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Protective Activity of Dexamethasone Against Cyclopospha mide-Induced Nephrotoxicity in Rats

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Key words: Cyclophosphamide, kidney, toxicity, dexamethasone, rat

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Abstract: The nephrotoxic effect of cyclophosphamide (CP) may involve inflammation. Dexamethasone (DEXA) is a glucocorticoid used for the treatment of inflammatory disorders. This study examined the protective potential of dexamethosane (DEXA) against CP-induced nephrotoxicity in albino rats. Thirty-six adult male albino rats divided in to six groups (n = 6) were used. Group I (Control) was treated with normal saline (0.2 mL day^{-1}) intraperitoneally (i.p.) for 24 h. Group 2 was treated with DEXA (1 mg/kg/day i.p.) for 24 h. Group 3 was treated with CP (150 mg/kg/day i.p.) for 24 h. Group 4 was pre-treated with DEXA (1 mg/kg/day) for 24 h before treatment with CP (150 mg/kg/day i.p.) for 24 h. Group 5 was co-treated with DEXA (1 mg/kg/day i.p.) and CP (150 mg/kg/day i.p.) ip for 24 h. Group 6 was treated with CP (150 mg/kg/day i.p.) for 24 h before post-treatment with DEXA (1 mg/kg/day i.p.) for 24 h. At the termination of treatments, the rats were euthanized and blood samples were assessed for serum renal function markers. Kidney samples were evaluated for histology, malondialdehyde and antioxidants (glutathione, catalase, superoxide dismutase and glutathione peroxidase). Treatment with CP produced significant ($p<0.001$) increases in serum uric acid, creatinine, urea and kidney malondialdehyde levels with significant ($p<0.001$) decreases in serum albumin, total protein and kidney antioxidants in relation to control. CP caused tubular necroses and increased Bowman's space in the kidneys of treated rats. CP-induced nephrotoxicity was significantly abrogated by post-treatment ($p<0.05$), co-treatment ($p<0.01$) and pre-treatment ($p<0.001$) with DEXA when compared to CP. DEXA may be effective against nephrotoxicity caused by CP.

INTRODUCTION

The kidneys are positioned on each side of the vertebral column around the lumbar region of the body. They are associated with homeostatic functions such as maintenance of fluid and chemical components of the intracellular environment by controlling the quantities of phosphate, sodium, potassium, chloride, water and

other chemical substances[1]. The kidneys also perform excretory function exposing them to milieu of drugs, waste products of metabolism and other chemical substances making them vulnerable to toxicity[2].

Cyclophosphamide (CP) is an anticancer agent used alone or in combination with other anticancer agents[3]. It has been very effective against malignancies such as Hodgkin's disease, breast cancer, leukemia and multiple myeloma. It is also effective against multiple sclerosis and systemic lupus erythematosus. Its use has reduced the incidence of death caused by cancer, but the development of toxicities including nephrotoxicity is a serious health challenge. Its nephrotoxic effect is characterized by glomerular and tubular dysfunctions[4]. It has been speculated that increased reactive oxygen species (ROS) activity leading to oxidative stress plays a primary function in CP-induced nephrotoxicity[5]. Also, the induction of inflammation through increased expression of pro-inflammatory mediators including cytokines and cyclooxygenase-2 has been speculated as a pathogenic factor in CP-induced nephrotoxicity[6].

Dexamethasone (DEXA) is a frequently used glucocorticoid for the treatment of inflammatory and auto-immune conditions. It also has anti-allergic, anti-shock and anti-endotoxin effects. DEXA enters the cytoplasm where it interacts with glucocorticoid receptor forming a ligand-receptor complex which translocates to the nucleus. In the nucleus, the ligand-receptor complex interacts with genomic DNA thereby regulating the expression of pro-inflammatory and anti-inflammatory genes at the transcriptional level. Comparatively, its anti-inflammatory activity is 25-30 times higher than most glucocorticoids and has stabilizing ability on lysosomal membrane[7-9]. In addition to the aforementioned effects, studies speculated that it can inhibit oxidative stress and prevent lipid peroxidation (LPO) that can arise as a consequence of the excess activities of ROS[10]. Furthermore, it has shown beneficial activity in animal models of renal injury[11, 12]. However, there is no literature on its protective effect against CP-induced nephrotoxicity in any animal model which this study assessed in albino rats.

MATERIALS AND METHODS

Drugs and experimental animals: DEXA (Ranbaxy Laboratory Ltd, India) and CP (Biochem Pharm Industries Ltd. India) were used. This study was conducted in the Department of Pharmacology and Toxicology, Faculty of Pharmacy, Niger Delta University, Nigeria. The rats used were housed in cages under natural light and temperature with unlimited access to diet and water. The rats were conditioned for two weeks prior to the study. The protocol for animal handling used was prepared by the National Academy of Science.

Drug administration: Group 1 (control) (n = 6): Rats were treated with a dose of normal saline (0.2 mL) intraperitoneally (i.p.) for 24 h; Group 2 (n = 6): Rats were treated with DEXA (1 mg/kg/day i.p.) for 24 h; Group 3 (n = 6): Rats were treated with CP (150 mg/kg/day i.p.)[13] for 24 h; Group 4 (n = 6) (Pre-treatment): Rats were treated with DEXA (1 mg/kg/day i.p) for 24 h before CP (150 mg/kg/day i.p.) for 24 h; Group 5 (n = 6) (Co-treatment): Rats were treated with DEXA (1 mg/kg/day i.p.) and CP (150 mg/kg/day i.p.) for 24 h. Group 6 (n = 6) (Post-treatment): Rats were treated with CP (150 mg/kg/day i.p.) for 24 h before treatment with DEXA (1 mg/kg/day i.p.) for 24 h.

Animal sacrifice and evaluation of biochemical parameters: At the end of treatment, the rats were anesthetized in a diethyl ether chamber. Blood samples were obtained from the heart and allowed to clot. The clots were centrifuged and serum samples were collected and evaluated for albumin, total protein, uric acid, creatinine and urea concentrations using commercial test kits (Randox Laboratories UK). After dissection, kidney samples were collected, rinsed in normal saline and homogenized in 0.1 M Tris-HCl (buffer, pH 7.4). The homogenates were collected; centrifuged (1500 rmp for 20 min) and the supernatants were decanted. The supernatants were used for the evaluation of total protein as reported by Gornall *et al.*[14], Superoxide Dismutase (SOD) as described by Sun and Zigman[15] and Catalase (CAT) according to Aebi[16]. The method described by Buege and Aust was used for the assessment of Malondialdehyde (MDA)[17]. Glutathione (GSH) was assayed as reported by Sedlak and Lindsay[18]. Glutathione Peroxidase (GPx) was assayed as reported by Rotruck *et al.*[19].

Histological evaluation of the kidney: Harvested kidney samples were fixed in 10% neutral buffered formalin for 24 h. The kidney samples were rinsed in serial dilutions of alcohol and embedded in paraffin block. Five µm thick sections were cut from the paraffin block and deparaffinzed. The deparaffinzed sections, were stained (hematoxylin and eosin) and assessed on a light microscope for histological changes.

Statistical analysis: Data expressed as mean±standard error of mean (SEM). One-way analysis of variance (ANOVA) and Tukey's test were used for data analysis. A $p<0.05$; 0.01 and 0.001 was considered significant.

RESULTS

Effect on renal function markers: Figure 1-3 showed significant ($p<0.001$) elevations in serum uric acid, creatinine and urea levels in CP-treated rats in comparison to control. But serum uric acid, creatinine and urea levels were decreased in rats pre-treated ($p<0.001$), co-treated

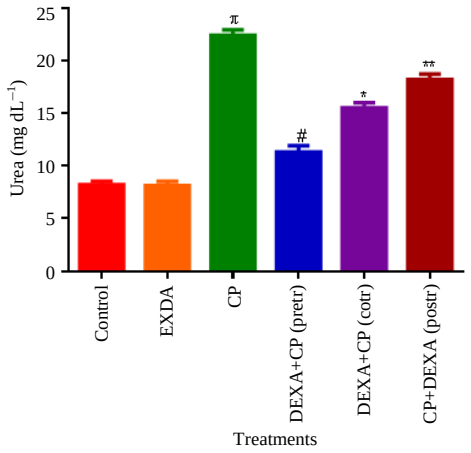

Fig. 1: Effect of dexamethasone on serum urea of cyclophosphamide-treated albino rats. DEXA: Dexamethasone, CP: Cyclophosphamide. Prêtr: Pre-treatment, Cotr: Co-treatment, Postr: Post-treatment. Data as mean±standard error of mean, n = 6. $^{\pi}$p<0.001 when compared to control. $^{\#}$p<0.001, *p<0.05 and **p<0.001 when compared to CP

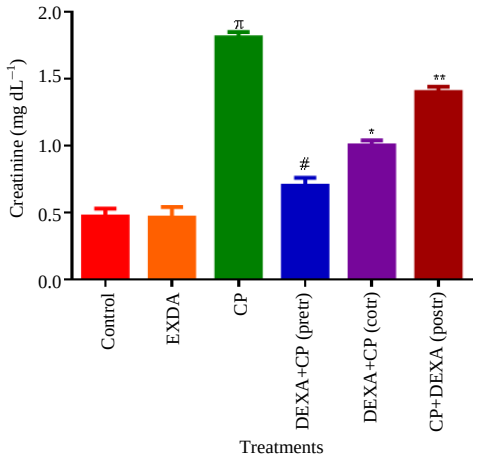

Fig. 2: Effect of dexamethasone on serum creatinine of cyclophosphamide-treated albino rats. DEXA: Dexamethasone, CP: Cyclophosphamide. Pretr: Pre-treatment, Cotr: Co-treatment, Postr: Post-treatment. Data as mean±standard error of mean, n = 6. $^{\pi}$p<0.001 when compared to control. $^{\#}$p<0.001, *p<0.05 and **p<0.001 when compared to CP

(p<0.01) and post-treated (p<0.05) with DEXA when compared CP. Significant (p<0.001) decreases in serum albumin and total protein levels occurred in CP-treated rats in comparison to control (Fig. 4 and 5). On the other hand, serum albumin and total protein levels were significantly increased in DEXA

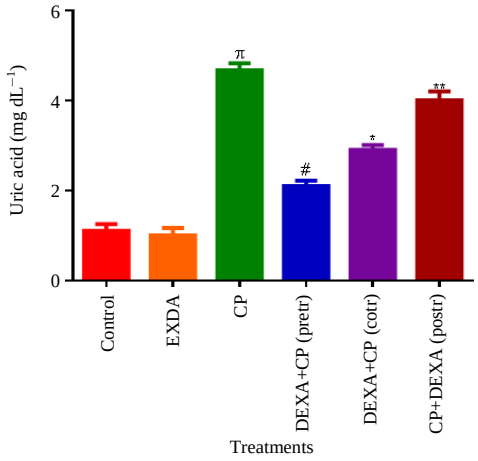

Fig. 3: Effect of dexamethasone on serum uric acid of cyclophosphamide-treated albino rats. DEXA: Dexamethasone, CP: Cyclophosphamide. Pretr: Pre-treatment, Cotr: Co-treatment, Postr: Post-treatment. Data as mean±standard error of mean, n = 6. $^{\#}$p<0.001, *p<0.05 and **p<0.001 when compared to CP

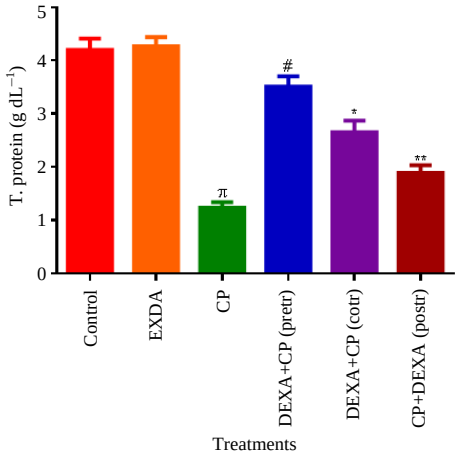

Fig. 4: Effect of dexamethasone on serum total protein of cyclophosphamide-treated albino rats. DEXA: Dexamethasone, CP: Cyclophosphamide. Pretr: Pre-treatment, Cotr: Co-treatment, Postr: Post-treatment. Data as mean±standard error of mean, n = 6. $^{\pi}$p<0.001 when compared to control. $^{\#}$p<0.001, *p<0.05 and **p<0.001 when compared to CP

pre-treated (p<0.001), co-treated (p<0.01) and post-treated (p<0.05) rats in comparison to CP (Fig. 4 and 5).

Effects on kidney oxidative stress markers and kidney histology: Results in Fig. 6-10 showed that CP significantly (p<0.001) decreased kidney GSH, CAT, SOD and GPx levels and significantly (p<0.001)

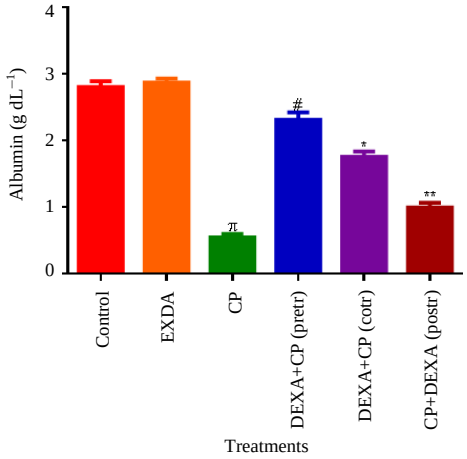

Fig. 5: Effect of dexamethasone on serum albumin of cyclophosphamide-treated albino rats. DEXA: Dexamethasone, CP: Cyclophosphamide. Pre-tr: Pre-treatment, Co-tr: Co-treatment, Postr: Post-treatment. Data as mean±standard error of mean, n = 6. $^{\pi}$p<0.001 when compared to control. $^{\#}$p<0.001, *p<0.05 and **p<0.001 when compared to CP

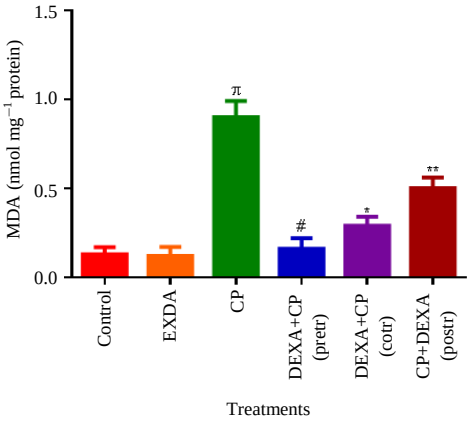

Fig. 6: Effect of dexamethasone on kidney malondialdehyde of cyclophosphamide-treated albino rats. MDA: Malondialdehyde, DEXA: Dexamethasone, CP: Cyclophosphamide. Pretr: Pre-treatment, Cotr: Co-treatment, Postr: Post-treatment. Data as mean±standard error of mean, n = 6. $^{\pi}$p<0.001 when compared to control. $^{\#}$p<0.001, *p<0.05 and **p<0.001 when compared to CP

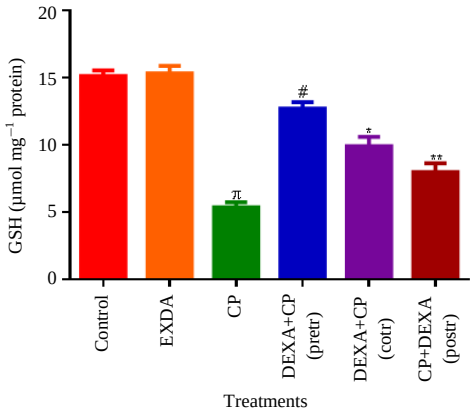

Fig. 7: Effect of dexamethasone on kidney glutathione of cyclophosphamide-treated albino rats. GSH: Glutathione, DEXA: Dexamethasone, CP: Cyclophosphamide. Pretr: Pre-treatment, Cotr: Co-treatment, Postr: Post-treatment. Data as mean±standard error of mean, n = 6, $^{\pi}$p<0.001 when compared to control. $^{\#}$p<0.001, *p<0.05 and **p<0.001 when compared to CP

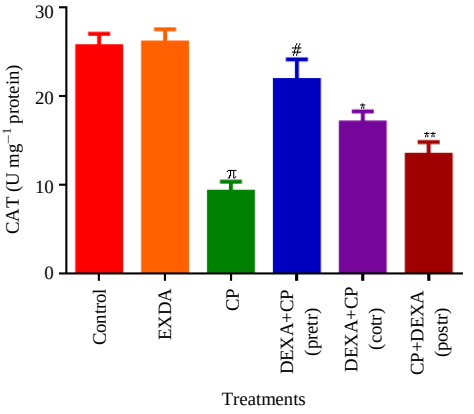

Fig. 8: Effect of dexamethasone on kidney catalase of cyclophosphamide-treated albino rats. CAT: Catalase, DEXA: Dexamethasone, CP: Cyclophosphamide. Pretr: Pre-treatment, Cotr: Co-treatment, Postr: Post-treatment. Data as mean±standard error of mean, n = 6. $^{\pi}$p<0.001 when compared to control. $^{\#}$p<0.001, *p<0.05 and **p<0.001 when compared to CP

increased MDA level in comparison to control. However, GSH, CAT, SOD and GPx levels were increased significantly, whereas MDA levels were decreased significantly in DEXA pre-treated (p<0.001), co-treated (p<0.01) and post-treated (p<0.05) rats in comparison to CP. The kidney of rat in the control group showed normal glomerulus and renal tubule (Fig. 11a). On the other hand, the kidney of rat in CP treated group showed increased Bowman's space and tubular necrosis (Fig. 11b). The kidney of rat in DEXA co-treated group showed normal glumerulus and renal tubule (Fig. 11c). However, the kidney of DEXA post-treated rat showed normal glomerulus and tubular necrosis (Fig. 11d). The kidney of DEXA pre-treated rat showed normal glomerulus and renal tubule (Fig. 11e).

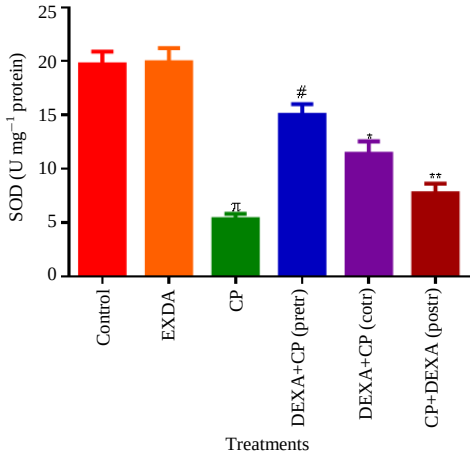

Fig. 9: Effect of dexamethasone on kidney superoxide dismutase, of cyclophosphamide-treated albino rats. SOD: Superoxide dismutase, DEXA: Dexamethasone, CP: Cyclophosphamide. Pretr: Pre-treatment, Cotr: Co-treatment, Postr: Post-treatment. Data as mean±standard error of mean, n = 6. $^{\pi}p<0.001$ when compared to control. $^{\#}p<0.05$ when compared to CP. $^{\#}p<0.001$, *p<0.05 and **p<0.001 when compared to CP

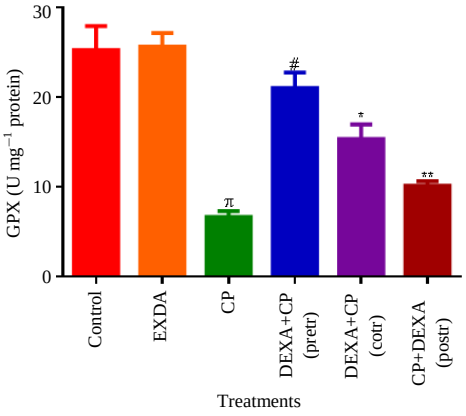

Fig. 10: Effect of dexamethasone on kidney glutathione peroxidase of cyclophosphamide-treated albino rats. GPx: Glutathione peroxidase, DEXA: Dexamethasone, CP: Cyclophosphamide. Pretr: Pre-treatment, Cotr: Co-treatment, Postr: Post-treatment. Data as mean±standard error of mean, n = 6, $^{\pi}p<0.001$ when compared to control. $^{\#}p<0.001$, *p<0.05 and **p<0.001 when compared to CP

DISCUSSION

CP is a broad anti-neoplastic drug that has synergistic action with other anti-neoplastic drugs. It is use alone or with other anti-neoplastic drugs for the treatment of various forms of malignancies. However, its clinical use has been affected by a number of toxicities including nephrotoxicity[20]. DEXA is a glucocorticoid with anti-allergic, anti-inflammatory, anti-shock and speculated antioxidant activities[21]. This study examined the possibility of DEXA to protect against CP-induced nephrotoxicity in rats. In this study, serum renal function markers (serum uric acid, urea, creatinine, albumin and total protein) were normal in DEXA-treated rats. In contrast, renal function was impaired in CP-treated rats.

This was characterized by elevated serum uric acid, urea and creatinine concentrations with decreased total protein and albumin concentrations. This is an evidence of renal perturbation caused by CP which is consistent with earlier reports[22, 23]. The increases in uric acid, urea and creatinine concentrations caused by CP may be due to the reduction in glomerular filtration rate stimulating decreased renal excretion of the aforementioned parameters[24-27]. However, renal function was restored in rats post-treated, pre-treated and co-treated with DEXA. This was characterized by decreased serum uric acid, urea and creatinine concentrations with increased albumin and total protein concentrations. Interestingly, renal function was most restored in rats pre-treated with DEXA.

In addition to alterations in serum renal function markers, kidney redox status was also impaired in CP-treated rats. This was marked by decreases in kidney antioxidant levels with increases in MDA levels. This may due to the induction of OS through ROS production by CP. The observed increase in MDA level attests to the oxidation of poly unsaturated fatty acid in the kidney by CP. These observations support earlier reports[28]. However, kidney redox status was restored in rats post-treated, post-treated and co-treated with DEXA as evidenced by increased kidney antioxidant levels with decreased MDA levels. Studies have shown that the nephrotoxic effect of CP also includes alteration in kidney histology[29]. The current study observed tubular necrosis and increased Bowman's space in rats treated with DEXA. This observation correlates with observed changes in serum biochemical indices and kidney OS markers. However, CP-induced histological alterations in the kidney were ameliorated in rats pre-treated, post-treated and co-treated with DEXA. The observation in this study showed that supplementation with DEXA may have benefit in CP-induced nephrotoxicity.

A number of mechanisms have been speculated to be involved in CP-induced nephrotoxicity. This includes the induction of OS by one of the metabolites of CP

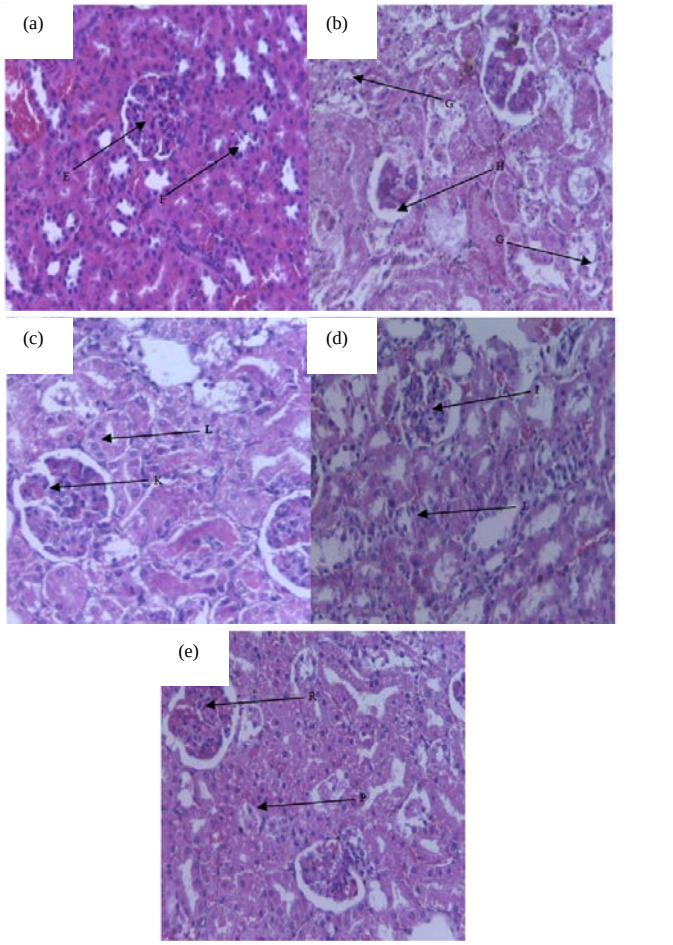

Fig. 11(a-e): (a) The kidney of rat in the control group shows normal glomerulus (E) and renal tubule (F); (b) The kidney of rat in cyclophosphasmide-treated group shows increased bowman's space (H) and tubular necrosis (G); (c) The kidney of rat in dexamethasone co-treated shows normal glumerulus (L) and renal tubule (K); (d) The kidney of rat in dexamethasone post-treated shows normal glumerulus (I) and tubular necrosis (J) and (e) The kidney of rat in dexamethasone pre-treated shows normal glumerulus (R) and renal tubule (P) (Hand EX 400)

(acrolein) produced during its hepatic bioactivation[30]. Acrolein has been associated with the generation of free radicals such as ROS stimulating OS causing protein adduction, DNA and mitochondrial damage[33]. Also, the induction of inflammation has been speculated as one of the mechanisms by which CP causes nephrotoxicity[31]. DEXA might have abrogated the induction of nephrotoxicity by CP by inhibiting OS and increasing kidney antioxidant defense capacity which can be correlated with increased kidney antioxidant levels. It might have decreased the induction of LPO by CP due to decreased kidney MDA level observed in this study. The aforementioned actions of DEXA might be attributed to its free radical scavenging activity. Also, DEXA might have restored kidney function by inhibiting the induction of inflammation by CP.

CONCLUSION

This study shows that DEXA may be repurposed as treatment for nephrotoxicity caused by CP.

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