

Reno-protective effect of berberine

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Abstract

Objectives: To assess the reno-protective effect of berberine on diclofenac-induced acute kidney injury in rats.

Methods: The experimental study was conducted at the College of Medicine, Mustansiriyah University, Baghdad, Iraq, from January to March 2018, and comprised Sprague Dawley male rats which were divided into 3 equal groups. Group1 rats were treated with distilled water plus normal saline for 14 days, Group2 rats were treated with distilled water plus diclofenac for 14 days and Group3 rats were treated with berberine plus diclofenac for 14 days. Parameters measured were blood urea, serum creatinine, serum malondialdehyde, superoxide dismutase, glutathione reductase, neutrophil gelatinase associated lipocalin, kidney injury molecules-1, Interleukin-18 and cystatin-c. Anthropometric measurements and estimated glomerular filtration rate were also noted. SPSS 20 was used for data analysis.

Results: Of the 30 rats, the three groups had 10(33.3%) each. Berberine reduced blood urea, serum creatinine, malondialdehyde, neutrophil gelatinase associated lipocalin, kidney injury molecules-1 and Interleukin-18 significantly compared to diclofenac-induced acute kidney injury ($p < 0.01$ each). Berberine improved anti-oxidant capacity through significant elevation of superoxide dismutase and glutathione reductase sera levels ($p < 0.01$ each).

Conclusions: Berberine was found to be an effective agent in the attenuation of diclofenac-induced acute kidney injury through the modulation of pro-inflammatory and oxidative stress biomarkers.

Keywords: Berberine, Diclofenac, Acute kidney injury. (JPMA 69: S-83 (Suppl. 3); 2019)

Introduction

Acute kidney injury (AKI) is a renal-specific condition in which the excretion of toxic metabolites does not depart proficiently due to toxic agents and drugs.¹

Diclofenac-induced AKI is due to the toxic effect of diclofenac on renal glomeruli and tubules. Diclofenac provokes tubular dilatation and loss of brush border.² Long-term therapies with diclofenac causes dose-dependent diminution of renal prostaglandins, leading to disturbances of glomerular function and reduction in glomerular filtration rate (GFR).³

It has been postulated that diclofenac may exert its toxic effects depending on its dose and kidney-related parameters like plasma coagulation parameters, oxidative damage, and adenosine deaminase enzyme activity. Moreover, diclofenac may cause exhaustion of adenosine triphosphate (ATP), lipid peroxidase, mitochondrial dysfunction and reduction of intracellular calcium concentrations.⁴

The kidney injury caused by diclofenac is mainly due to

the induction of renal vasoconstriction. Besides, oxidative damage induced by diclofenac toxicity causes noteworthy lipid peroxidation of cellular membranes.⁵

Additionally, diclofenac provokes renal mitochondrial dysfunction through the inhibition of mitochondrial complex, causing a reduction in ATP generation and apoptosis. Therefore, anti-oxidants are part of the problem in diclofenac-induced AKI.⁶ Similarly, diclofenac-induced AKI is linked to the induction of pro-inflammatory cytokines, including interleukin-6 (IL-6), IL-1, IL-33 and tumour necrosis factor-alpha (TNF- α) that induce renal nuclear fragmentation and condensation.⁷

Berberine belongs to the family of isoquinoline alkaloids derived from berberis plants. It has anti-oxidant and anti-inflammatory effects.⁸

Berberine improves mitochondrial function via reduction of oxygen consumption rate and by increasing mitochondrial membrane potential to protect against oxidative damage.⁹

The reno-protective effect of berberine was thought to happen through a mitochondrial-dependent pathway in rats with renal ischaemia-reperfusion model. Besides, berberine re-organises renal interstitial fibrosis in an

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obstructed kidney and protects against the renal damage caused by hypertension in rats.¹⁰

The current study was planned to assess the reno-protective effect of berberine on diclofenac-induced AKI in rats.

Materials and Methods

The experimental study was conducted in the Department of Pharmacology, College of Medicine, Mustansiriyah University, Baghdad, Iraq from January to March, 2018, and comprised Sprague Dawley male rats aged 2-3 months and weighing 200-350g. The animals were kept at a suitable room temperature with artificial 12/12hrs light-dark cycle. After acclimatisation period of one week, the rats were randomly divided into three equal groups. The experimental procedures were done according to the ethics for the care and use of laboratory animals in research and permission was obtained from the institutional ethical committee. All care and efforts were done to reduce animal suffering. The study procedure and method for induction of AKI was in line with literature.¹¹

Group 1 rats were treated with distilled water 5ml/kg for 14 days and on days 6-12 they received daily intra-peritoneal injection of normal saline 5ml/kg. Group 2 rats were treated with distilled water 5ml/kg for 14 days and on days 6-12 they received daily intra-peritoneal injection of diclofenac 15ml/kg (voltarin 15mg, Medopharm, Czech Republic). Group 3 rats were treated with berberine 100mg/kg (Naturegenics, USA) for 14 days and on days 6-12 they received daily intra-peritoneal injection of diclofenac 15ml/kg.

Length was measured by graduated tape measure from nose to the anus (naso-anal length in cm). Rat

bodyweight was measured by specific digital balance in gram. Body mass index (BMI) was calculated as per the standard procedure.^{2,12}

Estimated GFR (eGFR) was measured according to Schwartz formula, $eGFR = k \times \text{height (cm)}/\text{serum creatinine (mg/dl)}$, $K=0.55$.¹³

On day 14, chloroform was used to anaesthetise the rats and sharp scissors were used to exudate the rats. The blood sample was centrifuged for 10 minutes at 5000rpm at room temperature after which the supernatant layer was isolated as the serum sample and kept in freezer at -20°C to be assessed later.

Blood urea and serum creatinine were estimated by using an auto-analyser (ILab-300-Biomerieux Diagnostic, Milano, Italy) and they were expressed as mg/dL. Serum malondialdehyde (MDA), superoxide dismutase (SOD), glutathione reductase (GSH), Neutrophil Gelatinase Associated Lipocalin (NGAL), kidney injury molecules-1 (KIM-1), interleukin-18 (IL-18), and cystatin-c (Cyst-c) were measured by enzyme-linked immunosorbent assay (ELISA) kit method according to the instructions given by the kit manufacturer (Myo-bio source, USA).

Data was analysed using SPSS 20 and was presented as mean \pm standard deviation (SD). Unpaired or paired student t test was used to determine the differences. Analysis of variance (ANOVA) followed by Benferroni post-hoc test were used to compare the variables in different groups. The level of significance was set at $p < 0.05$.

Results

Of the 30 rats, the three groups had 10(33.3%) each. During AKI induction, the bodyweight increased non-

Table: Effect of diclofenac and/or berberine on renal parameters during diclofenac-induced acute kidney injury (AKI).

Variables	Control (n=10)	AKI (n=10)	Berberine (n=10)	A	B	C	ANOVA
Weight (g)	268.00 \pm 25.01	286.87 \pm 27.24	248.62 \pm 42.16	Ns	0.03	0.3	0.04
Height (cm)	21.50 \pm 0.83	21.51 \pm 0.53	21.37 \pm 1.76	Ns	Ns	Ns	Ns
BMI (g/ cm ²)	0.57 \pm 0.02	0.64 \pm 0.03	0.54 \pm 0.01	0.0003*	0.0001	0.01	0.0001
Blood urea (mg/dL)	41.83 \pm 7.46	70.50 \pm 12.53	45.62 \pm 10.86	0.0003*	0.0001	0.7	0.0001
S.cr (mg/dL)	0.70 \pm 0.14	1.93 \pm 0.49	0.99 \pm 0.13	0.001*	0.0001	0.1	0.0001
e GFR (ml/min/1.73)	16.89 \pm 4.21	6.12 \pm 1.7	11.87 \pm 3.78	0.0001*	0.002	0.007	0.0001
MDA(ng/mL)	289.85 \pm 44.18	427.65 \pm 51.39	339.9 \pm 45.87	0.0001*	0.0001	0.06	0.0001
SOD(pg/mL)	48.12 \pm 9.92	30.62 \pm 7.54	39.57 \pm 11.33	0.0003*	0.11	0.14	0.0018
GSH(μ g/mL)	15.94 \pm 2.39	8.88 \pm 1.02	14.93 \pm 2.95	0.0001*	0.0001	0.58	0.0001
IL-18(pg/mL)	29.79 \pm 3.27	39.37 \pm 3.74	28.28 \pm 4.56	0.0001*	0.0001	0.66	0.0001
KIM-1(pg/mL)	73.78 \pm 16.29	269.03 \pm 29.61	89.00 \pm 29.63	0.0001*	0.0001	0.4	0.0001
NGAL (pg/mL)	15.78 \pm 3.07	22.76 \pm 4.13	16.13 \pm 2.95	0.0004*	0.0003	0.9	0.0001

* $p < 0.05$; unpaired t-test, ANOVA: Analysis of variance; BMI: body mass index; S.cr: Serum creatinine, GFR: glomerular filtration rate; MDA: malondialdehyde; SOD-1: superoxide dismutase; GSH: glutathione reductase; IL-18: interleukin-18; KIM-1: kidney injury molecule-1; Cys-C: cystatin; NGAL: neutrophil gelatinase associated lipocalin. A: AKI vs control; B: berberine vs AKI; C: berberine vs control.

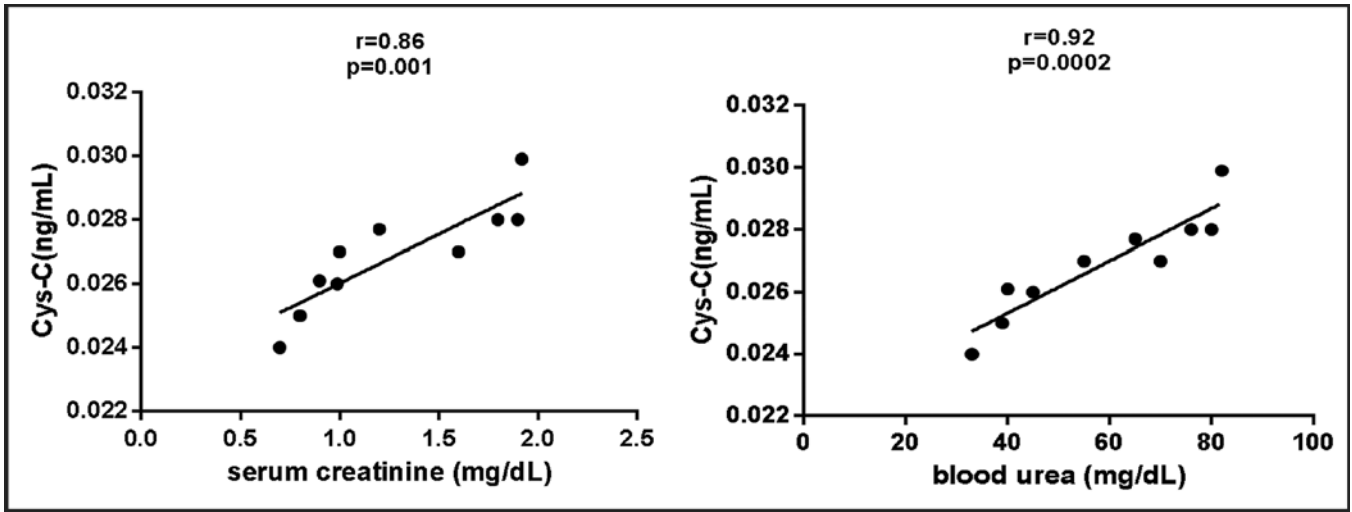


Figure-1: Significant positive correlation of blood urea and serum creatinine with cystatin (Cys-c) serum levels during acute kidney injury (AKI).

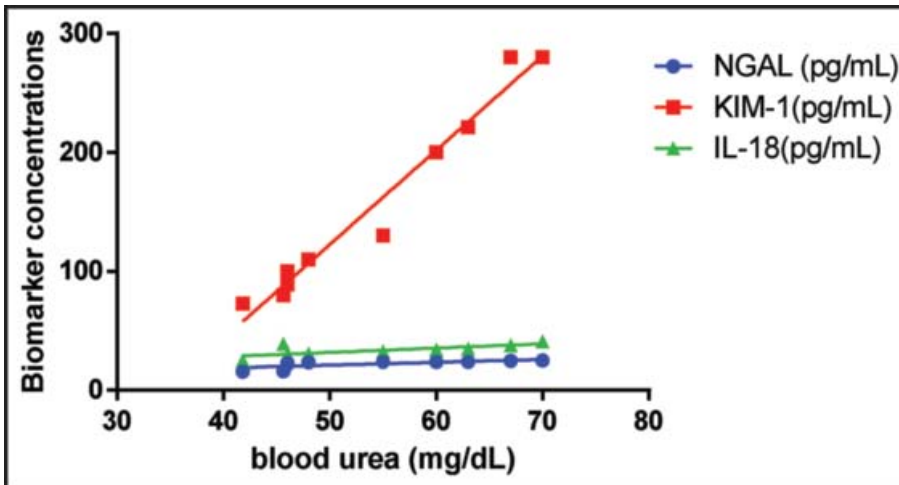


Figure-2: Positive correlation of blood urea with renal tubular injury and pro-inflammatory biomarkers during diclofenac-induced acute kidney injury (AKI).

significantly compared to the controls ($p > 0.05$). BMI increased significantly in the diclofenac group compared to the controls ($p = 0.0003$). Blood urea and serum creatinine were elevated significantly in the diclofenac group compared to the control $p < 0.01$). The estimated eGFR reduced significantly in the diclofenac group ($6.12 \pm 1.7 \text{ ml/min/1.73}$) compared to the controls ($16.89 \pm 4.21 \text{ ml/min/1.73}$) ($p = 0.0001$).

There was significant increase in the MDA serum levels in the diclofenac group compared to the controls ($p = 0.0001$), while both SOD and GSH serum levels were reduced in the diclofenac group compared to the controls ($p = 0.0018$). IL-18 and KIM-1 serum levels increased significantly in the diclofenac group compared to the

controls ($p = 0.0001$ each).

Co-administration of berberine significantly reduced biochemical changes, inflammatory and oxidative stress through the reduction of blood urea, serum creatinine, MDA, IL-18, KIM-1 and NGAL sera levels ($p < 0.01$ each). Besides, berberine improved renal function through the elevation of eGFR compared to the controls ($p < 0.0001$). Also, berberine improved the anti-oxidant potential through elevation of SOD and GSH sera levels significantly compared to the AKI model (Table).

Cys-c serum levels were elevated during the induction of AKI to $0.0288 \pm 0.005 \text{ ng/ml}$ compared to the controls $0.024 \pm 0.0005 \text{ ng/ml}$ ($p = 0.018$), while berberine significantly reduced Cys-c serum levels to 0.0248 ± 0.001 compared to the AKI model ($p = 0.02$). There was insignificant difference in Cys-c serum level compared to the controls ($p = 0.05$).

During AKI, blood urea and serum creatinine were significantly correlated with Cys-c serum levels (Figure-1). Moreover, high blood urea was significantly correlated with renal tubular injury and pro-inflammatory biomarkers during diclofenac-induced AKI ($p < 0.05$). The correlation of blood urea with KIM-1, NGAL and IL-18 was significant ($p < 0.05$ each) (Figure-2).

Discussion

The present study illustrated that diclofenac led to

significant AKI through elevation of blood urea and serum creatinine compared to the controls in the experimental rats as supported by Alabi et al. study which illustrated that diclofenac leads to significant renal tubular necrosis.¹⁴ The induced AKI in the present study also showed significant reduction in eGFR due to the development of acute tubular necrosis and glomerular damage. Tomohiro et al. confirmed that short-term administration of diclofenac leads to acute glomerular damage due to potential inhibition of renal prostaglandins which reduces the renal blood flow and causes renal ischaemia.¹⁵

BMI of the experimental rats were high in AKI compared to the controls which was perhaps due to daily bodyweight gain or fluid retention caused by renal damage. Moreover, reduced GFR also takes part in raised BMI via reduction of urine output and development of peripheral oedema as a number of rats developed ascites. An observational study suggested that fluid retention is the main element of AKI.¹⁶

Oxidative stress and diminution of anti-oxidant capacity are the chief mechanisms of diclofenac-induced AKI since high doses of diclofenac administration lead to significant lipid peroxidation which reflect through the elevation of MDA serum levels and reduction of SOD.¹⁷

Moreover, in diclofenac-induced AKI, the inflammatory process in the renal tissues is increased, so IL-18 serum levels significantly increase within a short period of renal injury¹⁸ as revealed in our study. Likewise, diclofenac in the present study increased serum levels of NGAL significantly as NGAL level is correlated with renal tubular damage in diabetic nephropathy.¹⁹

Furthermore, KIM-1 was significantly elevated in the present study compared to the controls. Lan et al. confirmed significant elevation of KIM-1 in cadmium-induced AKI. KIM-1 serum levels are elevated during the initiation of AKI as it increases in both serum and urine as it sheds from the renal proximal tubules. KIM-1 serum is significantly correlated with different grades of renal damage in AKI because it is highly sensitive and specific.²⁰

Cys-c is a sensitive biomarker for the detection of acute renal damage as it is more sensitive than blood urea and serum creatinine. Harman et al. confirmed that Cys-c is a surrogate biomarker for AKI and should be incorporated in the estimation of GFR.²¹ Therefore, Cys-c serum levels in diclofenac-induced AKI of the present study were elevated significantly compared to the controls.

Berberine, when combined with diclofenac, produced significant reduction in the rats' bodyweight and BMI

compared to the diclofenac group due to the attenuation of diclofenac-induced nephrotoxicity and amelioration of oedematous status through the improvement of rat GFR, given that berberine improves renal functions with significant protection of renal tubules against diclofenac-induced renal tubules injury.²²

In addition, berberine reduced MDA serum levels and improved the endogenous anti-oxidant capacity via elevation of GSH serum levels, as supported by a study which confirmed significant reno-protective effect of berberine against nephrotoxicity in rats due to anti-oxidant and anti-inflammatory properties.²³

Therefore, anti-oxidant effect is the chief mechanism of berberine in the attenuation of diclofenac-induced AKI. Different berberine derivatives have phenolic groups which activate and up-regulate SOD and GSH in a dose-dependent manner.²⁴

Berberine attenuates renal injury and inflammation via significant reduction of KIM-1 since it plays a potential role in the attenuation of gentamicin-induced nephrotoxicity through the reduction of oxidative and inflammatory pathways.²⁵

Likewise, berberine was successful in the reduction of Cys-c serum levels compared to the diclofenac group which might have been due to significant nephron-protective effect.

In sum, berberine attenuates diclofenac-induced AKI through the inhibition of inflammatory and oxidative stress mediated pathways with potential augmentation of endogenous anti-oxidant capacity.

Conclusions

Berberine was found to be an effective agent in the attenuation of diclofenac-induced AKI through the modulation of pro-inflammatory and oxidative stress biomarkers.

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