximal segment is the most enriched in mitochondria and the site of the secretion and the reabsorption. Moreover, the proximal

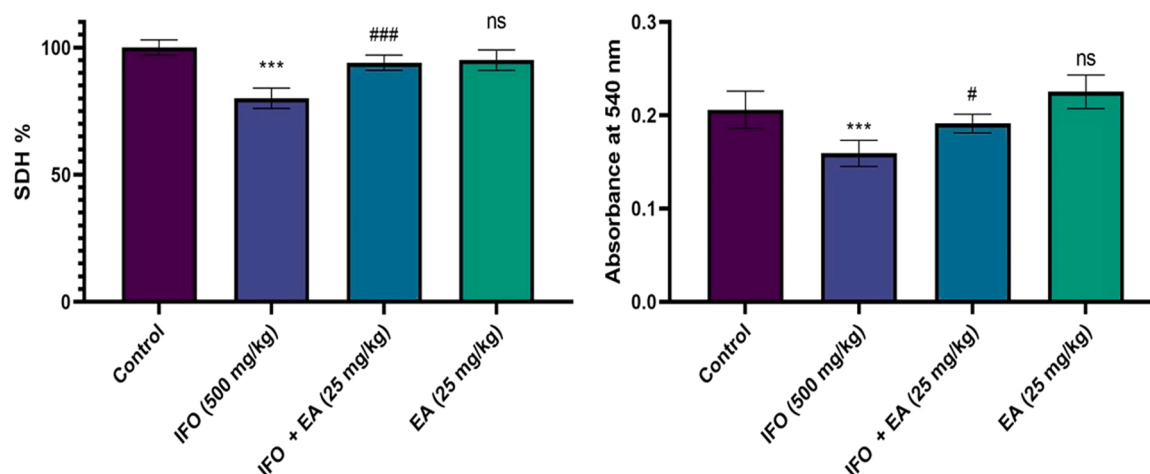


Fig. 2. The graphs represent the mitochondrial SDH activity (A) and mitochondrial swelling (B) in isolated mitochondria obtained rat kidney tissue. Columns represent mean \pm SEM, $n = 6$. *** $p < 0.001$ compared with the control group; ### $p < 0.001$ compared with the ifosfamide group. IFO, ifosfamide; SDH, succinate dehydrogenase; EA, ellagic acid.

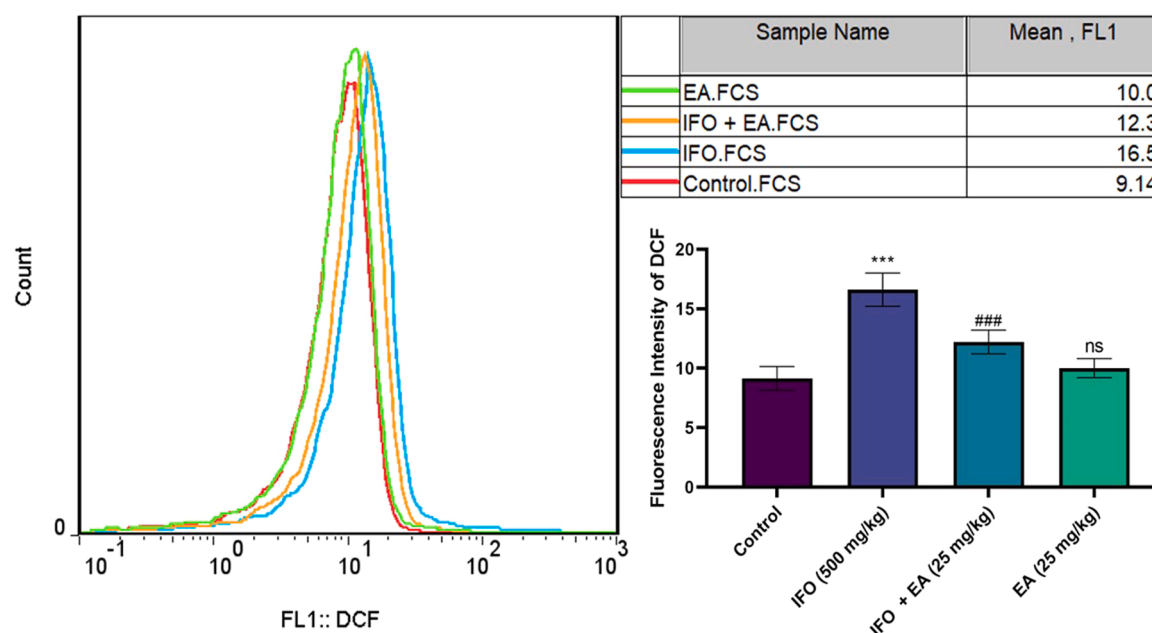


Fig. 3. Changes of the mean of fluorescence intensity of DCF as reflection of ROS formation in isolated mitochondria obtained from rat kidney tissue, among the groups, control group, IFO-treated group (IFO), EA + IFO-treated group (EA + IFO) and EA-treated group (EA). Columns represent mean \pm SEM, $n = 6$. *** $p < 0.001$ compared with the control group; ### $p < 0.001$ compared with the ifosfamide group. IFO, ifosfamide; EA, ellagic acid; ROS, reactive oxygen species; DCF, dichlorofluorescein.

tubular cells have a series of polyspecific solute transporters, which are involved in transportation of drugs from the peritubular blood into the urine or contrariwise [31]. Therefore, toxic effects on renal mitochondria can result in alterations in the mitochondrial function, decreased production of energy, increased formation of harmful ROS cell injury and tissue death. In different experimental and clinical studies have shown that drugs can induce of acute kidney injury (AKI), chronic kidney injury (CKI), nephrotoxicity and kidney damages via alterations mitochondrial functions [32–34]. Toxic effects of drugs and chemicals are associated with increased ROS production, reduced electron transport chain (ETC) activity, a loss of membrane potential, mitochondrial swelling and oxidative stress [34]. Our results in the current study confirmed that IFO-induced nephrotoxicity is associated with reduced ETC activity (SDH), ROS formation, the loss of MMP, mitochondria swelling in mitochondria, which resulted to GSH depletion, oxidative

stress and histopathological abnormality in kidney tissue, as final consequence. Previous studies consistent with our results have suggested that IFO can promote tubulo-interstitial injury, proximal tubular dysfunction (PTD), complete or incomplete Fanconi syndrome through mitochondrial damages, especially in proximal tubular injury [35,36]. As mentioned above, among of the segments of the nephron, proximal tubular cells have a high number of mitochondria, consumes large amounts of energy and are exposed to high concentrations of IFO and its toxic metabolites [37]. IFO penetrates to the proximal tubular cells via human organic cation transporter 2, and metabolized to into an active nitrogen mustard (DNA damage) and two nephrotoxic agents (acrolein and chloroacetaldehyde) [38]. Acrolein and chloroacetaldehyde can damage to mitochondria and cellular energy metabolism through inhibition of ETC and increase cellular vulnerability to oxidative stress [39, 40]. In our work, histopathological studies and oxidative stress markers

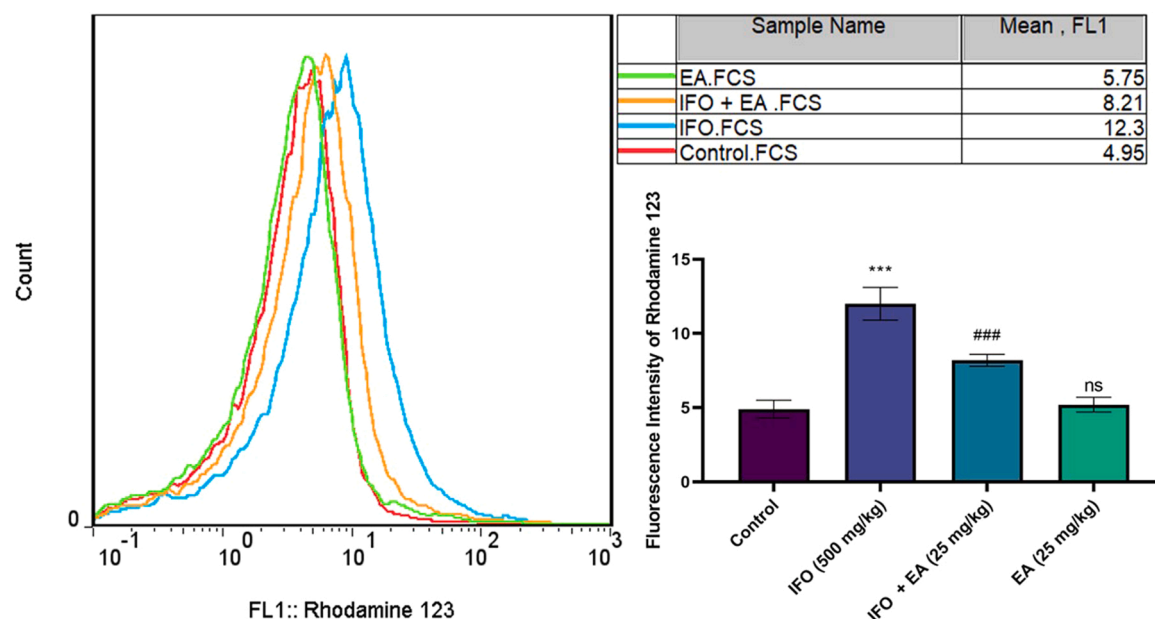


Fig. 4. Changes of the mean of fluorescence intensity of rhodamine 123 as reflection of MMP collapse in isolated mitochondria obtained from rat kidney tissue, among the groups, control group, IFO-treated group (IFO), EA + IFO-treated group (EA + IFO) and EA-treated group (EA). Columns represent mean \pm SEM, $n = 6$. *** $p < 0.001$ compared with the control group; ### $p < 0.001$ compared with the ifosfamide group. IFO, ifosfamide; EA, ellagic acid.

showed that IFO induced interstitial hemorrhage and tubular necrosis in the kidney tissue, which was associated with GSH depletion and lipid peroxidation.

Our results in this study and previous evidence demonstrate that the restoring mitochondrial functions and blocking excessive ROS production is a suitable complementary therapeutic strategy for IFO-induced nephrotoxicity. Accordingly, increasing evidence indicates that polyphenolic compounds found in fruits and vegetables can reduce mitochondrial dysfunction and oxidative stress in nephrotoxicity models caused by drugs and chemicals [11,12]. We found that EA as well-known polyphenolic compound, is able to reduce kidney damages caused by IFO via mitochondrial protection and antioxidant properties, which is associated with the increase of SDH activity, inhibition of MMP collapse and the decrease of ROS formation, mitochondria swelling, depletion of GSH and oxidative stress in the kidney tissue. Consistent with our study, several studies have demonstrated that EA can protect the mitochondria and reduce oxidative stress and toxicity induced by different chemicals and drugs. A recent study has reported that EA, due to its antioxidant properties, attenuates Cr(VI)-induced renal alterations by preserving mitochondrial ultrastructure and function, supporting enzymatic activities, preventing oxidative stress, suppressing TNF- α [41]. A study similar with our finding has demonstrated that EA can reduce gentamicin-induced nephrotoxicity via protecting mitochondria by preventing of MMP loss, decreasing the mitochondrial ROS content, decreasing cytochrome c release and reducing mitochondrial swelling [20]. Further, two recent studies have shown that EA can be used as a phyto-chelator to reduce hepato-renal oxidative damages and pathophysiological changes induced by lead exposure, due to its potent antioxidant, anti-apoptotic, and anti-inflammatory effects [19,42]. Consistent with the last study, our histopathological evaluations demonstrated that EA can reduce pathophysiological alterations induced by IFO. Many studies have reported that EA can protect the kidneys against toxic agents and renal diseases through various mechanisms such as the chelating, antifibrotic, autophagic, anti-inflammatory, antiapoptotic, antioxidant and mitochondrial protective properties [43]. The findings of our study highlighted the role of EA as a mitochondrial protective antioxidant agent against. It seems that EA can act like a double-edged sword and in high concentrations cause renal toxicity through mitochondrial dysfunction, which should be

considered in future studies, especially clinical studies [44].

In summary, the current investigation demonstrated that EA can provide significant protective effects against IFO-induced nephrotoxicity. EA protected histopathological abnormalities, depletion of GSH and oxidative stress, as well as mitochondrial toxicity parameters which are the features of IFO and its toxic metabolite nephrotoxicity. Our findings support that the pretreatment of EA before IFO administration may be effective for clinical purposes, due to the mechanism of action IFO (alkylating agent) as well as the anticancer properties of EA. The beneficial effect of ellagic acid against IFO-induced nephrotoxicity should be further studied by using cellular, animal and clinical trial studies. This study suggested that ellagic acid has the potential protective against IFO-induced mitochondrial toxicity and nephrotoxicity. It seems that this natural compound be benefit for medical uses such as nephroprotective agent with antioxidant and mitochondrial protection activities.

Funding

This study was supported by Ardabil University of Medical Sciences, Deputy of Research with ethics code IR.ARUMS.AEC.1400.027.

Author statement

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties. Ahmad Salimi conceived and designed research, analyzed data and wrote the manuscript. Mohammad Shabani, Deniz Bayrami, Amin Ashena Moghadam and Zhaleh Jamali conducted experiments. All authors read and approved the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

References

- [1] R. Ralhan, J. Kaur, Alkylating agents and cancer therapy, *Expert Opin. Ther. Pat.* 17 (9) (2007) 1061–1075.
- [2] J.S. McCune, D.L. Friedman, S. Schuetze, D. Blough, M. Magbulos, D.S. Hawkins, Influence of age upon ifosfamide-induced nephrotoxicity, *Pediatr. Blood Cancer* 42 (5) (2004) 427–432.
- [3] H.-Y. Han, M.-S. Choi, S. Yoon, J.-W. Ko, S.-K. Kim, T.-W. Kim, Investigation of ifosfamide toxicity induces common upstream regulator in liver and kidney, *Int. J. Mol. Sci.* 22 (22) (2021) 12201.
- [4] A. Di Cataldo1ABCDEF, M. Astuto2C, G. Rizzo2BE, G. Bertuna1D, G. Russo1F, G. Incorpora3F, Neurotoxicity during ifosfamide treatment in children, *Med. Sci. Monit.* 15 (1) (2009) 25.
- [5] I. Kashoor, D. Battle, Proximal renal tubular acidosis with and without Fanconi syndrome, *Kidney Res. Clin. Pract.* 38 (3) (2019) 267–281.
- [6] R. Chugh, T. Wagner, K.A. Griffith, J.M. Taylor, D.G. Thomas, F.P. Worden, et al., Assessment of ifosfamide pharmacokinetics, toxicity, and relation to CYP3A4 activity as measured by the erythromycin breath test in patients with sarcoma, *Cancer* 109 (11) (2007) 2315–2322.
- [7] I. Willits, L. Price, A. Parry, M. Tilby, D. Ford, S. Cholerton, et al., Pharmacokinetics and metabolism of ifosfamide in relation to DNA damage assessed by the COMET assay in children with cancer, *Br. J. Cancer* 92 (9) (2005) 1626–1635.
- [8] X. Wu, W. Cui, W. Guo, H. Liu, J. Luo, L. Zhao, et al., Acrolein aggravates secondary brain injury after intracerebral hemorrhage through Drp1-mediated mitochondrial oxidative damage in mice, *Neurosci. Bull.* 36 (2020) 1158–1170.
- [9] S. Singh, A. Kumar, Protective effect of edaravone on cyclophosphamide induced oxidative stress and neurotoxicity in rats, *Curr. Drug Saf.* 14 (3) (2019) 209–216.
- [10] P. Duann, P.-H. Lin, Mitochondria damage and kidney disease, *Mitochondrial Dyn. Cardiovasc. Med.* (2017) 529–551.
- [11] D. Mafra, E.K. Gidlund, N.A. Borges, D.A.C. Magliano, B. Lindholm, P. Stenvinkel, et al., Bioactive food and exercise in chronic kidney disease: targeting the mitochondria, *Eur. J. Clin. Invest.* 48 (11) (2018), e13020.
- [12] F. Ashkar, K.S. Bhullar, J. Wu, The effect of polyphenols on kidney disease: targeting mitochondria, *Nutrients* 14 (15) (2022) 3115.
- [13] J. Sharifi-Rad, C. Quispe, C.M.S. Castillo, R. Caroca, M.A. Lazo-Vélez, H. Antonyak, et al., Ellagic acid: a review on its natural sources, chemical stability, and therapeutic potential, *Oxid. Med. Cell. Longev.* 2022 (2022).
- [14] P. Chen, F. Chen, B. Zhou, Antioxidative, anti-inflammatory and anti-apoptotic effects of ellagic acid in liver and brain of rats treated by D-galactose, *Sci. Rep.* 8 (1) (2018) 1–10.
- [15] A. Salimi, E. Baghal, H. Ghobadi, N. Hashemidanes, F. Khodaparast, E. Seydi, Mitochondrial, lysosomal and DNA damages induced by acrylamide attenuate by ellagic acid in human lymphocyte, *PLoS One* 16 (2) (2021), e0247776.
- [16] R. Ahangari, S. Khezri, A. Jahedsani, S. Bakhshii, A. Salimi, Ellagic acid alleviates clozapine-induced oxidative stress and mitochondrial dysfunction in cardiomyocytes, *Drug Chem. Toxicol.* 45 (4) (2022) 1625–1633.
- [17] E. Mohammad Khanlou, S. Atashbar, F. Kahrizi, N. Shokouhi Sabet, A. Salimi, Bevacizumab as a monoclonal antibody inhibits mitochondrial complex II in isolated rat heart mitochondria: ameliorative effect of ellagic acid, *Drug Chem. Toxicol.* 45 (1) (2022) 456–463.
- [18] S. Atashbar, T. Sabzalipour, A. Salimi, Stabilization of mitochondrial function by ellagic acid prevents celecoxib-induced toxicity in rat cardiomyocytes and isolated mitochondria, *Drug Res.* 71 (04) (2021) 219–227.
- [19] A. Bhattacharjee, V.H. Kulkarni, M. Chakraborty, P.V. Habbu, A. Ray, Ellagic acid restored lead-induced nephrotoxicity by anti-inflammatory, anti-apoptotic and free radical scavenging activities, *Heliyon* 7 (1) (2021), e05921.
- [20] M.R. Sepand, M.H. Ghahremani, K. Razavi-Azarkhiavi, M. Aghsami, J. Rajabi, H. Keshavarz-Bahaghighat, et al., Ellagic acid confers protection against gentamicin-induced oxidative damage, mitochondrial dysfunction and apoptosis-related nephrotoxicity, *J. Pharm. Pharmacol.* 68 (9) (2016) 1222–1232.
- [21] E.T. Mohammed, K.S. Hashem, A.Z. Abdelazem, F.A. Foda, Prospective protective effect of ellagic acid as a SIRT1 activator in iron oxide nanoparticle-induced renal damage in rats, *Biol. Trace Elem. Res.* 198 (1) (2020) 177–188.
- [22] H. Çelik, S. Kucukler, S. Çomaklı, S. Özdemir, C. Caglayan, A. Yardım, et al., Morin attenuates ifosfamide-induced neurotoxicity in rats via suppression of oxidative stress, neuroinflammation and neuronal apoptosis, *Neurotoxicology* 76 (2020) 126–137.
- [23] N. Al-Kharusi, H. Babiker, S. Al-Salam, M. Waly, A. Nemmar, I. Al-Lawati, et al., Ellagic acid protects against cisplatin-induced nephrotoxicity in rats: a dose-dependent study, *Eur. Rev. Med. Pharm. Sci.* 17 (3) (2013) 299–310.
- [24] E. Wills, Mechanisms of lipid peroxide formation in animal tissues, *Biochem. J.* 99 (3) (1966) 667–676.
- [25] L.R. Shirmard, M. Shabani, A.A. Moghadam, N. Zamani, H. Ghanbari, A. Salimi, Protective effect of curcumin, chrysin and thymoquinone injection on trastuzumab-induced cardiotoxicity via mitochondrial protection, *Cardiovasc. Toxicol.* 22 (7) (2022) 663–675.
- [26] T. Micakovic, W.Z. Banczyk, E. Clark, B. Kränzlin, J. Peters, S.C. Hoffmann, Isolation of pure mitochondria from rat kidneys and western blot of mitochondrial respiratory chain complexes, *Bio-Protocol* 9 (19) (2019) e3379-e3379.
- [27] M. Sadighara, Z. Amirshardost, M. Minaian, V. Hajhashemi, P. Naserzadeh, A. Salimi, et al., Toxicity of atorvastatin on pancreas mitochondria: a justification for increased risk of diabetes mellitus, *Basic Clin. Pharmacol. Toxicol.* 120 (2) (2017) 131–137.
- [28] A. Eirin, A. Lerman, L.O. Lerman, The emerging role of mitochondrial targeting in kidney disease, *Pharmacol. Mitochondria* (2017) 229–250.
- [29] P. Bhargava, R.G. Schnellmann, Mitochondrial energetics in the kidney, *Nat. Rev. Nephrol.* 13 (10) (2017) 629–646.
- [30] J.B. Spinelli, M.C. Haigis, The multifaceted contributions of mitochondria to cellular metabolism, *Nat. Cell Biol.* 20 (7) (2018) 745–754.
- [31] P. Fisel, O. Renner, A.T. Nies, M. Schwab, E. Schaeffeler, Solute carrier transporter and drug-related nephrotoxicity: the impact of proximal tubule cell models for preclinical research, *Expert Opin. Drug Metab. Toxicol.* 10 (3) (2014) 395–408.
- [32] E. Kwiatkowska, L. Domański, V. Dziedzic, A. Kajdy, K. Stefańska, S. Kwiatkowski, The mechanism of drug nephrotoxicity and the methods for preventing kidney damage, *Int. J. Mol. Sci.* 22 (11) (2021) 6109.
- [33] M. Gyurászová, A.G. Kovalčíková, E. Renczés, K. Kmeťová, P. Celec, J. Bábíková, et al., Oxidative stress in animal models of acute and chronic renal failure, *Dis. Markers* (2019) 2019.
- [34] Z. Gai, T. Gui, G.A. Kullak-Ublick, Y. Li, M. Visentin, The role of mitochondria in drug-induced kidney injury, *Front. Physiol.* 11 (2020) 1079.
- [35] G. Ensergueix, N. Pallet, D. Joly, C. Levi, S. Chauvet, C. Trivin, et al., Ifosfamide nephrotoxicity in adult patients, *Clin. Kidney J.* 13 (4) (2020) 660–665.
- [36] M.M. Ommati, O. Farshad, V. Ghanbarinejad, H.R. Mohammadi, M. Khadijeh, A. Negar, et al., The nephroprotective role of carnitine against ifosfamide-induced renal injury and electrolyte imbalance is mediated via the regulation of mitochondrial function and alleviation of oxidative stress, *Drug Res.* 70 (01) (2020) 49–56.
- [37] G. Ciarimboli, S.K. Holle, B. Vollenbroëcker, Y. Hagos, S. Reuter, G. Burckhardt, et al., New clues for nephrotoxicity induced by ifosfamide: preferential renal uptake via the human organic cation transporter 2, *Mol. Pharm.* 8 (1) (2011) 270–279.
- [38] J. Springate, K. Chan, H. Lu, S. Davies, M. Taub, Toxicity of ifosfamide and its metabolite chloroacetaldehyde in cultured renal tubule cells, *Vitr. Cell. Dev. Biol. Anim.* (1999) 314–317.
- [39] H.-T. Wang, J.-H. Lin, C.-H. Yang, C.-H. Huang, C.-W. Weng, A.M.-Y. Lin, et al., Acrolein induces mtDNA damages, mitochondrial fission and mitophagy in human lung cells, *Oncotarget* 8 (41) (2017) 70406.
- [40] B. Knouzy, L. Dubourg, G. Baverel, C. Michoudet, Targets of chloroacetaldehyde-induced nephrotoxicity, *Toxicol. Vitro* 24 (1) (2010) 99–107.
- [41] M.Y. Cuevas-Magaña, C.C. Vega-García, J.C. León-Contreras, R. Hernández-Pando, C. Zazueta, W.R. García-Niño, Ellagic acid ameliorates hexavalent chromium-induced renal toxicity by attenuating oxidative stress, suppressing TNF- α and protecting mitochondria, *Toxicol. Appl. Pharmacol.* 454 (2022), 116242.
- [42] R.M. Bidanchi, L. Lalrindika, M. Khushboo, B. Bhanushree, R. Dinata, M. Das, et al., Antioxidative, anti-inflammatory and anti-apoptotic action of ellagic acid against lead acetate induced testicular and hepato-renal oxidative damages and pathophysiological changes in male Long Evans rats, *Environ. Pollut.* 302 (2022), 119048.
- [43] W.R. García-Niño, L. Ibarra-Lara, M.Y. Cuevas-Magaña, A. Sánchez-Mendoza, E. Armada, Protective activities of ellagic acid and urolithins against kidney toxicity of environmental pollutants: a review, *Environ. Toxicol. Pharmacol.* 95 (2022), 103960.
- [44] G.-E. Hurtado-Núñez, C. Cortés-Rojas, S.-G. Sánchez-Ceja, H.-E. Martínez-Flores, R. Salgado-Garciglia, M.-C. Bartolomé-Camacho, et al., Gallic, ellagic acids and their oral combined administration induce kidney, lung, and heart injury after acute exposure in Wistar rats, *Food Chem. Toxicol.* 170 (2022), 113492.