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Nephroprotective Effects of *Ipomoea cairica* Leaf Extract against Diclofenac Induced Renal Damage in Male Wistar Rats

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ABSTRACT

Ipomoea cairica is a medicinal plant traditionally employed in the treatment of malaria, viral illnesses, and bacterial infections. It is also applied therapeutically in the treatment of inflammatory and oxidative-related diseases. The study investigated the ameliorative potential of *Ipomoea cairica* leaf extract (ICE) against diclofenac-induced toxicity was investigated in rats. The rats were evenly assigned into five experimental groups (A, B, C, D, and E) of seven rats each. Group A was administered vehicle (control group); Group B was orally administered 100 mg/kg of diclofenac (DCF). Group C was orally administered 100 mg/kg of *I. cairica* leaf extract (ICE) for 14 consecutive days before exposure to DCF; group D was administered 250 mg/Kg of ICE for 14 consecutive days before administration of DCF. The rats were sacrificed 24 hrs after last administration and the blood and kidney were collected and processed for biochemical assays. The serum concentration of creatinine, urea, albumin, and electrolyte (sodium, potassium, chloride) were assessed. In addition, some markers of oxidative stress (malondialdehyde and protein carbonyl), enzymatic (glutathione peroxidase, and glutathione transferase, superoxide dismutase, and catalase) and non-enzymatic antioxidant (glutathione). The markers of oxidative enzymes (, NADP(H) oxidase, xanthine oxidase) and inflammatory enzyme (myeloperoxidase) were determined in the renal tissue of rats. DCF significantly increased serum creatinine (53.92%) ($P<0.001$) and no significant effect on urea and uric acid. In addition, the results also showed that DCF caused a significant decrease in electrolyte concentration (16.31% for sodium ion, 50.08% for potassium ion, and 44.71% for chloride ion) in the blood. With respect to oxidative stress, the results shows that DCF caused a significant increase in MDA concentration, decrease in antioxidant level and increase in inflammatory enzymes. ICE was able to reverse the induction of renal damage by DCF. The investigation gives credence to the neuropathic effect of the methanolic extract of the leaf extract against DCF-induced renal damage.

Keywords: NSAID; toxicity; oxidative stress; inflammation; medicinal plants

Introduction

Diclofenac is a widely used nonsteroidal anti-inflammatory drugs to relieve pains and aches, especially joint pains. It is often prescribed for the treatment of rheumatism, pyretic, analgesic, and

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inflammatory-related diseases. The only side effect under a normal dose is gastrointestinal discomfort. It often acts by inhibiting the cyclooxygenase pathway for the synthesis of prostaglandin (Singh *et al*., 2017). Due to its ready availability, its abuse is inevitable. The abuse and high ingestion of the drug have been identified as one of the primary causes of renal injury (Ungprasert *et al*., 2015). Renal damage remains a major health challenge affecting people. Ranco *et al*. (2019) reported that more than 50% admitted in the intensive unit are due to renal impairment. DCF is metabolized to its reactive metabolite N, 5-hydroxy diclofenac and 5-hydroxy diclofenac, which when accumulated can cause oxidative stress, attacking lipids and proteins in tissues and organs, leading to organ failure (Tang, 2003). The kidney is involved in DCF metabolism, therefore, under overdose, it is one of the organs that is affected.

In the absence of clinically approved synthetic drugs to prevent and treat renal failure, attention has shifted to natural therapy (Luo *et al*., 2014). The role of natural products as a source of novel drugs for the treatment, management, and prevention of various ailments has received a massive boost globally (Luo *et al*., 2014; Atanasov *et al*., 2021).

Despite the health benefits of *Ipomoea cairica*, there have been few scientific reports on the nephroprotective effect of *I. cairica*. It is of the Convolvulaceae family with more than 500 species identified at different part of the world it is commonly called morning glory. Various part of the plant is used for both medicine and ornamental purpose. The plant can be found in various part of the world, where its different parts *I. cairica* are used for various purposes. While the leaves and the roots are edible, the flower is used for beautification purpose. Various parts of the plant are ethnomedicinally used for the treatment of malaria, virus and bacterial infection, in addition, it is also applied therapeutically in the management of disease related to inflammation and oxidative stress. Thus, this experiment is designed to investigate the nephroprotective effect of *I. cairica* leaf extract against diclofenac-induced nephrotoxicity.

Materials and Methods

Collection and identification of Plant

Fresh leaves of *Ipomoea cairica* were harvested from a community in Nembe Local Government, Bayelsa State, Nigeria on the 8th of October, 2021. A section of the plant was taken to the department of Botany, University of Benin for identification and authentication by a plant Taxonomist. A voucher number- UBH-1561 was allotted to it.

Animal handlings and experimental design

Thirty-five rats were randomly divided into five groups (A, B, C, D, and E) of seven rats each.

Rats were acclimatized for two weeks under standard laboratory conditions with ad libitum access to food and water. .

Group A: administered distilled water

Group B: were orally administered mg/kg diclofenac bromate (DCF). DCF dissolved in distilled water and the volume was given according to the wight of each rat

Group C: administered 100 mg/kg of methanolic extract of *Ipomoea cairica* leaf (MEIC) for 14 consecutive days before exposure to DCF. Calculated volume of MEIC in ml according to the weight of each rat were orally administered daily for 14 days before a single dose administration of DCF

Group D: administered 250 mg/kg of methanolic extract of *Ipomoea cairica* leaf (MEIC) for 14 consecutive days before exposure to DCF. Calculated volume of MEIC in ml according to the weight of each rat were orally administered daily for 14 days before a single dose administration of DCF.

Group E; administered 250 mg/kg of methanolic extract of *Ipomoea cairica* leaf (MEIC) for 14 consecutives. This group was only administered MEIC for the duration of the experiment.

Ethical Considerations

The study adhered to the Helsinki Declaration of 1975 for ethical animal treatment. The experimental design was approved by the Federal University Otuoke's Ethics Committee on Animal Research and Treatment (ART2023008).

Collection of blood and tissue samples

Food was withdrawn overnight on the last day of administration prior to sacrifice. Animals were sacrificed via mild anaesthesia and the blood collected through cardiac puncture. The blood was processed for markers of renal functions and electrolyte. The kidney was excised and processed for markers of oxidative stress and other biochemical parameters.

Determination of renal function

Serum urea concentration was assessed using the method of Tietz, 1976), which involves a photometric measurement of ammonia via Barthelot's reaction. Serum creatinine concentration was determined following the protocol outlined by Henry, 1974.

Determination of electrolyte in the serum

Sodium concentration was determined by precipitating it as sodium magnesium uranyl acetate. Excess uranium was reacted with ferrocyanide to form a chromophore, whose absorbance is inversely proportional to the sodium concentration in the sample. Serum level of sodium ion was determined according to the modified method of Natelson, 1957. Briefly, the reaction mixtures contain magnesium uranyl acetate, ferrocyanide, which was allowed to stay for 5 minutes before reading the absorbance with a spectrophotometer. The serum concentration of potassium was determined by the modified method of Terri and Sesin, 1958. This involved the reaction of potassium ion with sodium tetraphenyl boron.

Oxidative stress parameters

The concentration of Malondialdehyde (MDA) in the renal tissues was evaluated according to the modified method of Ohkawa *et al*., 1979. Briefly, the reaction mixture contains kidney homogenates, trichloroacetic acid, thiobarbituric acid and incubated in waterbath for 45 min. the mixture was allowed to cool before centrifuging. The absorbance of the clear supernatant was read at 532nm. The concentration of glutathione (GSH) in the renal tissues was determined according to the modified method of Beutler *et al*., 1963. Briefly, the tissue was deproteinized using 4% sulphosalicyclic acid, centrifuged and Ellman's reagents was added to the clear supernatant and the absorbance read at 412 nm. Renal superoxide dismutase (SOD) activity was assessed using a modified method of Misra and Fridovich (Weydert *et al*., 2010). The reaction mixture was constituted as follows: 0.2 mL diluted sample and 2.5 mL 0.05 M carbonate buffer (pH 10.2), and initiated by adding 0.3 mL freshly prepared 0.3 mM adrenaline. Absorbance was read after rapid mixing. The activity of catalase in the kidney was measured according to the modified method of Weydert *et al*. (2010). Briefly, the reaction mixture, containing 20 µL phosphate buffer (50 mM, pH 7.0), 10 µL 19 mM H_2O_2, and 50 µl sample, was incubated for 3 minutes. The reaction was terminated by adding 20 µL dichromate in acetic acid solution, followed by a 10-minute incubation in a boiling water bath. The optical density of the cooled solution was measured at 570 nm.

Anti-inflammatory enzymes parameters

The anti-inflammatory effect was evaluated by determining the activity of myeloperoxidase (MPO) according to the modified methods of Nishikimi *et al*., 19972. Briefly, the reaction

mixture consists of 200 µl O-dianisidine, 50 µl diluted H_2O_2, and 7 µl sample. The absorbance was read at 470 nm for 3 minutes at 30s intervals.

Results

Effect of MEIC on serum renal function and electrolytes concentration in rats intoxicated with diclofenac

The renal function evaluated are creatinine (CREA), urea, and uric acid. The result shows that diclofenac caused a significant increase in creatinine and urea concentrations, while it has no significant change in the concentration of uric acid when compared with the rats in the control group. Treatment with 100- and 250 mg/kg caused a significant decrease in the serum concentration of creatinine and urea when compared with the rats in the untreated group (diclofenac). Administration of 250 mg/kg of MEIC had no significant effect on the renal parameters evaluated when compared with the rats in the control group. In addition, the results also showed that diclofenac caused a significant decrease in the concentration of sodium, potassium, and chloride ions concentration as compared to the rats in the control group. Administration of 100 mg/kg of MEIC did not affect the concentration of sodium and potassium ions, while there was a significant increase in the concentration of chloride ions when compared with the rats in the untreated group. However, 250 mg/kg of MEIC caused a significant increase in the concentration of sodium and chloride ions, with no significant effect on potassium ions when compared to the untreated group (diclofenac). Furthermore, the result shows that administration of 250 mg/kg of MEIC alone had no significant effect on the serum electrolyte when compared to the rats in the control group.

Table 1: Effect of Methanolic extract of *Ipomoea cairica* (MEIC) leaf on the serum concentration of creatinine (CRT), urea, and uric acid in male Wistar rats exposed to diclofenac.

	CRT µmol/l	UREA mmol/l	URIC ACID mmol/l
Control	41.79.38	6.02±0.40	0.13±0.01
Diclofenac	64.32±1.22	6.32±0.82	0.12±0.02
100 mg/kg MEIC+ Diclofenac	40.93±0.80	4.93±0.80	0.12±0.01
250 mg/kg MEIC+ Diclofenac	40.18±0.87	4.69±0.74	0.10±0.01
250 mg/kg MEIC	24.86±3.24	6.28±0.50	0.13±0.01

Values are expressed as mean±SD (n=7).

Table 2: Effect of Methanolic extract of *Ipomoea cairica* (MEIC) leaf on the serum concentration of electrolyte (sodium, potassium, chloride in male Wistar rats exposed to diclofenac.

	Na mmol/l	K_2 mmol/l	Cl_2 mmol/l
Control	290.33±49.99	12.10±0.84	134.18±16.16
Diclofenac	242.98±56.75	6.04±1.96	74.08±18.65
100 mg/kg MEIC+ Diclofenac	233.61±v52.35	6.21±1.43	92.23±10.8
250 mg/kg MEIC+ Diclofenac	290.43±56.00	6.70±0.87	107.48±13.26
250 mg/kg MEIC	383.49±59.54	12.58±3.82	120.52±15.98

Values are expressed as mean±SD (n=7).

Effect of MEIC on the antioxidant parameters in the renal tissue of rats intoxicated with diclofenac

The outcome of the experiment reveals that diclofenac significantly decreased ($P<0.001$) the activities of superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST), and glutathione peroxidase (GPx) in the kidney of rats in comparison to the rats in the control group. Treatment of the rats with MEIC caused a dose-dependent increase in the antioxidant enzymes in

comparison to the rats in the untreated group (diclofenac). In addition, diclofenac intoxication caused an insignificant decrease (P>005) in the concentration of GSH and a significant increase (P<0.05) in MDA concentration when compared to the rats in the control group. In administration of 250 mg/kg of MEIC had no significant effect on all the parameters evaluated when compared to the rats in the control group.

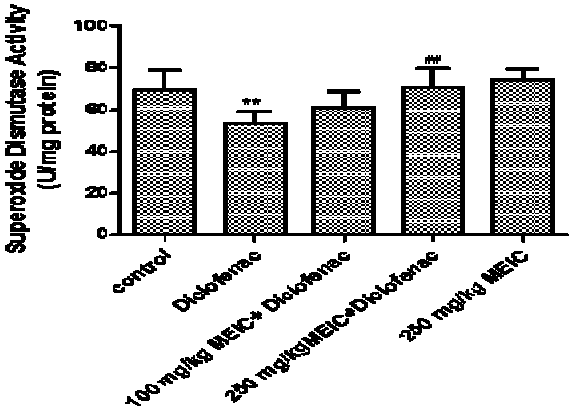


Figure 1: The effects of extracts of *I. cairica* leaf (MEIC) and diclofenac (DCF) on superoxide dismutase activity in the kidneys of male Wistar rats. *Each bar represents the average ± standard deviation (n=5), with symbols **and ## representing differences from control values p< 0.01 and p< 0.01 respectively.*

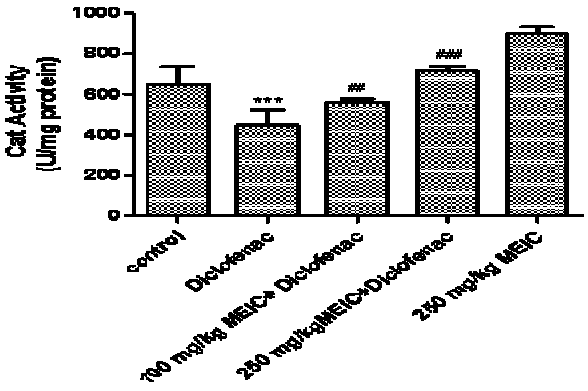


Figure 2: The effects of extracts of *I. cairica* leaf (MEIC) and diclofenac (DCF) on catalase activity in rats renal tissue. *Each bar represents the average ± standard deviation (n=5), with symbols ***, ##, and ### representing differences from control values p< 0.001, p< 0.01, and p<0.001 respectively.*

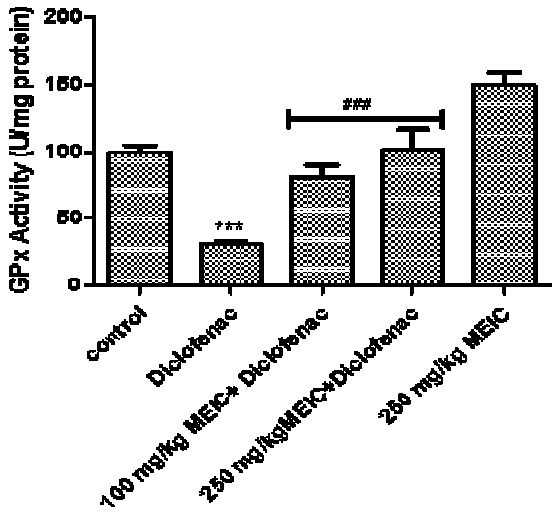


Figure 3: The effects of extracts of *I. cairica* leaf (MEIC) and diclofenac (DCF) on glutathione peroxidase activity in rats renal tissue. *Each bar represents the average ± standard deviation (n=5), with symbols ***, and ### representing differences from control values p< 0.001 and p<0.001 respectively.*

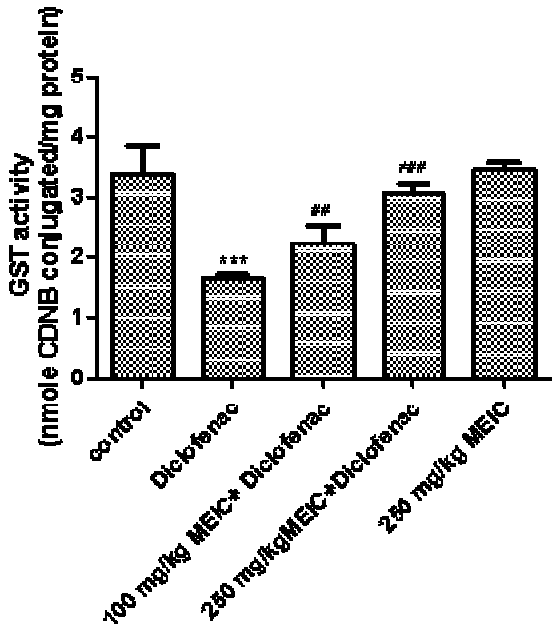


Figure 4: The effects of extracts of *I. cairica* leaf (MEIC) and diclofenac (DCF) on glutathione transferase activity in rats' renal tissue. *Each bar represents the average ± standard deviation (n=5), with symbols ***, ##, and ### representing differences from control values p< 0.001, p<0.01, and p<0.001 respectively.*

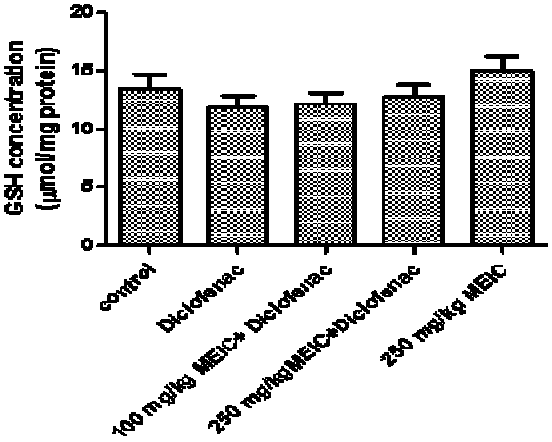


Figure 5: The effects of extracts of *I. cairica* leaf (MEIC) and diclofenac (DCF) on the concentration of glutathione rats renal tissue. *Each ar represents the average ± standard deviation (n=5).*

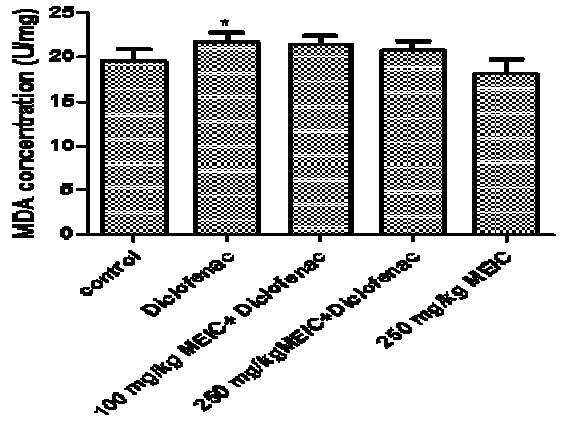


Figure 6: The effects of extracts of *I. cairica* leaf (MEIC) and diclofenac (DCF) on the concentration of malondialdehyde (MDA) rats renal tissue. *Each bar represents the average ± standard deviation (n=5), with symbol * representing differences from control values p< 0.05.*

Effect of MEIC on renal inflammatory enzyme (myeloperoxidase) and oxidative enzymes (NADPH oxidoreductase and xanthine oxidase) in diclofenac-intoxicated rats

The experimental results show that diclofenac induced renal inflammation as observed in elevated level of myeloperoxidase (MPO) (P<0.001) in comparison to the control. The oxidative effect of diclofenac was also confirmed with elevated activities of NADPH oxidoreductase and xanthine oxidase (P<0.001) in comparison to the control. MEIC treatment dose-dependently reversed diclofenac-induced oxidative stress in rat kidneys.

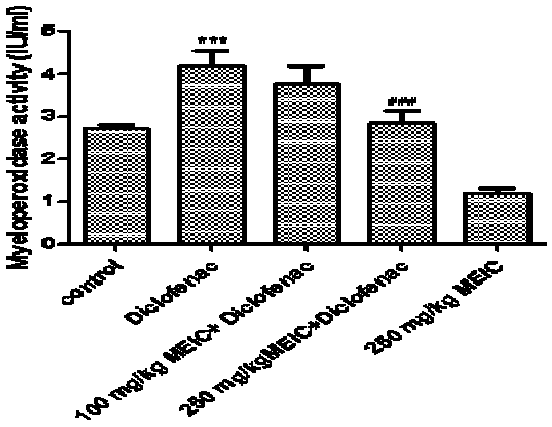


Figure 7: The effect of *I. cairica* leaf extract (MEIC) and diclofenac (DCF) on activity of myeloperoxidase in the kidney of male Wistar rats. *Each bar represents mean value ± standard deviation (SD) (n=5) and the symbols- *** and ### indicates significantly different at from control at p<0.001 and p<0.001 respectively.*

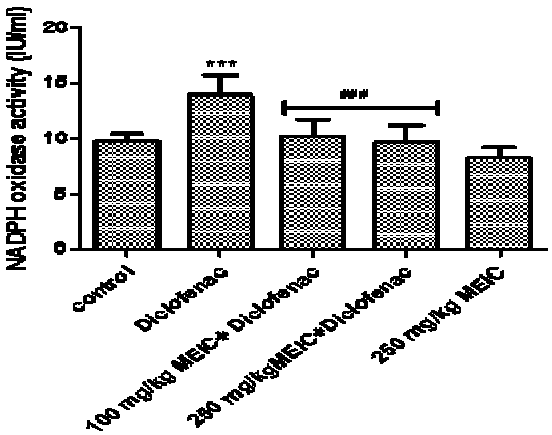


Figure 8: The effect of *I. cairica* leaf extract (MEIC) and diclofenac (DCF) on activity of NADPH oxidase in the kidney of male Wistar rats. *Each bar represents mean value ± standard deviation (SD) (n=5) and the symbols- *** and ### indicates significantly different at from control at p<0.001 and p<0.001 respectively.*

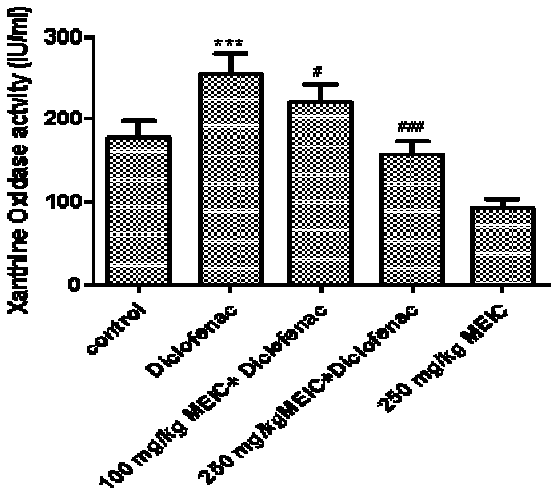


Figure 9: The effect of *I. cairica* leaf extract (MEIC) and diclofenac (DCF) on activity of xanthine oxidase in the kidney of male Wistar rats. *Each bar represents mean value ± standard deviation (SD) (n=5) and the symbols- *** # and ### indicates significantly different at from control at p<0.001, p<0.05 and p<0.001 respectively.*

Discussion

Acute kidney injury is one of the non-communicable diseases globally, this is further worsened with high consumption of non-steroidal anti-inflammatory drugs (NSAID) such as diclofenac (DFC) (Kristensen *et al*., 2018; Simon and Prince, 2017; Singh *et al*., 2016). Unregulated ingestion of DFC has been reported to trigger ulcers, liver, and kidney damage. The increasing knowledge and availability of medicinal plants, rich in phytochemical constituents, have established them as a potential alternative approach to treating and preventing chemical-induced organ toxicity. (Torres-Maravilla *et al*., 2022). This research is aimed at investigating the protective effect of *Ipomoea cairica* leaf extract against diclofenac nephrotoxicity. The current investigation confirms the toxicity of DFC in the renal tissue. This is based on the high concentration of creatinine in the serum, and the mild increase and decrease in the concentration of urea and uric acid respectively. Creatinine, urea, and uric acid are some of the major biomarkers of renal damage. The results are similar to the work of Famurewa *et al*., 2020 and Nouri and Heidarian, 2019), who also reported an increase in urea and creatinine concentration due to diclofenac toxicity. When their concentration is high, it is a sign of renal damage (Kolangi *et al*., 2018). The high serum concentration of creatinine can be due to several factors including a defect in the glomerular filtration rate (GFR). The mild alteration in the concentration of uric acid and urea could mean, that DFC at the administered dose does not have much effect on the leakage of urea and uric acid into the bloodstream. Creatinine level is a better indicator of kidney injury

Another major marker of kidney disease is electrolyte imbalance, whereby some minerals are either in excess or deficient. electrolyte plays an important role in fluid homeostasis in the blood and tissues (Goddard *et al*., 2003). Sodium ions and potassium ions play important roles in regulating fluids as well as assisting in efficient performance of nerves and muscles while chloride ions assist in maintaining blood pressure and body fluids. The low concentration of all the electrolytes in the experiment is proof that DCF caused hypernatremia, hypokalaemia, and chloremia. Excessive intake of NSAID has been reported to cause intoxication by depleting the body of electrolytes (Pakravan *et al*., 2007). The result of the experiment corroborates this statement. The nephroprotective effect of ICE was reflected in the low concentration of serum creatinine and high concentration of sodium and chloride ions. This can be due to the plant's ability to retain fluid or prevent the excretion of electrolytes from the blood and tissues (Christie *et al*., 2001). Thus, ICE can be included in plants that can be used to maintain the homeostasis of electrolytes.

One of the confirmed mechanisms of DCF renal toxicity is through oxidative stress, which is reflected in the elevated level of malondialdehyde

(MDA), protein carbonyl (PC), and oxidized macromolecules (Famurewa *et al*., 2020). This was corroborated in the results, however, treatment of ICE at 100- and 250 mg/kg significantly reverses the oxidative effect of DCF in the renal tissue. Further investigations show that DCF in the renal tissues caused a significant decrease in the activities of antioxidant enzymes (catalase, superoxide dismutase, glutathione-S-transferase). This shows that apart from the induction of the oxidative process, DCF also depletes the renal tissue of its defence system, further exposing the tissue to oxidative damage (Moradi *et al*., 2021). This further gives credence to the free radical generation ability of DCF. The antioxidant effect of ICE was observed in the experiment as the extract was able to improve the activities of all the antioxidant enzymes. Previous reports have shown the abundance of bioactive phytochemicals in the leaves of *I. cairica* (Arora and Shiba, 2013). These phytochemicals might contribute to the ability of ICE to counter the oxidative effect of DCF. In addition, some enzymes linked to inflammation were evaluated-xanthine oxidase, myeloperoxidase, and NAD(P)H oxidase. These enzymes are involved in fighting infected cells and eliminating them from the body, however, under high concentration, they attack healthy cells causing organ injury and death. The pro-inflammatory effect of DCF has been highly pronounced in the renal tissues as the drug-induced high expression of the inflammatory enzymes, despite being classified as an anti-inflammatory drug (El-Yazbi *et al*., 2017; Sánchez *et al*., 2007; Owumi and Dim (2019). Our results demonstrate that administration of a high dose of DCF can lead to inflammation. However, treatment with ICE reversed this inflammatory response, further supporting its anti-inflammatory potential, as previously observed in an in-vivo investigation of the plant (Ilesanmi and Inala, 2023)..

Conclusion

The investigation gives credence to the renoprotective activity of *Ipomoea cairica* leaf extract against diclofenac toxicity. the nephroprotective effect of *I. cairica* leaf extract against diclofenac toxicity. *I. cairica* exhibited a protective effect against diclofenac-induced nephrotoxicity by reducing oxidative stress and inflammation. Thus, *I. cairica* protective properties can be linked to phytochemicals present inside it that are antioxidant and anti-inflammatory characteristics.

Conflict of Interest

The authors have no financial or non-financial conflicts of interest to declare that could have influenced the design, conduct, analysis, or interpretation of this study.

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