ORIGINAL ARTICLE

Modulation of renal FOXO3 gene expression by Urolithin A in a rat model of renal ischemia-reperfusion injury

Munther Abosaooda[1], Qayssar Joudah Fadheel[2,]
[1]THE ISLAMIC UNIVERSITY, COLLAGE OF PHARMACY, NAJAF, IRAQ
[2]DEPARTMENT OF PHARMACOLOGY AND TOXICOLOGY, COLLEGE OF PHARMACY, UNIVERSITY OF BABYLON, BABYLON, IRAQ

ABSTRACT

Aim: This work uses a male Wistar Albino rat model of experimentally induced renal ischemia-reperfusion injury (IRI) to examine the nephroprotective potential of Urolithin A.

Materials and Methods: Twenty-eight rats (N=7) were randomly assigned to four groups: DMSO pre-injection as a vehicle, bilateral renal IRI for 30 minutes followed by two hours of reperfusion as a control, sham (laparotomy without IRI), and treatment (Urolithin A pre-injection for three days). ELISA and histological analysis were used to assess biomarkers of kidney injury, oxidative stress, inflammation, and apoptosis.

Results: While GSH levels were enhanced, Urolithin A therapy primarily decreased TNF-α, IL-1β, MDA, Caspase-3, and KIM-1 levels. Furthermore, the group treated with Urolithin A showed a substantial downregulation of FOXO3 expression. Histopathological results verified that the therapy group had less kidney damage.

Conclusions: These findings suggest that Urolithin A produces nephroprotective effects against IRI via modulating oxidative stress, inflammation, and apoptosis, particularly through the control of FOXO3. This study suggests that Urolithin A is a good treatment candidate for renal IRI control.

KEY WORDS: Urolithin A, IRI, IL-1β, TNF-α, KIM-1, FOXO3

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ABBREVIATIONS

UA: urolithin A
I/R: ischemia reperfusion),
KIM-1: kidney injury molecule 1
TNF-α: tumor necrosis factor alpha
IL-1β: interleukin -1 beta
FOXO3: Forkhead Box O3
DMSO: dimethylsulfoxide

INTRODUCTION

One of the main causes of acute kidney injury (AKI) is renal ischemia-reperfusion damage (IRI), and the inflammatory response has a significant impact on the pathophysiology of this condition [1]. Reactive oxygen species (ROS) overproduction is the primary cause of ischemia-reperfusion injury after reoxygenation, which can lead to a variety of cellular consequences, including necrosis, apoptosis, and the release of injury-associated proteins that trigger the inflammatory response [2–3]. Two main mechanisms mediate apoptosis: the extrinsic pathway, which is initiated by death receptors belonging to the tumour necrosis factor (TNF) receptor family, and the intrinsic pathway, which is principally controlled by mitochondrial signals [4]. The dynamic process of ischemia-reperfusion-induced kidney injury is marked by complex interactions between oxidative stress, lipid peroxidation, and inflammatory mediators, all of which exacerbate tissue damage [5]. Because it phosphorylates FOXO3, a transcription factor that controls apoptosis, the oxidative stress response, and cell cycle progression, the PI3K/AKT signaling pathway is essential to this process [6]. ROS overproduction is a sign of oxidative stress, which damages renal cells by causing lipid peroxidation and depleting vital antioxidants like glutathione (GSH). As a byproduct of lipid peroxidation, the buildup of malondialdehyde (MDA) serves as a biomarker for oxidative stress and is linked to the degree of kidney damage. These reactive oxygen species have the potential to cause apoptosis, energy imbalances, and mitochondrial malfunction [7]. Furthermore, the inflammatory response is exacerbated by inflammatory cytokines including interleukin-1 beta (IL-1β) and tumour necrosis factor-alpha (TNF-α), which are important mediators in the development of acute kidney injury (AKI). Biomarkers such as kidney injury

molecule (KIM-1), which provide insight into the degree of ischemia-reperfusion injury, are commonly used to assess renal function. Multifunctional compounds, particularly those with anti-inflammatory and antioxidant properties, are thought to be highly effective in reducing kidney damage. Their capacity to alter intracellular pathways linked to the dynamics of oxidative stress is primarily responsible for their protective role [8–10]. Antioxidant therapy has also shown promise in shielding cells from oxidative damage brought on by infrared radiation. These substances may provide protective advantages by restoring compromised intracellular functions associated with oxidative stress [11]. Prophylactic measures can lessen the impact of IRI, which often causes inflammation and may exacerbate kidney impairment. The pathophysiology of renal disorders is significantly influenced by the main pro-inflammatory cytokines, specifically interleukin-1 beta (IL-1β), interleukin-6 (IL-6), and tumour necrosis factor-alpha (TNF-α). In a rat model of ischemia-reperfusion injury, this study aimed to assess the renal protective effectiveness of UA through the analysis of multiple biomarkers, such as Kidney Injury Molecule-1 (KIM-1), IL-1β, TNF-α, GSH, malondialdehyde (MDA), Caspase-3, Foxo3 gene expression, and histopathological analysis.

AIM

This work uses a male Wistar Albino rat model of experimentally induced renal ischemia-reperfusion injury (IRI) to examine the nephroprotective potential of Urolithin A.

MATERIALS AND METHODS

PREPARATION OF ANIMALS

In this study, 28 adult male Wistar Albino rats weighing ([20–22] weeks, 300 ± 50 g). The animals were kept under standard laboratory conditions, which comprised a 12-hour light/dark cycle, unfettered access to food and water, a controlled temperature of 25°C, and a relative humidity of 60–65%. Every step involved in the care and experimentation of animals is carried out in compliance with accepted ethical standards for the use of animals in research. The rats were kept in the University of Kufa's College of Sciences' animal facility. The sources of the experimental materials were Macrogen (Korea), Sunlong Technology Lab (China), and Med-Chem-Express.

ETHICAL STATEMENT

This study was carried out at the University of Kufa's Middle Euphrates Unit for Cancer Research, Faculty of Medicine, and Department of Pharmacology and Toxicology, Faculty of Pharmacy. Ethical approval was granted by the Central Bioethics Committee at the University of Kufa.

All procedures involving the handling and experimentation on rats, as well as the conducted tests, were carried out in compliance with the applicable guidelines and regulations for the ethical use of animals, Kufa University (20545 in 29/8/2024). The animals were housed in the animal facility at the College of Sciences, University of Kufa.

UA

UA is a secondary metabolite of ellagic acid, a polyphenolic antioxidant, exhibiting antiproliferative, anti-inflammatory, and antioxidant properties [13]. The pomegranate is a significant source of ellagitannins, a category of natural polyphenols that are converted to Urolithin by gut microbiota [14]. Walnuts and raspberries are abundant in ellagitannins [15]. The pure powder of UA was gutted from med-Chem-express, USA Company. Formal Name: 3,8-dihydroxy-6H-dibenzo[b,d] pyran-6-one, Chemical Name: UA, Molecular Formula: C13H8O4, CAS Number: 1143-70-0, Molecular Weight: 228.2, Physical Description: A crystalline solid, Color: Yellow, Solubility: In DMSO: 30 mg/ml. DMSO was considered the standard vehicle for preparation this drug before use. The dose of drug that used was 10 mg\kg of rat weight intraperitoneally [16].

EXPERIMENTAL DESIGN

Four groups of seven Wistar Albino rats each were randomly assigned to this experiment, and each group was given a different set of treatment procedures. The ischemia-reperfusion injury (IRI) technique was not performed on the sham group, which served as the negative control. By clamping the renal pedicles for 30 minutes, the control group which served as the positive control—was subjected to bilateral renal ischaemia. For two days in a row before sacrifice, the vehicle group received intraperitoneal injections of DMSO once *per* 24 hours. On the third day, they also received a dose an hour before the induction of ischaemia. This group also underwent 30 minutes of renal ischemia followed by two hours of reperfusion for two days prior to sacrifice, the UA-treated group received an intraperitoneal injection of 10 mg/kg of UA once daily, with an additional dosage administered an hour before ischaemia was produced on the third day. These injections were given at the same intervals as the vehicle group.

RENAL ISCHEMIA REPERFUSION INJURY RAT MODEL

Rats were kept on a heat plate at 37°C after being sedated with intraperitoneal ketamine (100 mg/kg) and xylazine (10 mg/kg). To reveal the renal pedicles, a midline abdominal incision was done following shaving and antiseptic washing. Clamping both renal pedicles for 30 minutes caused bilateral renal ischemia. For hydration, one milliliter of warm, sterile saline was injected intraperitoneally. Clamps were taken off for reperfusion after ischemia, and the incision was sutured and covered with sterile gauze soaked in saline to avoid dehydration. Following bilateral nephrectomy, the kidneys were rinsed with ice-cold phosphate-buffered saline (PBS) to remove residual blood. The rats were then euthanized via cardiac puncture [17]. The left kidney was sectioned sagittal into two halves: one half was immediately stored at −80 °C for subsequent bimolecular analysis, while the other half was fixed in 10% formalin and later embedded in paraffin for histopathological examination.

PREPARATION OF TISSUE FOR MEASUREMENT OF IL-1B, TNF-A, GSH, MDA, CASPASE-3 AND KIM-1 BY ELISA

After completing the surgical procedure, the left kidney was carefully removed from the sacrificed rat and rinsed thoroughly with chilled normal saline to clear any remaining blood. The kidney was then bisected, and one half was processed for biochemical analysis. This tissue was homogenized in a 1:10 (w/v) ratio of phosphate-buffered saline supplemented with 1% Triton X-100 and a protease inhibitor cocktail, using a high-intensity ultrasonic liquid processor [18]. The prepared homogenate was subjected to centrifugation at 17,530 ×g for 20 minutes at a temperature of 4°C. The supernatant was collected to quantify interleukin-1 beta (IL-1β), tumor necrosis factor-alpha (TNF-α), GSH, malondialdehyde (MDA), KIM-1, and Caspase-3 using the enzyme-linked immunosorbent assay (ELISA) technique, conducted with a Bio-Elisa Reader (Bio-Tek Instruments, USA).

KITS AND REAGENTS

Human IL-1β (Thermo Fisher/Invitrogen, cat. KAC1211), human TNF-α (Abcam, cat. ab46087), total GSH (Elabscience, cat. E-EL-0026), MDA (competitive ELISA; ELK Biotech, cat. ELK8658), human KIM-1 (Elabscience, cat. E-EL-H6029), and human caspase-3 (Elabscience, cat. E-EL-H0017) were procured via regional distributors servicing Iraq and stored at 2-8 °C. All kits were research-use only. Substrates (TMB), stop solution, wash buffers, and plate sealers were those supplied with each kit.

TISSUE SAMPLING FOR HISTOPATHOLOGY ANALYSIS AND DAMAGE SCORES

After preserving a portion of the left kidney in 10% neutral buffered formalin (NBF), tissues were processed by standard methods (graded alcohol dehydration, xylene clearing, and paraffin embedding). Sections (4–5 μm) were cut and stained with hematoxylin and eosin (H&E). Microscopic examination was performed by a pathologist blinded to group allocation, and semi-quantitative scoring was performed by a second investigator, also blinded. Tubular injury was assessed at the corticomedullary junction in 10 non-overlapping fields per section at 40x total magnification. Injury was scored on a 0–4 scale adapted from established IRI scoring systems [20–21]: 0 = normal histology; 1 = focal injury in <10% of tubules (loss of brush border, epithelial swelling/blebbing); 2 = injury in 10–25% of tubules; 3 = injury in 26–50% of tubules with occasional casts; 4 = injury in >50% of tubules with widespread casts and frank necrosis. Each animal's score was the mean of all fields examined. Additional qualitative features (e.g., tubular epithelial enlargement, vacuolar degeneration, necrotic tubules, and cast formation) were documented as supportive findings [19].

ASSESSMENT OF TISSUE FOXO3 GENE EXPRESSION BY RT-QPCR

1. Total RNA extraction. Total RNA was isolated from ~30 mg renal cortex using [Easy-spin™ (DNA-free) Total RNA Extraction Kit; Manufacturer, Country; Cat. No. XXXX] following the manufacturer's protocol (including on-column DNase treatment). RNA quantity and purity were assessed by spectrophotometry (A260/A280 and A260/A230), and integrity was verified by agarose gel electrophoresis.
2. cDNA synthesis. [X μg] of RNA per sample was reverse-transcribed using [AddScript (or equivalent) cDNA Synthesis Kit; Manufacturer, Country; Cat. No. XXXX] in a [final volume X μL] with random hexamers/oligo(dT)/mixed primers [choose] according to the manufacturer's instructions ([temperature/time profile]). No-RT controls (minus reverse transcriptase) were included to exclude genomic DNA amplification
3. Primers and amplicons. Primers for Foxo3 (target) and [reference gene; e.g., Gapdh or Actb] were

Table 1. Primers used in this study

Host	Gene		5′-3′	Product (bp)	Accession number	Reference
Rattus	FOXO-3	F	AAAGGGGAAATGGGCAAAGC	83	XM_032888496.1	[22-23]
		R	GGCTGAGAGCAGATTTGGCA			
Rattus	GAPDH	F	ATGACTCTACCCACGGCAAG	89	NM_017008	
		R	CTGGAAGATGGTGATGGGTT			

Source: Own materials

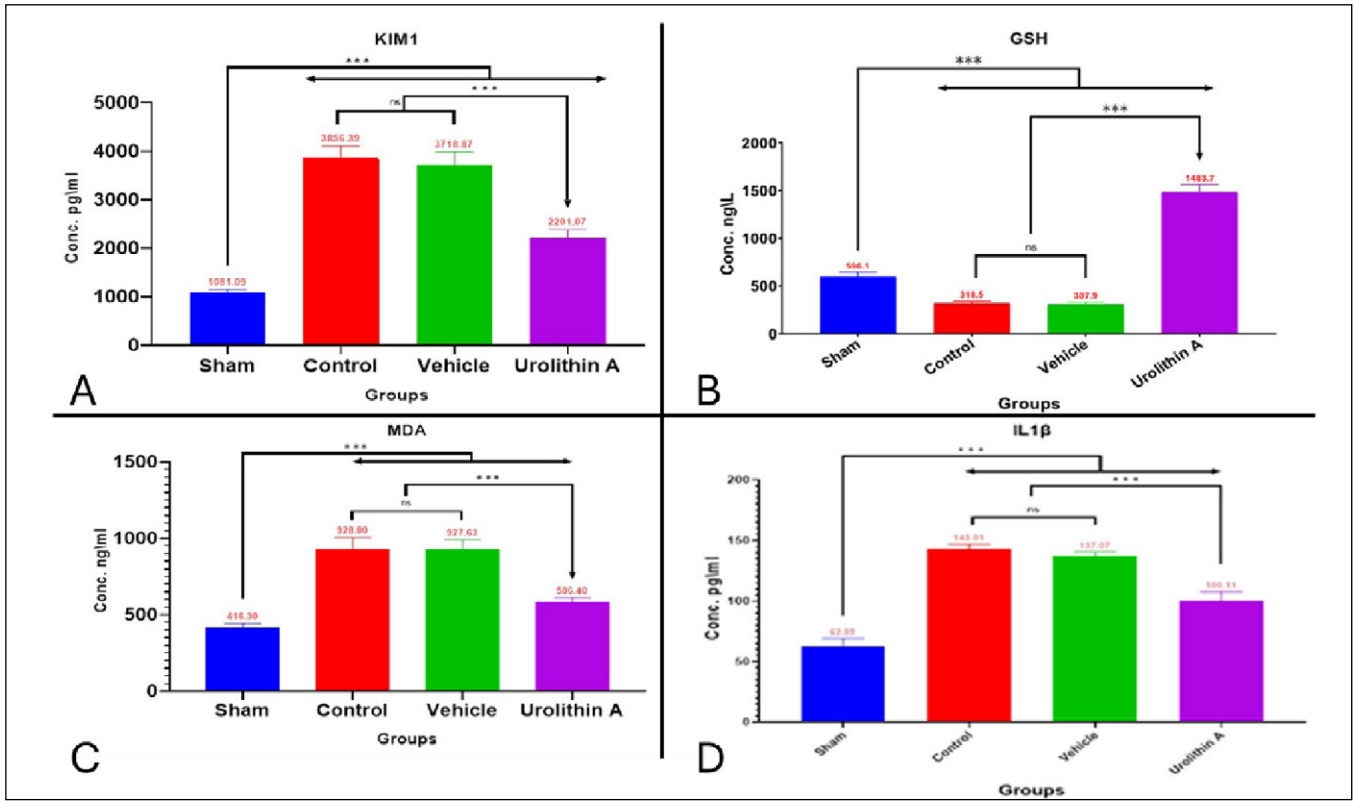

Fig. 1. Statistical of KIM1, GSH, MDA, and IL-1β; **A:** Statistical analysis of KIM-1 conc. (pg/mL) in renal tissues was made across the four experimental groups, each consisting of seven rats. The results showed a highly significant difference between the sham group and both the vehicle and control groups (***P < 0.001). Similarly, the UA-treated group showed a statistically significant decrease in KIM-1 levels when compared to the vehicle and control groups (***P < 0.001), **B:** The mean conc. of GSH in renal tissues, measured in ng/L, were statistically analyzed among the four experimental groups, each comprising seven rats. The sham group showed significantly higher GSH levels compared to the vehicle and control groups (***P < 0.001). Likewise, the UA-treated group exhibited a marked increase in GSH concentrations relative to the vehicle and control groups (***P < 0.001), showing a strong antioxidative effect, **C:** The statistical evaluation of malondialdehyde (MDA) conc., expressed as mean values in ng/mL, was conducted across the four experimental groups, each consisting of seven rats. The sham group showed significantly low MDA levels compared to both the vehicle and control groups (***P < 0.001). Similarly, rats treated with UA exhibited a notable reduction in MDA conc. relative to the vehicle and control groups (***P < 0.001), indicating a strong attenuation of oxidative stress, **D:** The mean concentrations of interleukin-1 beta (IL-1β) in renal tissues, measured in pg/mL, were statistically analyzed at the conclusion of the study across four animal groups, each containing seven rats. A highly significant increase in IL-1β levels was observed in both the vehicle and control groups compared to the sham group (***P < 0.001). Conversely, treatment with Urolithin A resulted in a significant reduction in IL-1β concentrations compared to the vehicle and control groups (***P < 0.001), demonstrating its anti-inflammatory potential.
Source: Own materials

synthesized by [Vendor, Country; Cat./Order No. XXXX]. Amplicon lengths were [X] bp (Foxo3) and [X] bp (reference). Primer specificity was confirmed by melt-curve analysis and a single band on gel. Primer efficiencies (standard curve, $R^2 \geq 0.99$) were [90–110]%. Full sequences, amplicon sizes, accession numbers, and efficiencies are provided in Table 1.

4. Primers Used in this Study [1, 2]Stefanie</author><-author>Wolloscheck, Tanja</author><author>Hölter, Philip</author><author>Wengert, Alexander</author><author>Grether, Markus</author><author>Sticht, Carsten</author><author>Weyer, Veronika</author><author>Wolfrum, Uwe</author><author>Spessert, Rainer %J Journal of neurochemistry</author><</authors></contributors><titles><title>Transcriptional

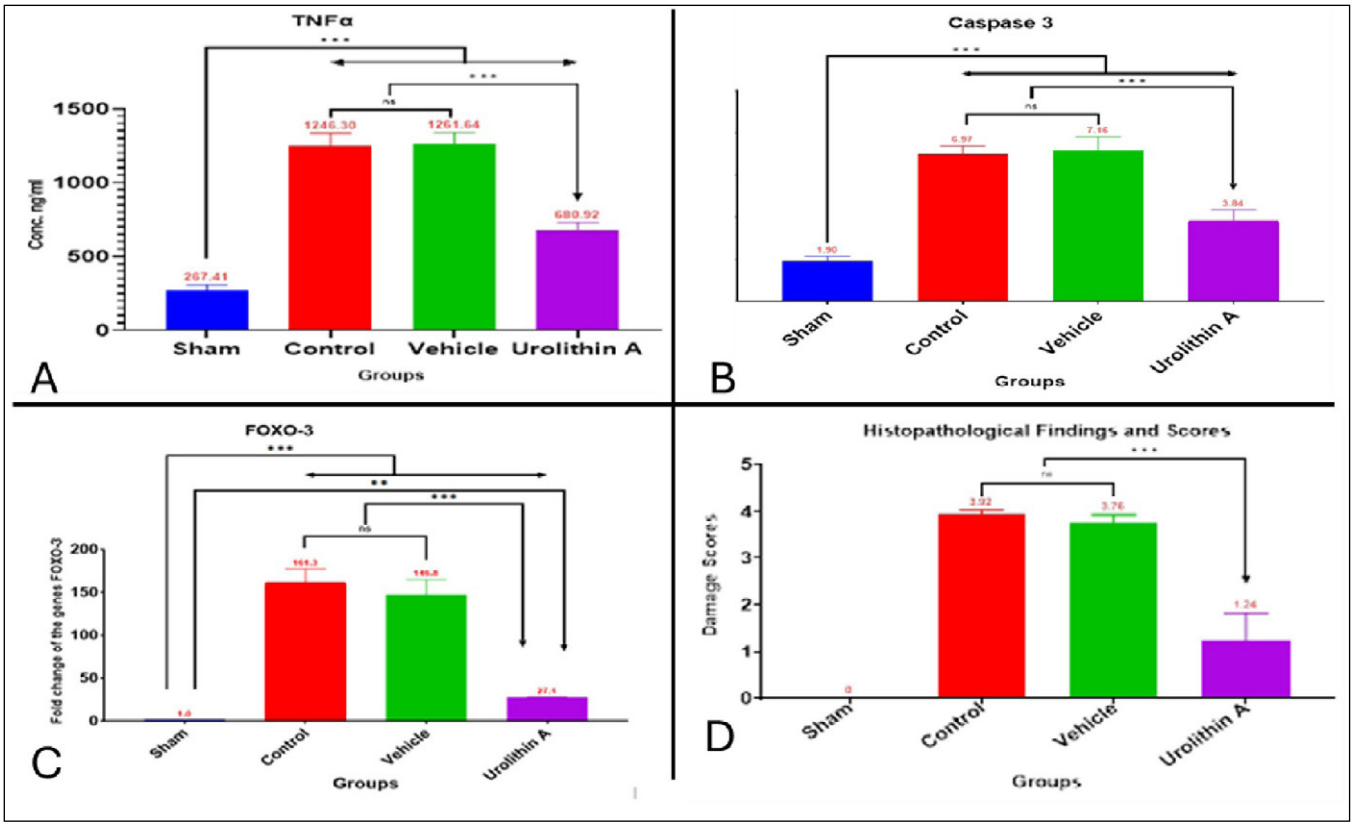

Fig. 2. Statistical of TNFα, caspase-3, Mean of fold change of FOXO3, and Score severity mean of renal tissue histopathology
Statistical comparisons of TNF-α, Caspase-3, FOXO3 gene expression, and renal histopathological scores among experimental groups; **A:** Mean concentrations of TNF-α (ng/L) in renal tissues across the four study groups at the end of the experiment (n = 7 per group). Significant differences were observed between the sham group and both the vehicle and control groups (***P < 0.001), as well as between the UA-treated group and the vehicle and control groups (***P < 0.001), **B:** Mean Caspase-3 levels (ng/L) in renal tissues among the four experimental groups (n = 7 each). Both the sham and UA groups showed significantly lower levels compared to the vehicle and control groups (***P < 0.001); **C:** Fold change in FOXO3 gene expression in renal tissue across the four groups at the study endpoint (n = 7 per group). Significant downregulation was observed in the UA and sham groups compared to vehicle and control groups (***P < 0.001); **D:** Mean histopathological severity scores of renal tissue in the four groups (n = 7 each). Both the sham and UA-treated groups exhibited significantly reduced scores relative to the vehicle and control groups (***P < 0.001).
Source: Own materials

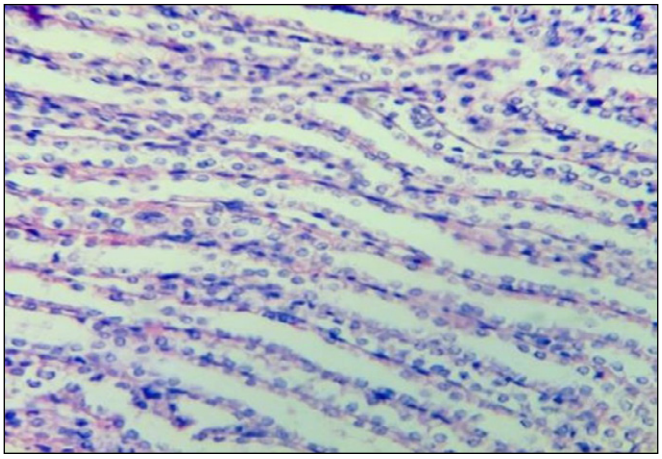

Fig 3. Sham group, a microscopic cross section of left kidney represented normal tissues histology, normal renal tubules, and normal cell size and there are no cast formation, cells edema or loss of brush boarder. Sham group. H & E stain × 400.The mean of the severity score was zero (score severity mean = 0 and represent 0% of damage renal tubules)
Source: Own materials

analysis of rat photoreceptor cells reveals daily regulation of genes important for visual signaling and light damage susceptibility</title></titles><pages>757-769</pages><volume>124</volume><number>6</number><dates><year>2013</year></dates><isbn>0022-3042</isbn><urls></urls></record></Cite></EndNote>

5. Protocol of GoTaq® RT-qPCR System for Real-Time qPCR (Gene expression assay).

STATISTICAL ANALYSIS

Statistical analysis was conducted using GraphPad Prism and SPSS version 28.0 for Windows. Results were presented as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was employed to compare differences among the groups, followed by Bonferroni post-hoc tests for pairwise comparisons. For evaluating histopathological changes in kidney tissue,

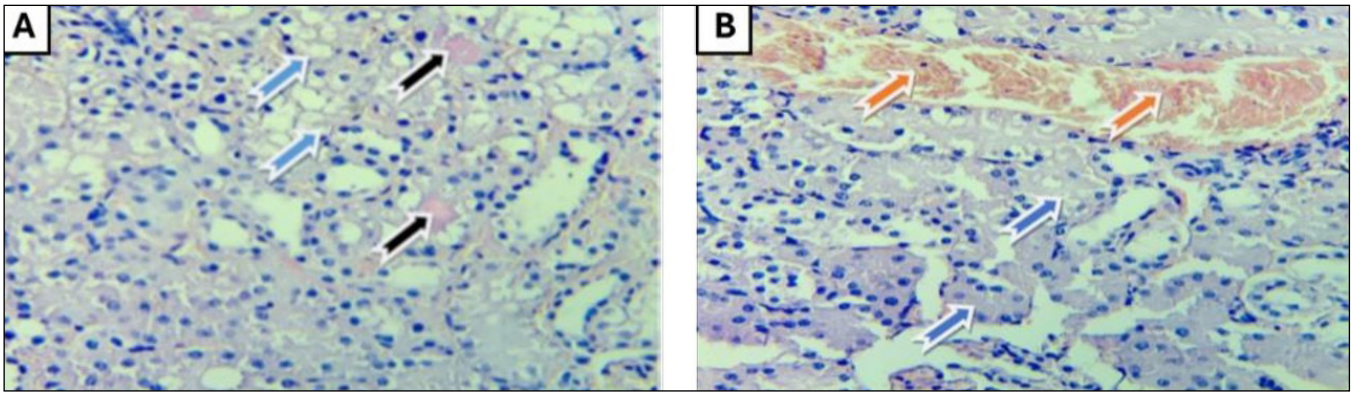

Fig. 4. (A) Control group. Rat kidney, renal tubules with score 4 damage involving 100% of the examined tubules. Cytoplasmic vacuoles (blue arrows) and eosinophilic cast (black arrows). H&E. X400, figure (**B**) Control group. Rat kidney, renal tubules with score 4 damage involving 100% of the examined tubules. Vascular congestion (orange arrows) and cytoplasmic eosinophilia (blue arrows). H&E. X400
Source: Own materials

the Kruskal-Wallis test was applied to determine the significance of differences in the overall severity scores across groups. A p-value of ≤ 0.001 was considered to indicate a statistically significant difference.

RESULTS

EFFECTS OF UA ON-KIDNEY FUNCTION PARAMETERS (KIM-1)

The renal tissue level of (KIM-1) in control and vehicle groups were significantly ($p<0.001$) higher than that in the sham group, On the other hand, the level in the UA treated group is significantly ($p<0.001$) lower than that in the control group, figure (1A).

EFFECT OF UA ON THE OXIDATIVE STRESS MARKER GSH

The mean GSH levels were significantly lower in the Control and control vehicle group compared to the UA treated groups ($P<0.001$) while slightly elevation when compare with sham group, figure (1B).

EFFECT OF UA ON THE OXIDATIVE STRESS MARKER MALONDIALDEHYDE (MDA)

The renal tissue level of MDA in control and vehicle groups were significantly ($p<0.001$) higher than that in the sham group, On the other hand, their levels in the UA were significantly ($p<0.001$) lower than that in the control group, figure (1C).

EFFECT OF UA ON INFLAMMATORY MARKER INTERLEUKIN -1 BETA

There was a significant ($P<0.001$) difference in IL-1β levels among the studied groups. The sham group had the lowest levels than all other groups ($P<0.001$ for all comparisons). Conversely, IL-1β levels were significantly ($P<0.001$) lower in the UA treated group compared with control groups, figure (1D).

EFFECT OF UROLITHIN A ON THE INFLAMMATORY MARKER TUMOR NECROSIS FACTOR-ALPHA (TNF-A)

The renal tissue level of (TNF-α) in control and vehicle groups were significantly ($p<0.001$) higher than that in the sham group. On the other hand, their levels in the UA were significantly ($p<0.001$) lower than that in the control group, figure (2A).

EFFECTS OF UROLITHIN A ON THE MARKER OF APOPTOSIS, CASPASE3

The renal tissue level of Caspase3 in control and vehicle groups were significantly $p<0.001$ higher than that in the sham group, On the other hand, their levels in the UA were significantly ($p<0.001$) lower than that in the control group, figure (2B).

THE EFFECT OF UROLITHIN A ON THE INTRACELLULAR SIGNALING (FOXO3) AFTER IRI BY PCR TECHNIQUE

The renal tissue levels of FOXO3 expression in control and vehicle groups were significantly ($p<0.001$) higher than that in the sham group, Meanwhile, the expression level of FOXO3 in the UA treated group is significantly ($p<0.001$) lower than that in the control group, figure (2-C).

HISTOPATHOLOGY FINDINGS

Results of the histopathological examination of renal tissues from the four study groups are shown in figures (2D, 3, 4A-B, 5-6)

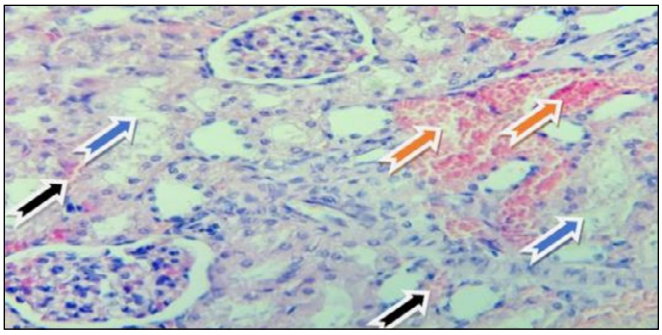

Fig. 5. Vehicle treated group. Rat kidney, renal tubules with score 4 damage involving 95% of the examined tubules. Cytoplasmic swelling and increased cytoplasmic eosinophilia (black arrows), cytoplasmic vacuoles (blue arrows), hemorrhage (orange arrows). H&E. X400
Source: Own materials

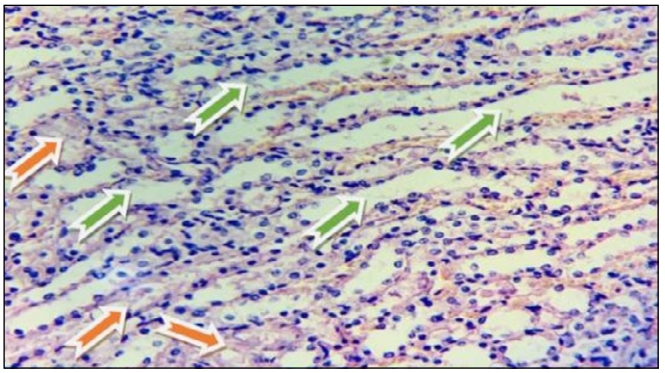

Fig. 6. UA treatment group. Rat kidney with score 1 damage involving 25% of the examined tubules, Damaged tubules (orange arrows) and normal tubules (green arrows). H&E. X400
Source: Own materials

DISCUSSION

Acute kidney injury (AKI), a syndrome commonly associated with substantial morbidity and mortality, is largely caused by renal ischemia-reperfusion injury (IRI). The overproduction of ROS is a key contributor to the development of this injury. ROS worsen the condition by causing inflammatory responses, encouraging the release of vasoconstrictive substances, and causing renal tubular cells to undergo both necrosis and apoptosis [24–25]. As a result, treatment approaches that lower ROS levels have attracted a lot of attention from researchers. By evaluating important biomarkers, this study assessed UA's possible nephroprotective benefits in IRI. KIM-1, a sensitive measure of kidney injury, was markedly decreased by UA therapy, indicating a preventive effect. Additionally, UA showed anti-inflammatory properties by reducing levels of Tumour Necrosis Factor-alpha (TNF-α) and Interleukin-1 beta (IL-1β), which in turn decreased inflammatory responses. Malondialdehyde (MDA) and other indicators of oxidative stress were reduced by UA, while GSH and other antioxidant defenses were strengthened. The study also showed that UA altered the expression of FOXO3, indicating its involvement in cellular survival pathways, and decreased the apoptotic marker Caspase-3, indicating anti-apoptotic effects. These results demonstrate that UA has the potential to reduce IRI-induced kidney damage via anti-inflammatory, anti-apoptotic, and antioxidant mechanisms, making it a promising therapeutic option for the treatment of AKI.

EFFECTS OF IRI AND UA ON KIM-1

Notably, the UA-treated groups in this experiment showed significantly $P \leq 0.001$ lower levels of KIM-1 than the vehicle and control groups. On the other hand, compared to the sham group, the control and vehicle groups showed a significant rise in KIM-1 levels in renal ischemic tissues. These outcomes are consistent with research by [26], which demonstrated that renal tissue expresses KIM-1, which is markedly upregulated in response to tubular injury. Due to its excellent sensitivity and specificity in identifying kidney injury, KIM-1 has been acknowledged and authorised by the U.S. Food and Drug Administration (FDA) as a trustworthy biomarker for assessing nephrotoxicity. Previous studies have shown that KIM-1 expression occurs on the apical surface of renal proximal tubule epithelial cells in response to ischaemia and toxic damage [27–28]. A possible nephroprotective effect of UA in IRI is suggested by the decreased KIM-1 levels seen in the treatment groups in this investigation. The effect of UA on KIM-1 levels in relation to IRI has not been examined in any prior research. This agent's anti-inflammatory and antioxidant qualities are probably responsible for the decrease in KIM-1 levels seen in this investigation. These results are consistent with the findings of [29], who showed that pioglitazone has an antioxidant effect by lowering oxidative stress, inflammation, and renal tubular damage by targeting multiple cellular pathways, such as TNF-α, NF-κB signaling, KIM-1, and NGAL pathways.

EFFECTS OF IRI AND UA ON-TUMOR NECROSIS FACTOR-ALPHA (TNF-A)

The findings of this study demonstrated that administering UA prior to the onset of ischemia led to a significant reduction ($P \leq 0.001$) in TNF-α levels within renal tissues subjected to ischemia, when compared to the ischemia-reperfusion (IR) control and vehicle-treated groups. Previous experimental research has reported elevated TNF-α concentrations following 30 minutes of renal ischemia and a subsequent 2-hour reperfusion period in rats. This increase has been associated with

marked alterations in endothelial function, with the rise in TNF-α contributing to the observed endothelial dysfunction [30]. Another study in a rat model showed that the level of the TNF-α gene was highly elevated in injured kidney tissues in rats that are undergone nephrectomy of the right kidney and ischemia in the left kidney for 45 min then reperfusion [31]. On the other hand, growing evidence suggests that UA possesses diverse and potent pharmacological properties, including antioxidant, anti-inflammatory, and anticancer effects. Studies have shown that UA can effectively suppress the activation of NF-κB triggered by pro-inflammatory cytokines such as IL-1β and TNF-α, thereby exerting anti-inflammatory effects in colon fibroblast cells [32]. These observations are in agreement with the results of the present study.

EFFECTS OF IRI AND UA ON INTERLEUKIN-1B (IL-1B)

The results in the present study, indicated that the control and vehicle group, which underwent IRI without any therapeutic intervention, exhibited significantly $P \leq 0.001$ elevated levels of IL-1β. These results are consistent with the previous experimental studies that shown a substantial increase in serum creatinine, Urea, IL-1β and caspase-3 levels after 30 min of ischemia followed by 2hrs of reperfusion in rats [33]. Conversely, the groups treated with UA demonstrated significantly $P \leq 0.001$ lower levels of IL-1β compared to both the control and vehicle groups. The observed decrease suggests that UA might have anti-inflammatory properties, possibly by inhibiting the production of IL-1β. Previous findings agreed with our, demonstrating that UA attenuated the pro-inflammatory factors production (IL-6, IL-1β and others) in vitro studies [34]. By lowering IL-1β levels, UA may lessen the inflammatory response linked to IRI and prevent additional renal damage.

EFFECTS OF IRI AND UA ON OXIDATIVE STRESS MARKERS MALONDIALDEHYDE (MDA)

The control and vehicle group, which received IRI without therapeutic intervention, exhibited significantly greater levels of MDA ($P \leq 0.001$) compared to the sham group, as indicated by the results of this experimental rat model study. This rise reflects the oxidative stress induced by ischemia damage. These findings correspond with those reported by [35], indicating that heightened levels of ROS, diminished antioxidant defenses, reduced superoxide dismutase (SOD) activity, and increased malondialdehyde (MDA) collectively exacerbate oxidative stress and tissue damage in renal IRI. The MDA concentrations in the UA-treated groups were considerably lower ($P < 0.001$) compared to the control and vehicle groups, suggesting a beneficial antioxidant activity. The decrease in MDA levels suggests that UA may possess antioxidant properties, possibly via processes that include the neutralization of free radicals or the augmentation of intrinsic antioxidant defenses. These findings align with several research that validate the antioxidant properties of UA. A study indicated that UA alleviates oxidative stress and inflammation in HepG2 cells, a model for hepatocellular carcinoma. UA diminished ROS production by approximately 50%, augmented antioxidant defenses (superoxide dismutase and glutathione peroxidase), and lowered lipid peroxidation (malondialdehyde levels). The findings indicate that UA safeguards against oxidative and inflammatory damage, which are principal factors in the progression of liver cancer. Additional investigations demonstrated the protective effect of UA against oxidative stress in rats experiencing DOX-induced hepatotoxicity. DOX treatment substantially elevated tissue malondialdehyde (MDA) levels, indicating increased lipid peroxidation, whereas UR mitigated this increase and dramatically enhanced antioxidant enzyme activity.

EFFECTS OF IRI AND UA ON ANTIOXIDANT MARKER GSH

GSH is a critical endogenous antioxidant that plays a vital role in protecting cells from oxidative stress by neutralizing free radicals and reactive oxygen species. Previously found data revealed that that the level of kidney glutathione in untreated IRI rats was significantly $P \leq 0.001$ lower than that of control rats [37]. Other studies confirmed that the level of GSH significantly decreased, which was accompanied with a significant increase in MDA level of kidney tissue following I/R [38]. Our investigation revealed markedly reduced GSH levels in the control group exposed to IRI, but UA pretreatment resulted in a substantial elevation of GSH levels relative to the control and vehicle groups. This indicates that enhancing GSH production or reducing oxidative stress augments renal antioxidant capability. Cásedas et al. (2020) indicate that these findings align with previous research demonstrating UA's in vitro antioxidant activity, encompassing its antiproliferative and protective effects on HepG2 hepatic cancer cells [39]. The potential antioxidative and neuroprotective properties of UA have been concurrently examined utilizing the Neuro-2a neuroblastoma cell line in mice. The findings indicated that UA exhibited many significant cytoprotective effects alongside robust antioxidant activity, as demonstrated by its results in the ORAC assay (13.1 μmol TE/mg). UA significantly enhances mitochondrial performance in cells exposed to hydrogen

peroxide-induced oxidative stress ($H2O_2$), suggesting a protective role in maintaining cellular energy metabolism. Moreover, it markedly reduced lipid peroxidation, an essential indicator of membrane damage generated by ROS. Moreover, UA enhanced the activity of critical antioxidant enzymes essential for neutralizing ROS and preserving cellular redox equilibrium, including glutathione reductase (GR), catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx). Significantly, it was discovered that UA might enhance the antioxidant defense system by upregulating the expression of peroxiredoxins 1 and 3 (Prx1 and Prx3), enzymes that mitigate peroxides and protect cells from oxidative harm, in a dose-dependent manner.

EFFECT OF IRI AND UA ON APOPTOTIC MARKER (CASPASE3)

This experimenzztal study confirmed a significant $P \leq 0.001$ increase in Caspase3 levels in renal tissue of both the I/R control and vehicle groups relative to the sham group following IRI.These findings supported earlier research by [18] who found that the level of Caspase3 was increased after 30 minutes of ischemia followed by 2 hours of reperfusion in rats. Another studies in a rat model appeared that the level of Caspase3 was highly elevated in injured kidney tissues in rats that undergone ischemia for 45 minutes then reperfusion for 24 hours and ischemia for 60 minutes then reperfusion for 2 hours, respectively [40-41]. In contrast, this study revealed that the pretreatment with UA before ischemia induction can significantly $P < 0.001$ downregulate the expression of caspase-3 in injured renal tissues in comparison with those in both control and vehicle groups. The results of the present study align with the findings of [42], which demonstrated that UA significantly enhanced cell viability and reduced intracellular ROS levels in a dose-dependent fashion in SK-N-MC cells. Additionally, UA was shown to lower the Bax/Bcl-2 ratio and suppress the expression of key apoptotic markers, including cytochrome c, cleaved caspase-9, cleaved caspase-3, and cleaved PARP. This reduction in Caspase-3 levels indicates UA may exert anti-apoptotic effects, potentially through mechanisms that involve the inhibition of pro-apoptotic signaling pathways or the enhancement of cell survival pathways. By decreasing the levels of Caspase-3.

EFFECT OF IRI AND UA ON THE MOLECULAR PROTEINS FOXO3

In the present study we found that the renal tissues from the control and vehicle groups, which underwent IRI without any treatment, exhibited significantly $P \leq 0.001$ higher levels of FOXO3 expression compared to the sham group. In contrast, the groups treated with UA exhibited a significant reduction ($P \leq 0.001$) in FOXO3 gene expression within renal tissues. FOXO3 is a transcription factor broadly expressed across various tissues in the body and plays a critical role in regulating cellular homeostasis, including responses to oxidative stress and apoptosis. Its activity is tightly controlled by the PI3K/AKT signaling pathway; when phosphorylated by this pathway, FOXO3 becomes inactivated and is retained in the cytoplasm. Upon dephosphorylation, however, it translocate into the nucleus, where it functions as a transcriptional regulator of genes involved in cell survival, oxidative defense, and apoptosis. The observed downregulation of FOXO3 in the UA-treated groups may reflect modulation of this pathway, possibly contributing to reduce apoptotic signaling and enhanced cellular protection in renal tissues [43] several studies have demonstrated that antioxidants can activate the PI3K/AKT pathway, leading to enhanced phosphorylation of AKT at specific sites such as Ser473 and Thr308. This activation results in downstream effects that promote cell survival, reduce apoptosis, and enhance cellular antioxidant capacity. Meng et al., 2021, demonstrated that the activation of the PI3K/AKT signaling pathway plays a critical role in reducing renal cell apoptosis, which is a significant factor in the development of AKI after AOLT. Specifically, the nuclear FoxO3 -mediated transcription of pro-apoptotic genes Bim and FasL was implicated in renal cell apoptosis, and the activation of PI3K/AKT was shown to counteract this effect. Also [44] showed that Oleanolic Acid has been shown to reduce oxidative stress, which is a significant contributor to renal injury during ischemia and reperfusion. By activating the PI3K/AKT pathway, OA may enhance the expression of antioxidant enzymes and reduce the production of ROS, thereby protecting renal cells from oxidative damage. Another study reported by [45] Previous research found that UA enhances cardiac function in myocardial ischemia/reperfusion (I/R) injury by activating the PI3K/AKT signaling pathway. UA treatment increased phosphorylation of PI3K and AKT, an effect blocked by the PI3K inhibitor LY294002, suggesting its anti-apoptotic role in cardiomyocytes during hypoxia/reoxygenation injury. These findings align with our study, which explored UA's Reno protective effects in AKI caused by renal IRI. Notably, UA had not been previously investigated in this model. Our results demonstrated significant kidney protection, highlighting UA's potential as a novel therapeutic agent for ischemia-reperfusion-induced renal injury.

EFFECT OF IRI ON KIDNEY PARENCHYMA

Histological analysis revealed an increase in tissue damage after IRI, as shown by the presence of Cytoplasmic swelling and increased cytoplasmic eosinophilia, vacuolization, dilated renal tubules, glomerular alterations and hemorrhage, as well as a dilatation of the Bowman's capsule and a lack of brush boundaries. These changes had been in agreement with some other studies [46-49] that showed the same histopathological changes.

EFFECT OF UA ON KIDNEY PARENCHYMA

This investigation revealed that UA pretreatment significantly reduced kidney injury severity ($P < 0.001$) in comparison to the control and vehicle groups. Histological research revealed modest kidney damage in the UA-treated group, while the control and vehicle groups displayed severe harm. These findings align with previous research on UA's protective properties against cisplatin-induced nephrotoxicity, demonstrating that UA maintained renal architecture and markedly reduced tubular injury. The efficacy of UA as a therapeutic agent for renal preservation during chemotherapy and ischemia-reperfusion injury was further substantiated by quantitative histological assessments. These findings underscore UA's capacity to mitigate cisplatin-induced nephrotoxicity, which is crucial for enhancing patient safety during oncological therapies [50]. Another study cited in [51] demonstrated that UA has considerable protective benefits against kidney damage generated by D-galactose in aging rats. The model group exhibited indications of apoptosis, including tubular necrosis and basement membrane thickening, alongside significant glomerular and tubulointerstitial damage. The high-dose UA (150 mg/kg) restored these changes, restoring renal morphology to values nearly equivalent to those of the control group. The efficacy in mitigating renal failure induced by aging and oxidative stress was evidenced by histological scoring, which revealed decreased inflammatory cell infiltration and necrosis in the treated animals. These results indicate that UA holds potential as a therapeutic agent for preserving kidney health in age-related conditions. The [52] indicated that UA exhibited dose-dependent protective effects against DOX-induced hepatic injury in rats. Histopathological enhancements including decreased necrosis, inflammation, and vacuolization, alongside near-normal liver architecture at elevated doses (5 mg/kg). These observations underscore UR's potential as a therapeutic drug for maintaining hepatic tissue integrity under toxic stress.

CONCLUSIONS

Together, these findings support UA's potential as a therapeutic agent for the treatment of AKI. Its numerous protective benefits are highlighted by its capacity to lower kidney injury indicators, inhibit inflammation, boost antioxidant defenses, and alter apoptotic pathways. These findings' translational significance emphasizes the necessity of additional research to prove UA as a workable medical treatment, which will ultimately enhance patient outcomes in critical care and nephrology settings.

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AVAILABILITY OF DATA AND MATERIALS

All data are available upon request

CONFLICT OF INTEREST

The Autors declare no conflict of interest

CORRESPONDING AUTHOR

Munther Abosaooda
The Islamic University, Collage of Pharmacy,
Najaf, Iraq,
e-mail: munther.abosaooda@iunajaf.edu.iq

ORCID AND CONTRIBUTIONSHIP
Munther Abosaooda: 0000-0003-1755-6278 B C D E
Qayssar Joudah Fadheel: 0000-0003-3301-8357 A F

A – Work concept and design, B – Data collection and analysis, C – Responsibility for statistical analysis, D – Writing the article, E – Critical review, F – Final approval of the article

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