Current Issues in Pharmacy and Medical Sciences
Formerly ANNALES UNIVERSITATIS MARIAE CURIE-SKLODOWSKA, SECTIO DDD, PHARMACIA
https://czasopisma.umlub.pl/curipms

Alimentary protein deficiency aggravates mitochondrial dysfunction in animals with acetaminophen-induced kidney injury

Oksana Voloshchuk*, Halyna Kopylchuk

Department of Biochemistry and Biotechnology, Yuriy Fedkovych Chernivtsi National University, Chernivtsi, Ukraine

ARTICLE INFO

Received 06 April 2024
Accepted 31 January 2025

Keywords:
acetaminophen,
kidney,
mitochondria,
low protein diet.

ABSTRACT

We investigated the indicators of kidney function, the degree of ROS-mediated damage to lipids and proteins of mitochondria, the activity of antioxidant enzymes and the I and II complexes of the respiratory chain enzymes in animals with APAP-induced kidney damage and alimentary protein deficiency. Accordingly, in rats with APAP kidney toxic injury against protein deficiency, one of the mechanisms of impaired kidney function is the activation of oxidative damage to mitochondrial biomolecules. This is followed by a dysfunction of mitochondrial complexes I (NADH dehydrogenase: ubiquinone oxidoreductase) and II (succinate: ubiquinone oxidoreductase), and leads to histopathological changes in the kidney structure. Our work demonstrated that protein deficiency in the diet is a critical factor in determining the degree of structural and functional changes in the kidneys in animals with APAP kidney injury. The obtained results substantiate the rationale for assessing the organism's supply of protein when developing a plan for nephrotoxicity management in patients with an acute acetaminophen overdose.

INTRODUCTION

Acetaminophen (APAP, N-acetyl-p-aminophenol, paracetamol) is the most commonly used antipyretic and analgesic. Its self-administration increased significantly during the COVID-19 pandemic [1,2]. APAP is generally considered free of adverse effects when used per the recommended therapeutic doses. However, acetaminophen overdose is among the most frequent causes of intentional and unintentional drug poisoning worldwide.

Under normal conditions, the primary routes of APAP metabolism within the hepatic milieu encompass glucuronidation and sulfation reactions. The ensuing formation of water-soluble metabolites is subsequently subjected to renal excretion. In instances of APAP overdose, a discernible depletion of glutathione is evident, concomitant with the accrual of noxious metabolites. This occurrence precipitates metabolic perturbations, instigates the induction of apoptosis or necrosis, and results in compromised organ functionality [3]. An acute acetaminophen overdose may cause a potentially lethal acute kidney injury [4]. The majority of the evidence for APAP-induced kidney injury came from clinical trials and case reports. As of today, the mechanism(s) of APAP-induced kidney damage remains incompletely elucidated. One under consideration involves the formation of N-acetyl-p-benzoquinonimine (NAPQI) or *p*-aminophenol with the involvement of CYP or COX enzymes. The generated conjugates of glutathione or cysteine and NAPQI deplete the glutathione pool in the kidneys. Consequently, acetaminophen metabolites exhibit an enhanced propensity to bind to renal cell proteins, intensifying nephrotoxicity [5]. It has been demonstrated that acute kidney injury due to APAP overdose may occur even in the absence of liver damage symptoms [6].

Not all patients exposed to toxic doses of APAP develop kidney injury. That is, the manifestation of drug-induced nephrotoxicity may depend on a person's metabolic status, age, nutrient supply and the presence of concomitant diseases enhancing the nephrotoxic potential of the drug [7]. Numerous interrelated factors contribute to acetaminophen-induced nephrotoxicity, including inflammation and generation of reactive oxygen species (ROS) [8]. Although the exact way in which APAP leads to kidney injury is not entirely researched, it is known the generation of ROS and oxidative stress induced by NAPQI lead to kidney damage caused by acetaminophen. At the same time, the relationship between the organism's protein supply and APAP toxicity remains unstudied.

* **Corresponding author**
e-mail: o.voloschuk@chnu.edu.ua

Therefore, we investigated the indicators of kidney function and the markers of ROS-mediated damage to lipids and proteins of mitochondria in animals with APAP-induced intoxication under different dietary protein supplies.

MATERIALS AND METHODS

Experimental animals and protocols

The study used 11-12 week old white nonlinear rats (n=36) weighing 130-140 g. During the experiment, the test subjects were housed in cages with unrestricted access to water and were housed at a controlled temperature of 20°C ± 1°C with a 12-hour light-dark cycle under constant humidity of 60% to 70%. All experiments were carried out according to the National Institute of Health Guidelines for the care and use of laboratory animals and the European Council Directive on 24 November 1986 for Care and Use of Laboratory Animals (86/609/EEC) and approved by the bioethics commission of the Educational and Scientific Institute of the Biology, Chemistry and Biological Resources of Yuriy Fedkovych Chernivtsi National University (protocol No 2 of 24.12.2021).

The rats were divided into four groups of 9 animals as follows: C – control; LP – animals receiving a low-protein diet for four weeks; TD – animals with APAP-induced toxic damage; LP/TD – animals with low-protein diet/APAP-induced toxic damage.

The animals of groups C and TD received a standard ration containing 14% protein (casein), 10% fat and 76% carbohydrates, balanced by all the essential nutrients. The animals of the groups LP and LP/TD received an isoenergetic ratio containing 4.7% protein, 10% fat and 85.3% carbohydrates.

The control ration (C) was the AIN-93 diet [9]. All ingredients are presented in Table 1.

Table 1. Ingredient composition of the diets (g/kg diet)

Ingredient	Diet	
	C; TD	LP; LP/TD
Cornstarch, g/kg	620.7	714.1
Casein, g/kg	140	46.6
Sugar, g/kg	100	100
Fiber (cellulose microfiber), g/kg	50	50
Mineral mix, g/kg[1]	35	35
Vitamin Mix, g/kg[1]	10	10
L-Cystine, g/kg	1.8	1.8
Choline bitartrate, g/kg	2.5	2.5
Soy Oil, g/kg	40	40

C – control; LP – animals receiving low-protein ration; TD – toxic damage; LP/TD – animals receiving low-protein and toxic damage
[1] Mineral and Vitamin Mix – Based on the AIN-93G vitamin and mineral mixes

The APAP-induced injury was modeled by *per os* administration of 2% starch suspension of APAP in a daily dose of 1250 mg/kg of the body weight for 2 days [10].

Histopathologic examination of renal tissues

The harvested kidneys were rinsed twice with cold PBS and subsequently placed in 10% formaldehyde for histopathological examination. Paraffin blocks were prepared and 5 μm thick sections were then counterstained with hematoxylin and eosin using standard procedure. All hematoxylin and eosin-stained kidney sections from different experimental groups were evaluated histologically.

Kidney function markers

Rats were fasted for 12 hours, and urine samples were collected to determine cystatin C, creatinine, GGT, and protein. The levels of cystatin C in the serum were determined by the cystatin C ELISA kit. Serum, urinary creatinine, protein concentrations and urinary GGT were analyzed using a clinical automatic analyzer and standardized colorimetric method. The amount of urine excreted was assessed so as to obtain creatinine clearance (CrCl), which was determined according to the following formula: CrCl = UV/P, where U is the creatinine concentration in urine (in mg/dL), V is the volume of urine excretion by minute (in mL/min), and P is the serum creatinine concentration (in mg/dL) [11].

Preparation of mitochondria

Kidneys were homogenized in an extraction buffer containing 250 mM sucrose, 10 mM Tris·HCl and 1 mM EDTA (pH 7.4) at 4°C. A first centrifugation (Heraeus Biofuge, Germany) was performed at 600 g for 10 min at 4°C to sediment cellular debris [11]. The supernatant was then recovered and centrifuged at 10000 g for 10 min to sediment the mitochondria. Afterwards, the pellet was resuspended and was centrifuged at 10 000 g for 10 min at 4°C. The pellet containing the mitochondrial fraction was subsequently resuspended in 1 mL extraction buffer, and proteins were determined according to Bradford's method.

Oxidative Markers

The performed analysis included enzymatic antioxidants (superoxide dismutase (SOD), catalase (CAT)), thiobarbituric acid reactive substances (TBARS), protein carbonyls (PC) and protein SH-group.

Lipid peroxidation was measured in the mitochondria through TBARS assay. The concentration of TBARS was resolved by the reaction with 2-thiobarbituric acid (TBA), occurring at high temperature in an acidic environment and forming the colored complex, determined spectrophotometrically (Agilent Technologies, USA) at λ 532 nm ($\varepsilon = 1.56 \cdot 10^5$ $M^{-1} \cdot cm^{-1}$) [12]. The concentration of TBARS was expressed as nmol per mg of protein.

Protein carbonylation was assessed via 2.4-dinitrophenylhydrazone derivatives, produced in reactions of oxidized amino acid residues with 2.4-dinitrophenylhydrazine. This was expressed as nmol of carbonyl protein derivatives per mg of protein [13]. The final protein pellet was dissolved in 1.25 ml 6M-guanidine hydrochloride and the absorbance of both solutions (DNPH and HCl) was measured at 370 nm. The carbonyl content was calculated in terms of nmol/mg protein [14].

Protein SH-group content was determined using a method based on the reaction of Ellman's reagent (5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB)) with SH-groups, the products being disulfide and thionitrophenyl anion (TNPA) with the amount of TNPA being directly proportional to the amount of SH-groups of proteins that reacted with DTNB.

The content of free SH-groups was calculated based on a molar extinction coefficient of $11.4 \cdot 10^3$ $M^{-1} \cdot cm^{-1}$ and expressed as nmol per mg of protein [15].

Superoxide dismutase (SOD) activity was assayed colorimetrically by measuring the mitochondrial activity of SOD and by inhibiting the oxidation of adrenaline to adrenochrome [16]. The autooxidation was monitored in a Spectrophotometer at 480 nm every 30 secs for 5 min.

Catalase activity was assessed by incubating the sample in 1.0 ml substrate (65 mmol/ml hydrogen peroxide in 60 mmol/l sodium-potassium phosphate buffer, pH 7.4) at 37°C for three minutes. The reaction was stopped with ammonium molybdate. The absorbance of the yellow complex of molybdate and hydrogen peroxide was measured at 374 nm against the blank [17].

Enzyme assays

NADH: Q oxidase activity was determined spectrophotometrically; reactions were monitored at 340 nm with 200 mM NADH as the substrate at 20°C, using a molar absorption coefficient of $6.22 \cdot 10^3$ $M^{-1} \cdot cm^{-1}$ to calculate enzymatic activity [15,18].

The succinate dehydrogenase activity was measured based on the restoration of potassium ferricyanide ($K_3[Fe(CN)_6]$) to potassium ferrocyanide ($K_4[Fe(CN)_6]$). Herein, 0.1 mg of protein of mitochondria were suspended in 50 mM (K) phosphate buffer (pH 7.4) with 3 mM potassium ferricyanide (III). A decrease in the absorption (420 nm) upon adding 50 mM succinate was followed to measure the rate of potassium ferrocyanide (II) formation at 30°C for 2 min. The reaction rate was calculated as nmol ferrocyanide formed per minute per mg protein [15,19].

Statistical analysis

The results are presented as mean±standard error of the mean (SEM). Statistical comparisons were made relative to the appropriate intact group using the Student's t-test and analysis of variance. Values were considered statistically significant at $P < 0.001$ and $P < 0.05$.

RESULTS

Kidney function

The levels of cystatin C, serum creatinine, serum total protein, urine GGT and protein, as well as creatinine clearance are shown in Table 2. We found that animals on a protein-deficiency diet had decreased creatinine clearance and increased activity of GGT in urine, but there was no protein in the urine (Table 2). In animals with APAP-induced toxic injury, serum creatinine level and creatinine clearance did not differ from controls, urinary GGT activity was, however, increased, while proteinuria was found. At the same time, in animals with an APAP injury against protein deficiency, we found a decreased creatinine clearance, increased GGT activity and serum cystatin C level, and pronounced proteinuria (Table 2).

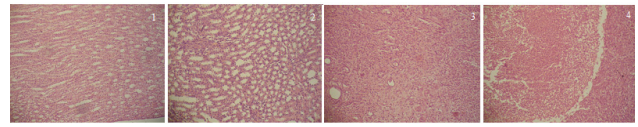
Histopathologic examination

Kidney sections of control animals demonstrate the standard tissue architecture of the glomerulus and surrounding structures. Moreover, histological examination of the kidneys of rats from protein deficiency or APAP overdose-induced toxic damage groups revealed no significant morphological changes. Kidney histology showed normal histoarchitecture of renal parenchyma with intact glomerulus and proximal/distal tubules with a typical structure. Significant pathological changes were found only in animals that received toxic doses of APAP and consumed a low-protein diet (Figure 1). In particular, kidney sections show signs of acute interstitial nephritis, manifested by interstitium swelling, accumulation of lymphocytes, macrophages, proliferation of fibroblasts, as well as papillary necrosis.

Table 2. General characteristics and kidney function

Parameter	Group			
	C	LP	TD	LP/TD
Final body weight, g	190±6[a]	153±5[b]	188±4[a]	152±5[b]
Serum total protein, g/L	63±5[a]	35±4[b]	61±6[a]	33±5[b]
Creatinine clearance (CrCl), mL/min	0.78 ±0.016[a]	0.48 ±0.008[b]	0.81 ±0.021[a]	0.26 ±0.006[c]
Serum creatinine, mg/dL	0.46 ±0.014[a]	0.42 ±0.012[ab]	0.40 ±0.010[b]	0.63 ±0.020[c]
Serum Cystatin C, µg/ml	0.51 ±0.017[a]	0.62 ±0.007[b]	0.58 ±0.033[c]	0.93 ±0.019[d]
Urine gamma-glutamyl-transferase (GGT), U/mL	0.043 ±0.011[a]	0.262 ±0.032[b]	1.015 ±0.118[c]	1.684 ±0.155[d]
Proteinuria, mg/mL	0.022 ±0.003[a]	0.024 ±0.005[a]	0.099 ±0.007[c]	0.165 ±0.018[d]

C – control; LP – animals receiving low-protein ration; TD – toxic damage; LP/TD – animals receiving low-protein and toxic damage
Values are means ± SD; n=9 different preparations from 9 different animals for each group. The values for all variables were obtained at the end of the experiment. Different letters indicate statistically significant differences ($P \leq 0.05$)



1 – control; 2 – low-protein ration (LP); 3 – APAP-induced toxic damage (TD); 4 – APAP-induced toxic damage/low-protein ration (LP/TD) (×200)

Figure 1. Kidney of a rat under conditions of toxic damage and protein deficiency

Oxidative stress markers

Analysis of the oxidative stress markers has shown the accumulation of TBARS and carbonyl derivatives in the kidney mitochondria and a decrease of CAT activity in rats kept on a protein deficiency (Table 3). In animals with APAP overdose-induced toxic kidney damage, an increased level of TBARS and carbonyls of mitochondrial proteins and

Table 3. Oxidative stress markers of the mitochondrial fraction of kidney

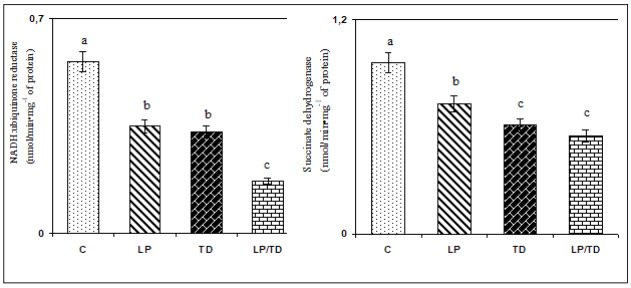
Parameter	Group			
	C	LP	TD	LP/TD
TBARS nmol/mg protein	7.58 ±0.84[a]	11.85 ±1.27[b]	15.05 ±3.64[c]	15.25 ±2.86[c]
Protein carbonyls nmol/mg protein	131.58 ±22.12[a]	157.31 ±18.87[b]	206.48 ±33.48[c]	2260.86 ±35.29[d]
Protein SH-groups nmol/mg protein	5.24 ±0.77[a]	4.21 ±0.61[a]	2.34 ±0.26[c]	1.88 ±0.17[d]
CAT nmol/min per mg protein	8.21 ±1.26[a]	5.94 ±1.14[b]	6.58 ±0.88[bc]	4.37 ±0.51[d]
SOD U/mg protein	38.47 ±4.16[a]	35.22 ±5.33[a]	29.51 ±4.71[b]	18.62 ±3.15[c]

C – control; LP – animals receiving low-protein ration; TD – toxic damage; LP/TD – animals receiving low-protein and toxic damage
Values are means ± SD; n=9 different preparations from 9 different animals for each group; TBARS, thiobarbituric acid-reactive substances; CAT, catalase; SOD, superoxide dismutase.
Different letters indicate statistically significant differences ($P \leq 0.05$)

decreased protein SH-groups level and SOD activity were found. Under the conditions of APAP intoxication against protein deficiency, the indicators of oxidative stress in kidney mitochondria reached the maximal levels: the most significant increase in protein carbonyls and reduction of protein SH-groups activity of CAT and SOD.

Enzyme assays

Figure 2 shows a decrease in succinate dehydrogenase and NADH-ubiquinone oxidase activity in the mitochondria of the kidney in both protein deficiency and APAP overdose-induced toxic damage. The most significant reduction in NADH-ubiquinone oxidase activity was found in animals from the low-protein/toxic damage group.



Note (here and forwards): C – control; LP – animals receiving low-protein ration; TD – APAP-induced toxic damage; LP/TD – APAP-induced toxic damage/low-protein ration.

Different letters indicate statistically significant differences (P≤0.05)

Figure 2. NADH-ubiquinone oxidase and succinate dehydrogenase activity in kidney cell mitochondria of rats with toxic kidney damage and protein deficiency

DISCUSSION

About 20% of all cases of acute kidney failure in the general population can be attributed to medication overdose; however, among the elderly, the incidence of such consequences can reach 66% [20]. In cases of acetaminophen overdose, instances of acute renal impairment are documented in 1-2% of all patients. The toxicity of APAP is the result of metabolism in the hepatocytes and extrahepatic tissues. Approximately 1% of the acetaminophen is excreted unchanged in the urine.

Hua H *et al.* have shown that "with therapeutic dosing in adults, approximately 63% of acetaminophen is metabolized via glucuronidation and 34% by sulfation. These phase II reactions occur primarily in the liver and result in water-soluble metabolites excreted via the kidney" [21]. In the liver, the mechanism of APAP toxicity is well known, but in the kidney, this is less understood.

The analysis of the results showed a reduction in the filtration capacity of the kidneys of rats consuming a low-protein ratio. The absence of changes in the plasma creatinine level against the background of a decrease in creatinine clearance is probably associated with a reduction in muscle mass associated with a lack of dietary protein and, as a result, reduced activity of glomerular apparatus and decreased number of functioning nephrons in the kidneys of rats of the experimental group. Fotheringham AK *et al.*, for example, showed that "low protein intake led to greater kidney tubular structural injury and inflammation; Lower protein intake decreased kidney mass and glomerular filtration capacity" [22].

In turn, the administration of the toxic acetaminophen doses does not cause any impairment of the kidney filtration function, which is evidenced by the absence of changes in both the serum creatinine and creatinine clearance. However, the detected proteinuria and increased urinary GGT activity indicate an increase in the permeability of the glomerular membrane and primary damage to the renal tubular cells. It has been shown that the acetaminophen metabolite N-acetyl-*p*-benzoquinoneimine (NAPQI) is capable of forming covalent bonds with macromolecules of cellular proteins, including those of the kidneys, thereby initiating tissue necrosis and organ dysfunction [23,24]. At the same time, the absence of changes in creatinine clearance in animals with APAP-induced toxic injury that were kept on a full-value diet, indicates that the supply of protein in the ration is the limiting factor for preserving the functional activity of the glomerular apparatus of the kidneys.

The research results show that the lack of protein in the ration of rats with toxic kidney injury leads to the aggravation of kidney dysfunction due to the impairment of the filtration and reabsorption capacity of the kidneys. Elevated serum creatinine levels are indicators of APAP-induced acute tubular necrosis [25]. The detected proteinuria is probably caused by both a disruption of the integrity of the renal tubular epithelium and an increase in the permeability of the renal filter under the studied conditions. An increase in proteinuria is a sign of deterioration of both reabsorption capacity and the filtration capabilities of the kidneys, as well as renal dysfunction in general.

Cystatin-C levels in animals LP/TD showed significant enhancement compared to all groups. Cystatin C is considered a reliable marker of kidney dysfunction. An increase in the activity of urine GGT indicates the mass death of renal tubular cells in protein-deficient animals with acetaminophen-induced intoxication. It is known that this enzyme is of renal origin: it is released into the urine from the destroyed cells of the proximal tubules, which contain it in a high concentration [26]. In the kidneys, the enzyme plays a significant role in the reabsorption of amino acids from primary urine [27]. An increase in the GGT activity is a marker of the early stages of kidney injury. Therefore, an increase in its activity in the urine of protein-deficient animals with toxin-induced kidney injury indicates damage to the proximal tubules due to the death of tubular cells.

It should be noted that the absence of histological changes in the kidneys of animals from the low-protein and APAP toxic damage groups and the presence of the signs of acute interstitial nephritis and papillary necrosis on kidney sections of animals with APAP-induced kidney damage and dietary protein deficiency indicate that the latter is a critical factor for kidney damage due to acetaminophen overdose. This fact can explain the various degrees of nephrotoxicity in different patients.

Previous research has demonstrated that acetaminophen can induce oxidative stress (particularly lipid peroxidation), disturbance of the antioxidant status and inactivation of a series of enzymes [3]. Simultaneously, there some suggestion that oxidative stress, akin to inflammation, contributes

to acetaminophen-induced renal damage. However, the precise mechanisms remain incompletely understood. Mitochondrial dysfunction is, however, considered a significant mechanism in the onset of acute kidney damage associated with acetaminophen-induced renal impairment [28]. It is known that a substantial number of mitochondria are present in renal tubules, playing an essential role in adenosine triphosphate (ATP) production in the process of oxidative phosphorylation (OXPHOS) [6]. Additionally, mitochondria play an essential role in signal transduction, cell death and cellular growth within the kidney. Therefore, damage to mitochondria can lead to an imbalance in energy processes and excessive reactive oxygen species (ROS) generation, ultimately resulting in renal cell damage [29].

Determination of the oxidative stress markers in kidney mitochondria has shown that under protein deficiency in the diet, in rats with APAP-induced toxic damage, there is a pronounced accumulation of products of ROS-induced damage to lipids and proteins accompanied by a reduction of CAT and SOD activity. CAT and SOD reduction in the LP/TD group can be interpreted as a manifestation of oxidative stress in the kidney cells because the LP group and TD group also revealed decreased catalase and SOD levels. It also may be associated with protein deficiency in the diet. Elevated concentrations of TBARS in renal mitochondria indicate kidney impairment [29]. Other studies have demonstrated that lipid peroxidation (LPO) product accumulation and the depletion of antioxidant defenses induce oxidative stress in the kidneys [25]. Also, under APAP-induced injury and protein deficiency: "the most pronounced intensification of free radical-induced processes in liver mitochondria [has been observed], associated with increased production of superoxide anion radical, hydroxyl radical and hydrogen peroxide that provide a more comprehensive understanding of the mitochondrial dysfunction observed in the LP/TD group" [30]. At the same time, an increase in the diet of cornstarch can worsen oxidative stress, induce metabolic strain and contribute to mitochondrial dysfunction, resulting in a more severe decline in kidney function through acetaminophen (APAP)-induced toxicity. By increasing ROS production, impairing protein metabolism and promoting hyperglycemia, excessive cornstarch consumption may exacerbate the toxic effects of acetaminophen on the kidneys. Beyond the aforementioned, a decrease in succinate dehydrogenase and NADH-ubiquinone oxidase activity in animals with acute toxic injury and protein deficiency indicates an impairment of the functional activity of mitochondria.

In the pathogenesis of renal diseases, mitochondrial damage has a well-established role [28] in that suppression of the activity of mitochondria reduces ROS production, thereby contributing to the preservation of renal functional activity. Accordingly, inhibiting the activity of respiratory chain enzymes, particularly complex I, is a protective reaction against renal damage [31]. As complex I is considered the primary site of ROS generation within the mitochondria, inhibiting complex I activity in abnormal mitochondria in obstructive kidney disease diminishes manifestations of organ damage [32]. Considering that normally about 90% of the ATP required for the reabsorption of ions, glucose and metabolites in the kidney is produced by oxidative phosphorylation [33], the disturbances in the filtration capacity and reabsorption of the kidneys primarily accompany the imbalance of energy production processes.

CONCLUSIONS

In the present study, we found that one of the mechanisms of impaired functional activity of the kidneys in animals with APAP-induced injury and protein deficiency is the activation of oxidative damage to mitochondrial biomolecules, with the subsequent disruption of the complexes I and II of the respiratory chain activity and development of histopathological changes in the kidneys. At the same time, dietary protein deficiency is a critical factor in determining the degree of structural and functional changes in the kidneys of animals with APAP-induced toxic injury. The study results substantiate the rationale for assessing the organism's supply of protein when developing a plan for nephrotoxicity management in patients with an acute acetaminophen overdose. Dietary adjustments, antioxidants or mitochondrial protectants should prevent or reduce oxidative stress and be administered to counteract the combined effects of low protein intake and APAP-induced renal damage. This issue, however, requires further study.

ORCID iDs

Oksana Voloshchuk https://orcid.org/0000-0002-6005-3732
Halyna Kopylchuk https://orcid.org/0000-0002-2906-4927

REFERENCES

1. Kanchanasurakit S, Arsu A, Siriplabpla W, Duangjai A, Saokaew S. Acetaminophen use and risk of renal impairment: A systematic review and meta-analysis. *Kidney Res Clin Pract*. 2020;39:81-92.
2. Mostafa EMA, Tawfik AM, Abd-Elrahman KM. Egyptian perspectives on potential risk of paracetamol/acetaminophen-induced toxicities: Lessons learnt during COVID-19 pandemic. *Toxicol Rep*. 2022;9:541-8.
3. Roy S, Pradhan S, Das K, Mandal A, Mandal S, Patra A, et al. Acetaminophen induced kidney failure in rats: a dose response. *J Biol Sci.* 2015;15:187-93.
4. Naggayi M, Mukiibi N, Iliya E. The protective effects of aqueous extract of Carica papaya seeds in paracetamol induced nephrotoxicity in male wistar rats. *Afr Health Sci*. 2015;15:598-605.
5. Shao QH, Yin XD, Liu HX, Zhao B, Huang JQ, Li ZL. Kidney injury following ibuprofen and acetaminophen: a real-world analysis of post-marketing surveillance data. *Front Pharmacol.* 2021;12:750108.
6. Hall AM, Schuh CD. Mitochondria as therapeutic targets in acute kidney injury. *Curr Opin Nephrol Hypertens*. 2016;25:355-62.
7. Perazella MA. Pharmacology behind Common Drug Nephrotoxicities. *Clin J Am Soc Nephrol.* 2018;13:1897-08.
8. El-Hashash SA, Gaballah WA, Faramawy AA, Rizk NI, Alsuwat MA, Alshehri MA, et al. Mitigating acetaminophen-induced Kidney Injury: The protective role of grape seed and peanut skin extracts through the iNOS/CYP2E1 pathway. *ACS Omega*. 2024;9(32):35154-69.
9. Reeves P, Nielsen F, Fahey G. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent. *J Nutr.* 1993;123(11):1939-51.
10. Gao Y, Cao Z, Yang X, Abdelmegeed MA, Sun J, Chen S, et al. Proteomic analysis of acetaminophen-induced hepatotoxicity and identification of heme oxygenase 1 as a potential plasma biomarker of liver injury. *Proteomics Clin Appl.* 2017;11:10.1002/prca.201600123.

11. Ruiz-Ramírez A, Barrios-Maya M, Quezada-Pablo H, López-Acosta O, El-Hafidi M. Kidney dysfunction induced by a sucrose-rich diet in rat involves mitochondria ROS generation, cardiolipin changes, and the decline of autophagy protein markers. *Am J Physiol Renal Physiol*. 2020;318:F53-F66.
12. Rodrigues T, de França LP, Kawai C, de Faria PA, Mugnol KC, Braga FM, et al. Protective role of mitochondrial unsaturated lipids on the preservation of the apoptotic ability of cytochrome C exposed to singlet oxygen. *J Biol Chem*. 2007;282:25577-87.
13. Voloshchuk OM, Kopylchuk GP, Mishyna YI. Activity of the mitochondrial isoenzymes of endogenous aldehydes catabolism under the conditions of acetaminophen-induced hepatitis. *Ukr Biochem J*. 2018;90:42-7.
14. Parihar MS, Pandit MK. Free radical induced increase in protein carbonyl is attenuated by low dose of adenosine in hippocampus and mid brain: implication in neurodegenerative disorders. *Gen Physiol Biophys*. 2003;22: 29-39.
15. Voloshchuk OM, Ursatyy MS, Kopylchuk GP. The NADH-ubiquinone reductase and succinate dehydrogenase activity in the rat kidney mitochondria under the conditions of different protein and sucrose content in the diet. *Ukr Biochem J*. 2022;94:105-13.
16. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J Biol Chem*. 1972;247:3170-5.
17. Hadwan MH, Abed HN. Data supporting the spectrophotometric method for the estimation of catalase activity. *Data Brief*. 2015;6: 194-9.
18. Grivennikova VG, Kapustin AN, Vinogradov AD. Catalytic activity of NADH-ubiquinone oxidoreductase (complex I) in intact mitochondria. evidence for the slow active/inactive transition. *J Biol Chem*. 2001;276:9038-44.
19. Ahmad F, Alamoudi W, Haque S, Salahuddin M, Alsamman K. Simple, reliable, and timeefficient colorimetric method for the assessment of mitochondrial function and toxicity. *Bosn J Basic Med Sci*. 2018;18:367-74.
20. Naughton CA. Drug-induced nephrotoxicity. *Am Fam Physician*. 2008;78:743-50.
21. Mazer M, Perrone J. Acetaminophen-induced nephrotoxicity: pathophysiology, clinical manifestations, and management. *J Med Toxicol*. 2008;4:2-6.
22. Fotheringham AK, Solon-Biet SM, Bielefeldt-Ohmann H, McCarthy DA, McMahon AC, Ruohonen K, et al. Kidney disease risk factors do not explain impacts of low dietary protein on kidney function and structure. *iScience*. 2021;24:103308.
23. Hiragi S, Yamada H, Tsukamoto T, Yoshida K, Kondo N, Matsubara T, et al. Acetaminophen administration and the risk of acute kidney injury: a self-controlled case series study. *Clin Epidemiol*. 2018;10: 265-76.
24. Ściskalska M, Śliwińska-Mossoń M, Podawacz M, Sajewicz W, Milnerowicz H. Mechanisms of interaction of the N-acetyl-p-aminophenol metabolites in terms of nephrotoxicity. *Drug Chem Toxicol*. 2015;38:121-5.
25. Ahmed OM, Fahim HI, Ahmed HY, Mahmoud B, Aljohani SAS, Abdelazeem WH. The nephropreventive and antioxidant effects of navel orange peel hydroethanolic extract, naringin and naringenin in n-acetyl-p-aminophenol-administered wistar rats. *Adv Anim Vet Sci*. 2018;6:96-105.
26. Mancinelli E, Shaw DJ, Meredith AL. γ-Glutamyl-transferase (GGT) activity in the urine of clinically healthy domestic rabbits. *Vet Rec*. 2012;171:475.
27. Crivellenti LZ, Mesa JS, Meirelles AE, Borin Crivellenti S, Mireya EG, Canola JC, et al. False positivity of gamma-glutamyl transpeptidase measurement inurine. *Ren Fail*. 2014;36:581-4.
28. Hua H, Ge X, Wu M, Zhu C, Chen L, Yang G, et al. Rotenone protects against acetaminophen-induced kidney injury by attenuating oxidative stress and inflammation. *Kidney Blood Press Res*. 2018;43: 1297-09.
29. Mandal A, Patra A, Mandal S, Roy S, Mahapatra SD, Mahapatra TD, et al. Therapeutic potential of different commercially available synbiotic on acetaminophen-induced uremic rats. *Clin Exp Nephrol*. 2015;19:168-77.
30. Kopylchuk GP, Voloshchuk OM. Peculiarities of the free radical processes in rat liver mitochondria under toxic hepatitis on the background of alimentary protein deficiency. *Ukr Biochem J*. 2016; 88(2):66-72.
31. Ding W, Xu C, Wang B, Zhang M. Rotenone attenuates renal injury in aldosterone-infused rats by inhibiting oxidative stress, mitochondrial dysfunction, and inflammasome activation. *Med Sci Monit*. 2015;21:3136-43.
32. Sun Y, Zhang Y, Zhao D, Ding G, Huang S, Zhang A, Jia Z. Rotenone remarkably attenuates oxidative stress, inflammation, and fibrosis in chronic obstructive uropathy. *Mediators Inflamm*. 2014;2014:670106.
33. Braga PC, Alves MG, Rodrigues AS, Oliveira PF. Mitochondrial pathophysiology on chronic kidney disease. *Int J Mol Sci*. 2022; 23:1776.