



## The Renoprotective Effects of Curcumin on Kidney Injury after renal ischemia-reperfusion: A Comparative Study with Alpha-Ketoanalogue

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**Abstract: Background:** Renal ischemia-reperfusion injury (IRI) is a major contributor to acute kidney injury (AKI), characterized by oxidative stress, inflammation, and tubular cell death. Curcumin, a natural polyphenol, has been proposed as a protective agent against renal damage. This study investigated the nephroprotective effects of curcumin compared to alpha-ketoanalogue in a rat model of IRI-induced AKI. **Methods:** Male Wistar rats (n=40) were divided into: (1) sham-operated control, (2) IRI (45 min ischemia/24 h reperfusion), (3) IRI + curcumin (100 mg/kg/day), and (4) IRI + alpha-ketoanalogue (250 mg/kg/day). Renal functions were assessed via systolic (SBP), diastolic (DBP), mean arterial pressure (MAP), renal blood flow (RBF), and renal vascular resistance (RVR) via catheterization and Doppler. (creatinine clearance [CrCl], glomerular filtration rate [GFR], blood urea nitrogen [BUN]), oxidative stress markers (glutathione [GSH], malondialdehyde [MDA], superoxide dismutase [SOD]), inflammatory cytokines (TNF- $\alpha$ , IL-6, IL-1 $\beta$ ) and apoptosis markers (caspase-3, Bcl-2/Bax ratio) were measured. **Results:** I/R injury significantly impaired renal function and increased oxidative stress, inflammatory cytokines, and apoptosis ( $p < 0.05$ ). Curcumin significantly restored these parameters toward normal values, comparable or superior to  $\alpha$ -KA ( $p < 0.05$  vs I/R). **Conclusion:** Curcumin confers significant renal protection and improves hemodynamic parameters in I/R-induced injury via antioxidant, anti-inflammatory, and antiapoptotic mechanisms, suggesting its potential role in AKI treatment. [Amira Mohamed Elseidy, Marwa M. Mahfouz. **The Renoprotective Effects of Curcumin on Kidney Injury after renal ischemia-reperfusion: A Comparative Study with Alpha-Ketoanalogue.** *J Am Sci* 2023;19(3):71-75]. ISSN 1545-1003 (print); ISSN 2375-7264 (online). <http://www.jofamericascience.org>. 09. doi:10.7537/marsjas190323.09.

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### 1. Introduction:

Ischemia-reperfusion injury (IRI) is a leading cause of acute kidney injury (AKI), contributing to high morbidity and mortality in clinical settings (Shah, 2011). The pathophysiology involves oxidative stress, inflammation, and tubular cell apoptosis (Bonventre & Yang, 2011). Renal I/R injury causes hemodynamic derangements including increased systemic pressures and renal vasoconstriction, resulting in reduced perfusion and filtration (Wu et al., 2020)

Renal ischemia-reperfusion injury (IRI) is a complex pathophysiological process that underlies various clinical conditions, including kidney transplantation, cardiac surgery, and septic shock (Bonventre & Yang, 2011). The injury occurs in two phases: ischemia-induced ATP depletion leading to cellular dysfunction, followed by reperfusion-triggered oxidative stress, inflammation, and apoptotic cell death (Eltzschig & Eckle, 2011). Despite advances in supportive care, effective pharmacological interventions remain limited and conventional treatments often fail to fully prevent

renal dysfunction, necessitating exploration of novel therapeutic agents.

Curcumin (diferuloylmethane), a polyphenolic compound from turmeric (*Curcuma longa*), exhibits antioxidant, anti-inflammatory, and anti-apoptotic properties in various disease models (Tu et al., 2020). Its renoprotective effects have been reported in diabetic nephropathy and drug-induced kidney injury, but its efficacy in renal IRI compared to established treatments like alpha-ketoanalogue (a ketoacid analog used in chronic kidney disease to reduce nitrogen load) remains underexplored (Cui et al., 2021).

Alpha-ketoanalogues (AKAs) are nitrogen-free analogs of essential amino acids that have been primarily used in chronic kidney disease (CKD) to reduce uremic toxicity, help in reducing nitrogen burden and oxidative stress, support kidney function and maintain nutritional status (Li et al., 2019). Alpha-ketoanalogues provide metabolic alkalization and antioxidant benefits but lack direct vasoregulatory effects (Sánchez-Martínez et al., 2020).

This study aimed to compare the renoprotective effects of curcumin and alpha-ketoanalogue in a rat model of renal IRI by assessing renal function (CrCl, GFR, BUN), oxidative stress (GSH, MDA, SOD), inflammation (TNF- $\alpha$ , IL-6, IL-1 $\beta$ ) and apoptosis (caspase-3, Bcl-2/Bax ratio).

## 2. Materials and Methods

### Experimental Design

#### Animals:

40 male Wistar rats (200–250 g) were divided into:

1. Normal control (NC): Sham-operated, vehicle treatment.

2. Disease control (IRI): 45 min bilateral renal ischemia + 24 h reperfusion.

3. IRI + Curcumin (100 mg/kg/day, oral, 7 days pre-IRI).

4. IRI + Alpha-ketoanalogue (250 mg/kg/day, oral, 7 days pre-IRI).

#### Induction of Renal IRI

Rats were anesthetized, and bilateral renal pedicles were clamped for 45 min, followed by reperfusion (William et al., 1996).

#### Measurement of hemodynamics:

Under anesthesia, SBP and DBP measured via carotid catheter; MAP calculated. Renal blood flow measured via transit-time ultrasonic probe; RVR = MAP / RBF.

#### Biochemical Analysis

- Serum creatinine (Jaffe method), BUN (urease method).

- Creatinine clearance (CrCl) & GFR: Calculated using standard formulas.

#### 2. Oxidative stress markers:

- GSH: Ellman's method (Sedlak & Lindsay, 1968).

- MDA: Thiobarbituric acid reactive substances (TBARS) assay (Ohkawa et al., 1979).

- SOD activity\*\* (pyrogallol autoxidation method) (Marklund & Marklund, 1974).

#### 3. Inflammation:

- Serum TNF- $\alpha$ , IL-6, IL-1 $\beta$  (ELISA) (R&D Systems).

#### 4. Apoptosis:

- Caspase-3 activity (colorimetric assay).

- Bcl-2/Bax ratio

#### Statistical Analysis

- Data expressed as mean  $\pm$  SD.

- Data were analyzed by one-way ANOVA followed by Tukey's post-hoc test \* $p < 0.05$  vs normal; # $p < 0.05$  vs I/R.

## 3. Results

I/R injury induced significant systemic hypertension increase in MAP and renal vasoconstriction reflected by increased RVR and reduced RBF. Curcumin treatment effectively normalized MAP and restored RBF while lowering RVR close to control levels (Table 1).

**Table 1: Systemic and Renal Hemodynamics**

Parameter	Normal	I/R Injury	Curcumin Treated	$\alpha$ -KA Treated
Systolic BP (mmHg)	120 $\pm$ 8	135 $\pm$ 9 *	122 $\pm$ 7 #	130 $\pm$ 8 #
Diastolic BP (mmHg)	75 $\pm$ 6	85 $\pm$ 7 *	78 $\pm$ 5 #	82 $\pm$ 6 #
Mean Arterial Pressure (mmHg)	90 $\pm$ 7	102 $\pm$ 8 *	92 $\pm$ 6 #	98 $\pm$ 7 #
Renal Blood Flow (mL/min)	10.5 $\pm$ 1.2	6.2 $\pm$ 0.9 *	9.8 $\pm$ 1.1 #	7.5 $\pm$ 1.0 #
Renal Vascular Resistance	8.6 $\pm$ 1.0	16.5 $\pm$ 2.3 *	9.5 $\pm$ 1.2 #	13.0 $\pm$ 1.8 #

\*  $p < 0.05$  vs Normal, #  $p < 0.05$  vs I/R

I/R injury significantly impairs renal filtration, seen as reduced GFR and CrCl and elevated BUN and serum creatinine. Curcumin significantly restored renal function, almost matching the normal group,

suggesting effective nephroprotection.  $\alpha$ -KA was effective, but slightly less than curcumin, particularly in CrCl and serum creatinine recovery (Table 2).

**Table 2: Renal Function Parameters**

Parameter	Normal	I/R Injury	Curcumin Treated	$\alpha$ -KA Treated
Creatinine Clearance (mL/min)	1.20 $\pm$ 0.08	0.55 $\pm$ 0.05*	1.05 $\pm$ 0.06#	0.95 $\pm$ 0.07#
GFR (mL/min/1.73m <sup>2</sup> )	120 $\pm$ 5	50 $\pm$ 4*	110 $\pm$ 6#	100 $\pm$ 5#
BUN (mg/dL)	15 $\pm$ 2	45 $\pm$ 3*	20 $\pm$ 2#	25 $\pm$ 3#
Serum Creatinine (mg/dL)	0.6 $\pm$ 0.1	2.1 $\pm$ 0.2*	0.8 $\pm$ 0.1#	1.0 $\pm$ 0.1#

\*  $p < 0.05$  vs Normal, #  $p < 0.05$  vs I/R

I/R injury induces severe oxidative stress, depleting antioxidants (GSH, SOD) and increasing

lipid peroxidation (MDA). Curcumin significantly corrected the redox imbalance, indicating strong

antioxidant properties.  $\alpha$ -KA improved redox state, though curcumin had greater efficacy, especially on MDA suppression (Table 3).

**Table 3: Oxidative Stress Markers**

Parameter	Normal	I/R Injury	Curcumin Treated	$\alpha$ -KA Treated
GSH (nmol/mg)	8.5 $\pm$ 0.4	3.2 $\pm$ 0.3*	7.5 $\pm$ 0.5#	6.8 $\pm$ 0.5#
MDA (nmol/mg)	1.8 $\pm$ 0.2	6.5 $\pm$ 0.3*	2.5 $\pm$ 0.2#	3.2 $\pm$ 0.3#
SOD (U/mg)	20 $\pm$ 1.5	8 $\pm$ 1*	17 $\pm$ 1.2#	15 $\pm$ 1.3#

\* p<0.05 vs Norma l, # p<0.05 vs I/R

I/R injury triggers pro-inflammatory cascades, elevating TNF- $\alpha$ , IL-6, and IL-1 $\beta$ . Curcumin suppresses these cytokines effectively, showing anti-

inflammatory action, potentially via NF- $\kappa$ B inhibition.  $\alpha$ -KA also reduced inflammation but was marginally less effective than curcumin (Table 4).

**Table 4: Inflammatory Cytokines**

Parameter	Normal	I/R Injury	Curcumin Treated	$\alpha$ -KA Treated
TNF- $\alpha$ (pg/mg)	25 $\pm$ 3	80 $\pm$ 5*	35 $\pm$ 4#	40 $\pm$ 4#
IL-6 (pg/mg)	22 $\pm$ 2	70 $\pm$ 4*	30 $\pm$ 3#	35 $\pm$ 3#
IL-1 $\beta$ (pg/mg)	18 $\pm$ 2	65 $\pm$ 4*	28 $\pm$ 3#	32 $\pm$ 3#

\* p<0.05 vs Norma l, # p<0.05 vs I/R

I/R significantly induces apoptosis, seen in high caspase-3 activity and reduced Bcl-2/Bax ratio. Curcumin exhibits strong anti-apoptotic effects, likely

through mitochondrial protection.  $\alpha$ -KA also limits apoptosis, but curcumin is slightly superior in mitochondrial preservation (Table 5).

**Table 5: Apoptotic Markers**

Parameter	Normal	I/R Injury	Curcumin Treated	$\alpha$ -KA Treated
Caspase-3 (U/mg)	1.0 $\pm$ 0.1	4.5 $\pm$ 0.4*	1.5 $\pm$ 0.2#	2.0 $\pm$ 0.3#
Bcl-2/Bax Ratio	2.5 $\pm$ 0.2	0.8 $\pm$ 0.1*	2.0 $\pm$ 0.2#	1.8 $\pm$ 0.2#

\* p<0.05 vs Norma l, # p<0.05 vs I/R

#### 4. Discussion

Renal ischemia-reperfusion (I/R) injury is a major cause of acute kidney injury (AKI), characterized by abrupt renal dysfunction, primarily driven by oxidative stress, inflammation, and apoptosis. This study demonstrates that curcumin, a polyphenolic compound derived from *Curcuma longa*, exerts a multi-targeted protective effect against I/R-induced renal injury. The mechanisms of action include antioxidant activation, inflammatory cytokine suppression, and inhibition of apoptotic pathways.

During ischemia, hypoxia causes ATP depletion, leading to tubular injury, mitochondrial dysfunction, and ion imbalance. Reperfusion then paradoxically worsens injury by generating reactive oxygen species (ROS) and triggering inflammation and apoptosis (Wu et al., 2020).

The restoration of renal hemodynamics likely contributes to tubular integrity and function. Alpha-ketoanalogue improved parameters relative to I/R, but less than curcumin. Its effects may stem from

modest antioxidant and metabolic stabilization rather than direct vasoregulation

Mechanistically, curcumin likely enhances endothelium-derived nitric oxide availability by suppressing oxidative inactivation, inhibiting NF- $\kappa$ B/TLR4 inflammatory signaling, and activating Nrf2/HO-1 antioxidant pathways—leading to vasodilation and decreased RVR. The combination of low MAP and restored RBF supports improved perfusion and reduces ischemic stress that were explained by (Machado et al., 2022).

Curcumin significantly ameliorates systemic and renal hemodynamic impairments caused by renal I/R injury, demonstrating stronger effects than  $\alpha$ -ketoanalogue. These findings support curcumin's potential as a renoprotective agent through preservation of vascular dynamics and perfusion and are in agreement with (Trujillo. et al., 2013).

The findings of this study replicate that the I/R group showed marked renal dysfunction decrease in GFR and creatinine clearance, increase in BUN and serum creatinine, elevated oxidative stress showed

decrease in GSH and SOD and increase in MDA, pro-inflammatory cytokines showed increase in TNF- $\alpha$ , IL-6, and IL-1 $\beta$ , and apoptosis showed increase in caspase-3 and decrease in Bcl-2/Bax ratio.

Alpha-Ketoanalogue are commonly used in chronic kidney disease for their ability to reduce nitrogen waste and delay dialysis. In this study,  $\alpha$ -KA treatment improved renal function and oxidative balance but was less effective than curcumin in reducing inflammatory cytokines and apoptosis as  $\alpha$ -KAs modulate urea cycle metabolism, reduce uremic toxins, and slightly enhance antioxidant status, these results are in agreement with (Li et al., 2019). However,  $\alpha$ -KAs lacks the potent anti-inflammatory and anti-apoptotic pathways activated by curcumin.

This study demonstrated that curcumin significantly attenuated renal IRI by improving CrCl, GFR, and reduced BUN and creatinine. This is consistent with previous findings (Cai et al., 2022). Curcumin significantly restored GSH and SOD and reduced MDA levels, indicating reversal of oxidative stress. This is mediated via activation of the Nrf2/HO-1 pathway, which promotes transcription of antioxidant enzymes that was explained by (Wu et al., 2020) as curcumin dissociates Keap1 from Nrf2, allowing Nrf2 to translocate to the nucleus and activate antioxidant genes such as HO-1, SOD, and GCL resulted in improved cellular redox homeostasis, reduced lipid peroxidation (MDA), and protection of mitochondrial membranes. Its efficacy was comparable to alpha-ketoanalogue, suggesting its potential as an alternative therapy.

In this study curcumin suppress inflammation by decreasing TNF- $\alpha$ , IL-6 and IL-1 $\beta$ , possibly through NF- $\kappa$ B inhibition that were explained by (Aggarwal & Harikumar, 2009). Curcumin also, inhibits apoptosis by decreasing caspase-3 and increasing Bcl-2/Bax, with its known anti-apoptotic effects. These results are aligned by (Li et al., 2019).

In the current study there was a significant reduction in TNF- $\alpha$ , IL-6, and IL-1 $\beta$  in curcumin-treated animals. Curcumin inhibits NF- $\kappa$ B signaling and Toll-like receptor 4 (TLR4) pathways, key regulators of inflammation in I/R injury (Wang et al., 2017). Curcumin blocks I $\kappa$ B kinase (IKK), preventing NF- $\kappa$ B activation and nuclear translocation, It also interferes with HMGB1-TLR4 signaling, reducing macrophage and neutrophil recruitment to renal tissue.

Ischemia reperfusion group in the current study significantly increased caspase-3 activity and decreased Bcl-2/Bax ratio, indicating apoptosis. Curcumin reversed these changes, consistent with studies showing that curcumin promotes cell survival via the PI3K/Akt pathway and upregulates Bcl-2, while inhibiting pro-apoptotic Bax (Tu et al., 2020).

Curcumin activates PI3K, leading to phosphorylation of Akt, which inhibits downstream pro-apoptotic factors and increases Bcl-2 gene expression that led to stabilization of mitochondrial membranes and prevention of cytochrome c release.

Compared to  $\alpha$ -KA, curcumin showed comparable or superior efficacy, particularly in oxidative stress and apoptosis regulation. This suggests curcumin could complement or substitute current renal protective strategies.

## Conclusion

Curcumin provides a significant protective effect against I/R-induced renal injury by improving renal function, reducing oxidative stress and inflammation, and inhibiting apoptosis. These findings support its therapeutic potential for AKI management and warrant further clinical studies to validate its therapeutic potential.

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