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Full length Research Article

Mitigation of Diclofenac-Induced Hepatorenal Toxicity by Methanolic Extract of *Cymbopogon citratus*

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Summary: Despite the wide range of advantages of non-steroidal anti-inflammatory drugs (NSAIDs) such as diclofenac, their abuse can have a deleterious impact on the hepatorenal system. The phytocompounds in methanolic extracts of *Cymbopogon citratus* have pharmacological properties such as antioxidant and anti-inflammatory properties that can enhance health by preventing and reducing NSAID-induced toxicity. This study evaluated the histopathological and molecular effects of diclofenac-induced hepatorenal toxicity on the expression of TGFβ and Nrf2 in the liver and kidneys of adult male Wistar rats treated with methanolic leaf extracts of *C. citratus* (lemon grass). Twenty adult male Wistar rats were randomly divided into four groups. The first group were the unexposed control rats administered with distilled water only, while the test groups (II-IV) were orally administered with diclofenac at a standard dosage of 5mg/kg/BW. The rats in groups III and IV were administered with methanolic extract of *C. citratus* at 100mg/kg/BW and 200mg/kg/BW respectively. After 28 days, the rats were euthanized by cervical dislocation, and the liver and kidneys were histologically processed. mRNA expression of nuclear factor erythroid 2-related factor 2 (Nrf-2) and transforming growth factor beta (TGF-β) was analyzed using the reverse transcription polymerase chain reaction (RT-PCR). The data obtained was statistically analysed using the One-way analysis of variance (ANOVA), and Duncan's multiple range test was employed in comparing categorical variables ($p < 0.05$). The results revealed significant pathological alterations in the histoarchitecture of the livers and kidneys of the untreated diclofenac-exposed rats with the administration of *C. citratus* significantly repressing the expression of TGF-β in the liver and upregulating Nrf-2 levels in the kidneys of the treated rats ($P<0.05$), while also ameliorating the histological derangement observed in the studied organs. *Cymbopogon citratus* modulates the expression of TGF-β and Nrf-2 in the liver and kidneys of the experimental animals consequently mitigating diclofenac-induced oxidative damage in the studied organs.

Keywords*: Diclofenac, drug abuse, lemon grass, medicinal plants, oxidative stress.*

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INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) primarily operate by inhibiting cyclooxygenase (COX) enzymes (Stiller and Hjemdahl 2022). Cyclooxygenase- 1 (COX-1) maintains physiological functions with COX-2 involved in inflammation (Faki and Er 2021). They are widely utilized for their antinociceptive, antipyretic, anti-inflammatory, and analgesic properties (Sriuttha *et al*., 2018; Bindu *et al*., 2020; Kushner *et al*. 2022). NSAIDs, including diclofenac and ibuprofen, are commonly prescribed cyclooxygenase inhibitors that have been associated with hepatotoxicity, manifesting as increased liver enzymes, hepatitis, and, in severe cases, acute liver failure. Additionally, they can also cause renal toxicity by directly damaging the renal tubular and glomerular systems, which is primarily caused by impeding prostaglandin synthesis (Sarges *et al*., 2016; Sriuttha, *et al*. 2018; Zoubek *et al*. 2020; Stiller and Hjemdahl, 2022; Boggula *et al*. 2023; Liu and Liu 2024). While NSAIDs effectively alleviate pain and inflammation, they also inhibit COX-1 causing gastrointestinal ulcers and

bleeding. Moreover, NSAIDs have been linked to kidney damage, including acute kidney injury (AKI) and chronic kidney disease (CKD), especially in the elderly (Wongrakpanich *et al*. 2018; Drożdżal *et al*. 2021; Dreischulte *et al*. 2022; Ward *et al*. 2022). The immunotoxic and hematotoxic effects of NSAIDs have also been documented (Gomaa 2017; Gomaa 2018). The mechanism by which oxidative stress impairs kidney function has been described by Nezu and Suzuki (2020). This mechanism involves the formation of reactive oxygen species (ROS) and electrophiles, which cause damage to the renal tubule epithelial cells and result in the production of damage-associated molecular patterns (DAMPs). In addition, the release of inflammatory cytokines initiates inflammation, which is subsequently mediated by myeloid cells. Furthermore, the conversion of interstitial fibroblast into myofibroblast further promotes kidney fibrosis. The two main syndromes among the various forms of renal failure are CKD and AKI with the likelihood of AKI associated with NSAIDs escalating with prolonged use, particularly in the presence of predisposing factors such as advanced age, diabetes mellitus, and cardiovascular conditions (Ohsaki *et al*. 2012; Patschan and Müller 2016; Nelson *et al*. 2019; Kaur *et al*. 2023; Weng *et al*. 2024). A cohort study conducted by Lipworth *et al*. (2016) documented an increase in NSAID use among acute kidney injury survivors. Furthermore, the chronic use of NSAIDs is linked to a higher prevalence of CKD, however, the exact relationship between NSAID use and CKD progression remains somewhat unclear (Wan *et al*. 2021).

Nuclear factor erythroid 2-related factor 2 or Nrf2 is a transcription factor crucial in inflammation and the regulation of pro-inflammatory genes such as COX-2, that is involved in producing inflammatory mediators, they further play a significant role in cellular defence against toxic and oxidative stress (Ahmed *et al*. 2017; Nezu and Suzuki 2020; Guerrero-Hue *et al*. 2021; Ngo and Duennwald, 2022). The activity of Nrf2 is regulated at both transcriptional and post-transcriptional levels, involving protein stability, post-transcriptional modifications, and binding partner availability (Tonelli *et al*. 2018). Consistent exposure to toxicants can lead to the generation of oxidative stress which is linked to the development of several disorders with studies suggesting that reduction in oxidative stress can inhibit various disease progressions including toxic-induced nephropathy, diabetic nephropathy and hypertension-associated kidney disease (Ratliff *et al*. 2016; Clarke *et al*. 2016; Moronkeji and Akinbo 2024).

Transforming growth factor beta (TGF-β) is a pleiotropic cytokine that plays a pivotal role in pro and anti-inflammatory activities. In addition to influencing a variety of biological processes like development, carcinogenesis, immunological responses, wound healing and fibrosis, this regulatory molecule also affects cell differentiation, migration, proliferation, and survival (Sanjabi *et al*. 2017; Baba *et al*. 2022). As an anti-inflammatory marker, TGF-β is involved in the biological response to injury and autoimmune diseases, initiating the healing process, which aids wound healing and the resolution of immune responses (Deng *et al*. 2024).

The increased usage of medicinal plants may be attributed to their acceptability, compatibility, adaptability, and minimal or non-existent negative effects on human health (Oladeji *et al*. 2019; Mokhtar *et al*. 2023). Chronic use of major NSAIDs has been demonstrated to be toxic, and there is evidence that acids exacerbate NSAID-induced injury in patients. In addition to lifestyle modifications, proton pump inhibitors, H2-receptor antagonists, and antioxidants are used to treat NSAID toxicity (Lazzaroni and Porro 2009; Solomon *et al*. 2017; Bindu *et al*., 2020). *Cymbopogon citratus* (lemon grass) is a member of the Poaceae family that is known for its lemony taste and aroma (Oladeji *et al*. 2019). *C. citratus* is a tall, monocotyledonous aromatic perennial plant with slender sharp-edge green leaves and a pointed apex. It is valued for its anti-inflammatory anti-mutagenicity and antioxidant properties which stem from its various phytoconstituents which include flavonoids, alkaloids and terpenoids and phenolic compounds which are responsible for the plant's therapeutic properties (Umar *et al*. 2016; Saenthaweesuk *et al*. 2017; Schabauer *et al*. 2017; Fahmy *et al*. 2020; Elekofehinti *et al*., 2020). The phytocompounds found in *C. citratus* have pharmacological activities that include antioxidant, anti-inflammatory, antiparasitic, anti-obesity, antibacterial, antifungal, antinociceptive and anti-diarrheal qualities. These compounds may improve health by providing a protective and ameliorative effect related to NSAID-induced toxicity (Moronkeji *et al*. 2024; Okere *et al*., 2014; Oladeji *et al*. 2019; Saenthaweesuk *et al*. 2017; Tarkang *et al*. 2014). This study evaluates the attenuative potential of methanolic extract of *C. citratus* in NSAID-induced hepatorenal toxicity via modulating the TGF β and Nrf2 signalling pathway.

MATERIALS AND METHODS

Ethical approval: Ethical approval with reference number MNR/V.384/41 was received from the Ministry of Agriculture, Akure while strictly adhering to the established guidelines for the use of experimental animals as stipulated by the research ethics committee which is in alignment with the international Humane Animal Care Standards (Hau and Van Hoosier 2002).

Plant collection, extraction and preparation: Fresh leaves of *Cymbopogon citratus* were harvested from a farm in Ondo town, Ondo state, Nigeria. An expert from the University of Medical Sciences Ondo's (UNIMED) plant biology and biotechnology unit identified the plant and assigned it herbarium identity number 033. The leaves were rinsed in tap water to get rid of any contaminants, shade-dried and further dried in an oven at 60°C and then processed into a powder using an electric blender. The powder was dissolved in methanol and allowed to stand for 24 hours with intermittent shaking ensured. The mixture was first filtered with cheesecloth and the filtrate was filtered again using Whatman filter paper No. 1. The filtrate was then evaporated to dryness using a rotary evaporator under reduced pressure at 40°C. Appropriate concentrations were then diluted in distilled water before being administered throughout the experiment (Moronkeji *et al*. 2024).

Experimental animals: Twenty adult male Wistar rats weighing 150 and 200 g were purchased from UNIMED's animal holding. The rats were maintained in a clean, well-

ventilated environment and given unrestricted access to water and standard rat pellets produced by New Hope Agriculture and Technology, Nigeria. The experimental animals were acclimatised for two weeks and were randomly divided into four groups consisting of seven rats each (n = 7). The first group (Group I) were the unexposed negative control group administered with distilled water only while the second group (Group II) were the diclofenac administered-untreated rats at a dose of 5mg/kg/bw. Rats in groups III and IV received a standard dose of diclofenac, followed by daily treatment with methanolic extract of *C. citratus* at doses of 100 mg/kg BW and 200 mg/kg BW, respectively. The exposure to diclofenac and treatment duration of this administration spanned 28 days and at the end of the experiment, the rats were euthanized and the livers and kidneys were excised and immediately transferred into 10% neutral buffered formalin for histopathological processing for microscopic evaluation. Samples for mRNA expression of TGF-β and Nrf-2 were washed and homogenized in TRIzol for nucleic acid extraction and processed accordingly as described by Molehin *et al.* (2023).

Histological examination: The excised livers and kidneys were fixed in 10% neutral buffered formalin for 48 hours and prepared for paraffin sectioning as described by Adeniyi *et al*., (2023). Thin sections of approximately 4mm thick were stained with Hematoxylin and Eosin (H&E) and the processed organs were then examined under a light microscope for pathological changes (Moronkeji and Akinbo 2024).

mRNA analysis: The RNA was extracted from the tissues using the Zymo Research Quick-RNA MiniPrepTM kit as directed by the manufacturer and gene expression levels were assessed using the RT-PCR method as outlined by Elekofehinti *et al*. (2020). The RNA purification was carried out from 100-200 mg of tissue using TRIZOL reagent (Inqaba Biotech West Africa Ltd), following the manufacturer's instructions (InvitrogenTM, Denmark). The complementary DNA (cDNA) was synthesized using the ProtoScript II First Strand cDNA Synthesis kit (Biolabs, New England), following a 3-step reaction as described by the manufacturer. The PCR for gene expression was conducted using the Luna Mastermix kit (Biolabs, New England) and Taqman kit probes obtained from TibM-01bio (Berlin, Germany), within a thermocycler. The Gel imaging was performed utilizing an electrophoresis gel imager, with β-actin serving as the reference gene. Standard gel electrophoresis was employed as the technique for analyzing the quality and yield of DNA products from the reaction. The cDNA primers utilized were procured from Inqaba Biotech (Hatfield, South Africa). The specific primers employed for PCR are as follows:

TGF-β:
forward (5′-ATACGCCTGAGTGGCTGTCT-3′)
reverse (5′-TGGGACTGATCCCATTGATT-3′).

NRF2:
forward (5′-GGGGAACAGAACAGGAAACA-3′)
reverse (5′-CCGTAATGCACGGCTAAGTT-3′).

β-actin:
forward (5’-CCCGCGAGTACAACCTTCT-3′)
reverse (5’-CGTCATCCATGGCGAACT-3′).

Statistical analysis: The results were pooled and expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) followed by Duncan's multiple tests for post-hoc analysis (DMRT) was employed to analyze the results. The statistical analysis was performed using the Statistical Package for Social Science (SPSS) version 17.0, with a 95% confidence interval ($p < 0.05$) used to determine the significance level.

RESULTS

Histopathological findings: Histopathological evaluation of the H&E-stained sections of the liver of the unexposed control rats appeared normal, with typical hepatocytes with a non-congested central vein or infiltrated sinusoid (Figure 1a). However, the liver of the diclofenac-exposed untreated rats had varying degrees of cytopathic lesions, evidenced by congested venules, and vacuolated hepatocytes with mildly congested sinusoids (Figure 1b). The liver of the *C. citratus* treated rats at 100mg/kg/bw had mildly congested central veins with normal appearing hepatocytes and sinusoids devoid of inflammation and congestion (Figure 1c). The rats treated with *C. citratus* at 200mg/kg/bw had a near normal histoarchitecture with the central vein devoid of congestion with uncongested sinusoids and normal appearing hepatocytes (Figure 1d).

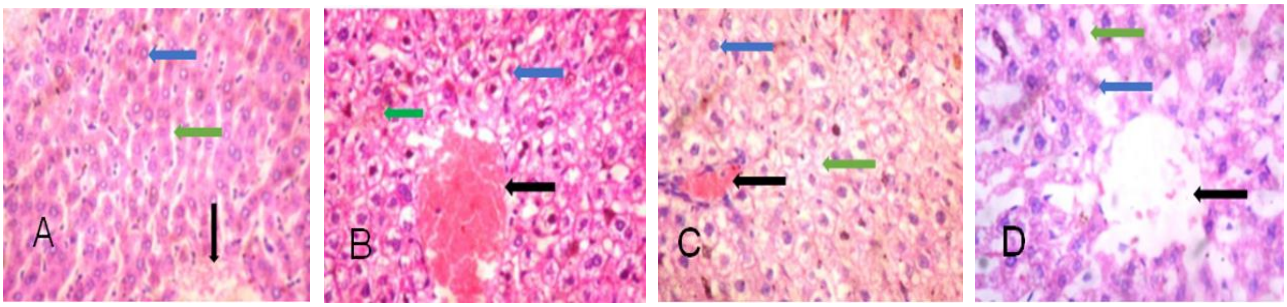

Plate 1
H and E-stained section of the liver of rats across the various groups. **A.** Central vein (black arrow), sinusoids (green arrow) hepatocytes (blue arrow) **B.** Congested venule (black arrow), vacuolated and hepatocytic necrosis (blue arrow), congested sinusoids (green arrow). **C.** Mildly congested central vein (black arrow), hepatocytes (blue arrow), sinusoids (green arrow). **D.** non-congested central vein (black arrow), hepatocytes (blue arrow), sinusoids (green arrow

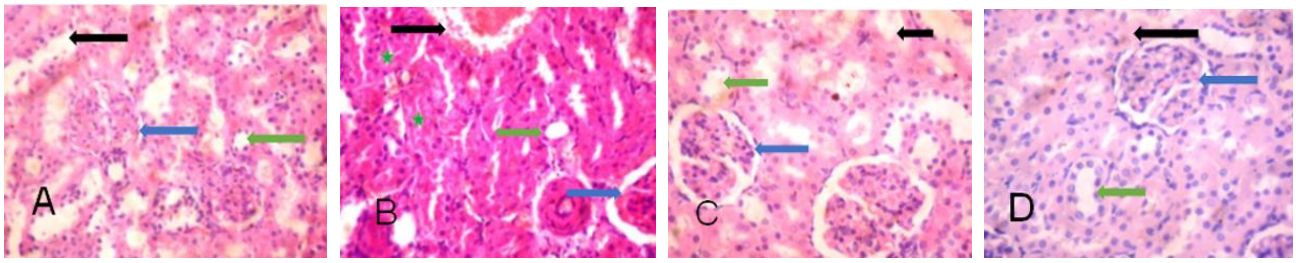

Plate 2.
H and E-stained sections of the kidneys of rats across the various groups. **A** glomerulus (blue arrow), renal tubules (green arrow) interstitial spaces (black arrow). **B.** Glomerulus (blue arrow), renal tubule (green arrow), renal tubule with reduced luminar space (starred green), congested interstitium (black arrow). **C.** glomerulus (blue arrow), renal tubule (green arrow), interstitial space (black arrow). **D.** Glomerulus (blue arrow) renal tubule (green arrow) non-congestion or inflammed interstitium (black arrow).

The kidneys of the unexposed control rats were normal and devoid of any pathological lesion with the glomerulus, renal tubules and interstitial spaces appearing normal (Figure 2a). Diclofenac-exposed untreated rats had congested interstitial spaces with mild tubular degeneration (Figure 2b). The treatment with 100mg/kg/bw of *C. citratus* showed better recovery compared to the diclofenac-exposed untreated rats showing normal glomeruli, and renal tubules with non-congested or inflammed interstitium (Figure 2c). The kidney sections of rats administered with 200mg/kg/BW of *C. citratus* were devoid of cytopathic lesions demonstrating a near normal histoarchitecture as the unexposed negative control rats (Figure 2d).

mRNA expression studies: Figure 3 shows the relative expression levels of TGF-β and Nrf-2 in the liver and kidneys of the NSAIDs-exposed untreated rats relative to the control and other treatment groups. TGF- β being a pleiotropic cytokine was significantly upregulated in the diclofenac-exposed untreated rats when compared to the control and other treatment groups indicating the induction of diclofenac-induced oxidative stress in the liver. However, the treatment with *C. citratus* mitigated the toxicity by repressing the expression levels of TGF-β with treatments at doses of 100mg/kg/bw and 200mg/kg/bw. Furthermore, comparative analysis across the 100mg/kg/bw and 200mg/kg/bw treatments with extracts of *C. citratus* was statistically insignificant (Figure 3a). The Nrf-2 expression pattern in the diclofenac-exposed untreated rats was downregulated when compared to other groups while treatment with *C. citratus* at a dosage of 100mg/kg/bw and 200/mg/kg/bw significantly elevated the expression levels of Nrf-2 relative to the diclofenac-exposed untreated rats indicating a reduction in the diclofenac induced oxidative stress in the kidneys of the treated rats (Figure 3b).

DISCUSSION

Prolonged exposure to NSAIDs has been shown to cause hepatorenal disorders, and inappropriate use of diclofenac can cause damage to the liver and kidneys (Sriuttha *et al*. 2018; Delungahawatta *et al*. 2023; Boggula *et al*., 2023; Liu and Liu 2024). Globally, over 850 million people suffer from kidney disease. Consequently, understanding the complex molecular pathology of kidney disease is crucial for creating novel treatments for renal diseases (Jager *et al*. 2019; Nezu and Suzuki 2020). Studies have demonstrated that the constituents of medicinal plants possess a variety of activity against various disorders and diseases. Additionally, these compounds have been shown to have analgesic, antioxidant, and immunomodulatory properties (Schabauer *et al*., 2017; Adeniyi *et al*., 2024; Moronkeji *et al*. 2024). By comparing the experimental animals treated with methanolic extracts of *C. citratus* to the untreated diclofenac-exposed rats, the histopathological examination in this study revealed remediation of cytopathic lesions associated with diclofenac-induced hepatorenal toxicity. This further provides insights into the protective effects of *Cymbopogon citratus* against diclofenac-induced hepatorenal toxicity. The findings in this study are consistent with previous studies showing that diclofenac caused notable structural alterations in the liver and kidneys of the experimental animals.

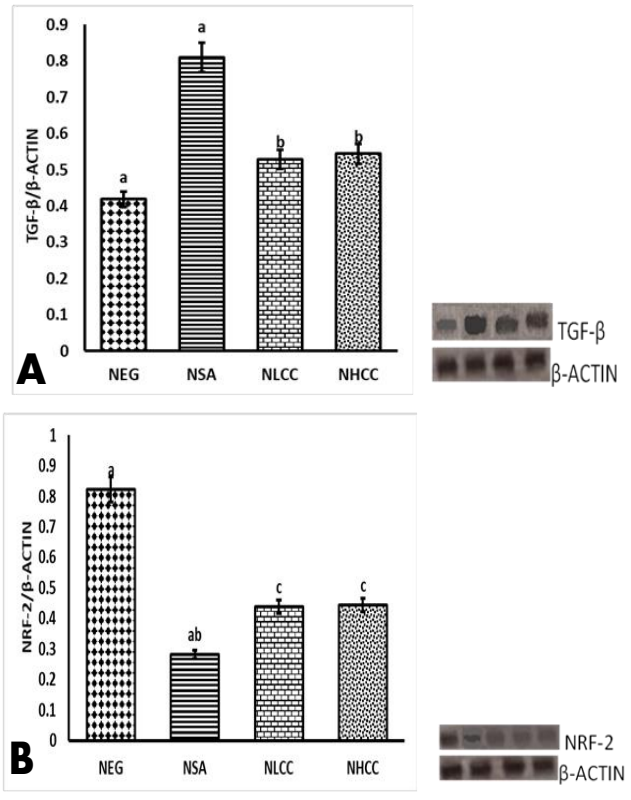

Figure 3.:
mRNA expression pattern of markers in the liver and kidney **a:** expression pattern of TGF-β in the liver. **b:** expression pattern of Nrf-2 in the kidney.
Keys: Neg; unexposed negative control; **NSA;** NSAIDs (diclofenac) exposed untreated rats; **NLCC;** NSAIDs (diclofenac) exposed low *C. citratus* treatment at 100mg/kg/bw; **NHCC:** NSAIDs (diclofenac) exposed high *C. citratus* treatment at 200mg/kg/bw.

Cytopathic lesions included hepatocytic vacuolation, inflamed and congested sinusoids combined with congested venules, periglomerular inflammation, and dilated interstitial spaces were evident in the diclofenac exposed untreated rats (Bindu *et al*. 2020; Delungahawatta *et al*. 2023; Adel *et al*. 2024). Furthermore, earlier studies by Sriuttha *et al*. (2018) had shown that NSAIDs like diclofenac had greater rates of hepatotoxic evidence than other NSAIDs like celecoxib and etoricoxib. However, treatment with *C. citratus* showed a dose-dependent improvement in liver histology, with treatments at 100 and 200 mg/kg/bw considerably ameliorating the lesions induced by diclofenac. Following treatment with 100 mg/kg/BW of *C. citratus*, liver sections exhibited uncongested sinusoids and slightly congested central veins with normal-appearing hepatocytes with better amelioration observed at treatment with 200 mg/kg/BW further corroborating the reports of Saenthaweesuk *et al*. (2017) who demonstrated the hepatoprotective effects of *C. citratus* extract against paracetamol-induced hepatotoxicity. Additional studies have also shown that *C. citratus* protects against NSAID-induced hepatotoxicity, owing to its ability to modulate TNF-α and IL-10 levels (Moronkeji *et al*., 2024). Moreover, studies on *C. citratus* have shown significant antioxidant, hepatorenal protection, and anti-mutagenic efficacy against hepatorenal damage generated by carbon tetrachloride (CCl4) (Fahmy *et al*. 2020; Molehin *et al*. 2023). Lesions typical of tubular degeneration coupled with congested interstitium were seen in the renal histoarchitecture of the untreated rats exposed to diclofenac. These findings are consistent with those of Bindu *et al*. (2020), who reported similar renal damage in NSAID-induced nephrotoxicity characterised by tubular injury and vascular congestion. Jarrar *et al*. (2019) also documented that NSAIDs also caused histological changes including sinusoidal dilatation, apoptotic hepatocytes, renal tubule hydropic degeneration, glomerular atrophy, and renal cell karyopyknosis that could affect organ function. In contrast to the diclofenac exposed untreated rats, treatment with *C. citratus* led to better recovery of the kidney as glomeruli and renal tubules appeared normal with interstitium devoid of inflammation and congestion, exhibiting near-normal histoarchitecture further suggesting a nephroprotective effect of C. citratus, in the context of NSAID-induced nephrotoxicity. The results of this investigation are in line with the general anti-inflammatory and antioxidant properties of *C. citratus* that have been reported by Oladeji *et al*. (2019) and Moronkeji *et al*. (2024). Studies have shown a correlation between NSAID usage and glomerulonephritis and acute kidney injury. NSAID use in the conventional sense is linked to a higher relative risk of nephrotic syndrome and a decrease in kidney haemodynamic functions, such as sodium excretion (Rivosecchi *et al*. 2016; Bakhriansyah *et al*. 2019). Nephrotic syndrome hypersensitivity mechanisms involving NSAIDs are caused by inhibiting prostaglandin synthesis from COX to lipoxygenase pathways, or by releasing lymphokines that raise leukotriene production and activate T-helper lymphocytes, which in turn affect glomerular permeability (Vega *et al*. 2012). The mRNA studies assessed the TGF-β and Nrf-2 expression pattern in NSAID-induced hepatorenal toxicity, offering an additional understanding of the molecular mechanism underlying the protective effects of *C. citratus*. TGF-β expression in the liver was upregulated in the diclofenac-exposed untreated rats, while treatments with *C. citratus* at doses of 100 and 200 mg/kg/bw repressed TGF-β expression levels. As a pleiotropic cytokine, TGF-β is essential for both pro- and anti-inflammatory processes (Sanjabi *et al*. 2017; Deng *et al*. 2024). As previously reported by Laskin *et al*. (2011), its increase in the diclofenac-exposed untreated rats suggests an inflammatory response and an effort at tissue repair via activation of hepatic stellate cells and stimulation of extracellular matrix protein synthesis. Studies have revealed that defects in TGF-β signalling, particularly in immune cells, tissue fibroblasts, and epithelial cells disrupt immunological tolerance, encourage inflammation, and are responsible for the aetiology of fibrosis and cancer which can contribute to the resistance of these conditions to therapy (Massagué and Sheppard, 2023; Deng *et al*. 2024). In the *C. citratus* treatment groups, TGF-β was found to be moderately expressed compared to the unexposed control and the diclofenac-exposed untreated rats. This suggests that TGF-β may contribute to reducing inflammatory responses while still facilitating tissue repair, further highlighting its potential to maintain an equilibrium in the inflammatory response and potentially prevent excessive fibrosis while promoting tissue repair. This is consistent with the report of Massagué and Sheppard (2023) who documented the intricate function of TGF-β in tissue homeostasis and disease. Furthermore, reports by Laskin *et al*. (2011) and Gauthier *et al*. (2023) reported that TGF-β suppressed the production of pro-inflammatory cytokines by activating the transcriptional coactivator SMAD3 and suppressing the activity of pro-inflammatory transcription factors NF-κB, STAT1, and AP-1. The findings in this study demonstrated a decrease in cytopathic lesions following treatment with *C. citratus* at doses of 100 mg/kg BW and 200 mg/kg BW along with moderately expressed TGF-β expression, further indicating the role in inflammation and tissue repair.

Nuclear factor E2-related factor 2 (Nrf2) is an essential transcription factor of cellular defense mechanism against xenobiotics and oxidative stress. It regulates the activation of several cytoprotective genes at both the basal and stress-induced levels (Tonelli *et al*., 2018). This study details the function of *C. citratus* in reducing oxidative damage associated with diclofenac-induced renal toxicity. This is achieved by upregulating the expression of genes related to cryoprotection, which helps mitigate the histoarchitectural distortion observed in the examined organs. The findings in this study are consistent with the reports of Nezu and Suzuki (2020) highlighted the role of Nrf2 in renal toxicity using murine model to emphasize the significance of Nrf2 in renal damage. Comparing the unexposed control group to the diclofenac-exposed untreated rats, it was observed that Nrf2 was significantly repressed while Nrf-2 was upregulated in rats treated with *C. citratus* at the administered doses. This finding is particularly significant when considered in light of a previous study by Ahmed *et al*. (2017) who described Nrf-2 as a crucial transcription factor in regulating cellular defense against oxidative stress and inflammation. Although, clinical research is still ongoing to develop therapies to treat kidney diseases with Nrf2 activators that block Keap1 (Kelch-like ECH-

associated protein 1), which represses Nrf2 in the absence of oxidative stress (Pergola *et al*. 2011; de Zeeuw *et al*. 2013; Nezu, 2017; Yamamoto *et al*., 2018; Ito *et al*., 2020). The repression of Nrf2 observed in the diclofenac-exposed untreated rats indicates compromised antioxidant defences, which is consistent with the known mechanisms of NSAID-induced toxicity involving oxidative stress (Stiller and Hjemdahl 2022; Delungahawatta *et al*. 2023). Studies using Knockout mice models have highlighted the protective roles of Nrf2 in various disease models such as respiratory disease (Cho *et al*. 2013; Eba *et al*. 2013; Sussan *et al*. 2015; Nagashima *et al*. 2019) cardiac disease (Katsumata *et al*. 2014), neurological disease (Linker *et al*. 2011; Uruno *et al*. 2020), diabetes mellitus (Yagishita *et al*. 2014; Yagishita *et al*. 2017; Yagishita *et al*. 2019), inflammation (Itoh *et al*. 2004; Maicas *et al*. 2011), liver disease (Taguchi *et al*. 2019; Okada *et al*. 2013) and sensory organ diseases (Knatko *et al*. 2015; Xu *et al*. 2014). Furthermore, studies indicate that the loss of Nrf2 exacerbates tissue damage and fibrosis in kidney disease models, including toxic injury, ureteral obstruction and podocyte injury (Tanaka *et al*. 2008; Tan *et al*. 2016). These findings suggest that renal damage may be prevented by quenching oxidative stress through Nrf2 activation in tubules, which may be a potential cause of kidney disease. According to reports, systemic Nrf2 activation in Keap1 hypomorphic mutant (Keap1 knockdown; Keap1-KD) significantly attenuates 8-hydroxydeoxyguanosine (8-OHdG) mitigating kidney injury progression relative to wild-type mice (Nezu *et al*. 2017). Additionally, Nrf2 functions as an integrator, promoting the expression of genes encoding antioxidant-producing enzymes like glutathione and NADPH and lowering pro-oxidants such as heme and quinonoids further protecting cells from oxidative damage (Yamamoto *et al*., 2018; Nezu, Suzuki, and Yamamoto 2017). Studies and clinical trials involving chemical compounds that activate Nrf2 in rats exposed to a toxic injury model have documented the beneficial role of Nrf2 activation in treating kidney disease. Compounds such as curcumin, dimethyl fumarate, farrerol, and sulforaphane have been shown to improve the outcome of toxic injury-induced kidney disease (Zhao *et al*. 2016; Wu *et al*. 2017; Li *et al*. 2019; Jin *et al*. 2020; Ma *et al*. 2019). In this study, the upregulation of Nrf-2 in the *C. citratus* treatment groups at 100mg/kg/bw and 200mg/kg/bw further suggests enhanced antioxidant mechanisms, potentially contributing to the protective effects against diclofenac-induced toxicity. Further evidence that maximal protection can be obtained at less toxic doses comes from our observation that the experimental animal treated with *C. citratus* at a lower dosage of 100mg/kg/bw experienced the same immunomodulatory effect on Nrf-2 as did the animal treated with a higher dose of 200mg/kg/bw. A possible mechanism and antioxidant properties of *C. citratus* for the observed nephroprotective effects as observed in this study further agree with the reports of Oladeji *et al*. (2019). Previous reports by Ma, (2013) and Guerrero-Hue *et al*. (2021) reported the protective function of Nrf2 in cellular defence against toxic and oxidative stress and renal disease.

In conclusion, *Cymbopogon citratus* demonstrated significant hepatorenal protective properties by reducing the damage induced by diclofenac. Studies on mRNA expression of the plant have also supported the anti-inflammatory potential of *C. citratus* by repressing the expression of TGF-β while upregulating Nrf-2 levels in the treated rats. Furthermore, the methanolic extracts of *C. citratus* can serve as TGF-β and Nrf2 activators and can be harnessed for proper management in hepatorenal disease treatment. This study further establishes the cytoprotective role of TGF-β and Nrf2 pathways and unveils the regulatory mechanism of their activities in response to diclofenac-induced hepatorenal toxicity. It is further suggested that caution needs to be exercised when NSAIDs are to be administered which should be limited to the lowest therapeutic doses, to prevent its harmful effect.

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