



PROTocatechuic acid RESCUES CYCLOPHOSPHAMIDE-MEDIATED OXIDATIVE STRESS IN WISTAR RATS

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Summary

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The objective of this study was to evaluate the effect of protocatechuic acid (PCA), a notable polyphenol, on cyclophosphamide (CPM)-induced oxidative stress in Wistar rats. Twenty male Wistar rats (175±25 g) were allotted into four equal groups (A to D) and treated as follows: Group A (control): 1 mL/kg body weight normal saline, group B: 25 mg/kg of CPM, group C: 50 mg/kg PCA, and group D received both PCA and CPM. The treatments were carried out orally for seven days, except for the CPM, which was administered intraperitoneally on days 6 and 7. Haematological and serum biochemistry parameters, malondialdehyde (MDA), H₂O₂ and glutathione (GSH) levels, superoxide dismutase (SOD), glutathione-S-transferase (GST), and glutathione peroxidase (GPx) activities were monitored in the liver, kidneys, and heart of the experimental rats. The CPM-treated group showed a reduction in body weight change compared to the control. This reduction was restored in the co-treated group by PCA. In the liver, CPM caused a significant (P<0.05) increase in H₂O₂ level compared to the control. There was a significant (P<0.05) decrease in GSH levels and activities of GPx and SOD in CPM-treated rats relative to the control group. In the kidney, CPM significantly (P<0.05) increased MDA level and decreased GSH level and SOD activity compared to the control. SOD activity was reduced significantly (P<0.05) by CPM in the cardiac tissues compared to the control group. PCA significantly reversed these parameters in the tissues of the co-treated rats. It was concluded that protocatechuic acid rescues cyclophosphamide-induced toxicity in rats via the suppression of oxidative stress and induction of the antioxidant system biomarkers.

Key words: antioxidant, cyclophosphamide, oxidative stress, protocatechuic acid

INTRODUCTION

Invasive chemotherapy and radiotherapy have been used in cancer management for many years. Unfortunately, chemotherapy is tissue-neutral and causes harm to both

healthy and cancerous cells via direct and indirect processes (Abdel Hafez *et al.*, 2017; Zhang *et al.*, 2024). Cyclophosphamide, a member of the nitrogen mus-

tard family that belongs to the oxazaphosphorine class of alkylating agents, is a notable chemotherapy used in clinical settings to manage malignant diseases (Bhat *et al.*, 2018). It still remains one of the most effective anti-tumour medications, and its immunosuppressive effects are also well-known (Lee *et al.*, 2024; Ma *et al.*, 2025). Cyclophosphamide is used in the treatment of several tumours, such as multiple myeloma, and its immunomodulatory quality at low doses has been explored to treat non-neoplastic conditions, including vasculitis, rheumatoid arthritis, and lupus erythematosus (Pugh *et al.*, 2018; Swan *et al.*, 2020; Shi *et al.*, 2023). Interestingly, cyclophosphamide use increases the risk of tumour and toxicity, including bladder toxicity (Bernatsky & Clarke, 2008; Mills *et al.*, 2019). Cyclophosphamide mediates toxicity mainly in tissues with frequently dividing cells, such as the bone marrow (Floyd *et al.*, 2005; Paul *et al.*, 2011). Also, cyclophosphamide toxicity has been reported in the liver, kidney, and testes (Turk *et al.*, 2010; Bhat *et al.*, 2018). Cyclophosphamide causes cellular damage, severe tissue oxidative stress, and apoptosis (Bhatia *et al.*, 2006; Abdel-Hafez *et al.*, 2017; Olukole *et al.*, 2020; Swan *et al.*, 2020). Cyclophosphamide treatment disrupts the markers of the oxidant-antioxidant system, such as superoxide dismutase, reduced glutathione, glutathione peroxidase, and alkaline phosphatase (Adikwu & Bokolo, 2018; Pavin *et al.*, 2018).

Some intervention methods, such as the use of polyphenols, which are rich in antioxidants, are employed to ameliorate the biochemical derangements mediated by most anticancer drugs, including cyclophosphamide. Polyphenols make up a major part of human diets. They have an-

ti-oxidant properties and are categorised into different classes, such as phenolic acids, flavonoids, lignans, and stilbenes (Kakkar & Bais, 2014; Rudrapal *et al.*, 2022). The use of protocatechuic acid (PCA), a dihydroxybenzoic acid, has drawn a lot of attention. Protocatechuic acid is a metabolite of complex polyphenols, including anthocyanins and procyanidins, which are abundant in edible fruits and vegetables and are absorbed by humans and animals (Masella *et al.*, 2012; Al Olayan *et al.*, 2020).

PCA has been reported for its beneficial properties and physiological activities. It has antioxidant, antibacterial, anti-diabetic, anti-inflammatory, and analgesic activities (Kakkar & Bais, 2014; Adedara *et al.*, 2019). The neuroprotective effect of PCA has been documented in rats (Al Olayan *et al.*, 2020), including its hepatorenal protective activity in Wistar rats (Owumi *et al.*, 2019). PCA is useful against oxidative stress-induced neurodegenerative diseases (Choi *et al.*, 2020). The immune modulatory effect of PCA has been reported in cyclophosphamide-induced brain damage (Salama *et al.*, 2022). This study, therefore, aims to investigate the potential protective effect of protocatechuic acid on cyclophosphamide-induced oxidative stress in the liver, heart, and kidney of male Wistar rats.

MATERIALS AND METHODS

Drugs and chemicals

Cyclophosphamide (CPM) (MW 279.10, As 57.6% CAS No 7784-46-5; Sigma-Aldrich, USA) was used at a dosage of 25 mg/kg body weight, equivalent to 1/10 of the oral LD₅₀ of the salt. The analytical grades of protocatechuic acid, xylenol orange, trichloroacetic acid, Tris Base, thiobarbituric acid, NaOH, 5,5'-dithio-bis-

2-nitrobenzoic acid, reduced glutathione, and 1,2-dichloro-4-nitrobenzene were purchased (Sigma-Aldrich, St. Louis, MO, USA) and used.

Experimental animals

Twenty male Wistar rats (150 g – 200 g) were purchased from the Experimental Animal Unit of the Faculty of Veterinary Medicine, University of Ibadan. The animals were accommodated in well-ventilated cages and provided with commercial rat chow and water *ad libitum*. All experiments were conducted without anaesthesia, and the protocol conformed to the guidelines of the National Institute of Health for laboratory animal care and use (National Institute of Health, 1985).

Experimental protocol

The rats were randomly and equally allocated into four experimental groups. The treatment modalities were as follows: Group A (control) was administered normal saline at 1 mL/kg body weight, Group B: 25 mg/kg of cyclophosphamide (CPM), Group C: 50 mg/kg protocatechuic acid (PCA), and Group D: PCA and CPM. The treatments were carried out orally for seven days, except for the CPM, which was administered intraperitoneally on days 6 and 7. The body weights of the rats were recorded every other day until the day of sacrifice.

Haematological analysis

Following the completion of treatment, blood samples were collected from the retro-orbital venous plexus into heparinised tubes and utilised for the estimation of packed cell volume (PCV), haemoglobin concentration (HB), red blood cell (RBC) count, total and differential white blood cell (WBC) count (Schalm *et al.*, 1975). The mean corpuscular volume

(MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were extrapolated from the PCV, RBC, and HB values (Ikewuchi & Ikewuchi 2013).

Biochemical analysis

Blood samples were collected into plain tubes, allowed to clot, and centrifuged (3000 rpm for 10 minutes) to obtain serum samples. The samples were refrigerated at -4°C until needed. Creatinine, blood urea nitrogen (BUN), total protein, albumin, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) levels were determined using the Randox laboratory kits (UK). Also, the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were monitored using Randox laboratory kits as described by the manufacturer (Randox Laboratories Ltd, Crumlin, UK).

According to the method described by Oyagbemi *et al.* (2017), the heart, kidney, and liver tissues were excised, gently rinsed with cold phosphate buffer solution, weighed, placed in phosphate buffer solution (0.1M, pH 7.4), and stored in ice before homogenisation. The tissues were homogenised, and the homogenate centrifuged (10,000 rpm, 10 min, -4°C). The resulting supernatant was obtained and used for oxidant-antioxidant assays.

Evaluation of oxidative stress markers. The level of malondialdehyde (MDA) to assess lipid peroxidation was determined as described by Varshney & Kale (1990). Hydrogen peroxide (H_2O_2) levels were determined as previously described (Wolff, 1994).

Evaluation of antioxidant markers. Superoxide dismutase (SOD) activity was determined according to the method of Misra & Fridovich (1972) with a slight

modification (Oyagbemi *et al.*, 2015). Reduced glutathione (GSH) level was assessed using the method described by Beutler *et al.* (1963). Glutathione-S-transferase (GST) and glutathione peroxidase (GPx) activities were determined according to the method described by Habig & Pabst (1974) and Rotruck *et al.* (1973), respectively.

Statistical analysis

The data generated were subjected to one-way analysis of variance (ANOVA) with Dunnett's multiple comparisons test using GraphPad Prism version 5. All values were expressed as mean \pm standard deviation (SD) with P-value <0.05 considered statistically significant.

RESULTS

The effect of CPM and PCA administered alone or in combination on body weight changes in Wistar rats was investigated (Table 1). The group treated with CPM showed a reduction in body weight change compared to the control. This was restored in the co-treated group by PCA. There was about two-fold increase in the body weight of PCA-treated rats compared to control animals. No significant difference was found in the relative organ weights of all experimental groups except for the significant ($P<0.05$) increase in the relative liver weight change in the co-exposed group (Fig. 1).

The mean values of the haematological parameters - packed cell volume (PCV), haemoglobin concentration (HB), red blood cell (RBC) count, total and differential white blood cell (WBC) count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) of rats treated with PCA and

Table 1. Body weight changes (%) in rats from different groups. Control: 1 mL/kg of distilled water, CPM: 25 mg/kg of cyclophosphamide; PCA: 50 mg/kg of protocatechuic acid; PCA + CPM: 25 mg/kg CPA and 50 mg/kg PCA. All results were expressed as the mean \pm standard deviation (n=5).

Group	Body weight change (%)
Control	8.95 \pm 0.086 ^{ab}
CPM	7.23 \pm 0.433 ^{ac}
PCA	18.37 \pm 0.120 ^{bcd}
PCA+CPM	8.73 \pm 0.088 ^{cd}

Means with the same superscript (a,b,c,d), within the column are significantly different.

CPM are shown in Table 2. There was no significant ($P>0.05$) difference in PCV, RBC, HB, WBC and its differentials for all the groups. However, total WBC counts in the PCA-treated rats were significantly increase ($P<0.05$) compared to the control rats.

Regarding the blood electrolytes of rats treated with PCA and CPM, there was no significant ($P>0.05$) difference in the concentrations of sodium, potassium, chloride, and bicarbonate of all the groups compared to the control. Similarly, total protein, albumin, urea and creatinine, and activities of ALP, AST, and ALT of CPM-treated rats were insignificantly increased than those in the control group. Also, there was no significant ($P>0.05$) difference in the average levels of HDL and LDL in all treated groups vs controls (Table 3).

The effects of CPM and PCA administered alone or in combination on liver markers of oxidant-antioxidant system in Wistar rats was investigated. In relation to controls, CPM increased significantly ($P<0.05$) H_2O_2 but insignificantly ($P>0.05$) MDA levels (Fig. 2). The PCA and the PCA+CPM groups showed a reduction in these parameters compared to

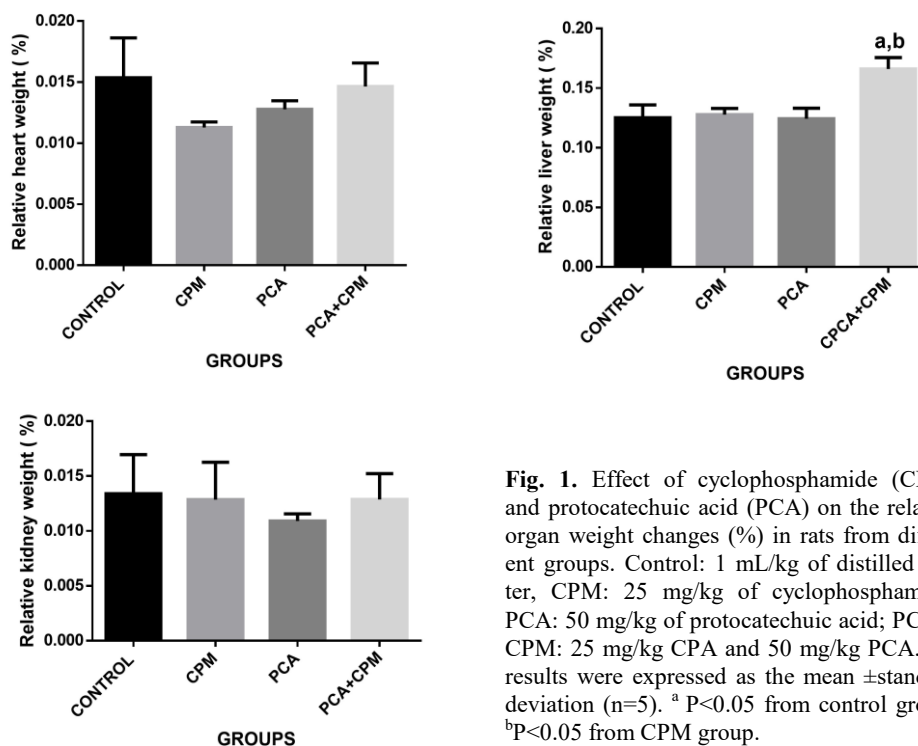


Fig. 1. Effect of cyclophosphamide (CPM) and protocatechuic acid (PCA) on the relative organ weight changes (%) in rats from different groups. Control: 1 mL/kg of distilled water, CPM: 25 mg/kg of cyclophosphamide; PCA: 50 mg/kg of protocatechuic acid; PCA + CPM: 25 mg/kg CPA and 50 mg/kg PCA. All results were expressed as the mean \pm standard deviation (n=5). ^a P<0.05 from control group; ^b P<0.05 from CPM group.

Table 2. Haematological indices of rats from different groups. Control: 1 mL/kg of distilled water, CPM: 25 mg/kg of cyclophosphamide; PCA: 50 mg/kg of protocatechuic acid; PCA + CPM: 25 mg/kg CPA and 50 mg/kg PCA. All results were expressed as the mean \pm standard deviation (n=5).

Parameters	Control	CPM	PCA	PCA+CPM
Packed cell volume (L/L)	0.50 \pm 0.012	0.51 \pm 0.015	0.52 \pm 0.015	0.52 \pm 0.015
Haemoglobin (g/L)	162.3 \pm 3.8	165.0 \pm 5.9	165.3 \pm 7.1	158.7 \pm 19.0
RBCs ($\times 10^{12}$ /L)	11.67 \pm 0.68	12.87 \pm 0.77	13.12 \pm 0.73	12.70 \pm 0.23
MCV (fL)	43.00 \pm 2.50	40.00 \pm 2.16	39.00 \pm 1.91	39.00 \pm 5.50
MCHC (g/L)	325.0 \pm 0.50	321.0 \pm 6.60	320.0 \pm 8.40	321.2 \pm 1.30
MCH (pg)	32.50 \pm 5.72	32.00 \pm 6.60	32.00 \pm 3.22	32.10 \pm 3.30
Platelets ($\times 10^9$ /L)	514 \pm 4.50	560 \pm 6.40	591 \pm 6.4	558 \pm 1.2
WBCs ($\times 10^9$ /L)	8.18 \pm 0.33 ^a	9.03 \pm 0.15	9.96 \pm 0.24 ^a	9.04 \pm 0.20
Neutrophils (%)	66.00 \pm 4.04	63.33 \pm 3.28	61.33 \pm 5.21	69.67 \pm 3.38
Lymphocytes (%)	34.00 \pm 4.04	34.67 \pm 2.91	37.33 \pm 5.24	29.67 \pm 3.84

Means with the superscript (a), within rows are significantly different at P<0.05.

the control and the CPM rats. In addition, the antioxidant system parameters (SOD, GPx, and GSH) were significantly reduced (P<0.05) in the group treated only

with CPM compared to control whereas these reductions were reversed in the co-exposed group by the PCA (Fig. 3).

In the kidney, CPM administration re-

Table 3. Serum biochemistry indices of rats from different groups. Control: 1 mL/kg of distilled water, CPM: 25 mg/kg of cyclophosphamide; PCA: 50 mg/kg of protocatechuic acid; PCA + CPM: 25 mg/kg CPA and 50 mg/kg PCA. All results were expressed as the mean \pm standard deviation (n=5).

Parameters	Control	CPM	PCA	PCA+CPM
Na ⁺ (mmol/L)	139.0 \pm 1.16	140.7 \pm 0.33	137.3 \pm 0.88	138.3 \pm 2.19
K ⁺ (mmol/L)	3.90 \pm 0.06	3.83 \pm 0.12	3.73 \pm 0.19	3.73 \pm 0.19
Cl ⁻ (mmol/L)	103.3 \pm 1.67	106.7 \pm 1.67	100.0 \pm 2.89	101.7 \pm 4.41
HCO ₃ ⁻ (mmol/L)	22.00 \pm 1.16	22.67 \pm 0.88	24.33 \pm 0.33	23.00 \pm 1.53
Urea (mmol/L)	4.77 \pm 0.147	5.66 \pm 0.510	4.33 \pm 0.193	4.55 \pm 0.683
Creatinine (μ mol/L)	55.70 \pm 2.65	61.89 \pm 5.30	50.40 \pm 2.65	55.70 \pm 7.96
Total protein (g/L)	68.7 \pm 0.90	70.7 \pm 1.30	67.0 \pm 0.60	68.7 \pm 2.6
Albumin (g/L)	38.3 \pm 1.50	40.0 \pm 1.50	36.7 \pm 0.90	39.3 \pm 1.80
AST (IU/L)	14.33 \pm 1.45	16.67 \pm 1.20	13.00 \pm 0.58	16.00 \pm 0.58
ALP (IU/L)	54.00 \pm 4.16	61.33 \pm 1.76	52.00 \pm 3.61	60.33 \pm 1.76
ALT (IU/L)	10.33 \pm 0.88	13.33 \pm 0.88	9.67 \pm 0.88	13.33 \pm 0.67
HDL (mmol/L)	0.474 \pm 0.057	0.500 \pm 0.067	0.517 \pm 0.091	0.440 \pm 0.065
LDL (mmol/L)	0.784 \pm 0.129	0.871 \pm 0.075	0.897 \pm 0.116	0.845 \pm 0.151

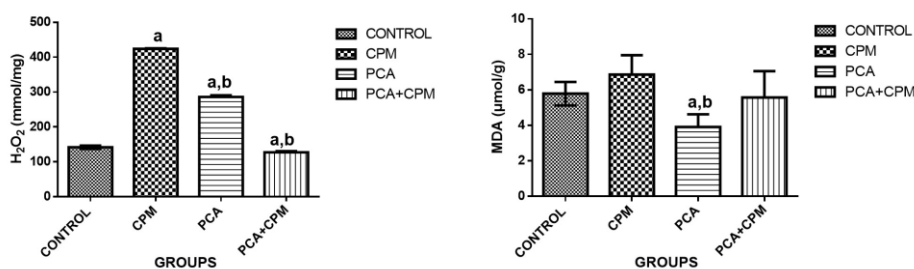


Fig. 2. Effect of cyclophosphamide (CPM) and protocatechuic acid (PCA) on the liver oxidative stress indices in rats from different groups. Control: 1 mL/kg of distilled water, CPM: 25 mg/kg of cyclophosphamide; PCA: 50 mg/kg of protocatechuic acid; PCA+CPM: 25 mg/kg CPA and 50 mg/kg PCA. All results were expressed as the mean \pm standard deviation (n=5). ^aP<0.05 from control group; ^bP<0.05 from CPM group.

sulted in a significant (P<0.05) increase in MDA level compared to the control. The MDA level was reduced significantly (P<0.05) in the PCA-only and co-exposed compared to CPM-challenged group (Fig. 4). Meanwhile, PCA significantly increased the GPx and SOD activities (P<0.05) compared to control and CPM groups. The GST and GSH levels however were slightly increased (P>0.05) by PCA compared to the controls. The PCA reversed the reduced activity of these enzymes in the co-treated group (Fig. 5).

In the cardiac tissue, CPM administration resulted in a non-significant (P>0.05) increase in the MDA and H₂O₂ contents in relation to saline-treated controls. Again, there was no significant (p>0.05) difference in the levels of MDA and H₂O₂ in the PCA and co-exposed groups compared to the control (Fig. 6). The CPM caused a significant (P<0.05) reduction in SOD activity compared to the control. Meanwhile, the PCA group displayed a non-significant (P>0.05) increase in GSH levels and GPx and GST activities compared to control rats (Fig. 7).

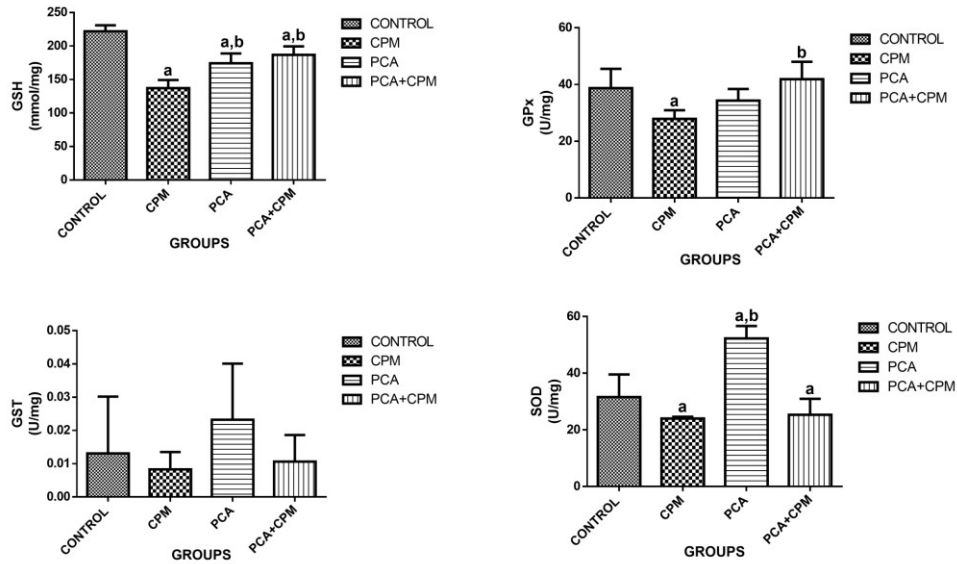


Fig. 3. Effect of cyclophosphamide (CPM) and protocatechuic acid (PCA) on the liver antioxidant system indices in rats from different groups. Control: 1 mL/kg of distilled water, CPM: 25 mg/kg of cyclophosphamide; PCA: 50 mg/kg of protocatechuic acid; PCA + CPM: 25 mg/kg CPA and 50 mg/kg PCA. All results were expressed as the mean \pm standard deviation (n=5). ^aP<0.05 from control group; ^bP<0.05 from CPM group.

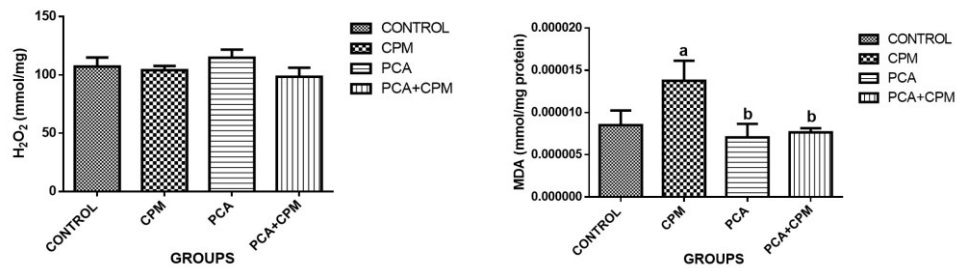


Fig. 4. Effect of cyclophosphamide (CPM) and protocatechuic acid (PCA) on the kidney oxidative stress indices in rats from different groups. Control: 1 mL/kg of distilled water, CPM: 25 mg/kg of cyclophosphamide; PCA: 50 mg/kg of protocatechuic acid; PCA + CPM: 25 mg/kg CPA and 50 mg/kg PCA. All results were expressed as the mean \pm standard deviation (n=5). ^aP<0.05 from control group; ^bP<0.05 from CPM group.

Protocatechuic acid rescues cyclophosphamide-mediated oxidative stress in Wistar rats

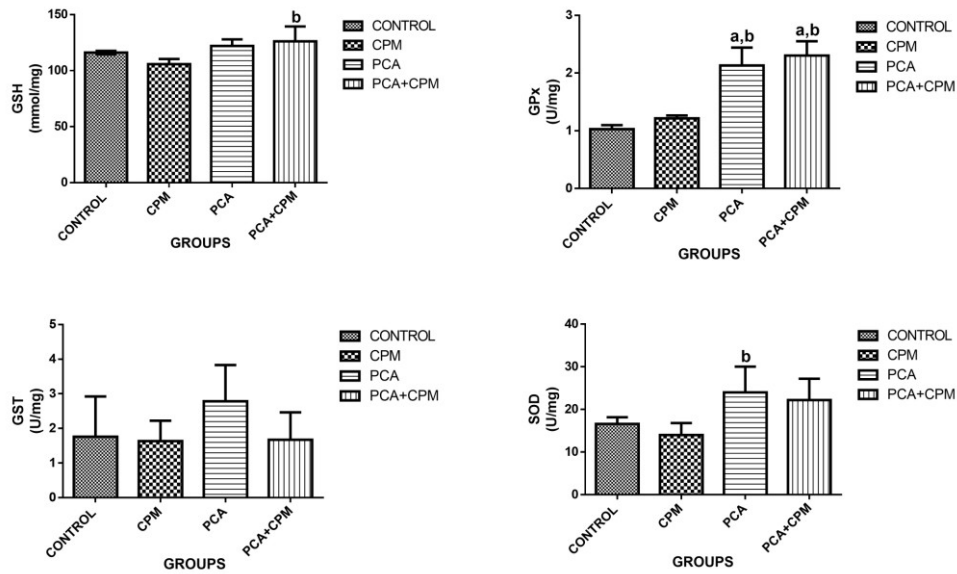


Fig. 5. Effect of cyclophosphamide (CPM) and protocatechuic acid (PCA) on the kidney antioxidant system indices in rats from different groups. Control: 1 mL/kg of distilled water, CPM: 25 mg/kg of cyclophosphamide; PCA: 50 mg/kg of protocatechuic acid; PCA + CPM: 25 mg/kg CPA and 50 mg/kg PCA. All results were expressed as the mean \pm standard deviation (n=5). ^aP<0.05 from control group; ^bP<0.05 from CPM group.

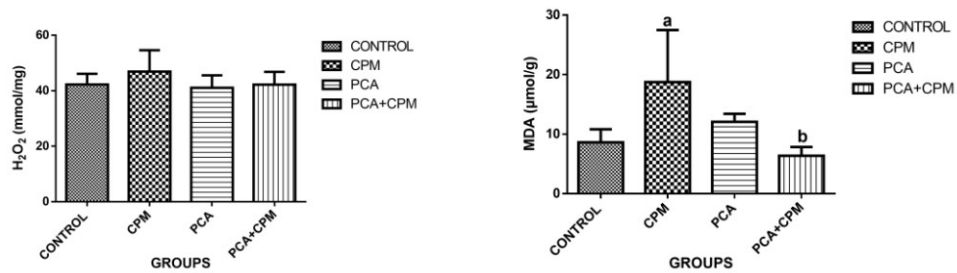


Fig. 6. Effect of cyclophosphamide (CPM) and protocatechuic acid (PCA) on the heart oxidative stress indices in rats from different groups. Control: 1 mL/kg of distilled water, CPM: 25 mg/kg of cyclophosphamide; PCA: 50 mg/kg of protocatechuic acid; PCA + CPM: 25 mg/kg CPA and 50 mg/kg PCA. All results were expressed as the mean \pm standard deviation (n=5). ^aP<0.05 from control group; ^bP<0.05 from CPM group.

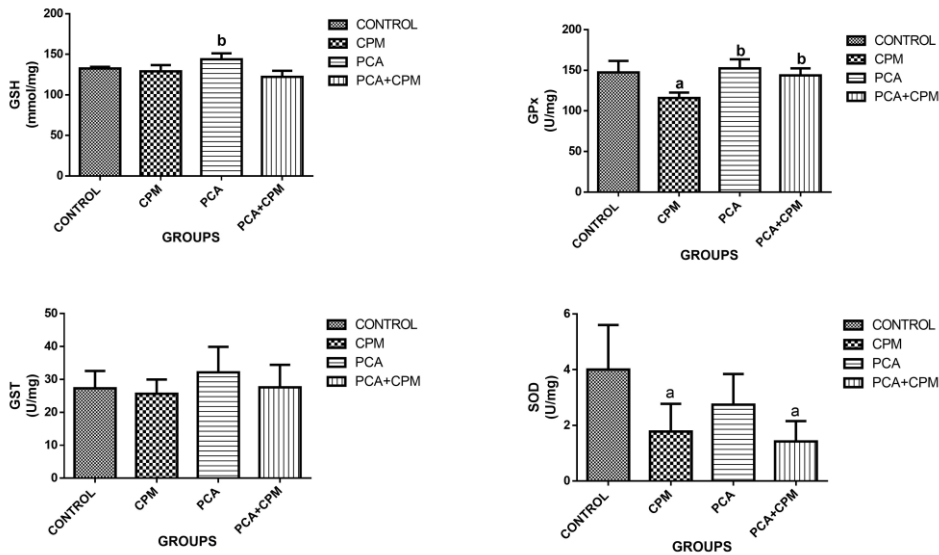


Fig. 7. Effect of cyclophosphamide (CPM) and protocatechuic acid (PCA) on the heart antioxidant system indices in rats from different groups. Control: 1 mL/kg of distilled water, CPM: 25 mg/kg of cyclophosphamide; PCA: 50 mg/kg of protocatechuic acid; PCA + CPM: 25 mg/kg CPA and 50 mg/kg PCA. All results were expressed as the mean \pm standard deviation (n=5). ^aP<0.05 from control group; ^bP<0.05 from CPM group.

DISCUSSION

Cyclophosphamide (CPM) is a typical chemotherapeutic drug used to treat tumours and functions primarily by suppressing the immune system (Ma *et al.*, 2025). Despite its numerous therapeutic uses, it has been recognised as a carcinogen, and its continued use increases the possibility of leukaemia, lymphomas, bladder and skin cancer (Bernatsky & Clarke, 2008; Mills *et al.*, 2019). This has prompted the need to find alternative substances which can serve as an adjunct to ameliorate its side effects. Protocatechuic acid (PCA) has been obtained from various plants and reported to show therapeutic effects (Wang *et al.*, 2024). PCA, a phenolic compound and major metabolite of anthocyanins, shows high bioavailabil-

ity in tissues and positive biological effects, such as amelioration of oxidative stress (Lubbers & deVries, 2021).

The findings of this current study align with and further corroborate the previously reported therapeutic potential of protocatechuic acid, as observed in the cyclophosphamide-induced toxicity model (Salama *et al.*, 2023). Our study is consistent with other studies which reported the CPM-induced reduction in body weight or weight gain in rats and mice (Hou *et al.*, 2007; Zhang *et al.*, 2021a). A similar result was reported that CPM was unable to alter relative organ weights, as we discovered in our study (Zhang *et al.*, 2021a). CPM and PCA administration did not significantly alter sodium, potassium, chloride, and bicarbonate levels compared to the control group. This suggests that

CPM might not cause a substantial disruption in ion homeostasis. However, there is a need to investigate whether the continuous and prolonged exposure to CPM could result in severe ion disruption. In this study, the red and white blood cell parameters measured in the rats were not affected by CPM exposure. This corroborates the previous findings (Zhang *et al.*, 2021a) supporting the fact that CPM does not negatively affect erythropoiesis.

The administration of CPM resulted in a non-significant increase in blood urea nitrogen and serum creatinine concentration. This slight increase following acute CPM treatment has been reported in acute renal injury (Kellum *et al.*, 2021). A more severe toxicity induced by CPM on urea and creatinine was confirmed in rats (Sakr & El-messady, 2017) and other animal models, such as mice (El-Naggar *et al.*, 2015; Bhat *et al.*, 2018). Disruptions in the levels of creatinine and urea have been associated with renal diseases (McWilliam & Macnab, 2009). In addition, the non-significant increase in ALP, AST, and ALT activities in the CPM-treated rats could suggest mild damage to the liver of the exposed rats (Kalas *et al.*, 2021). The liver is a major organ that metabolises CPM through biotransformation, which can result in the generation of toxic metabolites (Hyun *et al.*, 2021), ultimately resulting in oxidative stress-mediated hepatotoxicity. In a study by Mansour *et al.* (2017), CPM demonstrated a toxic effect in the liver of male Wistar rats. The authors reported elevated serum ALT and AST associated with increased hepatic oxidative stress and inflammatory biomarkers. Similarly, Jali *et al.* (2023) reported increased liver biomarkers, AST, ALT, ALP, and bilirubin in CPM-treated experimental rats. The pronounced elevation in these liver damage markers might

be due to the high dosages (150 mg/kg and 200 mg/kg) used. Furthermore, in our study, HDL and LDL levels were not significantly altered among all groups suggesting that both CPM and PCA did not alter lipid metabolism at given dosages. This is in contrast to previous studies that reported an association between CPM administration and lipid peroxidation and dyslipidaemia (Cengiz *et al.*, 2020).

Previous studies reported that CPM can induce oxidative stress and inflammation in various organs through its ability to generate acrolein, a toxic metabolite (Moghe *et al.*, 2015; Bhat *et al.*, 2018) from the metabolism of CPM by the Cytochrome P450 isozymes (CYP2B6, 2C9, and 3A4) (Zhao *et al.*, 2021). Acrolein is a reactive aldehyde that not only initiates oxidative stress but also has the potential to attack macromolecules and cause DNA damage (Hikisz & Jacenik, 2023). This cellular event in the kidney impairs normal function and disrupts the renal metabolism of urea and the excretion of creatinine, leading to accumulation in the blood.

In the cardiac tissue, an insignificant increase in the level of malondialdehyde (MDA) and hydrogen peroxide (H₂O₂) in groups treated with CPM – an indication of oxidative stress and lipid peroxidation was found, which may also be attributed to the generation of acrolein, the metabolite of CPM. The kidney and liver showed greater elevation in these parameters in the CPM-treated groups. This is consistent with the reports of previous researchers (Oyagbemi *et al.*, 2016; Sherif, 2018; Zhang *et al.*, 2021a). This result may also be attributed to the response of the endogenous antioxidant system that functions to modulate a balance between the oxidant-antioxidant system in living organisms, as supported by Ibrahim *et al.* (2023). The ability of the PCA to reverse these elevat-

ed levels of MDA and H₂O₂ in the PCA and co-exposed groups is a testament to the protective role of PCA against CPM-induced toxicity. PCA has been reported to be a potent therapeutic agent that can efficiently scavenge free radicals (Amić *et al.*, 2025) through the donation of electrons to neutralise ROS (Zhang *et al.*, 2021b). The antioxidant potential of PCA has been previously established by Cadena *et al.* (2024), who reported that the compound activates the transcription factor, Nrf2, resulting in increased expression of glutathione peroxidase and glutathione reductase, which are critical antioxidant enzymes in living organisms. In addition, PCA not only activate Nrf2 but it also stabilises it, hence, the association with an increase in cellular antioxidant system and a reduction in oxidative stress (Cadena *et al.*, 2024).

Superoxide dismutase (SOD) is the first-line antioxidant defence enzyme in the living organism that majorly function to convert superoxide radicals into normal molecular oxygen (O₂) and hydrogen peroxide (H₂O₂) (Younus, 2018). However, its activity was reduced following the CPM administration. According to Kopeřa *et al.* (2024), acrolein can inactivate antioxidant systems by forming a bond with their thiol (-SH) group. However, the administration of PCA played a protective role. A similar finding was observed for other markers of the antioxidant systems (GPx, GST, and GSH) across cardiac, kidney, and liver tissues.

CONCLUSIONS

The findings presented in this study establish the protective role of protocatechuic acid (PCA). This study revealed that one of the mechanisms of action of the cyclophosphamide induced toxicity is via oxi-

dative stress induction and antioxidant system suppression as revealed by elevated MDA and H₂O₂ levels, and reduced SOD, GPx, GST activities, and GSH level. However, CPM did not affect erythropoiesis negatively. Taken together, pre-exposure/co-administration of an antioxidant like protocatechuic acid with cyclophosphamide in cancer treatment might provide a good combination to protect against the undesirable effects of cyclophosphamide in cancer treatments.

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