

Protective assessment of cimetidine against cyclophosphamide-induced kidney injury



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ABSTRACT

Background: Nephrotoxicity is one of the frequent toxicities observed with cyclophosphamide (CP) use which may involve oxidative stress. Cimetidine is an antihistamine with anti-oxidative stress activity. **Aims and Objectives:** The study aimed to evaluate the effect of cimetidine on cyclophosphamide-induced kidney damage in albino rats. **Materials and Methods:** Forty eight adult rats randomised into 8 (A-H) groups of 6 rats per group were experimentally used for this study. Group A (control) was treated with water, while groups B-D were treated with 5, 10 and 20 mg/kg of cimetidine intraperitoneally (ip) daily for 5 days respectively. Group E was treated with 150 mg/kg of CP ip on the 5th day. Groups F-H were pretreated with 5, 10 and 20 mg/kg cimetidine ip daily for 5 days and treated with CP ip on the 5th day respectively. Rats were sacrificed serum was extracted from blood and evaluated for renal function markers, while kidneys were harvested and evaluated for oxidative stress markers and histology. **Results:** There were no significant effects ($p > 0.05$) on the body and kidney weights of CP-treated rats. However, impaired kidney functions in CP-treated rats were marked by significant ($p < 0.05$) increases in creatinine, urea, uric acid, sodium, potassium, chloride, bicarbonate, and malondialdehyde levels when compared to control. On the other hand, significant ($p < 0.05$) decreases in superoxide dismutase, catalase, glutathione, glutathione peroxidase, total protein and albumin were obtained in CP-treated rats when compared to control. Necrotic changes were observed in the kidneys of CP-treated rats. However, CP-induced nephrotoxic effects were significantly ($p < 0.05$; 0.01) reversed in cimetidine pretreated rats. **Conclusion:** Cimetidine shows potential as adjunct remedy for cyclophosphamide associated nephrotoxicity.

Key word: Cyclophosphamide; Kidney; Toxicity; Cimetidine; Prevention; Albino Rats

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INTRODUCTION

Cancer is a serious health challenge with a cosmopolitan incidence.¹² Treatment methods including the application of chemotherapy have been of tremendous therapeutic benefits in the reduction of morbidity and mortality associated with cancer. Cyclophosphamide (CP) is one of the commonly and most frequently used drugs in cancer chemotherapy. CP is an alkylating agent, belonging to the class of oxazaphosphorins.³ It is widely used in the treatments of various cancers, rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, and as an immunosuppressant in organ transplantation.⁴ The anticancer effect of CP has been attributed to its two

active metabolites (phosphoramidate mustard and acrolein) produced through metabolic activation by hepatic microsomal cytochrome P450 mixed functional oxidase.⁵

These metabolites interfere with the growth of susceptible rapidly proliferating malignant cells by the inhibition of DNA duplication. The reactive metabolites of CP chemically alkylate DNA and protein and produce cross-links, which are responsible for their cytotoxic effect.⁵ It is generally accepted that cancer cell death occurs as a result of the inhibition of DNA replication, as the interlinked strands do not allow separation of the two strands.⁶ However, the use of CP has been associated with the induction of nephrotoxicity characterised by tubular

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necrosis, tubular fibrosis and glomerular congestion. CP-induced nephrotoxicity has been attributed to the activities of its two primary metabolites (phosphoramidate mustard and acrolein).⁷ Phosphoramidate mustard produces antineoplastic effect while acrolein is a highly reactive metabolite that is electrophilic in nature.⁸ Recent studies suggest that these metabolites can alter kidney redox status in favour of oxidants production leading to kidney biomolecular damage. This action has been correlated with altered serum markers of renal function and kidney histo-architectural damage observed in CP treated rats⁹

Cimetidine is one of the most important H₂-receptor antagonists.¹⁰ It is an inhibitor of Cytochrome P 450 enzyme which plays an important role in nephrotoxicity. Cimetidine has imidazole and cyano groups that inhibit cytochrome P-450 by interacting with the heme moiety, which represents an important source for iron during cell and tissue injury.¹¹ Studies have also indicated that cimetidine is able to scavenge oxidative radicals suggesting that this effect may be related to its antioxidant activity. Cimetidine is able to effectively inhibit free radical generation *in vivo* and reduce oxidative damage in tissues.¹² Furthermore, studies have reported possible beneficial effects of cimetidine in animal model of drug-induced toxicities. Therefore, the current study examined the effect of cimetidine on cyclophosphamide-induced kidney damage in albino rats.

MATERIALS AND METHODS

Drugs and experimental animals

Cyclophosphamide (Biochem Pharmaceutical Industries India) and cimetidine (Shandong Shenglu Pharmaceutical India) were used for this study. Dose selection: Cyclophosphamide (150mg/kg)¹³ and cimetidine (5, 10 and 20mg/kg) were used for this study. Forty eight (48) adult albino rats with average weight of 220g ± 5g used for this study were obtained from the animal house of the Department of Pharmacology and Toxicology Madonna University, Elele, Rivers State. The rats were housed in 8 (A-H) cages of 6 rats per cage and were allowed to acclimatize for 2 weeks in a well-ventilated room, maintained at a room temperature of 28 °C, under natural lighting condition. The rats were fed with standard rodent chow and given water *ad libitum*.

Drug administration

- Group A was treated with water
- Group B was treated with 5mg/kg of cimetidine ip daily for 5 days
- Group C was treated with 10 mg/kg of cimetidine ip daily for 5 days
- Group D was treated with 20 mg/kg of cimetidine ip

daily for 5 days

Group E was treated with 150 mg/kg of cimetidine ip on the 5th day

Group F was treated with 5mg/kg of cimetidine ip daily for 5 days + CP on the 5th day

Group G was treated with 10 mg/kg of cimetidine ip daily for 5 days+ CP on the 5th day

Group H was treated with 20 mg/kg of cimetidine ip daily for 5 days+ CP on the 5th day

Sacrifice of animals

After the completion of 5 days of drug administration, on the 6th day the rats in all groups were sacrificed by ether inhalation. Blood was collected by intracardiac puncture, and centrifuged at 3000 rpm for 15 min. The serum was collected and used for the estimation of serum renal function biomarkers. Kidney of each rat was isolated, separated from their surrounding connective tissues, washed and cleaned with normal saline solution. Renal tissues were blotted using filter paper, weighed and rinsed in ice-cold saline. The kidney was homogenized for endogenous antioxidants analysis.

Evaluation of renal function parameters

Serum creatinine, urea, uric acid, albumin, total protein and bicarbonate were measured using standard laboratory test kits. Sodium, potassium, and chloride were evaluated as reported by Olurishe *et al.*, 2012¹⁴

Evaluation of oxidative stress indices

Kidney protein was evaluated as reported by Gornall *et al.*, 1945¹⁵ whereas superoxide dismutase was determined according to Sun and Zigman 1978.¹⁶ Catalase was assayed according to Aebi 1984¹⁷ whereas reduced glutathione was estimated as described by Sedlak and Lindsay (1968).¹⁸ Malondialdehyde was determined using the method of Buege and Aust 1978¹⁹ whereas glutathione peroxidase was determined according to Rotruck *et al.*, 1973²⁰

Histological examination of the kidney

Kidneys were fixed in 10% formalin for at least 24 h. Then, kidney tissues were dehydrated with a sequence of ethanol solutions, embedded in paraffin, cut into 5 μM sections and stained with Hematoxylin and Eosin dye. The stained sections were evaluated for pathology using a light microscope.

Statistical analysis

Data was analysed using SPSS 18 software (SPSS Inc, Chicago, IL). Results are expressed as mean ± standard error of mean (SEM). Mean differences were established using ANOVA followed by Tukey's *post hoc* test. Values of *p* < 0.05; 0.01 were considered significant

RESULTS

In the current study, treatment with 5, 10 and 20mg/kg of cimetidine did not produce significant ($p>0.05$) effects on serum renal function parameters and kidney oxidative stress indices when compared to control (Table 1-4). Treatment with 150 mg/kg of CP had no significant ($p>0.05$) effects of on the body and kidney weights of albino rats when compared to control (Table 1). However, the present study observed significant ($p<0.05$) increases in serum creatinine, urea and uric acid whereas serum albumin and total protein were significantly ($p<0.05$) decreased in rats treated with CP when compared to control (Table 2). Interestingly, significant ($p<0.05$; 0.01) decreases in serum creatinine, urea and uric acid with significant ($p<0.05$; 0.01) increases in serum albumin and total protein were observed in rats pretreated with cimetidine in a dose-dependent manner (Table 2). Furthermore, the administration of CP produced significant ($p<0.05$) effects on K^+ , Cl^- , Na^+ and HCO_3^- levels when compared to the levels of these parameters in the control group. However, this study observed that the serum levels of K^+ , Cl^- , Na^+ and HCO_3^- were significantly ($p<0.05$) restored in a dose-dependent manner in cimetidine pretreated rats (Table 3). The kidney levels of CAT, GSH, SOD and GPX were significantly ($p<0.05$) decreased whereas MDA levels were significantly ($p<0.05$) increased in CP-treated rats in comparison to control (Table 4). Interestingly, pretreatment with cimetidine significantly

($p<0.05$;0.01) increased kidney levels of CAT, GSH, SOD and GPX whereas MDA levels were significantly ($p<0.05$;0.01) decreased in a dose-dependent manner (Table 4). The kidneys of the control rat and rats treated with 5, 10 and 20 mg/kg of cimetidine showed normal histology (Figure 1a-d). However, the kidney of 150 mg/kg of CP-treated rat showed dilated tubules, tubular necrosis, interstitial edema and infiltration of interstitium by inflammatory cells (Figure 1e). Also, the kidney of rat treated with 5 mg/kg of cimetidine and 150mg/kg of CP showed dilated tubules, tubular necrosis, interstitial edema with infiltration of interstitium by inflammatory cells (Figure 1f). Furthermore, the kidney of rats treated with 10 mg/kg of cimetidine and 150mg/kg of CP showed tubular necrosis (Figure 1g). Also, the kidney of rat treated with 20 mg/kg of cimetidine and 150mg/kg of CP showed tubular necrosis (Figure 1h).

DISCUSSION

Cyclophosphamide (CP) is an alkylating agent used as an immunosuppressant in rheumatoid arthritis and in the treatment of several cancers.²¹ One of the most common limitations of the use of CP is nephrotoxicity. CP is associated with the induction of tubular necrosis, tubular fibrosis, glomerular congestion and inflammation, thereby causing renal dysfunction.²² Treatment with CP and cimetidine did not produce significant effects on body and kidney weights. Renal biochemical markers play important

Table 1: Effect of cimetidine on body and kidney weights of cyclophosphamide- treated albino rats

Dose(mg/kg)	Initial body weight (g)	Final body weight (g)	Kidney weight (g)	Relative kidney weight (%)
Control	226.6±14.9	235.6±15.6	1.23±0.03	0.52±0.02
CD 5	207.8±17.4	215.8±14.0	1.02±0.02	0.47±0.06
CD 10	209.6±14.7	217.0±13.8	1.05±0.06	0.48±0.03
CD 20	219.8±15.6	225.4±17.9	1.03±0.02	0.45±0.01
CP 150	214.6±10.8	207.6±15.1	1.06±0.04	0.51±0.07
CD 5+CP	217.6±17.9	213.0±14.4	1.11±0.03	0.52±0.03
CD 10+CP	232.4±12.6	228.6±12.1	1.13±0.03	0.54±0.06
CD 20+CP	211.2±14.3	206.6±15.7	1.23±0.01	0.55±0.05

CD=Cimetidine, CP=Cyclophosphamide, values are expressed as Mean±SEM, n=6

Table 2: Effect of cimetidine on renal function parameters of cyclophosphamide- treated albino rats

Dose (mg/kg)	Urea mg/dL	Creatinine mg/dL	Uric acid mg/dL	Total protein mg/dL	Albumin mg/dL
Control	33.3±3.00	1.41±1.08	1.34±1.11	7.90±1.61	3.69±0.34
CD 5	30.4±3.30	1.40±1.02	1.33±1.09	7.64±0.58	3.47±0.39
CD 10	32.6±2.02	1.38±0.15	1.31±0.31	7.59±0.50	3.55±0.44
CD 20	30.7±2.73	1.69±0.04	1.30±0.25	7.53±0.47	3.50±0.40
CP 150	120.5±7.80 ^a	3.19±0.13 ^a	3.71±0.13 ^a	2.43±0.22 ^a	0.74±0.17 ^a
CD 5+CP	88.1±6.38 ^b	2.68±0.15 ^b	2.98±0.20 ^b	3.05±0.17 ^b	1.19±0.19 ^b
CD 10+CP	65.7±3.96 ^b	2.06±0.90 ^b	2.23±0.22 ^b	4.15±0.24 ^b	2.00±0.11 ^b
CD 20+CP	50.8±4.69 ^c	1.60±0.13 ^c	1.62±0.10 ^c	5.60±0.44 ^c	2.88±0.23 ^c

CD=Cimetidine, CP=Cyclophosphamide, values are expressed as Mean±SEM, n=6, ^aSignificant ($p<0.05$) difference when compared to control, ^bSignificant ($p<0.05$) difference when compared to CP-treated rats, ^cSignificant ($p<0.01$) difference when compared to CP-treated rats

Table 3: Effect of cimetidine on serum electrolytes of cyclophosphamide-treated albino rats

Dose (mg/kg)	K ⁺ mmol/L	CL ⁻ mmol/L	Na ⁺ mmol/L	HCO ₃ ⁻ mmol/L
Control	4.79±0.46	149.5±13.1	128.6±23.1	27.6±2.10
CD 50	4.83±0.49	150.7±6.72	130.1±18.6	29.2±4.78
CD 10	4.80±0.50	152.5±9.78	129.9±10.8	28.0±3.07
CD 20	4.82±0.45	153.6±18.4	131.0±12.1	30.5±2.62
CP 150	1.22±0.15 ^a	50.9±6.56 ^a	60.6±9.79 ^a	10.5±0.07 ^a
CD 5+CP	2.88±0.11 ^b	70.4±5.63 ^b	84.6±11.2 ^b	15.7±0.88 ^b
CD 10+CP	3.42±0.27 ^c	95.7±9.91 ^b	100.9±10.4 ^b	22.6±4.11 ^c
CD 20+CP	4.04±0.74 ^c	144.3±18.0 ^c	118.6±15.2 ^c	28.0±1.24 ^c

CD=Cimetidine, CP=Cyclophosphamide, values are expressed as Mean±SEM, n=6, ^aSignificant (p<0.05) difference when compared to control, ^bSignificant (p<0.05) difference when compared to CP-treated rats, ^cSignificant (p<0.01) difference when compared to CP-treated rats.

Table 4: Effect of cimetidine on kidney oxidative stress indices of cyclophosphamide-treated rats

Dose (mg/kg)	MDA nmole/mg protein	GSH µg/mg/protein	CAT U/mg/protein	SOD U/mg/protein	GPX µg/mg/protein
Control	0.34±0.04	7.84±0.55	21.3±2.75	19.9±2.39	24.9±3.02
CD 5	0.30±0.14	7.90±0.67	22.5±2.35	19.5±2.21	26.4±1.87
CD 10	0.33±0.22	7.95±0.75	23.8±2.59	20.3±2.24	28.0±1.67
CD 20	0.32±0.52	7.97±0.74	23.4±2.67	22.7±2.30	28.2±1.69
CP 150	0.78±0.06 ^a	1.83±0.15 ^a	8.28±1.21 ^a	6.63±0.25 ^a	6.95±0.30 ^a
CD 5+CP	0.55±0.03 ^b	3.46±0.18 ^b	11.4±1.08 ^b	11.1±0.96 ^b	10.2±1.59 ^b
CD 10+CP	0.48±0.60 ^c	5.19±0.16 ^c	16.9±2.86 ^b	15.6±1.35 ^c	15.6±1.46 ^c
CD 20+CP	0.32±0.02 ^c	7.61±0.87 ^c	22.2±1.84 ^c	21.1±2.16 ^c	22.2±3.85 ^c

CD=Cimetidine, CP=Cyclophosphamide, values are expressed as Mean±SEM, n=6, ^aSignificant (p<0.05) difference when compared to control, ^bSignificant (p<0.05) difference when compared to CP-treated rats, ^cSignificant (p<0.01) difference when compared to CP-treated rats.

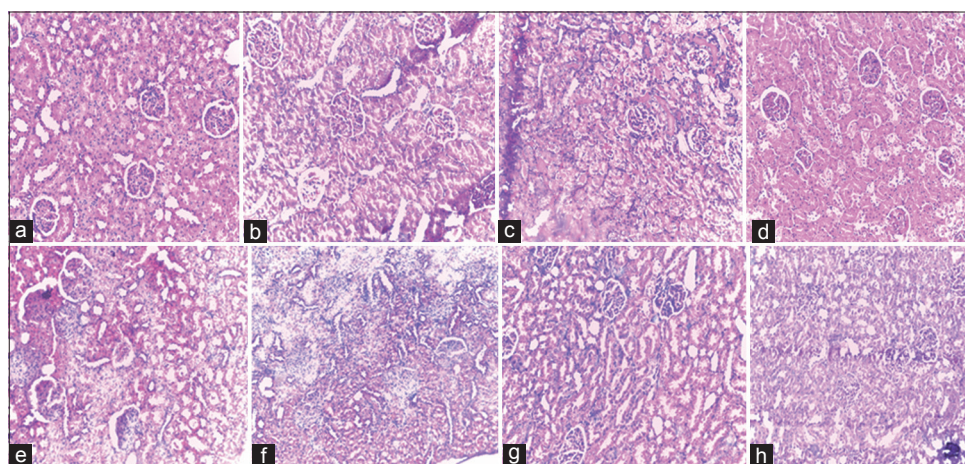


Figure 1: (a) Kidney of control rat treated with water showing normal histology. (b-d) Kidneys of rats treated with cimetidine 5, 10, 20 mg/kg daily for 5 days showing normal histology. (e) Kidney of rat treated with 150mg/kg of cyclophosphamide showing dilated tubules with necrosis, interstitial edema with infiltration of interstitium by inflammatory cells. (f) Kidney of rat treated with 150mg/kg of cyclophosphamide showing dilated tubules with necrosis, interstitial edema with infiltration of interstitium by inflammatory cells. (g) Kidney of rat treated with 10mg/kg of cimetidine and 150mg/kg of cyclophosphamide showing tubular necrosis. (h) Kidney of rat treated with 10mg/kg of cimetidine and 150mg/kg of cyclophosphamide showing tubular necrosis (H and Ex 200)

roles in accurate diagnosis of renal diseases. Instead of urine analysis which is relatively discomforting for patients, serum analysis of renal function markers which include urea, creatinine, uric acid and electrolytes are used routinely.²³ In the current study, evaluation of the serum levels of creatinine, urea and uric acid in CP-administered rats showed higher levels above normal. This observation is a common feature of CP associated nephrotoxicity.²⁴ The increase in serum levels of these parameters after CP administration

may be due to reduction in glomerular filtration rate and renal excretion of these parameters.²⁵ However, serum levels of creatinine, urea and uric acid were restored in a dose-dependent manner in rats pretreated with cimetidine. Electrolytes are positively and negatively charged ions that are found within cells and extracellular fluids. Electrolyte evaluation includes the measurement of sodium, potassium, chloride, and bicarbonate. These ions are measured to assess kidney function.²⁶ Serum electrolytes are the keys to

homeostasis and their regulation depend on renal function. Kidney disease is associated with aberrations in the regulation of serum electrolytes.²⁷ This study observed altered levels of serum electrolytes in CP-treated rats. This is consistent with observations reported by some authors.²⁸ In contrast; the serum levels of electrolytes were restored in a dose-dependent manner in rats pretreated with cimetidine. CP renal pathology could be associated with necrotic changes in the histo-architecture of the kidney. Studies have reported atrophy of glomerular tuft, tubular necrosis, dilation, edema and congestion of renal blood vessels.²⁹ The finding in this study showed tubular necrosis, edema and inflammatory cells infiltration in the kidneys of CP-treated rats. However, necrotic changes were ameliorated in cimetidine pretreated rats.

According to Park *et al.* (2013)³⁰ oxidative stress mediates a wide range of renal impairments through oxidative radical production and antioxidant depletions. Superoxide dismutase (SOD) is the first antioxidant enzyme to deal with oxy-radicals by accelerating the dismutation of superoxide to hydrogen peroxide. Catalase (CAT) is a peroxisomal hem protein that catalyzes the removal of hydrogen peroxide formed during the reaction catalyzed by SOD. Thus, SOD and CAT act as mutually supportive antioxidants that provide defence against oxidative radicals.³¹ Glutathione (GSH) is a major constituent of the detoxification pathway. The amino acid constituent of GSH (cysteine) is regarded as part of the first line of defence and neutralizes oxidative radicals and plays an important role against inflammation and oxidative stress.³² Studies suggest the role of oxidative stress in CP-induced renal toxicity and it is often accompanied by depletions and decreases in antioxidant activities.³³ The current study observed depletions in antioxidant activities characterised by lower levels of SOD, CAT, GSH and GPX in the kidneys of CP-treated rats. However, depletions were inhibited and the activities of these antioxidants were up-regulated in cimetidine pretreated rats. Lipid peroxidation is a free radical stimulated oxidative destruction of poly unsaturated fatty acid. It is the product of the reaction of free radicals with lipids that is considered as an integral feature of xenobiotic-induced renal injury.³⁴ Malondialdehyde (MDA) is one of end products of lipid peroxidation and is used to assess lipid peroxidation and free radical generation.³⁵ In consonance with finding by Golshahiet *al.*, 2018³⁶ the present study, observed lipid peroxidation marked by elevated kidney levels of MDA in CP-treated rats. However, there was decrease in lipid peroxidation marked by low levels of MDA in cimetidine pretreated rats.

The mechanism by which CP causes kidney damage is not well understood, but studies have attributed it to its

cytotoxic metabolites; phosphoramidate mustard and acrolein. Phosphoramidate mustard has antineoplastic activity while acrolein, is a highly reactive metabolite that can induce oxidative stress.³⁷ Furthermore, Abraham *et al.*, (2009)³⁸ reported that CP-induced renal damage may be due to nitrosative stress. Also, CP can induce the production of TNF- α in renal tissues. TNF- α is a pleiotropic cytokine that can induce cell death via apoptosis and necrosis pathway.³⁹ In the present study, the observed ameliorative effect of cimetidine could be attributed to its ability to inhibit CP-induced oxidative and nitrosative stress in renal tissues of treated rats. Cimetidine has the ability to scavenge free radicals, up-regulate antioxidant activities and inhibits the production of pro-inflammatory mediators.⁴⁰ Also, as an inhibitor of hepatic enzyme, cimetidine might have prevented the biotransformation of CP to its toxic metabolites by hepatic enzymes.

CONCLUSION

Cimetidine ameliorates cyclophosphamide-induced renal toxicity in albino rats.

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EA- Concept and design of the study, literature search, collection of data, statistical analysis, manuscript preparation and critical revision of the manuscript;
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