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Original scientific paper

COMPARATIVE EFFECTS OF CIPROFLOXACIN AND PREDNISOLONE ON RENAL FUNCTION MARKERS IN A TNBS-INDUCED CROHN'S DISEASE RAT MODEL

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ABSTRACT

Crohn's disease (CD) is a chronic inflammatory bowel disorder characterized by mucosal inflammation and systemic complications, including renal impairment. This study investigated the comparative effects of ciprofloxacin and prednisolone on renal function markers, electrolyte balance, oxidative stress, and renal histoarchitecture in a trinitrobenzene sulfonic acid (TNBS)-induced Crohn's disease rat model. Forty male rats were randomly assigned into four groups (n = 10 per group): Group A (normal control), Group B (TNBS-induced CD), Group C (CD + ciprofloxacin), Group D (CD + prednisolone). Serum creatinine, urea, and electrolyte levels (Na^+, K^+, Cl^-) were measured, alongside kidney malondialdehyde (MDA) levels as a marker of oxidative stress. Histopathological changes were assessed via hematoxylin and eosin staining. TNBS induction showed significant increases in serum creatinine (0.40±0.01 to 6.27±0.66 mg/dL), urea (16.67±0.88 to 45.33±1.55 mg/dL), kidney malondialdehyde (MDA) levels (0.03±0.014 to 1.28±0.04 µmol/mL), and significant decrease in electrolytes; sodium (139.67±2.33 to 84.34±1.49 mmol/L) and chloride (100.33±2.33 to 54.66±1.70 mmol/L) when compared to the control ($p < 0.05$). Treatment with ciprofloxacin and prednisolone significantly improved these parameters, although prednisolone produced greater reductions in creatinine (1.30±0.02 vs 5.90±0.03 mg/dL), urea (23.33±0.97 vs 37.53±1.18 mg/dL), and MDA (0.17±0.03 vs 0.51±0.03 µmol/mL), alongside more substantial restoration of sodium and chloride levels when compared with ciprofloxacin ($p < 0.05$). Histological analysis showed partial preservation of glomerular and tubular structures with ciprofloxacin and more pronounced architectural protection with prednisolone. Both agents attenuated TNBS-induced renal injury, but prednisolone demonstrated superior renoprotective effects, likely due to stronger suppression of inflammation and oxidative stress.

Keywords: Crohn's disease, ciprofloxacin, prednisolone, renal function, oxidative stress

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INTRODUCTION

Crohn's disease (CD) is a chronic inflammatory bowel disease (IBD) characterized by transmural inflammation of the gastrointestinal tract, often leading to systemic complications, including renal dysfunction (ALFREDSSON and WICK, 2020). The global prevalence of CD continues to rise, with significant increases reported across Europe, North America, Asia, and Africa, underscoring its growing public health relevance (NG et al., 2017). The disease is driven by a complex interplay of genetic susceptibility, dysregulated immune responses, impaired epithelial barrier function, and alterations in the intestinal microbiota. Persistent mucosal injury and immune activation lead not only to gastrointestinal manifestations but also to extraintestinal complications that significantly worsen patient outcomes. Among these complications, renal impairment has gained increasing attention, although it remains underexplored relative to other systemic effects.

Renal dysfunction in CD arises from multiple mechanisms, including chronic inflammation, immune-mediated injury, dehydration due to severe diarrhea, nephrolithiasis, and the nephrotoxic effects of certain medications (AMBRUZS and LARSEN, 2018). Inflammatory cytokines such as TNF-α, IL-1β, and IL-6, which are elevated in active CD, have been implicated in glomerular and tubular injury through promotion of oxidative stress and endothelial dysfunction (GUAN and ZHANG, 2017). Recent reports indicate that oxidative stress contributes significantly to renal damage in inflammatory bowel disease, as reactive oxygen and nitrogen species drive lipid peroxidation, protein modification, and structural distortion of renal tissue (TIAN et al., 2017, REZAIE et al., 2007). Emerging evidence also suggests that CD-associated inflammation and oxidative stress contribute to kidney damage, manifesting as altered renal biomarkers (e.g., elevated creatinine, urea) and histopathological changes (DE SIRE *et al.,* 2024).

Pharmacologic management of CD traditionally relies on corticosteroids, immunomodulators, and biological agents, each with distinct benefits and limitations. Prednisolone remains a widely used corticosteroid because of its potent ability to suppress inflammatory cytokines and leukocyte infiltration. However, prolonged steroid exposure is associated with adverse renal effects, including fluid imbalance, hypertension, and direct tubular toxicity (YASIR et al., 2023). In contrast, ciprofloxacin, a fluoroquinolone antibiotic, has been shown to reduce intestinal inflammation by altering gut microbiota composition, limiting bacterial translocation, and attenuating endotoxin-driven immune activation (NITZAN et al., 2016). Although ciprofloxacin is frequently used as adjunct therapy in IBD, its potential renoprotective properties in the context of CD-related oxidative stress have not been clearly defined. This knowledge gap provides a compelling rationale for comparative evaluation of both agents.

The trinitrobenzene sulfonic acid (TNBS) model of chronic colitis provides a reliable experimental platform for studying CD-like pathology. TNBS triggers a Th1-mediated immune response, mucosal ulceration, transmural inflammation, and systemic oxidative stress that closely resemble human disease (SILVA et al., 2019, LOEUILLARD et al., 2014). Because TNBS exposure is known to induce extraintestinal oxidative injury, including renal involvement, this model is suitable for assessing the interplay between intestinal inflammation and kidney damage. This present study investigates the comparative effects of ciprofloxacin and prednisolone on renal function biomarkers, electrolyte balance, oxidative stress indices, and renal histoarchitecture in TNBS-induced CD with the aim to determine whether ciprofloxacin offers renoprotective benefits comparable to or greater than prednisolone, thereby contributing to therapeutic optimization for patients with CD who are at risk of renal complications.

MATERIALS AND METHODS

Forty adult male Sprague-Dawley rats seven weeks old (weight= 180 - 200g) obtained from Animal House of the Olabisi Onabanjo University, Sagamu Campus, Ogun State, Nigeria were housed in a standard environment condition with 12 hours light/dark cycle and air filtration at a constant temperature and humidity, fed on standard died. Animals have free access to water and feed. Experimental protocols met the Guideline of Animal Experimentation approved by Olabisi Onabanjo University Teaching Hospital Human Research Ethics Committee (approval number: OOUTH/HREC/746/2023AP). The animals were divided into four groups of ten rats each:

Group A: Normal control group;
Group B: Crohn's Disease group;
Group C: Ciprofloxacin-treated group;
Group D: Prednisolone-treated group.

Induction of Crohn's Disease

The induction of Crohn's disease was done following the method of LOEUILLARD *et al.*, (2014). After 24 hours fast, the rats were anesthetized using 1.75 mL ketamine (1 g/mL) and 0.25 mL Xylazine (1 g/mL) intra-peritoneal prior to induction. Crohn's disease was induced by weekly administration of increasing dose of TNBS (15, 30, 45, 60, 60 and 60 mg) transrectally for 6 weeks. After instillation of TNBS, the rats were maintained in a head-down position for a few minutes to prevent leakage of the intracolonic instillate.

Administration of Ciprofloxacin and Prednisolone

100 mg of ciprofloxacin (Ratnamani Healthcare PVT. LTD., India) was dissolved in 2 mL of distilled water to give a solution of 50 mg/mL, and 50 mg/kg of ciprofloxacin was administered every 72 hours for forty-two days orally (PARASHURAM *et al*., 2017). 10 mg of prednisolone (Jiangsu Penyao Pharmaceutical Co. Ltd., China) was dissolved in 2.5 mL of distilled water to give a solution of 4 mg/mL, 4 mg/kg of prednisolone was administered every 72 hours for forty-two days orally (ABUOHASHISH *et al.,* 2023).

Kidney Function Biomarkers (Urea, Creatinine) Determination

Blood samples were collected from rat and centrifuged after coagulation at 3000 rpm for 10 minutes to separate the serum. Urea levels were measured using a commercially available kit (Urea Assay Kit, Abcam, UK) according to the manufacturer's instructions. The kit uses a colorimetric method to detect urea levels. Creatinine levels were measured using a commercially available kit (Creatinine Assay Kit, Cayman Chemical, USA) according to the manufacturer's instructions. The kit uses a colorimetric method to detect creatinine levels. The assays were calibrated, and quality control samples were run before and after each batch of samples to ensure accuracy and precision.

Blood Electrolyte Determination

Blood electrolyte levels (sodium, potassium, and chlorine) were determined using an automated electrolyte analyzer (Roche Cobas 6000). The analysis was performed according to the manufacturer's instructions. Blood samples were collected in tubes containing anticoagulants and centrifuged at 3000 rpm for 10 minutes to separate the plasma. The plasma samples were then analyzed using an automated electrolyte analyzer (Roche Cobas 6000), which uses ion-selective electrodes to measure the levels of sodium, potassium, and chlorine.

The analyzer was calibrated, and quality control samples were run before and after each batch of samples to ensure accuracy and precision.

Determination of Malondialehyde Activity (Lipid Peroxidation)

0.1 g of kidney tissue was homogenized in 4 mL ice cold phosphate buffer (pH 7.2) then centrifuged (at 3000 x g, for 10 min) and supernatant collected. One mL of tissue homogenate was combined with 2 mL of TCA-TBA-HCL and mixed thoroughly. The solution was heated for 15 minutes in a boiling water bath. After cooling, the flourescent precipitate was removed by centrifugation (at 1000 x g for 10 mins). The absorbance of the sample was determined at 535 nm against a blank that contains all the reagents minus the sample. The malondialdehyde concentration of the sample was calculated using an extinction coefficient of 1.56 $x10^5$ reciprocal molar centimeter. Calculation of lipid peroxidation

$$\text{MDA(nmol/mL)} = \boldsymbol{OD} \sum \times \frac{\boldsymbol{V}}{\boldsymbol{v}} \quad (1)$$

OD = Absorbance (optical density) of sample;
$\sum$ = Molar extinction coefficient;
V = Total volume of the reacting sample;
v = Volume of the sample.

Procedure for Histological Study

For histological analysis, tissue samples (kidney) were fixed in 10% neutral buffered formalin (NBF) for 24-48 hours, followed by dehydration in a series of ethanol solutions (70%, 80%, 90%, and 100%) and clearing in xylene. The tissues were then embedded in paraffin wax, sectioned into 5-µm thick slices using a microtome, and deparaffinized in xylene. The sections were rehydrated in a series of ethanol solutions, stained with Harris' hematoxylin solution for 5-10 minutes, and then stained with eosin Y solution for 1-2 minutes. After dehydration and clearing, the sections were mounted on glass slides using a mounting medium (DPX) and examined under a light microscope to observe tissue morphology and architecture.

Statistical analysis

All the values are expressed as mean ± standard error of mean (SEM). Analysis of data was done using graph pad prism version 8 for Windows. Differences between groups were analyzed by one-way ANOVA followed by Bonferroni post-hoc test. Differences were considered significant when $p < 0.05$.

RESULTS

Results presented in Table 1 showed a significant increase in serum creatinine and urea level in group B when compared with group A respectively. Ciprofloxacin treated group C and prednisolone treated group D showed a significant decrease in serum creatinine and urea level when compared with group B respectively. However, there was a significant decrease in serum creatinine and urea level in group D when compared with group C.

Results presented in Table 2 showed a significant decrease in blood electrolytes (Na^+, K^+, Cl^-) level in group B when compared with group A respectively. Ciprofloxacin treated group C and prednisolone treated group D showed a significant increase in blood electrolytes (Na^+ and Cl^-) level when compared with group B respectively. However, there was a significant increase in Na^+ and Cl^- level in group D when compared with group C.

Results presented in Table 3 showed a significant increase in kidney MDA level in group B when compared with group A respectively. Ciprofloxacin treated group C and prednisolone treated group D showed a significant decrease in kidney MDA level when compared with group B respectively. However, there was a significant decrease in kidney MDA level in group D when compared with group C.

Table 1: Comparative Effects of Ciprofloxacin and Prednisolone on Serum Urea and Creatinine Level in a TNBS-Induced Crohn's Disease Rat Model.

Groups	Creatinine (mg/dL)	Urea (mg/dL)
A	0.40±0.01	16.67±0.88
B	6.27±0.66^{a}	45.33±1.55^{a}
C	5.90±0.03^{ab}	37.53±1.18^{ab}
D	1.30±0.02^{abc}	23.33±0.97^{abc}

Each value is an expression of mean ± SEM (P<0.05)
a, b, c = values were significant when compared to group A, B and C respectively.

Table 2: Comparative Effects of Ciprofloxacin and Prednisolone on blood electrolyte in a TNBS-Induced Crohn's Disease Rat Model.

Groups	Na^{+} (mmol/l)	K^{+} (mmol/l)	Cl^{-} (mmol/l)
A	139.67±2.33	8.36±0.63	100.33±2.33
B	84.34± 1.49^{a}	3.14±0.02^{a}	54.66±1.70^{a}
C	102.00±2.13^{ab}	4.23±0.02^{a}	73.00±1.33^{ab}
D	124.00±2.18^{abc}	4.53±0.03^{a}	99.00±1.58^{bc}

Each value is an expression of mean ± SEM (P<0.05)
a, b, c = values were significant when compared to group A, B and C respectively.

Table 3: Comparative Effects of Ciprofloxacin and Prednisolone on oxidative stress marker (malondialdehyde, MDA) in a TNBS-Induced Crohn's Disease Rat Model.

Groups	MDA (µmol/ml)
A	0.03±0.014
B	1.28±0.04^{a}
C	0.51±0.03^{ab}
D	0.17±0.03^{ab}

Each value is an expression of mean ± SEM (P<0.05)
a, b, c = values were significant when compared to group A, B and C respectively.

In figure 1, the control group demonstrates a well-differentiated glomerulus, a distinct capsular space, and intact proximal and distal convoluted tubules lined with simple squamous epithelial cells. In contrast, the TNBS group exhibits thickened and constricted tubules, glomerular distortion and epithelial cell loss. Treatment with ciprofloxacin shows partial improvement, although thickened tubular walls, a distorted glomerulus and reduced epithelial cell populations are still evident. Prednisolone treatment results in comparatively better preservation of renal structures, showing constricted but identifiable tubules, a slightly distorted glomerulus, and a more prominent capsular space, although epithelial cell reduction persists.

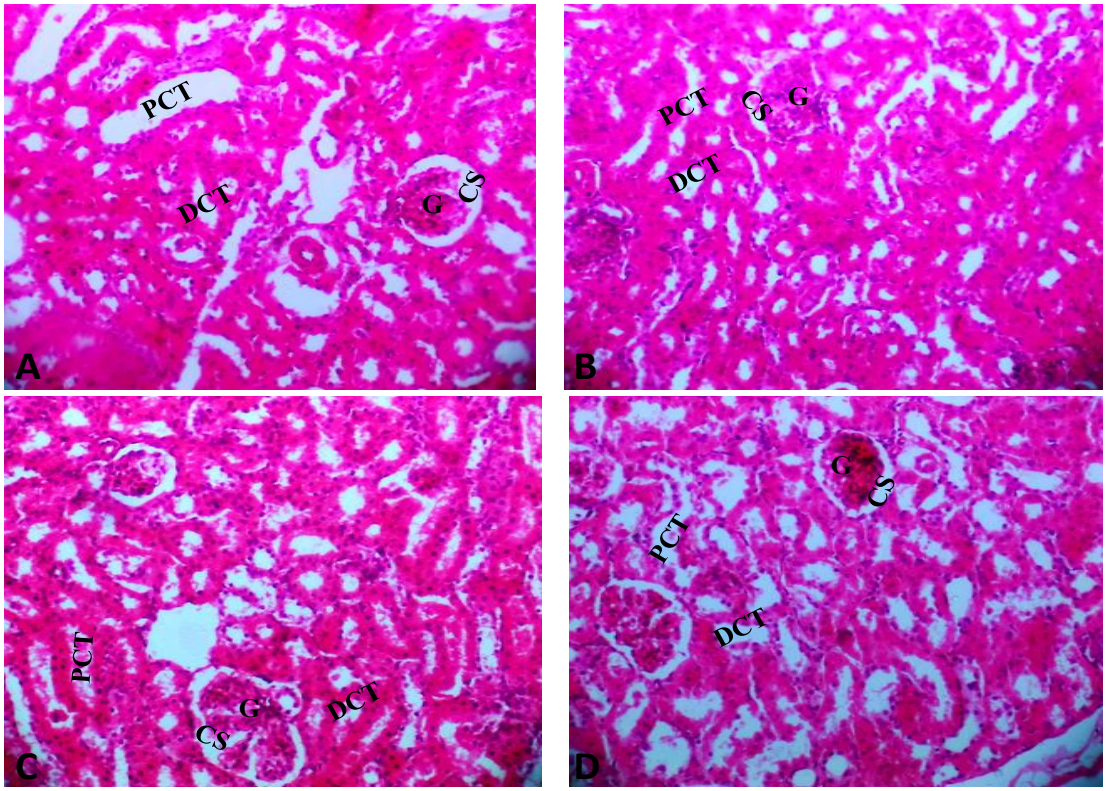

Figure 1: Comparative Effects of Ciprofloxacin and Prednisolone on kidney tissue histology in a TNBS-Induced Crohn's Disease Rat Model

(**A** - showed well-differentiated glomerulus (G), distinct capsular space (CS), proximal (PCT) and distal convoluted tubules (DCT) lined with simple squamous epithelial cells (red arrow). **B** - showed thickened and constricted PCT, DCT, glomerulus (G) with epithelial cell loss, and capsular space (CS). **C** - showed thickened walls of PCT and DCT, distorted glomerulus (G), capsular space (CS), and reduced epithelial cells. **D** - showed constricted PCT and DCT, slightly distorted glomerulus (G), dilated capsular space (CS) with reduced epithelial cells. H/E X 400).

DISCUSSION

In this study, TNBS-induced Crohn's disease caused substantial increases in serum creatinine and urea. These changes align with reports that intestinal inflammation contributes to extraintestinal organ injury through systemic immune activation and microbial translocation (De Sire *et al.,* 2024; Ambruzs and Larsen, 2018). Both ciprofloxacin and prednisolone reduced these biomarkers (serum creatinine and urea level), which may indicate improved renal clearance. Ciprofloxacin likely exerted its effect by lowering pathogenic gut bacterial load and reducing endotoxin-driven systemic inflammation (Sartor, 2004), while prednisolone's stronger effect can be attributed to its direct suppression of cytokine-mediated renal injury (Guan and Zhang, 2017; Alfredsson and Wick, 2020). The greater biochemical improvement observed with prednisolone aligns with its established role as a potent modulator of inflammatory signaling in IBD (Yasir *et al.,* 2023).

Electrolyte abnormalities in the TNBS group, particularly hyponatremia and hypochloremia, likely reflect intestinal fluid loss, impaired mucosal absorption, and inflammation-induced tubular dysfunction. Significant increase in sodium and chloride levels following treatment may suggest attenuation of intestinal inflammation and improved renal tubular handling. Ciprofloxacin's partial correction of electrolyte imbalance is consistent with its microbiota-modulating activity, which reduces inflammatory mediators that impair absorption and renal regulation (Park *et al.*, 2024). Prednisolone produced more complete normalization, consistent with its ability to reduce tubular interstitial inflammation and

edema, processes shown to interfere with electrolyte transport in inflammatory conditions (AMBRUZS and LARSEN, 2018; YASIR *et al.,* 2023).

Oxidative stress was evident from increased MDA levels in untreated TNBS-exposed animals, confirming significant lipid peroxidation associated with inflammatory injury. This pattern mirrors previous observations in Inflammatory Bowel Disease (IBD) models where reactive oxygen species generated by activated neutrophils and macrophages contribute to renal oxidative damage (REZAIE *et al.,* 2007; TIAN *et al.,* 2017). Both treatments lowered MDA levels, although prednisolone demonstrated a more pronounced reduction, reflecting its potent anti-inflammatory effect on cytokine pathways that drive ROS production. Ciprofloxacin's reduction in MDA likely arises from diminished microbial translocation and subsequent attenuation of immune activation. While MDA is a valid oxidative stress index, broader profiling using nitric oxide metabolites, myeloperoxidase activity, and antioxidant enzymes would provide a more complete assessment of redox balance.

Histological evaluation supported the biochemical findings. TNBS exposure resulted in glomerular distortion, tubular constriction, epithelial loss, and disrupted capsular space, all consistent with inflammatory and degenerative renal injury. Ciprofloxacin treatment partially preserved epithelial continuity and glomerular structure, reflecting reduced bacterial-driven immune activation (NITZAN *et al.,* 2016; SILVA *et al.,* 2019). Prednisolone produced more substantial improvements, including better preservation of glomerular and tubular architecture, which correspond with its ability to suppress leukocyte infiltration and limit structural damage associated with chronic inflammation (GUAN and ZHANG, 2017; YASIR *et al*., 2023).

CONCLUSION

This study indicates that TNBS-induced Crohn's disease causes significant renal dysfunction through inflammatory, oxidative, and structural mechanisms. Both ciprofloxacin and prednisolone offer renoprotective benefits, but prednisolone demonstrated superior efficacy in restoring renal function markers, correcting electrolyte imbalance, and reducing oxidative stress, likely due to its potent anti-inflammatory action.

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