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Research Article

BETTERMENT OF DICLOFENAC-INDUCED NEPHROTOXICITY BY PENTOXIFYLLINE THROUGH MODULATION OF INFLAMMATORY BIOMARKERS

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ABSTRACT

Objectives: Diclofenac-induced nephrotoxicity is caused by oxidative stress which leads to lipid peroxidation and formation of free radicals. Pentoxifylline can ameliorates renal tissue injury by its anti-inflammatory, antifibrotic, and antioxidant effects, so it mitigates the progression of renal diseases. Therefore, the aim of this study was to evaluate the nephroprotective effects of pentoxifylline on diclofenac-induced nephrotoxicity in rats.

Methods: A total of 30 male Sprague-Dawley rats were allocated into three groups, Group 1 (n=10): Rats treated with distilled water 5 ml/kg plus normal saline 5 ml/kg for 12 days, Group 2 (n=10): Rats treated with distilled water 5 ml/kg plus diclofenac 15 mg/kg for 12 days, and Group 3 (n=10): Rats treated with pentoxifylline 100 mg/kg plus diclofenac 15 mg/kg for 12 days. Blood urea, creatinine, malondialdehyde (MDA), superoxide dismutase (SOD-1), glutathione reductase (GSH), neutrophil gelatinase associated lipocalin (NGAL), kidney injury molecules (KIM-1) vitronectin (VTN), integrin (ITG) , interleukin-18 (IL-18) and cystatin-C were used to measure the severity of nephrotoxicity.

Results: Diclofenac-induced nephrotoxicity led to significant elevation in blood urea, serum creatinine, MDA, IL-18, KIM-1, NGAL, serum ITG, and VTN with decrease of SOD-1 and GSH sera levels $p<0.05$. Treatment with pentoxifylline showed no significant effect on all biomarker levels compared to diclofenac group except on serum level KIM-1 and serum VTN, $p<0.05$.

Conclusion: Pentoxifylline produced significant nephroprotective effect on diclofenac-induced nephrotoxicity through modulation of inflammatory biomarkers.

Keywords: Nephrotoxicity, Diclofenac, Pentoxifylline.

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INTRODUCTION

Nephrotoxicity is a renal-specific situation in which the toxic metabolites do not excrete normally due to toxic agents and drugs [1]. Nephrotoxic agents lead to interstitial nephritis and glomerulonephritis. Acute interstitial nephritis is caused by nonsteroidal anti-inflammatory drug (NSAIDs) and rifampicin, while chronic interstitial nephritis is caused by lithium and calcineurin inhibitors [2].

Proximal renal tubular cells are susceptible to the toxic effect of different drugs and agents due to extended exposure and reabsorbing process. Nephrotoxic drugs lead to damage of the proximal renal tubules through stimulation of oxidative stress, impairments of mitochondrial functions, and interfering with reabsorbing tubular transport process [3].

Diclofenac is a NSAID belongs to the structural family of acetic acid derivatives. Diclofenac is used as analgesic, anti-inflammatory, and antipyretic agent [4].

The foremost mechanism of diclofenac is through the inhibition of prostaglandin-endoperoxide synthase which also called cyclooxygenase-2 (COX-2). Diclofenac inhibits COX-1 about 10-fold than COX-2. It also inhibits lipoxygenase pathway that reduces the production of leukotrienes and other pro-inflammatory autacoids as well diclofenac sodium blocks phospholipase A2 lead to broad and potent anti-inflammatory effect [5].

Long-term treatment with diclofenac causes dose-dependent reduction in the glomerular function. In addition, diclofenac induces autolysis that increases renal intracellular osmolality which is responsible for dilatations of proximal renal tubules [6].

Toxic dose of diclofenac provokes renal mitochondrial dysfunction through the inhibition of mitochondrial complex causes a reduction in adenosine triphosphate (ATP) generation and apoptosis. Thus, antioxidants may play a potential role in prevention of diclofenac-induced nephrotoxicity through attenuation of free radical and oxidative stress effects [7].

Pentoxifylline is a xanthine derivative drug used in the treatment of peripheral vascular diseases [8]. Pentoxifylline has a beneficial effect in nephropathy through reduction of tumor necrosis factor (TNF)-α, proteinuria, and microalbuminuria in diabetic patients. Pentoxifylline inhibits leukotriene and TNF synthesis, increases intracellular cAMP, and decreases innate immunity and inflammation. Pentoxifylline also prevents the expression of mRNA levels of TNF-α and antagonize adenosine receptors [9].

Pentoxifylline impedes the reduction of estimated glomerular filtration rate (eGFR) by a mechanism independent of its effects. Pentoxifylline protects the kidney by inhibition of arachidonic acid metabolism. Pentoxifylline ameliorates renal injury through anti-inflammatory, antifibrotic, and antioxidant effects, so it mitigates the progression of renal diseases [10].

There is a positive significant relationship between pentoxifylline therapy and reduction in TNF-α level and albuminuria. Since TNF-α production occurs within the kidneys, So administration of pentoxifylline leads to modulation of its synthesis [11]. Pentoxifylline decreases proteinuria in patients with diabetic neuropathy. As well, pentoxifylline has the ability to reduce proteinuria by two main mechanisms. First, it has the ability to increase the deformability of red blood cells result in lowering of blood viscosity that decreases glomerular pressure and

proteinuria. The second mechanism is thought to be due to the ability of pentoxifylline to block adenosine receptors since hyperfiltration is associated with high production of renal adenosine [12].

In addition, pentoxifylline ameliorates nephrotic syndrome and membranous glomerulonephritis. Moreover, pentoxifylline reduces proteinuria in patients with advanced and early-onset diabetic nephropathy [13].

Therefore, the aim of this study was to evaluate the nephroprotective effects of pentoxifylline on diclofenac-induced nephrotoxicity in rats.

METHODS

Study design

A total of 30 male Sprague-Dawley rats were used, which were gained from the National Center for Drug Control and Research. Rats age ranged from 3 to 4 months and their body weight ranged from 200 to 400 g. The animals were isolated as three rats in each sterilized cage and placed with suitable temperature of 22–25°C and artificial 12/12 light-dark cycle. They left for 1 week for adaptation without any intervention with free access to normal chow pellets and water. This study was permitted by specific Scientific Adjudicators and Ethical Committee in the Medical Board College of Medicine, Al-Mustansiriya, Baghdad, Iraq. Humane care for animals was according to the guide to the care and the use of laboratory animal. All drugs were purchased from private pharmacy, diclofenac sodium ampule 75 mg/3 ml (Almiral®, Czech Republic), and pentoxifylline 400 mg tablet (Trental®, Canada). The study protocol and method for induction of nephrotoxicity were according to Singh *et al.* method [14].

Group 1 (*n*=10): Rats treated with distilled water 5 ml/kg plus normal saline 5 ml/kg for 12 days. Group 2 (*n*=10): Rats treated with distilled water 5 ml/kg plus diclofenac 15 mg/kg for 12 days. Group 3 (*n*=10): Rats treated with pentoxifylline 100 mg/kg plus diclofenac 15 mg/kg for 12 days.

At the 13th day of the study, animals were sacrificed and blood samples were centrifugated at 3500 rpm/15 min for biochemical analysis.

Assessment of renal injury biomarkers

Renal injury was evaluated by measuring specific biomarkers including blood urea, serum creatinine and biomarkers of nephrotoxicity including malondialdehyde (MDA), superoxide dismutase (SOD), glutathione reductase (GSH), Neutrophil Gelatinase Associated Lipocalin (NGAL), kidney injury molecules (KIM-1), interleukin-18 (IL-18), vitronectin (VTN), integrin (ITG) and cystatin-C were measured by enzyme-linked immunosorbent assay kit methods according to the instruction of the manufacture.

Assessment of anthropometric variables

Length was measured by graduated tape measure from nose to the anus (naso-anal length in cm). Rat body weight was measured by specific digital balance in gram. Body mass index was measured by specific equation, body mass index (BMI) = BW (grams)/length (cm)^2 [15].

Estimated eGFR was measured according to Schwartz formula, eGFR=k × height (cm)/serum creatinine (mg/dl), K=0.55 [16].

Statistics

Data of the present study were presented as mean ± SD. Unpaired Student's *t*-test was used to detect the level of significance between control and treated groups. P value was regarded as statistically significant when it <0.05.

RESULTS

During diclofenac-induced nephrotoxicity, rat body weight was increased to 286.87±27.24 g compared to the control group 268.00±25.01g but not significant, p=0.20. Rat BMI was increased significantly in diclofenac group compared to the control, p=0.0003. Blood urea was raised significantly in diclofenac group (70.5±12.53 mg/dl) compared to the control group (41.83±7.46 mg/dl), p=0.0003, also serum creatinine increased significantly (1.52±0.49 mg/dl) compared to control group (0.7±0.14 mg/dl), p=0.0019. The estimated GFR was reduced significantly in diclofenac group (7.59±1.7 ml/min/1.73) compared to the control (16.89±4.21 ml/min/1.37), p=0.0001.

Regarding the oxidative stress and endogenous antioxidant capacity, there was insignificant increase in the MDA serum levels in diclofenac group (427.65±210.39 ng/ml) compared to the control group (289.85±44.18 ng/ml), p=0.14; also, both SOD-1 and GSH serum level were reduced in diclofenac group compared to control group p=0.20 and p=0.49, respectively.

Concerning the inflammatory and pro-inflammatory biomarkers in diclofenac-induced nephrotoxicity, IL-18 serum level was increased but not significant in diclofenac group from 29.79±3.27 to 14.88±3.02 pg/ml compared to control group, p=0.36. Moreover, KIM-1 was significantly raised in diclofenac group (269.03±29.61 pg/ml) compared to the control group (73.78±16.29), p=0.0001. However, serum levels of NGAL, ITG, and VTN were insignificantly increased (p>0.05) compared to the control group as shown in Table 1.

Indeed, cystatin-C serum level was insignificantly increased during induction of nephrotoxicity by diclofenac from 0.024±0.0005 ng/ml in the control group to 0.0277±0.009 ng/ml in the experimental group, p=0.33 (Fig. 1).

The potential effect of pentoxifylline during diclofenac-induced nephrotoxicity showed a significant reduction in BMI (0.55±0.01g/cm^2) compared to diclofenac group (0.64±0.03 g/cm^2), p=0.0001. Pentoxifylline significantly improves estimated GFR from 7.59±1.7 ml/min/1.73 in diclofenac group to 12.22±4.33 ml/min/1.37, p=0.0138. In addition, pentoxifylline reduced KIM-1 from 269.03±29.61 pg/ml in diclofenac group to 71.6±31.36 pg/ml significantly p=0.0001. As well, pentoxifylline reduced vitronectin serum level to 1.35±0.699 ng/ ml compared to 2.47±0.89ng/ml in diclofenac group, p=0.0142. Accordingly, pentoxifylline illustrated insignificant effect on the other renal biomarkers p>0.05, as shown in Table 2.

In addition , pentoxifylline produced insignificant effect in reduction cystatin-c serum levels, p=0.67 (Fig. 2).

DISCUSSION

Diclofenac-induced nephrotoxicity has been broadly studied in the past and become one of imperative and usual model of drug-induced nephrotoxicity. However, numerous controversies are still about the major mechanism of toxicity and the latent role of different biomarkers concerning inflammatory and pro-inflammatory cytokines [17].

The present study illustrated significant nephrotoxicity which induced by diclofenac through elevation of blood urea and serum creatinine compared to the control in the experimental rats as supported by Alabi *et al.* [18].

Oxidative stress, free radical generations, lipid peroxidations, and diminution of endogenous antioxidant capacity are the chief mechanisms of diclofenac-induced nephrotoxicity as explained by Hickey *et al.* study that demonstrated a high dose of diclofenac administration lead to significant lipid peroxidation through elevation of MDA and reduction of SOD-1. As a result, free radicals lead to mitochondrial damage and reduction in the ATP formation causing DNA damage which eventually leads to acute renal injury [19]. However, in the present study, there was insignificant elevation in MDA serum level and insignificant reduction in the antioxidant capacity (SOD-1, GSH), respectively, which might due to insufficient diclofenac dose, small sample size, or short duration of experimental study.

Many cytokines are elevated during diclofenac-induced nephrotoxicity that reflects the inflammatory process in the renal tissues. Bunel

Table 1: Effect of diclofenac on the anthropometric variables, biochemical and inflammatory biomarkers in diclofenac-induced nephrotoxicity

Variable	Control (n=10)	Diclofenac (n=10)	p
Weight (g)	268.00±25.01	286.87±27.24	0.20
Height (cm)	21.50±0.83	21.51±0.53	0.19
BMI (g/cm^2)	0.57±0.02	0.64±0.03	0.0003*
Blood urea (mg/dL)	41.83±7.46	70.50±12.53	0.0003*
Serum creatinine (mg/dL)	0.70±0.14	1.52±0.49	0.0019*
Estimated GFR (ml/min/1.73)	16.89±4.21	7.59±1.7	0.0001*
MDA (ng/mL)	289.85±44.18	427.65±210.39	0.14
SOD-1 (pg/mL)	48.12±32.92	30.62±15.54	0.20
GSH (µg/mL)	15.94±2.39	14.88±3.02	0.49
IL-18 (pg/mL)	29.79±3.27	39.37±24.74	0.36
KIM-1 (pg/mL)	73.78±16.29	269.03±29.61	0.0001*
NGAL (pg/mL)	15.78±3.07	18.76±4.13	0.16
ITG (ng/mL)	1.47±0.71	2.11±0.65	0.10
VTN (ng/mL)	1.99±0.11	2.47±0.89	0.21

*p<0.01; unpaired *t*-test, BMI: Body mass index; 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; ITG: Integrin; vitronectin

Table 2: Effect of pentoxifylline on the anthropometric variables, biochemical and inflammatory biomarkers in diclofenac-induced nephrotoxicity

Variable	Diclofenac (n=10)	Pentoxifylline (n=10)	p
Weight (g)	286.87±27.24	281.37±29.712	0.70
Height (cm)	21.00±0.53	22.50±0.92	0.0013*
BMI (g/cm^2)	0.64±0.03	0.55±0.01	0.0001*
Blood urea (mg/dL)	70.50±12.53	64.75±27.48	0.59
Serum creatinine (mg/dL)	1.52±0.49	1.012±0.52	0.06
Estimated GFR (ml/min/1.73)	7.59±1.7	12.22±4.33	0.0138
MDA (ng/mL)	427.65±210.39	283.75±56.89	0.08
SOD-1 (pg/mL)	30.62±15.54	42.00±27.52	0.32
GSH (µg/mL)	14.88±3.02	20.64±8.89	0.10
IL-18 (pg/mL)	39.37±24.74	29.41±12.99	0.33
KIM-1 (pg/mL)	269.03±29.61	71.6±31.36	0.0001*
NGAL (pg/mL)	18.76±4.13	16.78±3.79	0.33
ITG (ng/mL)	2.11±0.65	2.01±1.81	0.88
VTN (ng/mL)	2.47±0.89	1.35±0.699	0.00142*

*p<0.01; p<0.05 unpaired *t*-test, BMI: Body mass index; 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; ITG: Integrin; vitronectin

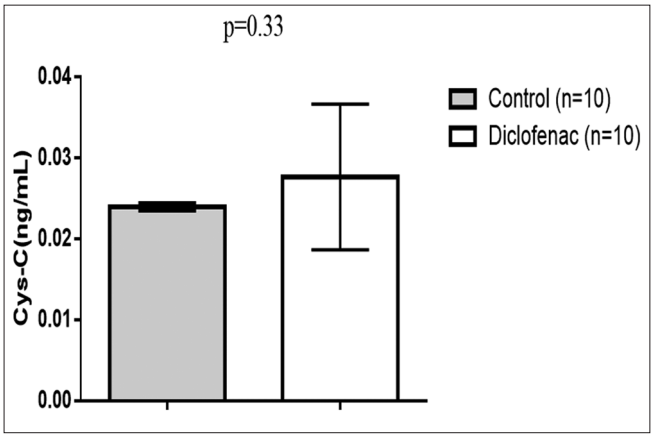

Fig. 1: Cystatin-C serum levels in diclofenac-induced nephrotoxicity

et al. study showed that IL-18, NGAL, ITG, and VTN sera levels were significantly increased within a short period of renal injury (within h) and return to the normal levels within few days due to partial regeneration of damaged renal tubules [20]. This may explain the insignificant elevated levels of these biomarkers in the present study. In addition, renal biomarkers are subjected to different inconsistency including dynamic of their excretion, diurnal variations, and baseline assessment as supported by Harrill *et al.* study that confirmed the variable results of the present study [21].

On the contrary, Fu *et al.* illustrated significant role of vitronectin and integrin in the detection of diabetes-induced renal injury [22]. As well, Kim *et al.* study showed that elevation of NGAL serum level is correlated with renal tubular damage in diabetic nephropathy; thus, NGAL reflects independently early proximal renal tubules damage [23]. Furthermore, KIM-1 was significantly elevated in the present study compared to the control. Lan *et al.* study confirmed a significant elevation of KIM-1 in cadmium-induced nephrotoxicity [24].

Harman *et al.* study confirmed that cystatin-C is a surrogate biomarker for acute renal injury and should be incorporated in the estimation of GFR [25], but cystatin-C serum levels in diclofenac-induced nephrotoxicity of the present study were elevated insignificantly compared to the control which might due to small sample size, biomarker variability, and short duration of the study. These factors may contribute into the insignificant elevation of cystatin-C in diclofenac-induced nephrotoxicity in rats' model of the current study.

Pentoxifylline once co-administrated with diclofenac it created a significant reduction in rat BMI compared to the diclofenac group due to the reduction of diclofenac-induced nephrotoxicity and amelioration of edematous status through enhancement of rat GFR, given that, pentoxifylline improves renal functions with significant protection of renal tubules against diclofenac-induced renal tubules injury. However, blood urea and serum creatinine were not reduced by the effect of pentoxifylline.

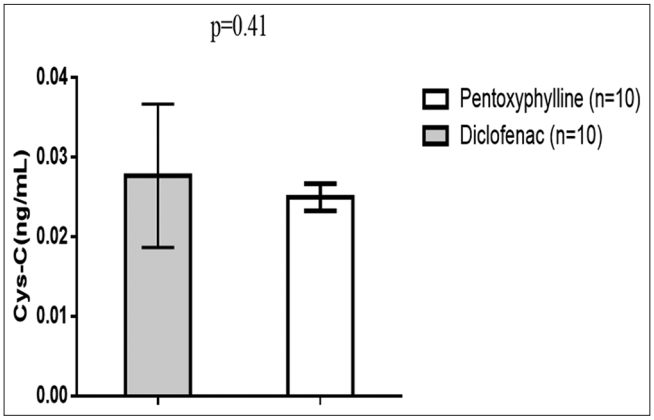

Fig. 2: Pentoxifylline produced insignificant reduction in cystatin-C serum levels in diclofenac-induced nephrotoxicity

Pentoxifylline in the present study produced insignificant attenuation of diclofenac-induced oxidative stress. This not corresponds with a recent study that confined the renoprotective effect of pentoxifylline through attenuation of oxidative stress in rate model study. The insignificant findings might due to short duration of the experimental study or inadequate dose of pentoxifylline to create a sufficient effect on oxidative stress since the duration of Ozturk *et al*. study was 4 weeks to show the protective effect of pentoxifylline on oxidative renal damage [26].

Indeed, pentoxifylline reduced KIM-1 and VTN serum levels significantly, but it produced insignificant effect on the other biomarkers compared to the diclofenac group. Thus, nephroprotective effect of pentoxifylline was mainly due to the reduction of KIM-1 in the present study as supported by Soni *et al.* study [27].

In addition, pentoxifylline produced insignificant reduction in cystatin-C serum levels in diclofenac-induced nephrotoxicity. Cystatin-C serum levels are correlated with the severity of acute renal injury and nephrotoxicity, so it used as a proxy biomarker for diagnosis and prognosis of acute renal injury [28]. However, pentoxifylline reduced cystatin-C in the present study but not significant level which might due to low dose or short period of the study which limits the cytoprotective effect of pentoxifylline as confirmed by Selvin *et al.* study that illustrated the variability in renal biomarkers [29].

CONCLUSION

Pentoxifylline produced significant nephroprotective effect on diclofenac-induced nephrotoxicity through modulation of inflammatory biomarkers.

AUTHORS' CONTRIBUTIONS

All authors contribute equally in data collection, experimental design, interpretation, statistical analysis, literature review, manuscript preparation, and review.

CONFLICTS OF INTEREST

There are no conflicts of interest to declare.

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