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Curcumin prevents tenofovir/lamivudine/efavirenz-induced nephrotoxicity in rats

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ABSTRACT

Background and Aims: Nephrotoxicity is an adverse effect, which may occur with the use of tenofovir/lamivudine/efavirenz (TLE) in the treatment of human immunodeficiency virus (HIV) infection. Curcumin (CUM), an isolate of *Curcuma longa* L. is used in folk medicine for the treatment of ailments. This study attempts to establish whether CUM supplementation can protect against a rat model of TLE-induced nephrotoxicity.

Materials and Methods: Adult male Wistar rats (n=40) were randomly grouped and supplemented orally with CUM (50, 100 and 200 mg/kg/day) prior to the oral administration of TLE (300/300/600 mg/kg/day) for 30 days. After the treatment, the rats were fasted overnight, weighed and anesthetized. Blood samples were collected, and sera were extracted for biochemical analyses. Kidney samples were excised, weighed and processed for oxidative stress markers and histology.

Results: Body weight was decreased (p<0.01) whereas kidney weight was increased (p<0.01) in TLE administered rats when compared to the control. Significant (p<0.001) increments in serum uric acid, creatinine, urea and kidney malondialdehyde levels were observed in TLE-administered rats. Significant (p<0.001) decreases in serum total protein, albumin, electrolytes, kidney superoxide dismutase, glutathione, catalase and glutathione peroxidase levels were observed in TLE administered rats when compared to the control. TLE produced tubular necrosis and hypercellular glomerulus with mesangial proliferation in the kidneys of treated rats. However, CUM (50, 100 and 200 mg/kg) supplementation abrogates TLE-induced nephrotoxicity in a dose-related manner at p<0.05, p<0.01 and p<0.001, respectively, when compared to TLE group.

Conclusion: CUM seems effective against TLE-induced nephrotoxicity.

Keywords: Antiretroviral, curcumin, nephrotoxicity, rat

INTRODUCTION

Highly active antiretroviral therapy (HAART) consists of three drugs active against human immununodeficiency virus (HIV) infection. HAART has significantly decreased HIV progression and prevents HIV-related infections. The success of HAART reflects on the reductions of HIV related morbidity and mortality in the world (Palella et al., 1998; Montaner et al., 2010). However, the use of HAART has been associated with a myriad of toxicities, especially nephrotoxicity. Acute kidney injury, tubulopathies, chronic kidney disease, and end-stage renal disease requiring renal replacement therapy have been documented with the use of HAART (Kalyesubula & Perazella, 2016).

Tenofovir -lamivudine -efavirenz (TLE) is an integral part of the preferred first-line regimens for the treatment of HIV in adolescents and antiretroviral-naive adults especially in resource-limited settings (WHO, 2013). It has reduced the incidence of HIV related death, but the incidence of nephrotoxicity attributed to its tenofovir component, which can be aggravated by partner drugs is a worrisome challenge. An incidence of 17–22% of tubular dysfunction was observed in tenofovir containing regi-

mens. Also, an incidence of 18.3% moderate renal impairment and 2.3% severe renal impairment were documented with the use of tenofovir containing regimens (Nartey et al., 2019). The primary clinical presentations of nephrotoxicity caused by tenofovir include proximal tubular dysfunction, electrolytes and acid-base disorder (Perazella, 2010). Kidney histological aberrations include chronic tubular-interstitial scarring, necrosis, tubular atrophy and interstitial fibrosis (Herlitz et al., 2010).

Curcumin (diferuloyl methane) is a low-molecular weight compound extracted from the roots of *Curcuma longa* L. (Zingiberaceae). It is traditionally used for centuries in Asia and other parts of the world for medicinal and culinary purposes. CUM has a diverse and wide range of targets at molecular and cellular levels (Noorafshan & Ashkani-Esfahani, 2013). Substantial number of *in-vitro* and *in-vivo* studies showed that it has essential pharmacological activities including anti-inflammatory, antispasmodic, antioxidant, anti-cancer, and antimicrobial effects (Akram et al., 2010). In folk medicine, it is used as treatments for sprains, liver disorders, anorexia, rheumatism, diabetes, cough, sinusitis and inflammation (Noorafshan & Ashkani-Esfahani, 2013). It has redox regulatory effects such as scavenging of free radicals and increased antioxidant activities (Hewlings & Kalman, 2007). Its anti-inflammatory activity has been characterized by reduction in pro-inflammatory cytokines including tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6) and the inhibition of NF-kappa B pathway (Noorafshan & Ashkani-Esfahani, 2013). CUM has been shown to have potential protective effect on experimentally induced toxicities including hepatotoxicity (Farghaly & Hussein, 2010) cardiotoxicity (Mohantya et al., 2004) and nephrotoxicity (El-Zawahry & Abu El Kheir, 2007). This study examined its protective activity against TLE-induced nephrotoxicity in Wistar rats, which is a novel study.

MATERIALS AND METHODS

Animals, drugs, chemicals and treatment
Tenofovir disoproxil fumarate/lamivudine/ efavirenz (TLE), *Curcuma longa* (CUM), and piperine were used.

Adult male Wistar rats (n=40) were randomly grouped into 8 of n=5/group and used. The rats were acclimated for 2 weeks in cages in a standardized condition (12 h light/day cycles, 25°C±5°C) with *ad libitum* access to food and water. The rats were purchased from the animal research unit of the Department of Pharmacology, Faculty of Basic Clinical Sciences, University of Port Harcourt, Rivers State, Nigeria. The guideline (2020/569/*EU)* on animal handling prepared by European Parliament and of the Council was used for this study. Ethical approval for this study (NDU/PHARM/PCO/AEC/064B) was provided by the Research Ethics Committee of the Department of Pharmacology/Toxicology, Faculty of Pharmacy, Niger Delta University on 4 August 2020.

Animal treatment
Group 1 (Control) received normal saline (0.2 mL/day/p.o). Groups 2-4 received CUM (50, 100 and 200 mg/kg/day/p.o) (Lee et al., 2016). Group 5 received TLE (300/300/600 mg/kg/day/p.o). Groups 6-8 received CUM (50, 100 and 200 mg/kg/day/p.o) before receiving TLE (300/300/600 mg/kg/day/p.o).

Piperine (20 mg/kg/p.o) was added to CUM to improve bioavailability (Shoba et al., 1998). All the rats were treated for 30 days. After the treatment, the rats were allowed to fast overnight, weighed and anesthetized through inhalation in a chamber of diethyl ether. Blood samples were collected from the heart, centrifuged (1500 rpm for 20 min) and sera were extracted for biochemical assessments. Kidney samples were collected, rinsed in cold saline and homogenized in 0.1 M Tris-HCl solution buffered (pH 7.4). The homogenates were centrifuged (3000 rpm for 15 min), supernatants decanted and assayed for oxidative stress markers.

Assessment of serum biochemical markers
Serum total protein, creatinine, albumin, uric acid, urea, sodium, bicarbonate potassium, and chloride and concentrations were measured using laboratory test kits.

Assessment of kidney oxidative stress markers
Malondialdehyde (MDA) was assayed as reported by Buege & Aust, 1978. Reduced glutathione (GSH) was assayed using the method reported by Sedlak & Lindsay, 1968. Catalase (CAT) was assayed as described by Aebi, 1984. Glutathione peroxidase (GPx) was measured according to Rotruck et al., 1973. Superoxide dismutase (SOD) was assessed as reported by Sun & Zigman, 1978.

Histological analysis
Kidney samples were collected, blotted and fixed in 10% buffered formaldehyde. Kidney samples were processed and embedded in paraffin. Sections (3 μm in thickness) were produced on slides and stained with hematoxylin and eosin (H&E). Stained sections were assessed for histological changes using a light microscope.

Statistical analysis
Mean±standard error of mean (SEM) for the results for all groups (n=5) was determined. Data was analyzed using one-way analysis of variance (ANOVA) followed by Tukey's *posthoc test*. Graph Pad Prism 5 Software (San Diego, CA USA) was used for data analysis. Significance was set at $p<0.05$, $p<0.01$ and $p<0.001$.

RESULTS

Effects of curcumin on body and kidney weights of tenofovir/lamivudine/efavirenz -treated rats
The effects of CUM on body and kidney weights were not significant ($p>0.05$) when compared to control (Table 1). The administration of TLE produced a significant ($p<0.01$) decrease in body weight with a significant increase in kidney weight ($p<0.01$) when compared to control (Table 1). However, body and kidney weights were restored in CUM (50, 100, and 200 mg/kg) supplemented rats at $p<0.05$, $p<0.01$, and $p<0.01$, respectively when compared to TLE group (Table 1).

Effect of curcumin on serum kidney function markers of tenofovir/lamivudine/efavirenz -treated rats
The administration of CUM had no significant ($p>0.05$) effects on serum total protein, potassium, chloride, sodium, bicarbonate, albumin, uric acid, creatinine and urea levels when

compared to control (Table 2) (Figures 1-5). In TLE administered rats, serum total protein, potassium, chloride, sodium, bicarbonate and albumin levels were decreased significantly (p<0.001) whereas uric acid, creatinine and urea levels were increased significantly (p<0.001) when compared to control (Table 2) (Figures 1-5). However, CUM (50, 100, and 200 mg/kg)

Table 1. Effects of curcumin on body and kidney weights of tenofovir/lamivudine/efavirenz -treated rats.

Dose (mg/kg)	FBW (g)	AKW(g)	RKW (%)
Control	255.8±17.7	0.65±0.06	0.25±0.06
CUM 50	250.1±15.1	0.67±0.01	0.27±0.03
CUM 100	252.7±13.0	0.63±0.09	0.25±0.01
CUM 200	250.2±15.9	0.66±0.04	0.26±0.06
TLE	161.5±17.6#	1.99±0.03#	1.23±0.09#
CUM 50 + TLE	180.3±16.6	1.70±0.02	0.94±0.07*
CUM 100 + TLE	200.7±16.1*	1.16±0.06*	0.58±0.05**
CUM 200 + TLE	250.8±18.6**	0.70±0.08**	0.28±0.01***

CUM: Curcumin, TLE: Tenofovir/lamivudine/efavirenz, FBW: Final body weight, AKW: Absolute kidney weight, RKW: Relative kidney weight, Data as mean±SEM, (Standard error of mean), n=5, #p<0.01 Significant differnce when compared to control, *p<0.05, **p<0.01, ***p<0.001 Significant difference when compared to TLE.

Table 2. Effect of curcumin on serum electrolytes of tenofovir/lamivudine/efavirenz-treated rats.

Dose (mg/kg)	Potassium (mmo/L)	Chloride (mmo/L)	Sodium (mmo/L)	Bicarbonate (mmo/L)
Control	3.70±0.19	111.02±12.0	125.63±11.3	12.86±1.45
CUM 50	3.71±0.04	112.11±13.9	126.16±12.0	12.67±2.17
CUM 100	3.73±0.30	114.12±10.7	128.87±13.2	12.52±3.63
CUM 200	3.78±0.06	116.76±12.8	129.74±14.8	12.35±2.33
TLE	1.57±0.17$^{\pi}$	48.10±5.57$^{\pi}$	48.03±4.66$^{\pi}$	5.00±0.76$^{\pi}$
CUM 50+TLE	2.18±0.06[a]	65.02±6.86[a]	67.16±6.92[a]	7.02±0.09[a]
CUM 100+TLE	2.89±0.43[b]	85.07±8.66[b]	88.34±8.55[b]	9.00±1.22[b]
CUM 200+TLE	3.60±0.36[c]	109.11±10.7[c]	118.78±11.1[c]	12.48±1.60[c]

CUM: Curcumin, TLE: Tenofovir/lamivudine/efavirenz, n=5, Data as mean ± SEM (Standard error of mean), πp<0.001 Significant differnce when compared to control, [a]p<0.05, [b]p<0.01, [c]p<0.001 Significant differnce when compared to TLE.

Table 3. Effect of curcumin on kidney oxidative stress markers of tenofovir/lamivudine/efavirenz-treated rats.

Dose (mg/kg)	MDA (mmol/mg protein)	GSH (μmole/ mgprotein)	CAT (U/mgprotein)	SOD (U/ mgprotein)	GPx (U/ mgprotein)
Control	0.17±0.06	20.03±3.89	29.55±3.24	23.05±3.77	30.34±4.87
CUM 50	0.16±0.09	20.27±2.45	29.73±2.56	23.37±2.98	30.51±3.96
CUM 100	0.15±0.05	20.61±2.40	30.04±3.01	23.54±4.70	30.70±4.33
CUM 200	0.13±0.07	20.90±3.73	30.53±4.66	24.06±3.66	31.05±4.16
TLE	2.51±0.73$^{\pi}$	4.66±0.73$^{\pi}$	7.14±0.67$^{\pi}$	7.36±0.72$^{\pi}$	8.05±0.56$^{\pi}$
CUM 50+TLE	1.80±0.81[a]	7.54±0.84[a]	10.27±1.09[a]	11.47±1.89[a]	11.67±1.22[a]
CUM 100+TLE	0.94±0.06[b]	11.64±1.02[b]	16.65±1.67[b]	16.50±1.89[b]	17.83±1.01[b]
CUM 200+TLE	0.20±0.08[c]	18.92±2.16[c]	26.92±4.27[c]	21.73±2.02[c]	27.42±3.50[c]

SOD: Superoxide dismutase, CAT: Catalase, GSH: Glutathione, MDA: Malondialdehyde, GPx: Glutathione peroxidase, CUM: Curcumin, TLE: Tenofovir/lamivudine/efavirenz, n=5, Data as mean±SEM (Standard error of mean), πp<0.001 Significant differnce when compared to control, [a]p<0.05, [b]p<0.01, [c]p<0.001 Significant differnce when compared to TLE.
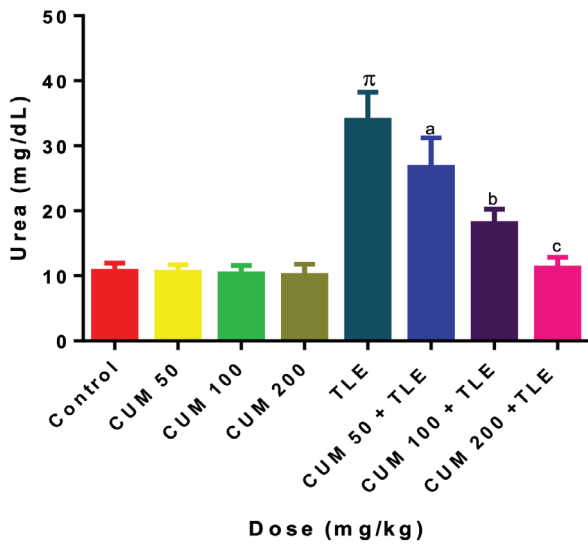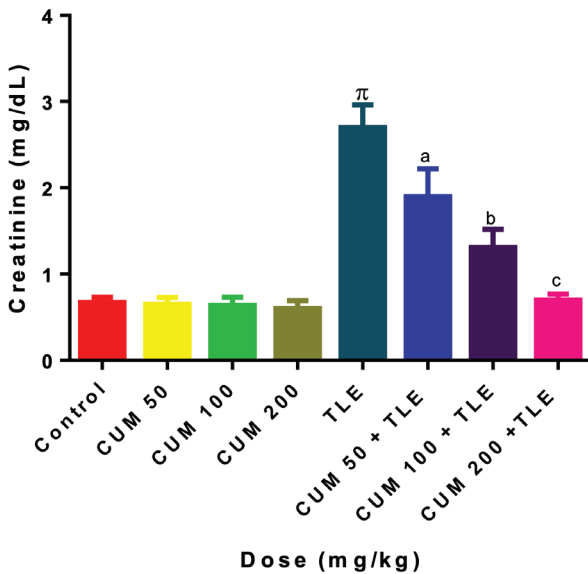
Figure 1. Effect of curcumin on serum urea levels of tenofovir/lamivudine/efavirenz -treated rats.

CUM: Curcumin, TLE: Tenofovir/lamivudine/efavirenz, Data as mean±SEM (Standard error of mean), n=5, $^{\pi}p<0.001$ significance when compared to control, $^{a}p<0.05$, $^{b}p<0.01$, $^{c}p<0.001$ significance when compared to TLE.

Figure 3. Effect of curcumin on serum uric acid levels of tenofovir/lamivudine/efavirenz -treated rats.

CUM: Curcumin, TLE: Tenofovir/lamivudine/efavirenz, Data as mean±SEM (Standard error of mean), n=5, $^{\pi}p<0.001$ significant difference when compared to control, $^{a}p<0.05$, $^{b}p<0.01$, $^{c}p<0.001$ significant difference when compared to TLE.

Figure 2. Effect of curcumin on serum creatinine levels of tenofovir/lamivudine/efavirenz -treated rats.

CUM: Curcumin, TLE: Tenofovir/lamivudine/efavirenz, Data as mean±SEM (Standard error of mean), n=5, $^{\pi}p<0.001$ significant difference when compared to control, $^{a}p<0.05$, $^{b}p<0.01$, $^{c}p<0.001$ significant difference when compared to TLE.

Figure 4. Effect of curcumin on serum total protein levels of tenofovir/lamivudine/efavirenz -treated rats.

CUM: Curcumin, TLE: Tenofovir/lamivudine/efavirenz, Data as mean±SEM (Standard error of mean), n=5, $^{\pi}p<0.001$ significant difference when compared to control, $^{a}p<0.05$, $^{b}p<0.01$, $^{c}p<0.001$ significant difference when compared to TLE.

supplementation increased serum total protein, potassium, chloride, sodium, bicarbonate and albumin levels significantly at p<0.05, p<0.01, and p<0.01, respectively when compared to TLE. CUM (50, 100, and 200 mg/kg) supplementation decreased serum uric acid, creatinine and urea levels in a dose-related manner at p<0.05, p<0.01, and p<0.001, respectively when compared to TLE (Table 2) (Figures 1-5).
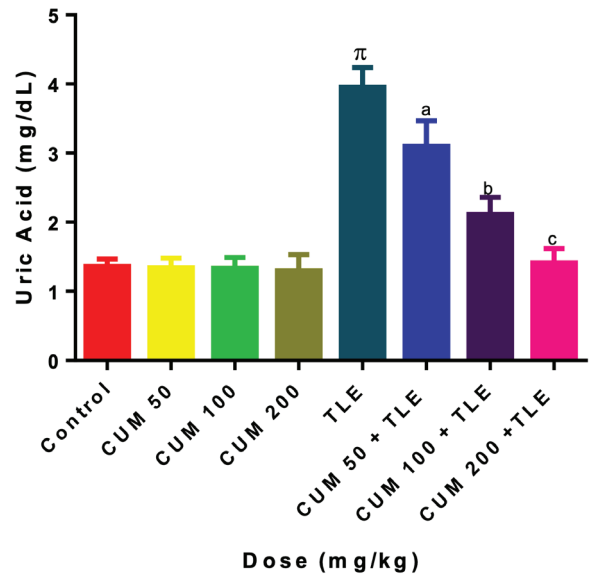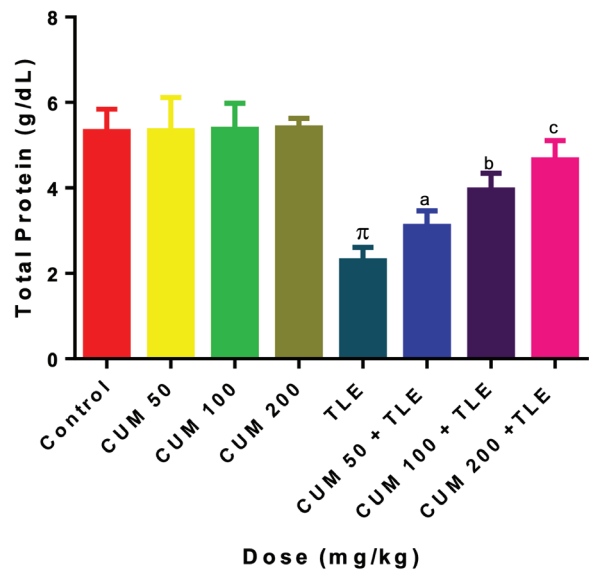
Effects of curcumin on serum kidney oxidative markers and histology of tenofovir/lamivudine/efavirenz -treated rats

Kidney antioxidants (SOD, CAT, GSH and GPx) and MDA levels were normal (p>0.05) in CUM-administered rats when compared to control. TLE administration significantly (p<0.001)
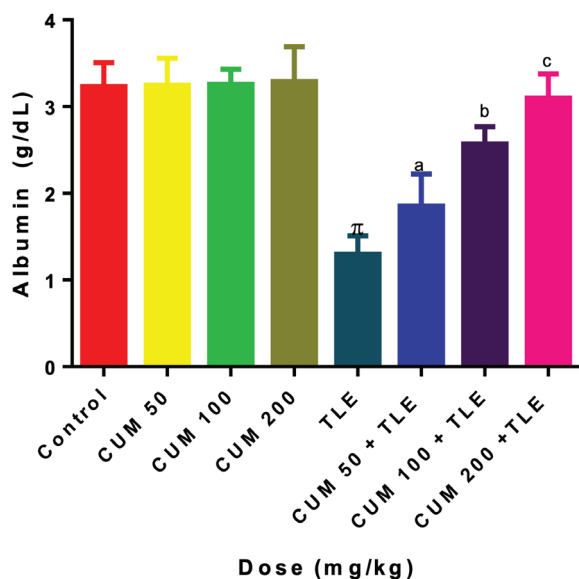
Figure 5. Effect of curcuminon serum albumin of tenofovir/lamivudine/efavirenz-treated rats.

CUM: Curcumin, TLE: Tenofovir/lamivudine/efavirenz, Data as mean±SEM (Standard error of mean), n=5, $\pi p<0.001$ significant difference when compared to control, [a]$p<0.05$, [b]$p<0.01$, [c]$p<0.001$ significant difference when compared to TLE.

decreased kidney antioxidants, but significantly (p<0.001) increased kidney MDA levels when compared to control (Table 3). However, CUM (50, 100, and 200 mg/kg) supplementation significantly increased kidney antioxidants, and significantly decreased kidney MDA levels in a dose-related manner at p<0.05, p<0.01, and p<0.001, respectively when compared to TLE treated rats (Table 3). The kidney of the control rats showed normal renal tubule and glomerulus (Fig 6A), but the kidney of TLE-treated rat showed tubular necrosis and hypercellular glomerulus with mesangial proliferation (Figure 6B). The kidney of CUM (25 mg/kg) and CUM (50 mg/kg) supplemented rats showed normal renal tubules and hypercellular glomeruli with mesangial proliferations as shown in (Figure 6C) and (Figure 6D), respectively. However, the kidney of CUM (100 mg/kg) supplemented rat showed normal renal tubules and glomerulus (Figure 6E).

DISCUSSION

TLE-related nephrotoxicity can add to HIV infection associated socio-economic burden (Perazella, 2010). CUM is an isolate of turmeric with a wide spectrum of biological activity that is used in folk medicine (Noorafshan & Ashkani-Esfahani, 2013). This study attempts to establish whether CUM supplementation can prevent TLE-induced nephrotoxicity in a rat model. In the current study, the administration of CUM had no effects on all evaluated parameters at the serum and tissue levels. On the other hand, the administration of TLE increased kidney weight, but decreased body weight, which supports earlier reports (Jang et al., 2010). The observed decrease in body weight may be ascribed to decreased appetite whereas increase in kidney weight may be predicated on the induc-

tion of inflammation by TLE. In this study, the conspicuous incapacitation of kidney function by TLE was marked by elevated serum uric acid, urea, and creatinine levels with decreased serum total protein, albumin and serum electrolytes. This observation supports previous reports (Fenandez-Fernandez et al., 2011). The health status of the kidney is ascribed to its capacity to functionally regulate the serum concentrations of the aforementioned indices (Gowda et al., 2010). In the midst of perturbations caused by chemical assaults or diseases the functional capacity of the kidney is impaired causing aberrations in serum uric acid, urea, creatinine, total protein, albumin and serum electrolytes (Gowda et al., 2010). In the present study, TLE caused dysfunction in kidney reduction/oxidation status of treated rats characterized by decreased antioxidants and increased MDA levels. This observation supports earlier findings (Adikwu & Apiakise, 2016). Antioxidants form defensive network that prevents oxidative damage by scavenging and neutralizing free radicals, but could be consumed and depleted as a consequence of increased free radicals production beyond antioxidants regulation causing oxidative stress (Adikwu & Apiakise, 2016). Hence, depleted kidney antioxidants observed in TLE-treated rats established oxidative stress. MDA is used experimentally to mirror the occurrence of lipid peroxidation (LPO) in a pathologic process or condition (Adikwu & Apiakise, 2016). Therefore, TLE-induced elevation in MDA level established the occurrence of LPO. In the current study, TLE-induced nephrotoxicity was characterized by kidney tubular necrosis and hypercellular glomerulus with mesangial proliferation. This observation is in agreement with earlier findings (Herlitz et al., 2010). In the current study, CUM supplementation abrogates TLE-induced nephrotoxicity in a dose-related manner. This was characterized by restored body and kidney weights and up-regulation of serum total protein, albumin, electrolytes and kidney antioxidants. CUM supplementation caused down-regulation of serum uric acid, creatinine, urea and kidney MDA levels. Also, tubular necrosis and hypercellular glomerulus with mesangial proliferation were absent in the kidneys of rats supplemented with the highest dose of CUM. This finding correlates with the reported protective activity of CUM against gentamicin-induced nephrotoxicity in rats (El-Zawahry & Abu El Kheir, 2007). In the present study, the protective impact of CUM on TLE-induced nephrotoxicity may be ascribed to its antioxidant and anti-inflammatory activities. Studies have associated most therapeutic effects of CUM to its antioxidant and anti-inflammatory activities (Hewlings & Kalman, 2007). CUM, as an antioxidant, inhibits oxidative stress and LPO by scavenging and neutralizing ROS (Menon &Sudheer, 2007). It can up regulate the activities of endogenous antioxidants (SOD, CAT, and GSH) and increase antioxidants gene expression. CUM can inhibit enzymes including xanthine hydrogenase/oxidase and lipoxygenase/cyclooxygenase, which are facilitators of free radicals production and can decrease the gene expression of such enzymes (Lin et al., 2007). The anti-inflammatory action of CUM includes inhibitory effects on pro-inflammatory mediators such as cytokines (TNF-α and IL-6). It can also inhibits NF-kappaB activation pathway, which is essential for inflammation (Noorafshan & Ashkani-Esfahani, 2013; Lin et al., 2007).
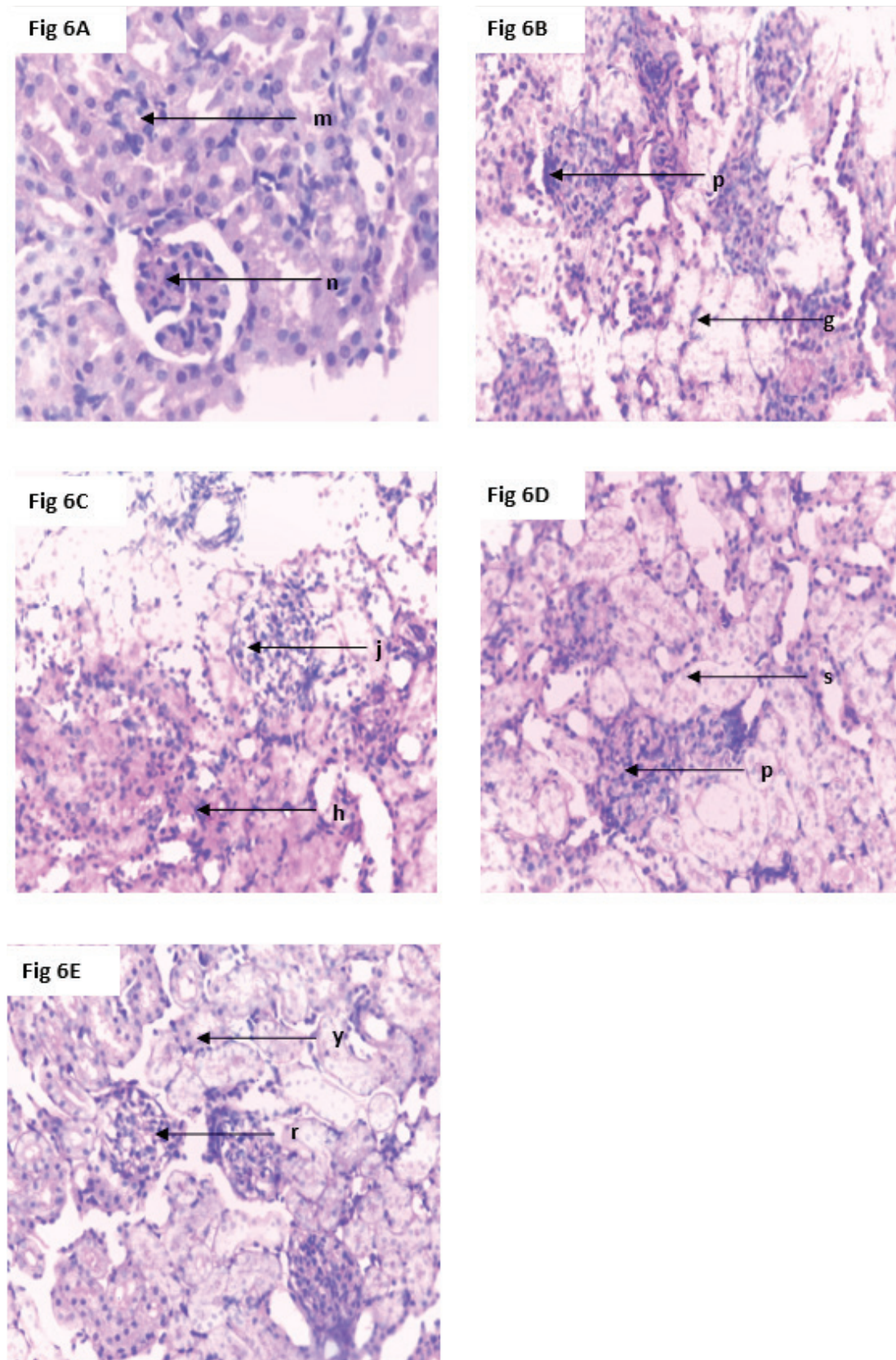
Figure 6. A: Kidney of control rat showed normal renal tubule (n) and glomerulus (m); B: Kidney of TLE-treated rat showed tubular necrosis (h) and hypercellular glomerulus with mesangial proliferation (j); C: Kidney of CUM (25mg/kg) supplemented rat showed normal renal tubule (h) and hypercellular glomerulus with mesangial proliferation (j); D: Kidney of CUM (50mg/kg) supplemented rat showed normal renal tubule (s) and hypercellular glomerulus with mesangial proliferation (p); E: Kidney of CUM (100mg/kg) supplemented rat showed normal renal tubule (y) and glomerulus (r).

CONCLUSION

Based on the observation in the current study, CUM may clinically protect against TLE-related nephrotoxicity.

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