International Journal of Medical Science and Clinical Research Studies

ISSN(print): 2767-8326, ISSN(online): 2767-8342

Volume 03 Issue 10 October 2023

Page No: 2221-2226

DOI: https://doi.org/10.47191/ijmscrs/v3-i10-19, Impact Factor: 6.597

Diclofenac-Induced Alterations in Renal Antioxidants and Cytokines in Male Wistar Rats

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ABSTRACT

Introduction: Diclofenac is a nonsteroidal anti-inflammatory drug (NSAID) that is widely used to treat pain and inflammation. However, diclofenac use has been associated with a number of side effects, including renal toxicity. The mechanisms underlying diclofenac-induced nephrotoxicity are not fully understood, but oxidative stress and inflammation are thought to play a role. This study investigated the effects of diclofenac on renal antioxidants and cytokines in male Wistar rats.

Method: Male Wistar rats were randomly divided into three groups: control group, low and high-dose diclofenac group (10 and 30 mg/kg/day) respectively. Treatment was administered for 10 days. At the end of the study, the rats were sacrificed, serum samples and kidney homogenates were analysed for markers of oxidative stress, lipid peroxidation and inflammation including assessment of renal function.

Result: Diclofenac treatment caused a significant decrease in renal antioxidants, including superoxide dismutase (SOD) and glutathione peroxidase (GPx) compared to the control, (0.78±0.11 versus 0.61±0.14), (214.80±46.37versus 149.70±39.43) P <0.05 respectively.

In addition, a significant increase in the level of MDA and renal cytokine (TNF-α) was observed between the control and treated group of rats (2.62±0.29 versus 8.74±4.34, p<0.001) and (1276.0±90.18 versus 222.90±38, P=<0.00) and respectively. The high-dose diclofenac caused a significant increase between the treated and control group of rats respectively and deranged renal function test (serum creatinine and renal KIM-1)

Conclusion: This study provides evidence that diclofenac may induce alterations in renal antioxidants, and mediates oxidative stress and inflammation with consequent kidney injury in male Wistar rats.

KEYWORDS: Diclofenac, antioxidant, inflammation, nephrotoxicity, cytokines

ARTICLE DETAILS

Published On:
10 October 2023

Available on:
https://ijmscr.org/

INTRODUCTION

Diclofenac is an analgesic and belongs to the non-steroidal anti-inflammatory drug (NSAID) that is widely used to treat pain and inflammation. It works by inhibiting the production of prostaglandins, which are responsible for inflammation. [1, 2]

However, diclofenac use has been associated with a number of side effects, including renal toxicity. Nephrotoxicity is a major concern, as it can lead to kidney failure. [1, 3, 4] The exact mechanism of diclofenac-induced renal toxicity is not fully understood. However, it is thought to be mediated by inflammation, oxidative stress, and depletion of renal antioxidant molecules such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx).[3, 5-7]

Oxidative stress is a condition in which there is an imbalance between the production of free radicals and the body's ability to neutralize them. Free radicals are unstable molecules that can damage cells and tissues. Renal damage associated with diclofenac has also been linked with inflammation, a complex biological process that is characterized by the release of inflammatory cytokines that play a significant role in the immune response. Tumour necrotic factor alpha and Interleukin-1β (TNF-α and IL-1β) are involved in the recruitment of inflammatory cells to the site of infection or injury. They also promote the release of other pro-

inflammatory cytokines. This study was designed to investigate the effects of diclofenac on renal antioxidants and cytokine in male Wistar rats.

MATERIALS AND METHODS

In this study, 15 male Wistar rats were selected. These rats are known for their docile nature, ease of handling, and genetic homogeneity, making them an ideal choice for controlled experiments. The rats were randomly divided into 3 groups of equal number, housed in separate compartments of plastic cages and allowed to acclimatize for a week. Standard laboratory setting with a natural light/dark cycle and room temperature and humidity were maintained and they were fed standard rat pellets and distilled water ad libitum. Ethical clearance was obtained from the ethical committee of the College of Medicine, Ekiti State University (EKSU/A67/2022/02/021). The rats were handled in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals detailed in the Guidelines for Animal Research.[8]

Experimental design: The rats were randomly divided into three groups: control group, low-dose diclofenac group (10mg/kg/day), and high-dose diclofenac (30mg/kg/day).

Drugs and vehicle: Diclofenac potassium tablet (Cataflam® 50mg by Novartis) was purchased from a private pharmaceutical outlet. The drug was dissolved in distilled water and the calculated dosages was administered orally for 10 successive days according to the study protocol using rat cannula. The dose of diclofenac that is sufficient to cause renal damage was determined from previous reports.[9-11]

Collection of tissue and blood samples for biochemical analysis

At the end of the experiment, the rats were sacrificed by cervical dislocation method. Whole blood samples were collected from each rat separately using cardiac puncture technique with sterile needles and syringes into clean anticoagulant-free bottles. The blood was then allowed to coagulate for about 30 minutes before the serum in each tube was separated by cold centrifuge at 4000rpm for 10 minutes to obtain sera. The serum was collected with the aid of a Pasteur pipette in plain bottles and kept in ice-cold pack for analysis. Also, the right kidneys were harvested immediately and rinsed in ice-cold physiological saline. About 10% of each kidney tissue was homogenized using a mortar and pestle in 100 mM potassium phosphate buffer at pH 7.4 and centrifuged at 10,000 rpm for 10 minutes at 4°C in a cold centrifuge. The supernatant was collected and used for the determination of enzyme activities in renal homogenates and marker of lipid peroxidation using appropriate method; Superoxide dismutase (SOD) [12], Malondialdehyde (MDA) [13] and Fortress kit (Fortress Diagnostic Limited, United Kingdom) was used for the quantitative determination of total Glutathione peroxidase (GPx) according to the manufacturer's instructions. ELISA kits (Fortress) were used for the determination of KIM-1 and renal cytokine (TNF- α) according to the manufactural instructions. Serum creatinine was analysed using the method described by Bartels and Bohmer. [14]

Statistical analysis

The data obtained were entered and analysed with GraphPad Prism 6.01 (GraphPad Software Inc., La Jolla, CA, USA. Values of all measured parameters were presented as the mean and standard deviations and were analysed using one-way analysis of variance followed by Tukey's multiple comparison test. The P values of less than or equal to 0.05 were considered statistically significant.

RESULTS

Effect of treatment on kidney function.

The serum creatinine level was significantly higher in high-dose DCF treated rats compared to the control (2.65±0.43 versus 0.80±0.11), P = <0.0001, (Figure1a). There was no significant difference between the control group and low dose DCF group, (0.80±0.11versus 1.28±0.37), P= 0.0630, Table 1. As shown in figure 1b and Table 1, the level of KIM-1 in the high-dose DCF treated group was significantly elevated compared to the control group, P=<0.0001. we observed no significant difference in the KIM-1 level between the low-dose DCF (10mg/kg) group and the control group.

Effect of treatment on renal cytokine.

A significant increase in the mean value of the TNF-α was observed in the high-dose diclofenac-treated group compared to the control (1276.0±90.18 versus 222.90±38) P=<0.000, (Figure 2, Table 1).

Effect of treatment on the level of antioxidants and markers of oxidative stress.

The effect of the low-dose diclofenac (10mg/kg) is shown in Tables 2. There was no significant difference in the mean level of these markers between the control and the low-dose DCF treated group. Compare with the control group, the high-dose DCF (30mg/kg) caused a significant increase in the level of MDA (2.62±0.29 versus 8.74±4.34), p<0.001 with a concomitant reduction in the level of SOD (0.78±0.11 versus 0.61±0.14) and GPx (214.80±46.37versus 149.70±39.43) P <0.05, (Table 2, Figure 3,4a and 4b).

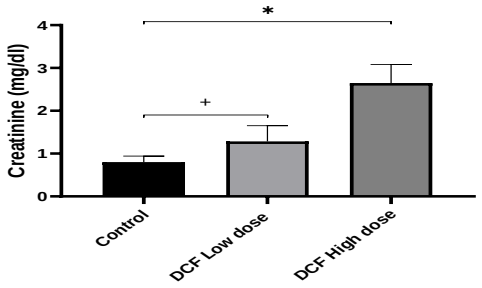

Figure 1a. Effect of treatments on level of serum creatinine. Data are expressed as mean ± SD. (*n* = 5/group). DCF=diclofenac,
***p< 0.05 versus control group.**
+p>0.05 DCF low dose versus control

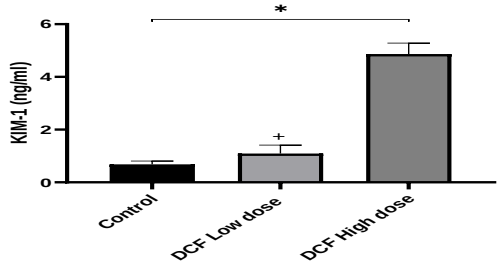

Figure 1b. Effect of treatments on level of KIM-1 in renal tissue. Data are expressed as mean ± SD. (*n* = 5/group). DCF=diclofenac.
***p< 0.05 versus control group.**
+p>0.05 versus control group

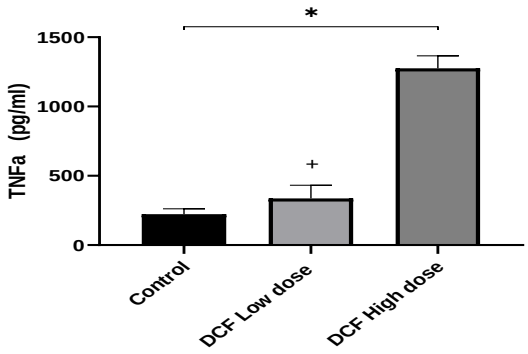

Figure 2: Effect of treatments on level of TNF-*α* in renal tissue. Data are expressed as mean ± SD. (*n* = 5/group). DCF=diclofenac.
***p< 0.05 versus control group**
+p>0.05 versus control group

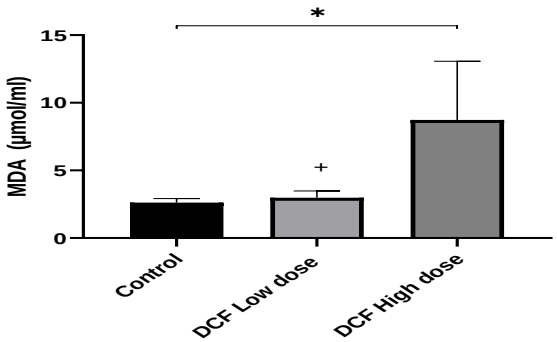

Figure 3. Effect of treatments on level of MDA in renal tissue.
Data are expressed as mean ± SD. (*n* = 5/group). DCF=diclofenac, MDA=malondialdehyde
***p< 0.05 versus control group.**
+p>0.05 versus control group

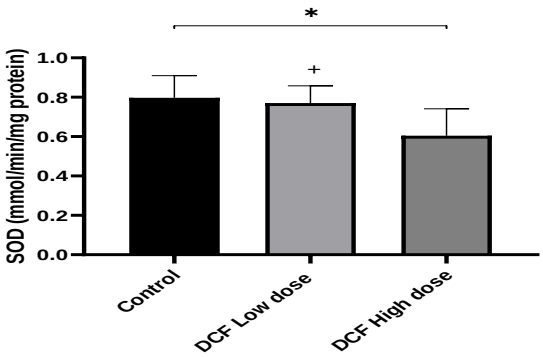

Figure 4a: Data are expressed as mean ± SD. (*n* = 5/group). DCF=diclofenac, SOD=superoxide dismutase
***p< 0.05 versus control group.**
+p>0.05 versus control group

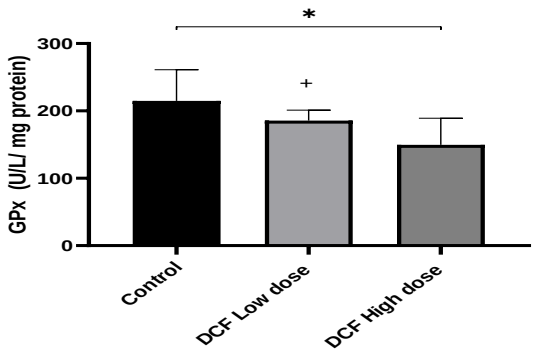

Figure 4b: Data are expressed as mean ± SD. (*n* = 5/group). DCF=diclofenac, GPx=glutathione peroxidase
***p< 0.05 versus control group.**
+p>0.05 versus control group

Figures showing the renal function tests, oxidative and Inflammation markers and antioxidants levels among the control and treated rats

Table 1. Level of biochemical parameters among the control and treated group of rats.

Groups	Creatinine (mg/dl)	KIM-1 (ng/ml)	TNF-α pg/ml
Control	0.80±0.11	0.68±0.13	222.90±38.43
DCF_L	1.28±0.37	1.09±0.32	337.0±95.47
DCF_H	2.65±0.43*	4.87±0.42*	1276.0±90.18*

Control group (distilled water only), DCF_H = (diclofenac 30mg/kg daily). DCF_L= (diclofenac 10mg/kg daily). Values are given as mean ± SD. subscript $_H$ and $_L$ denote high and low dose respectively.

*: Significant different from control group at $p < 0.05$.

Table 2. Level of renal Malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities in control and experimental groups of rats.

Groups	MDA (µmol/ml)	SOD (mmol/min/mg protein)	GPx (U/L/ mg protein)
Control group	2.62±0.29	0.78±0.11	214.80±46.37
DCF_L	2.99±0.49	0.77±0.09	185.60±15.63+
DCF_H	8.74±4.34*	0.61±0.14*	149.70±39.43*

Control group (distilled water only), DCF_H = (diclofenac 30mg/kg daily). DCF_L= (diclofenac 10mg/kg daily). Values are given as mean ± SD. subscript $_H$ and $_L$ denote high and low dose respectively.

*: Significant different from control group at $p < 0.05$

+: non-significant difference from control group, $p > 0.05$.

DISCUSSION

Nonsteroidal anti-inflammatory drug such as diclofenac are a class of drugs commonly used for their analgesic and anti-inflammatory effects.[1] In the medical literature, the use of diclofenac has been associated with different forms of kidney damage in both experimental animals and humans.

In this study, we found that administration of diclofenac at a dose of 30mg\kg\day resulted into deranged renal function, altered renal antioxidant, increase cytokine level in addition to elevated marker of lipid peroxidation. Non-steroidal anti-inflammatory drug has been linked to altered redox reaction, inflammation and development of renal injury. [1, 9]

Nonetheless, low levels of reactive oxygen species (ROS) and reactive nitrogen specie (RNS) are important for normal redox signalling in the kidney. This is essential for renal vasoreactivity, cell growth and survival while also providing sensor for hypoxia.[15] However, during injury or disease state, oxidative stress alters these processes and promotes proinflammatory state.[6]

As cells and tissues are stressed, the balance between ROS and RNS generation and elimination is lost due to upregulated ROS/RNS formation and/or reduced antioxidant activity. The resultant increase in the levels of ROS and RNS can result into cell damage and impair tissue functions which is evident in our study (elevated serum creatinine and renal KIM-1 as markers of renal damage) following the administration of diclofenac. Oxidative stress is a proinflammatory state which has been linked to kidney disease in the setting of diclofenac use as a result of both antioxidant depletion and increased ROS production.[15]

The result obtained from this research is similar to previous studies that investigated the nephrotoxic effect of diclofenac.[4, 16, 17] Diclofenac-induced oxidative stress in renal tissue may result in altered antioxidant mechanisms and increased production of inflammatory mediators causing cellular dysfunction and apoptosis of renal tissue, histopathological changes and ultimately nephrotoxicity.[5-7, 11, 18, 19]

Diclofenac was found to be a strong inducer of oxidative stress, as evidenced by the dose-dependent increase in MDA levels and reduction in SOD and GPx activities in our study. This finding is in agreement with the work of Hickey et al., who demonstrated the nephrotoxicity of diclofenac and documented the drug to be a strong inducer of oxidative stress in male ICR mice.[3] The role of oxidative stress, particularly production of reactive oxygen species (ROS) and antioxidant system dysfunction with administration of diclofenac has been well established.[2, 5, 9, 10, 17, 20-22] Diclofenac produced ROS mainly through targeting two subcellular organelles, microsomes and mitochondria. Mitochondria are essential organelles in the kidney they produce cellular energy for metabolic processes. During mitochondrial metabolism, ROS are produced which function as secondary messengers that induce post-translational modifications (PTM) in proteins in addition to activating or deactivating different cell signalling pathways. [23] However, in the presence of nephrotoxic substances, ROS overproduction may lead to oxidative stress inducing dysregulation of redox-sensitive signalling pathways, mitochondrial dysfunction and altered metabolism.[24, 25] These imbalances can ultimately lead to changes in the cell redox-sensitive signalling pathways, causing inflammation and apoptosis cell death.[4] Other target of ROS attack includes lipids proteins leading to increased malondialdehyde, and decreased enzymatic and non-enzymatic antioxidants activities (GPx, SOD, CAT and Glutathione). [3, 20, 26, 27]

CONCLUSION

This study provides evidence that diclofenac may induce alterations in renal antioxidants, mediates oxidative stress and

inflammation with consequent kidney injury in male Wistar rats.

LIMITATIONS

This study has some limitations. First, the sample size was relatively small. Second, the study was conducted in rats, so the results may not be directly applicable to humans.

CONFLICT OF INTEREST

The authors declare no interest of conflict.

FUNDING STATEMENT

The work did not receive any fund from any organisation or body.

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