



Nano ubiquinone: Promising candidate for treatment of renal toxicity induced by over dose of paracetamol



Laila Fadda^a, Hanaa M Ali^{b,c,*}, Ghada A Aldrees^d, Noura M Alquraishi^d

^a Department of Pharmacology and Toxicology, College of Pharmacy, King Saud University Riyadh, Saudi Arabia

^b Common First year Deanship, King Saud University, Riyadh, Saudi Arabia

^c Genetic and Cytology department, National Research Centre, Cairo, Egypt

^d Faculty of Pharmacy, King Saud University, Riyadh, Saudi Arabia

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ABSTRACT

Over doses of Paracetamol (panadol; acetaminophen) can cause life-threatening renal damage. This study compared the impact of nano-ubiquinone (Nubiq) with native ubiquinone (ubiq) reducing damage induced by Paracetamol-toxicity in rats. Paracetamol treatment produced an elevation in serum urea, uric acid, creatinine, C-reactive protein, renal nitric oxide, and lipid peroxide levels, and reductions in interleukin-10, superoxide dismutase, and glutathione levels. Meanwhile, c-Jun N-terminal kinases, vascular cell adhesion protein-1, cyclooxygenase-2 protein, and kidney injury molecule-1 were highly expressed, and NFE2-related factor 2 gene expression was down-regulated. Destruction of the epithelium, necrosis, and inflammatory cell infiltration could be observed in the renal tissue. Treatment with both ubiq a nubiq significantly ameliorated all of these signs. These findings suggest that Nubiq achieved the most significant amelioration in oxidative stress and inflammatory biomarkers in paracetamol -induced nephrotoxicity.

1. Introduction

N-acetyl-*p*-aminophenol (paracetamol or acetaminophen, marketed as Panadol, Tylenol, and other preparations) is an analgesic and anti-pyretic associated with hepatotoxicity and nephrotoxicity in overdose [1,2].

N-acetyl-*p*-benzoquinone imine (NAPQI) is a reactive toxic metabolite of paracetamol which induces the production of reactive oxygen species (ROS) in the kidney and liver, resulting in dysfunction, oxidative stress, altered permeability of the mitochondrial pores, damage to DNA damage, and cell death [3–6], sometimes leading to acute renal failure [7]. At present, there is no specific antidote for paracetamol-induced nephrotoxicity.

Ubiquinone (ubiq) is a natural antioxidant, associated with a 50% decrease in all-cause mortality. It is the first novel drug yielding a significant improvement in heart failure mortality introduced since 2009 and has been recommended as part of future standard treatment approaches [11–13]. It has been documented that Ubiq functions as important electron carriers within the cell which It suppresses oxidative stress [8–14] and improves doxorubicin-induced nephrotoxicity [15], and may be of use in addressing paracetamol-induced nephrotoxicity as

well.

Nanoparticles (NPs) play a vital role in drug targeting, by improving stability, bioavailability, and transport and release of active compounds to affected areas. Nano ubiquinone, or Nubiq, could be an effective means of drug provision for nephrotoxicity during paracetamol overdose. Therefore, we aimed to investigate the effects of Nubiq and ubiq against paracetamol overdose in rats and to describe novel molecular mechanisms underlying their protective effects.

2. Materials and methods

2.1. Experimental animals

Twenty four male albino rats (180–190 g) were supplied by the Animal House, College of Pharmacy, King Saud University. Animals were maintained under standard conditions. All procedures relating to animal care and treatments strictly adhered to the ethical procedures and policies approved by King Saud University (KSU, SE, 19–22).

* Corresponding author at: Common First year Deanship, King Saud University, Riyadh, Saudi Arabia.

E-mail addresses: lfadda@ksu.edu.sa (L. Fadda), hanali@ksu.edu.sa (H.M. Ali), Ghadah_aldrees@hotmail.com (G.A. Aldrees), NouraQur@hotmail.com (N.M. Alquraishi).

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Table 1
Serum creatinine, urea and uric acid levels in all treated groups.

Parameter Groups	Creatinine (mg/dl)	Urea (mg/dl)	Uric Acid (mg/dl)
Control	2.20 ± 0.11	2.04 ± 0.08	0.36 ± 0.01
Paracetamol	4.2 ± 0.2 ⁺⁺⁺	3.92 ± 0.18 ⁺	1.66 ± 0.14 ⁺⁺
Paracetamol & Ubiquitin	2.9 ± 0.12 ^{**}	3.07 ± 0.2	0.87 ± 0.12 ^{**}
Paracetamol & Nubiquitin	2.7 ± 0.2 ^{**}	2.6 ± 0.15 [*]	0.727 ± 0.08 ^{**}

Notes: Data are presented as mean ± SEM (N = 6). ⁺⁺⁺ $P \leq 0.001$ ⁺⁺ $P \leq 0.01$ and ⁺ $P \leq 0.05$ vs control, and ^{***} $P \leq 0.001$ ^{**} $P \leq 0.01$ and ^{*} $P \leq 0.05$ vs Paracetamol-intoxicated group.

2.2. Chemicals and animals preparation

All chemicals were the product of Sigma and Merck companies. Sigma Chemical Co. (Sigma, St. Louis, MO, USA). Rats were allowed to acclimate to laboratory housing for one week, then randomly divided into four groups of six. Group 1 served as an untreated control group. In Group 2, rats were administered a single oral dose of paracetamol at 1000 mg/kg [16], with no treatment following this. Group 3 was administered the same dose of paracetamol, then treated with 10 mg/kg ubiquitin [17] 2 and 6 h afterward. Group 4 was administered paracetamol, then treated with 10 mg/kg Nubiquitin [18] according to the same timeline. After 24 h, all rats were sacrificed. Blood samples were taken and serum was separated. Kidneys were dissected out, and three from each group were placed in formalin for histological examination. Three parts of kidneys were kept under nitrogen for western blot analysis. The remaining kidney tissues were homogenized in phosphate buffered saline (PBS) and centrifuged.

2.3. Blood and kidney tissue analysis

Serum creatinine, urea, and uric acid levels were measured using their respective kits (Randox). C-reactive protein (CRP) and interleukin-10 (IL-10) levels were estimated using enzyme-linked immunosorbent assay (ELISA) kits. In renal tissues, malondialdehyde (MDA) was measured using thiobarbituric acid [19] to indicate lipid peroxidation. Glutathione (GSH) was evaluated as per Ellman (1959) [20], and total nitrate and nitrite (NO) levels were measured indirectly as per the report by Moshage et al. (1995) [21].

Kidney injury molecule-1 (KIM-1) and nuclear factor erythroid-related factor 2 (Nrf2) were measured as indicators of tissue damage using real-time PCR in kidney samples prepared using PBS.

Total RNA was extracted, transcribed into cDNA, and amplified by PCR using RT-PCR kit (Stratagene, USA) according to Livak and Schmittgen [22], using the KIM-1 forward primer 5'-TATTTGGGGGAACAGGTTGC and reverse primer 5'-CAAGTCACTCTGGTTAGCCGTG, and Nrf2 forward primer 5'-CACATCCAGACAGACACCAGT and reverse primer 5'-CTACAAATGGGAATGTCTCTGC.

Western blot analysis of preserved kidney tissue was carried out as per Mahmoud et al. [23] to quantify c-Jun N-terminal kinases (JNK), vascular cell adhesion protein 1 (VCAM), and cyclooxygenase-2 (COX-

Table 2
Renal MDA, GSH, NO levels and SOD activity in all treated groups.

Parameter Groups	MDA (nmol/mg)	SOD (nmol/mg)	GSH (nmol/mg)	NO (nmol/mg)
Control	1.18 ± 0.15	7.29 ± 0.14	0.54 ± 0.01	2.14 ± 0.05
Paracetamol	3.78 ± 0.05 ⁺⁺⁺	4.63 ± 0.2 ⁺⁺⁺	0.24 ± 0.0 ⁺⁺⁺	5.38 ± 0.137 ⁺⁺
Paracetamol & Ubiquitin	1.54 ± 0.13 ^{***}	5.9 ± 0.11 [*]	0.28 ± 0.01	2.6 ± 0.02 ^{**}
Paracetamol & Nubiquitin	1.24 ± 0.09 ^{***}	5.8 ± 0.16 [*]	0.44 ± 0.02 ^{**}	2.35 ± 0.07 ^{**}

Notes: Data are presented as mean ± SEM (N = 6). ⁺⁺⁺ $P \leq 0.001$ ⁺⁺ $P \leq 0.01$ vs control, and ^{***} $P \leq 0.001$ ^{**} $P \leq 0.01$ and ^{*} $P \leq 0.05$ vs paracetamol-intoxicated group.

MDA: Malondialdehyde; GSH: glutathione; NO: total nitrate/nitrite concentrations levels and SOD: superoxide dismutase.

2).

Histological sections of kidneys were stained using hematoxylin and eosin (H&E) and examined using a light microscope.

2.4. Statistical analysis

Data are expressed as mean ± standard error (SEM) and analyzed using one-way analysis of variance (ANOVA) in SPSS (Statistical Package for the Social Sciences, version 16.0.1, Chicago, IL) software. Differences were regarded as significant at $p \leq 0.05$; $p \leq 0.01$ and $p \leq 0.001$.

3. Results

Group 2 rats administered paracetamol showed significantly greater serum urea, creatinine, and uric acid levels than Group 1 rats receiving no paracetamol, indicating predicted effects of the overdose. In Groups 3 and 4, these levels were lower, suggesting the ameliorated effects of ubiq and Nubiq (Table 1). Group 2 also showed increased renal MDA and NO and serum CRP, and reduced IL-10, SOD, and GSH. In Groups 3 and 4, these levels were comparable to those in Group 1 (Table 2, Fig. 1).

In Group 2 rats, paracetamol significantly reduced expression of Nrf2 and increased expression of KIM-1, with these findings significantly ameliorated in Groups 3 and 4 by ubiq and Nubiq (Fig. 2). Paracetamol overdose also increased protein production of JNK, VCAM, and COX-2 (Fig. 3), as well as DNA fragmentation (Fig. 4). Administration of ubiq and Nubiq significantly improved these former parameters, indicating that renal tissues were protected from DNA damage.

Kidney sections from Group 2 showed renal cortices with damaged or destroyed glomerular corpuscles. The proximal and distal convoluted tubules showed destroyed epithelial lining, vacuolization in tubular cells, focal necrosis, and infiltration of interstitial inflammatory cells. Meanwhile, sections from Groups 3 and 4 showed renal cortices with normal renal corpuscles and glomeruli, a normal pattern of proximal convoluted tubules lined by a thick columnar epithelium, and normal distal convoluted tubules lined by a lower cuboidal epithelium (Fig. 5), indicating that they were not subject to the same degree of damage.

4. Discussion

Rises in serum urea, uric acid, and creatinine were indicative of renal oxidative tissue damage, while ubiq or Nubiq administration successfully decreased these levels. Ubiquitin has been shown to similarly reduce oxidative stress and cardiorenal fibrogenesis induced by isoprenaline in rats [24].

Increased renal MDA and reduced GSH under paracetamol toxicity were also ameliorated by ubiq and Nubiq, indicative of their capacity for reducing lipid peroxidation. Reckziegel et al., [25] stated that ROS mediated oxidative damages can be detected by the rise in lipid peroxidation.

Ubiquitin was stated to show renoprotective powers against toxic chemicals-induced lipid peroxidation in rats [26].

Paracetamol triggers pathological inflammation, apoptosis, and

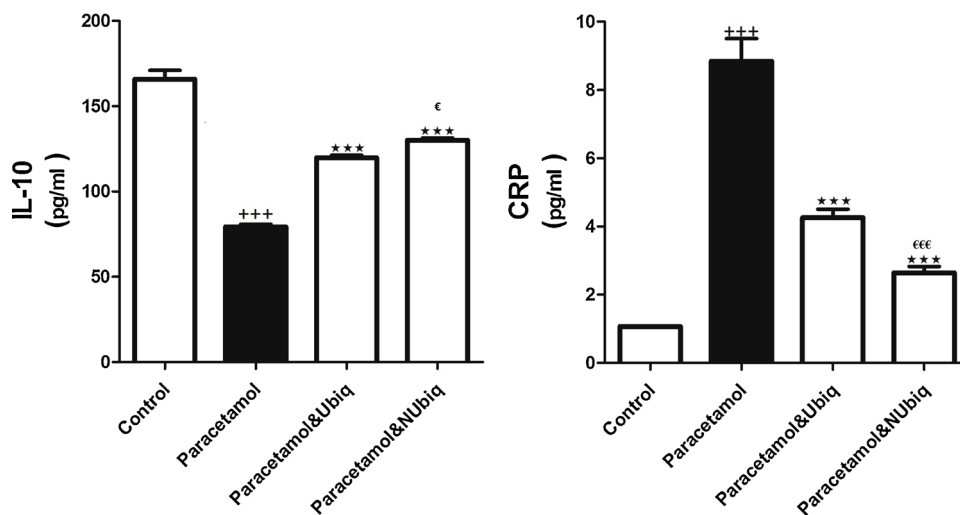


Fig. 1. Modulation of serum levels of IL-10 and CRP in all treated groups. Notes: Data are presented as mean ± SEM (N = 6). ⁺⁺⁺P ≤ 0.001 vs control, and ^{***}P ≤ 0.001 vs paracetamol -intoxicated group. ^εP ≤ 0.05 vs paracetamol and Ubiq treated group. IL-10: Interleukin 10 and CRP: C-Reactive Protein.

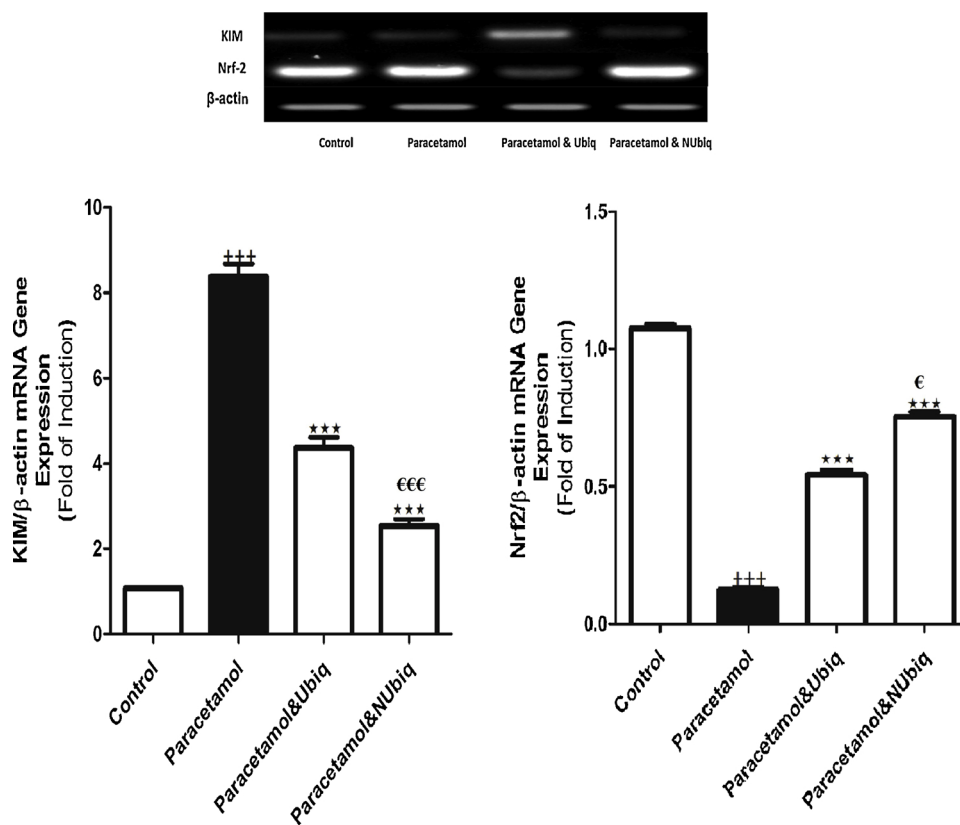


Fig. 2. Effects of ubiq and Nubiq on the KIM, Nrf-2 protein levels in paracetamol - induced nephrotoxicity in rats. Notes: Data are presented as mean ± SEM (N = 6). ⁺⁺⁺P ≤ 0.001 vs control, and ^{***}P ≤ 0.001 vs paracetamol -intoxicated group. KIM: kidney injury molecule-1 and Nrf-2: NFE2-related factor 2.

oxidative DNA-damage [27], reflected by the markedly raised CRP, renal NO, and lipid peroxide and decreased IL-10, SOD, and GSH levels observed in our study.

Ubiq and Nubiq significantly inhibited rises in CRP and NO, and increased IL-10, GSH, and SOD, suggesting a degree of protection related to their anti-inflammatory and antioxidant activities.

ROS-mediated prolongation of JNK activation is essential for JNK proapoptotic function via Bcl-2 inactivation, Bax promotion, and

caspase-8 translocation to mitochondria [28]. We found that paracetamol overdose significantly reduced Nrf2 expression, as previously reported [29], and increased VCAM, JNK, COX-2 protein expression and gene expression of KIM-1. Additionally, DNA fragmentation was evident compared to control rats. Urinary Kim-1 has been reported as a specific and very sensitive biomarker for the early stage diagnosis of nephrotoxicant-induced proximal tubular injury [30].

Ubiq or Nubiq administration significantly ameliorated these

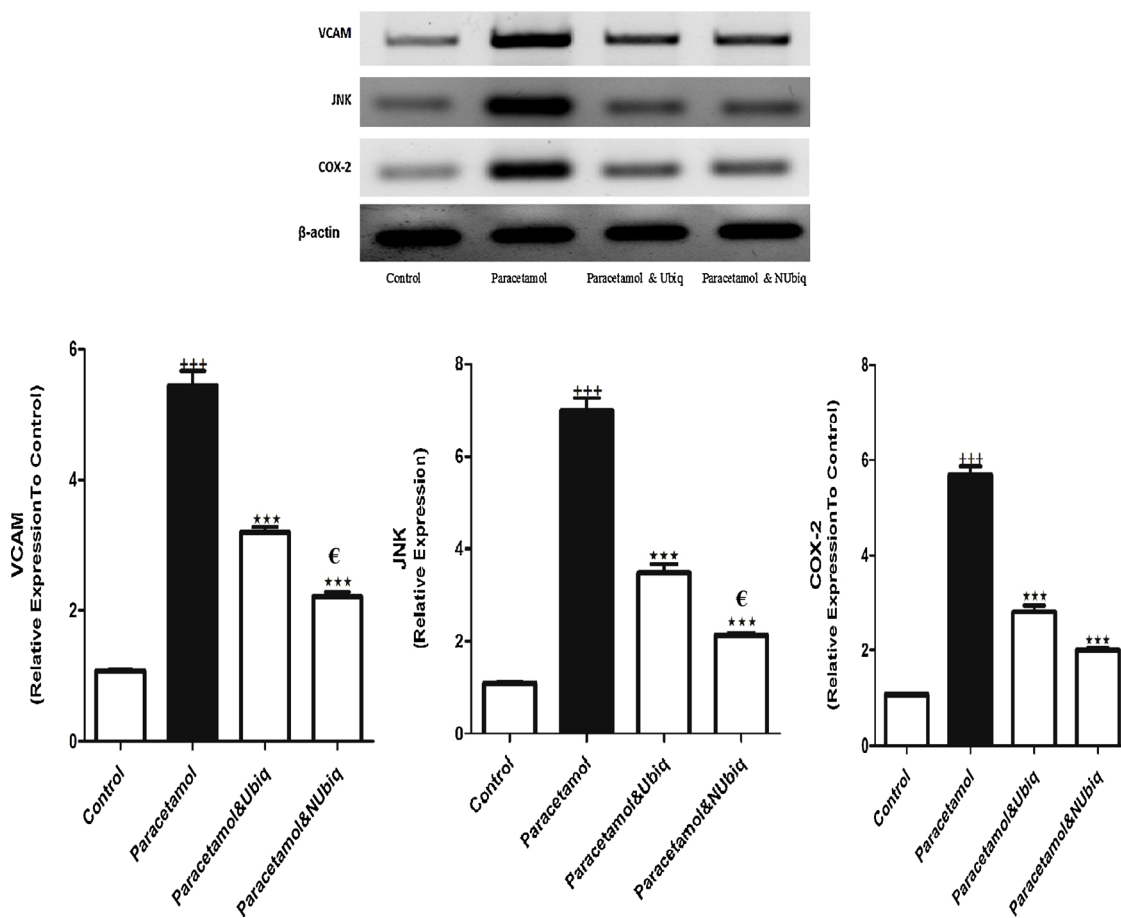


Fig. 3. Effects of ubiq and Nubiq on the VCAM, JNK, COX-2 mRNA gene expression levels in paracetamol- induced nephrotoxicity in rats. Notes: Data are presented as mean \pm SEM (N = 6). ⁺⁺⁺P \leq 0.001 vs control, and ^{***}P \leq 0.001 vs paracetamol -intoxicated group. VCAM: Vascular cell adhesion protein 1, JNK: c-Jun N-terminal kinases and COX-2: cyclooxygenase-2.

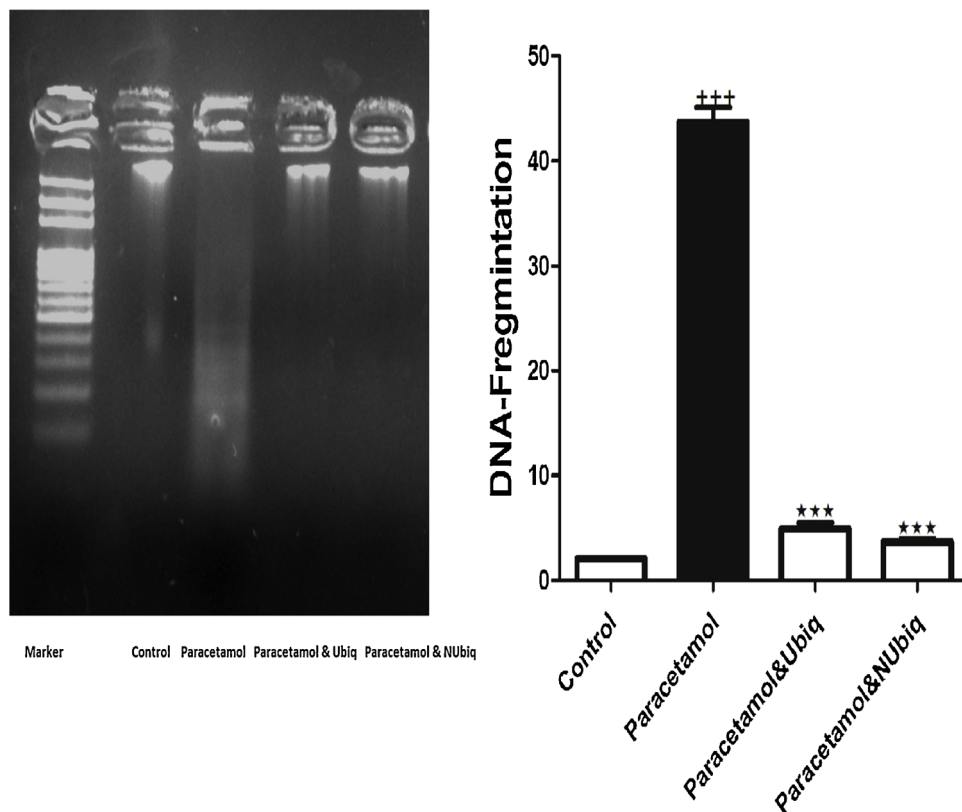


Fig. 4. Quantitative analysis of DNA fragmentation levels in control, paracetamol -intoxicated and all treated groups. Intoxication with paracetamol caused significant increase in DNA fragmentation; (The DNA was electrophoresed using agarose gel). Notes: Data are presented as mean \pm SEM (N = 6). ⁺⁺⁺P \leq 0.001 vs control, and ^{***}P \leq 0.001 vs paracetamol -intoxicated group.

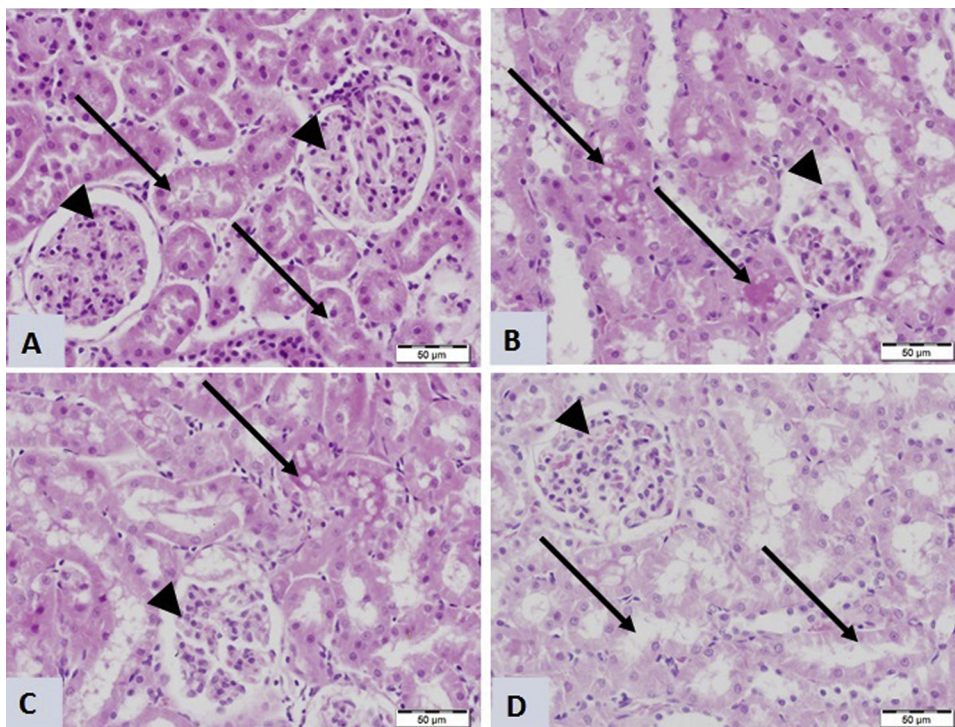


Fig. 5. Representative photomicrographs of kidney sections stained with H&E. Scale bar 50 µm.

(A) From control rat showing normal renal corpuscles and glomeruli (arrow heads) and normal sections of renal tubules (arrows). (B) from rat received a toxic dose of paracetamol showing sporadic degenerated renal corpuscles with shrunken glomeruli (arrow head). Most of cortical tubules are filled with eosinophilic casts with degeneration of their epithelial cell lining (arrows). (C) from rat received high dose of paracetamol and treated with ubiq showing few degenerated renal corpuscles (arrow head) and decrease of tubular epithelial degeneration and casts (arrow), while those received large dose of paracetamol and treated with Nubiq (D) shows marked decrease of degenerated renal corpuscles that becomes similar to the control one (arrow head). The cortical tubules show also normal epithelial lining and absence of intra-lumen casts (arrows).

conditions. Moreover, histological findings indicated that ubiq and Nubiq prevented pathological changes in renal tissue architecture.

Overall, these findings show that treatment with ubiq or Nubiq antioxidants protected the kidney against paracetamol-induced toxicity via CRP downregulation, inhibition of apoptosis via Nrf2 upregulation, suppression of VCAM and KIM-1 expression, and suppression of DNA damage. Interestingly, Nubiq showed superior protective efficacy compared to ubiq in ameliorating IL10, CRP levels, VCAM, JNK, Nrf2 and KIM-1 expressions.

Declaration of Competing Interest

The authors declare no conflict of interest.

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