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Moderation of doxorubicin-induced nephrotoxicity in Wistar rats by aqueous leaf-extracts of *Chromolaena odorata* and *Tridax procumbens*

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Abstract

Background: The major draw-back of doxorubicin's use in chemotherapy is its toxicity on various organs including the kidneys. This study investigated the potential protective role of aqueous leaf-extracts of *Chromolaena odorata* and *Tridax procumbens* against nephrotoxicity induced by doxorubicin.

Methods: To this end, their impact on plasma biomarkers of kidney function, as well as renal lipid profile, biomarkers of oxidative stress, electrolyte profile and activities of renal ATPases was monitored in doxorubicin treated rats. Metformin (250 mg/kg body weight, orally) and the extracts (50, 75 and 100 mg/kg, orally) were daily administered for 14 days; while nephrotoxicity was induced with doxorubicin (15 mg/kg, intra-peritioneally), once on the 12th day of study.

Results: The plasma concentrations of creatinine, and urea; as well as the renal malondialdehyde, cholesterol, calcium and sodium concentrations in the Test control, were significantly ($P < .05$) higher than those of all the other groups. However, the renal concentrations of ascorbic acid, chloride, magnesium and potassium, and the renal activities of catalase, glutathione peroxidase superoxide dismutase, Ca^{2+}-ATPase, Mg^{2+}-ATPase and Na^{+},K^{+}-ATPase in the Test control were significantly ($P < .05$) lower than those of all the other groups.

Conclusions: Pre-treatment with the extracts and metformin boosted endogenous antioxidants, and prevented doxorubicin-induced renal damage, as indicated by the attenuation of doxorubicin-induced renal oxidative stress, as well as the attenuation of doxorubicin-induced adverse alterations in renal cholesterol, ATPases and electrolyte balance, and plasma biomarkers of kidney function, and keeping them at near-normal values.

Keywords: ATPases, cholesterol, *Chromolaena odorata*, doxorubicin, electrolytes, kidney function markers, oxidative stress, *Tridax procumbens*

Introduction

Doxorubicin's toxicity affects various organs including the kidneys.[1–6] Numerous studies suggests that doxorubicin-induced toxicity may be a consequence of oxidative stress, which results in oxidation and cross-linking of cellular thiols and membrane lipid peroxidation.[1] Doxorubicin-induced oxidative stress in renal tissues is characterized by elevated malondialdehyde (a marker of lipid peroxidation) and lowered reduced glutathione levels[4,7,8]; as well as lowered activities of catalase,[2] glutathione peroxidase and superoxide dismutase.[3,8,9] In addition to oxidative damage, doxorubicin toxicity also induces inflammatory changes in kidney tissues.[8,10] Doxorubicin-induced nephrotoxicity causes increased capillary porousness and glomerular shrinking.[8] It is characterized by increased plasma levels of creatinine, urea[2,7] and uric acid,[7] and increased plasma lactate dehydrogenase activity[7]; as well as reduced renal Ca^{2+}-ATPase, Mg^{2+}-ATPase and Na^{+}, K^{+}-ATPase activities.[1,11]

Studies suggest that the healing effect of metformin (a widely used anti-hyperglycaemic drug for type 2 diabetes mellitus) is facilitated by its effect on adenosine monophosphate-activated protein kinase in tissues.[12,13] Numerous studies show that metformin lowers intracellular reactive oxygen species and regulates mitochondrial function.[12,14,15] The beneficial effects of metformin on renal injury with different aetiologies have been reported,[16] including the alleviation of diabetes-associated renal injury.[12,17] Metformin ameliorates tubular injury by regulating oxidative stress and restoring biochemical alterations in renal tubules,[12] as well as by anti-inflammatory and anti-apoptotic activities.[14,18]

Studies have shown that doxorubicin-induced oxidative damage to the kidney can be mitigated or prevented by treatment with natural antioxidants;[9,10,19] hence necessitating the investigation of various natural sources of antioxidants. The leaves of *Chromolaena odorata* and *T procumbens* are rich in potent antioxidants such as allicin, caffeic acid, ellagic acid, epicatechin, lycopene, naringenin, quercetin and silymarin.[20–26] These antioxidants have been variously reported to exert nephro-

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protective effects via attenuation of oxidative stress in the kidney, induced by doxorubicin, oxytetracycline, cadmium or gentamicin.[2,5,8,9,10,19,27–32]

Various studies have reported the anti-hypertensive, anti-dyslipidaemic, weight reducing,[22,24,25,33–39] hepato-protective and anti-diabetic activities of leaf-extracts of *C odorata* and *T procumbens*.[21,40–44] Their anticancer[45-47] and antioxidant[48–52] activities have also been reported. In this study the impact of aqueous leaf-extracts of *C odorata* and *T procumbens* on doxorubicin-induced renal damage was investigated in Wistar rats.

Materials and methods

Procurement of materials

Fresh samples of *C odorata* and *T procumbens* were collected from within the University of Port Harcourt, and were duly identified as earlier reported.[20–26,34–39,41,53] Forty-five Wistar rats (weight 120–190 g) were obtained from the Animal House of Department of Pharmacology, University of Port Harcourt, Nigeria. All chemicals used were of analytical grade and products of Sigma-Aldrich, St Louis, MO, USA. The cholesterol, triglyceride and calcium kits were products of Randox Laboratories Ltd, County Antrim, UK; the sodium and potassium kits were products of Atlas Medical, Cowley Rd, Cambridge, UK; while the chloride, magnesium, creatinine and urea kits were products of Agappe Diagnostics Switzerland, GmbH.

Preparation of extracts

The leaves were rid of dirt. Then 6 kg of *C odorata* and 5.5 kg of *T procumbens* were macerated. The resultant extracts were dried in a water bath, and their residues (127 and 116 g, respectively) were stored for use in the assay. The resultant leaf-extracts of *C odorata* and *T procumbens* (hereinafter referred to as COLE and TPLE, respectively), were weighed, reconstituted in distilled water and administered to the experimental animals, according to their groups' dosages and their individual weights.

Experimental design and sample collection

All experimental procedures in this study were performed in accordance with the ethical guidelines for investigations using laboratory animals, and complied with the guide for the care and use of laboratory animals.[54] The animals were weighed and sorted into 9 groups of five animals each, so that their average differences in weights were <3 g.[55] They were housed in cages at the Department of Pharmacology, and allowed water and feed ad libitum. After 1 week acclimatization, the treatment commenced and lasted for 14 days. Diabetmin^{TM} (metformin HCl) (dissolved in distilled water) was orally administered daily at 250 mg/kg body weight to the Metformin group. The extracts were administered via the same route at 50 mg/kg to COLE-50mg (COLE) and TPLE-50mg (TPLE); 75 mg/kg to COLE-75mg (COLE) and TPLE-75mg (TPLE); and 100 mg/kg to COLE-100mg (COLE) and TPLE-100mg (TPLE). The Normal and Test control groups received distilled water in place of the extract.

On day 12, doxorubicin was dissolved in normal saline and intra-peritioneally injected (15 mg/kg), into all the groups, except the Normal control which was given normal saline instead. The doxorubicin dosage was adopted from Song et al.[56] The dosages of administration of *C odorata* extract was adopted and modified from Ikewuchi et al[22,24,25]; that of *T procumbens* extract was from Ikewuchi et al[36,37]; while that of metformin was from Zilinyi et al.[57]

The animals were sacrificed on day 14, under chloroform anaesthesia and blood samples were collected into heparin bottles; their kidneys were harvested, and their weights and sizes were documented.[24] The blood samples were centrifuged at 1000 rpm for 10 min, and their plasma were removed and stored. The harvested organs were homogenized in distilled water (at 0.4 g per 5 mL), and the ensuing homogenates were stored for use in the assay. The kidney weights/sizes indices were determined using the formula below.[58]

$$\text{Kidney weight or size index (\%)} = \frac{\text{Kidney weight (g) or kidney size (cm}^3\text{)}}{\text{Body weight (g)}} \times 100$$

Assay of biochemical parameters

The homogenates' malondialdehyde concentrations were analysed according to the method of Gutteridge and Wilkins.[59] The "sample tubes" contained 1 mL of glacial acetic acid, 1 mL of 1% thiobarbituric acid solution and 0.2 mL of sample. They were read at 532 nm, after zeroing the spectrophotometer with a blank containing 0.2 mL of distilled water instead. The ascorbic acid contents were estimated by iodine titration.[60] Aliquot (1.0 mL) of the sample was added to 5 mL of reaction mix (31.746 mg% starch in 1.243% (v/v) HCl); and titrated with iodine solution, until the appearance of a permanent blue colour. Catalase activities were according to Beers and Sizer.[61] The "sample tubes" contained 2.50 mL of hydrogen peroxide, and 2.70 mL of distilled water was used to zero the spectrophotometer and absorbance read at 420 nm, exactly 1 minute after adding 0.20 mL of the sample. The "reference" contained 0.20 mL of distilled water in place of the sample. Superoxide dismutase activities were according to Misra and Fridovich.[62] The "sample tubes" contained 0.1 mL of sample, 1.25 mL of 0.05 M carbonate buffer. They were equilibrated at room temperature, and 1.5 mL of distilled water was used to zero the spectrophotometer and absorbance read at 520 nm, exactly 1 minute after adding 0.15 mL of 0.3 mM adrenaline. The "reference" contained 0.1 mL of distilled water in place of the sample. Glutathione peroxidase activities were according to Rotruck et al.[63] The assay mixture containing 0.5 mL of sodium phosphate buffer (0.1 M, pH 7.4), 0.1 mL of 10 mM sodium azide, 0.2 mL of 4 mM reduced glutathione, 0.1 mL of 25 mM hydrogen peroxide, 0.5 mL sample, and 0.6 mL distilled water was incubated at 37°C for 3 min, before adding 0.5 mL 10% TCA. After centrifugation, the residual glutathione contents of the supernatants, was determined by adding 0.5 mL of the supernatants, 4.0 mL of 0.3 M disodium hydrogen phosphate solution and 1 mL of 0.01 M DTNB reagent, and reading at 412 nm, against a reagent blank containing only 4.5 mL phosphate solution and 1 mL DTNB reagent. Half millilitre of the standard (4 mM glutathione solution) was treated in a similar way. The activities of the ATPases were determined by the method of Hesketh et al.[64] The quantity of inorganic phosphate was determined by the method of Fiske and Subbarow.[65] The homogenates' protein contents were determined by Lowry method.[66]

The calcium, chloride, cholesterol, magnesium, potassium, sodium and triglyceride contents of the homogenates were assayed according to the kits manufacturers' instructions; except that homogenates were used instead of plasma. The assay procedures for the plasma creatinine and urea concentrations

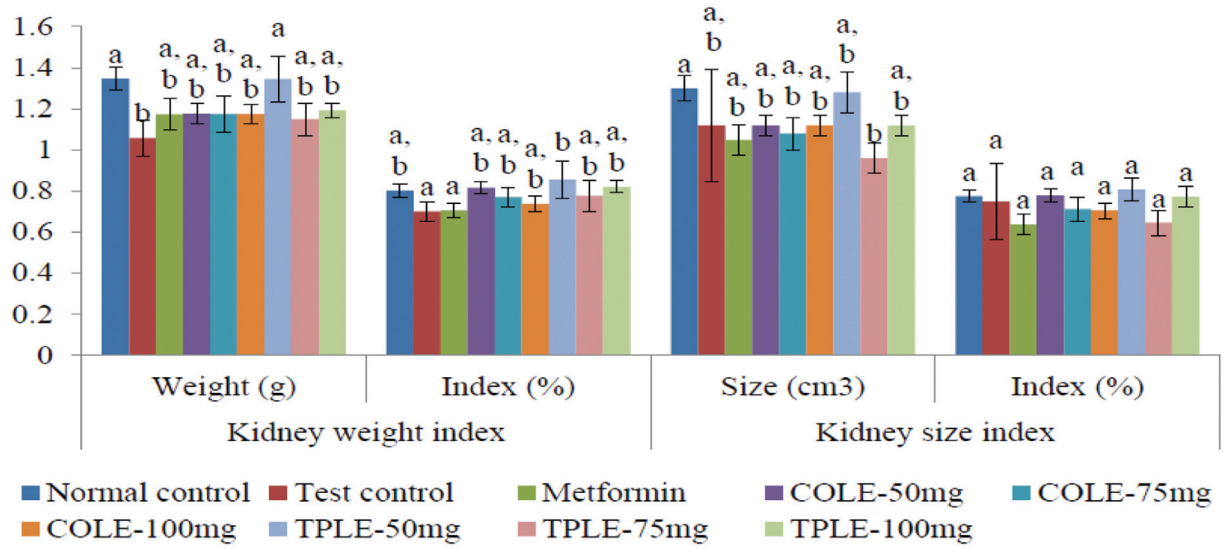

Figure 1. Effects of aqueous leaf-extracts of *C odorata* and *T procumbens* on the kidney weight and size indices of doxorubicin treated rats. Values are mean ± SEM, n = 5 animals, per group. Bars in the same block with different superscript letters differ significantly at $P < .05$.

were according to the kits manufacturers' instructions. The urea to creatinine ratio was calculated with the formula below.[67]

$$\text{Urea/creatinine ratio} = \frac{\text{Plasma urea concentration (mmol/L)}}{\text{Plasma creatinine concentration (mmol/L)}}$$

Determination of per cent protection

The per cent protection of the kidneys were calculated with the formula below.[21]

$$\text{Percent protection} = \frac{\text{Parameter}_{\text{test control}} - \text{Parameter}_{\text{treatment}}}{\text{Parameter}_{\text{test control}} - \text{Parameter}_{\text{normal control}}}$$

Statistical analysis of data

Statistical calculations were carried out with the Excel 2010 (Data Analysis Add-in) software. All data are expressed as mean ± standard error of the mean (SEM), and were analysed using 1-way analysis of variance (1-way ANOVA). Significant difference of the means was determined using a post-hoc analysis involving LSD (least significant difference) test; with $P < .05$ considered statistically significant.

Results

The effects of aqueous leaf-extracts of *C odorata* and *T procumbens* on the kidney weight and size indices of doxorubicin treated rats is presented in Figure 1. The kidney weight index and kidney size index of Test control were not significantly different from those of all the other groups, except TPLE-50mg. The renal malondialdehyde concentration of Test control was significantly ($P < .05$) higher than those of all the others (Table 1). The renal ascorbic acid concentration, and the renal catalase, glutathione peroxidase and superoxide dismutase activities of Test control were significantly ($P < .05$) lower than those of all the others (Table 1). The plasma concentrations of creatinine, urea and blood urea nitrogen of Test control were significantly ($P < .05$) higher than those of all the others (Fig. 2). However, the urea/creatinine ratio of Test control was significantly ($P < .05$) lower than those of COLE-100mg and TPLE-100mg, but not significantly different from the others.

The renal cholesterol concentration of Test control was significantly ($P < .05$) higher than those of all the others except

Table 1

Effects of aqueous leaf-extracts of *C odorata* and *T procumbens* on renal biomarkers of oxidative stress of doxorubicin treated rats

Treatments	Malondialdehyde (μmol/mg protein)	Ascorbic acid (μg/mg protein)	Glutathione peroxidase (μmol/min/mg protein)	Superoxide dismutase (units/mg protein)	Catalase (μmol/min/mg protein)
Normal control	1.221 ± 0.108^{a}	18.881 ± 0.270^{a}	0.192 ± 0.004^{a}	0.503 ± 0.056^{a}	3.382 ± 0.118^{a}
Test control	1.894 ± 0.093^{c}	7.925 ± 0.030^{c}	0.137 ± 0.008^{c}	0.281 ± 0.032^{c}	1.646 ± 0.083^{b}
Metformin	$1.157 \pm 0.096^{a,b}$	12.838 ± 0.222^{d}	0.210 ± 0.002^{d}	$0.754 \pm 0.042^{b,d}$	3.412 ± 0.313^{a}
COLE-50mg	$1.159 \pm 0.062^{a,b}$	15.324 ± 0.227^{e}	0.194 ± 0.007^{a}	0.554 ± 0.036^{a}	3.399 ± 0.281^{a}
COLE-75mg	$1.122 \pm 0.105^{a,b}$	15.580 ± 0.248^{e}	$0.171 \pm 0.004^{b,f}$	0.647 ± 0.038^{d}	2.664 ± 0.100^{c}
COLE-100mg	$1.003 \pm 0.059^{a,b}$	9.331 ± 0.177^{f}	$0.184 \pm 0.007^{a,f}$	0.787 ± 0.029^{b}	$3.607 \pm 0.104^{a,d}$
TPLE-50mg	$1.132 \pm 0.047^{a,b}$	9.402 ± 0.157^{f}	0.168 ± 0.002^{b}	$0.687 \pm 0.027^{b,d}$	$3.001 \pm 0.231^{a,c}$
TPLE-75mg	1.245 ± 0.140^{a}	8.603 ± 0.036^{g}	$0.177 \pm 0.003^{b,e,f}$	$0.736 \pm 0.068^{b,d}$	3.467 ± 0.086^{a}
TPLE-100mg	0.946 ± 0.085^{b}	10.687 ± 0.275^{b}	$0.187 \pm 0.002^{a,e}$	$0.719 \pm 0.061^{b,d}$	4.103 ± 0.238^{d}

Values are mean ± SEM, n = 5. Values in the same column with different superscript letters differ significantly at $P < .05$.

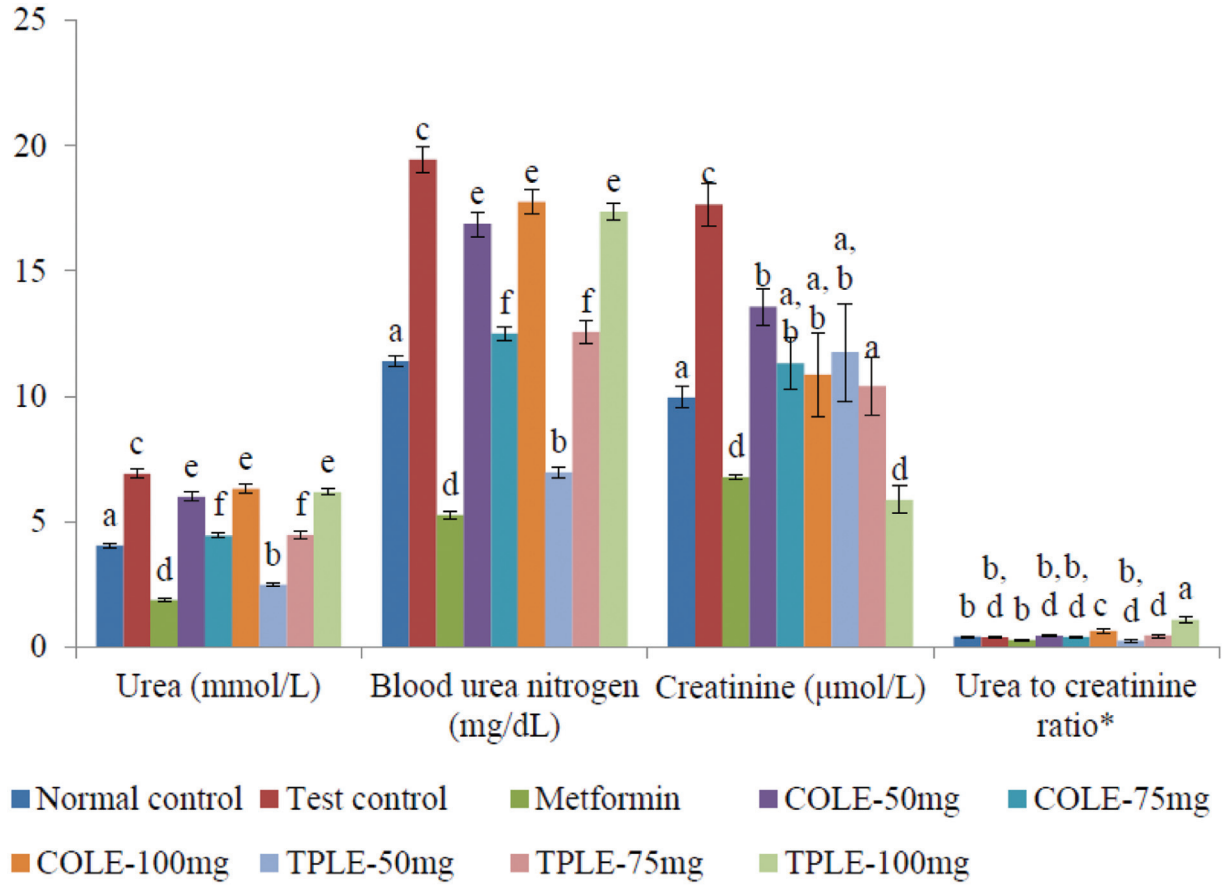

Figure 2. Impact of aqueous leaf-extracts of *C odorata* and *T procumbens* on plasma biomarkers of kidney function of doxorubicin treated rats. Values are mean ± SEM, n = 5. Bars in the same block with different superscript letters differ significantly at $P < .05$. *Has no unit.

Metformin (Table 2). The renal triglyceride concentration of Test control was not significantly different from those of all the others. The Mg^{2+}-ATPase, Na^{+},K^{+}-ATPase and Ca^{2+}-ATPase activities of Test control were significantly ($P < .05$) lower than those of all the other groups (Table 2). As shown in Table 3, the renal chloride, magnesium and potassium concentrations of Test control were significantly ($P < .05$) lower; while the calcium and sodium concentrations were significantly ($P < .05$) higher than those of all the other groups. The administration of the extracts and metformin prevented the doxorubicin-induced renal damage, as indicated by the attenuation of doxorubicin-induced adverse alterations in the plasma markers of renal functions/integrity, and renal markers of oxidative stress, and caused a subsequent protection towards normalization. The administration of the extracts also prevented the doxorubicin-induced reduction in activities of renal ATPases, and caused a subsequent protection towards normalization. These protections have been presented in Table 4 in the form of per cent protection of the parameters by the different test treatments.

Discussion

One of the major contributors to doxorubicin toxicity is oxidative stress, which leads to membrane lipid peroxidation,

Table 2

Effects of aqueous leaf-extracts of *C odorata* and *T procumbens* on renal lipid profiles and ATPase activities of doxorubicin treated rats

Treatments	Lipid profile (mmol/mg protein)		ATPase activities (mmol/min/mg protein)		
	Triglyceride	Cholesterol	Mg^{2+}-ATPase	Na^{+},K^{+}-ATPase	Ca^{2+}-ATPase
Normal control	0.237 ± 0.043^{a}	0.231 ± 0.056^{a}	3.721 ± 0.353^{a}	15.881 ± 1.601^{a}	$1.468 \pm 0.081^{a,f}$
Test control	0.306 ± 0.079^{a}	0.459 ± 0.052^{b}	1.965 ± 0.129^{c}	7.973 ± 1.250^{c}	0.697 ± 0.058^{c}
Metformin	0.250 ± 0.023^{a}	$0.401 \pm 0.022^{b,c}$	3.555 ± 0.212^{a}	28.399 ± 1.210^{b}	1.713 ± 0.068^{d}
COLE-50mg	0.212 ± 0.048^{a}	0.219 ± 0.043^{a}	13.060 ± 0.930^{d}	16.503 ± 1.928^{a}	$1.143 \pm 0.059^{b,e}$
COLE-75mg	0.216 ± 0.025^{a}	0.185 ± 0.045^{a}	4.257 ± 0.292^{a}	19.626 ± 2.474^{a}	$1.656 \pm 0.085^{d,f}$
COLE-100mg	0.169 ± 0.051^{a}	$0.252 \pm 0.059^{a,c}$	8.326 ± 0.481^{f}	16.178 ± 2.033^{a}	1.012 ± 0.087^{b}
TPLE-50mg	0.241 ± 0.040^{a}	0.243 ± 0.049^{a}	5.565 ± 0.182^{b}	17.365 ± 2.198^{a}	$1.297 \pm 0.051^{a,e}$
TPLE-75mg	0.195 ± 0.051^{a}	0.174 ± 0.059^{a}	4.192 ± 0.409^{a}	16.608 ± 2.579^{a}	1.908 ± 0.086^{d}
TPLE-100mg	0.233 ± 0.050^{a}	0.202 ± 0.070^{a}	5.853 ± 0.499^{b}	21.522 ± 2.274^{a}	1.420 ± 0.074^{a}

Values are mean ± SEM, n = 5. Values in the same column with different superscript letters differ significantly at $P < .05$.

Table 3

Effects of aqueous leaf-extracts of *C odorata* and *T procumbens* on renal electrolytes profiles of doxorubicin treated rats

Treatments	Calcium (μg/mg protein)	Chloride (μEq/mg protein)	Magnesium (μg/mg protein)	Potassium (μmol/mg protein)	Sodium (μEq/mg protein)
Normal control	9.557 ± 0.197^{a,d}	11.307 ± 0.262^{a}	3.894 ± 0.026^{a}	0.799 ± 0.020^{a}	7.090 ± 0.831^{a,c}
Test control	13.867 ± 0.487^{c}	6.877 ± 0.076^{c}	3.072 ± 0.027^{b}	0.416 ± 0.017^{c}	12.449 ± 0.443^{b}
Metformin	9.172 ± 0.462^{a,b,d}	8.914 ± 0.266^{d}	5.876 ± 0.030^{c}	0.474 ± 0.010^{d}	7.138 ± 0.163^{a,c}
COLE-50mg	10.027 ± 0.434^{d}	12.882 ± 0.387^{e}	5.083 ± 0.041^{d}	0.561 ± 0.012^{e}	8.017 ± 0.220^{a}
COLE-75mg	8.644 ± 0.508^{a,b}	8.597 ± 0.338^{d}	4.626 ± 0.040^{e}	0.471 ± 0.016^{d}	7.521 ± 0.144^{a,c}
COLE-100mg	8.163 ± 0.326^{a}	14.603 ± 0.323^{f}	4.351 ± 0.024^{f}	0.606 ± 0.009^{e,f}	8.019 ± 0.382^{a}
TPLE-50mg	8.197 ± 0.480^{a}	13.191 ± 0.362^{b,e}	5.250 ± 0.047^{g}	0.734 ± 0.029^{g}	8.107 ± 0.315^{a}
TPLE-75mg	8.176 ± 0.406^{a}	8.245 ± 0.071^{d}	3.732 ± 0.026^{h}	0.685 ± 0.008^{b,g}	6.516 ± 0.188^{c}
TPLE-100mg	8.718 ± 0.263^{a,b}	14.040 ± 0.442^{b,f}	5.056 ± 0.041^{d}	0.646 ± 0.027^{b,f}	8.161 ± 0.326^{a}

Values are mean ± SEM, n = 5. Values in the same column with different superscript letters differ significantly at $P < .05$.

and oxidation and cross-linking of cellular thiols.[1] That the doxorubicin administration produced oxidative stress in this study, is supported by the elevated renal malondialdehyde and lowered renal ascorbic acid levels, as well as the reduced renal activities of catalase, glutathione peroxidase and superoxide dismutase, observed in the Test control group. This result is in consonance with earlier reports.[2,7–9] Pre-treatment with the extracts and metformin attenuated the doxorubicin-induced oxidative stress, by lowering the renal malondialdehyde and raising the ascorbic acid levels, and activities of catalase, glutathione peroxidase and superoxide dismutase. This antioxidant protective effect is in agreement with the reports of the improvement of ocular antioxidant levels in alloxan-induced diabetic rats by *T procumbens* extract,[41] and improvement of antioxidant levels in the diaphragms of streptozotocin-induced diabetic rats by *C odorata* extract.[42] This result supports the suggestion by Lee et al[68] that significant enhancements of endogenous enzymatic antioxidants by plant extracts might be a good strategy for decreasing oxidative stress in tissues. So, these increases caused by the extracts, signify a consolidation of the endogenous antioxidant status of renal tissues, and their subsequent shielding from free radical damage.[41] The high ascorbic acid content in the renal tissues may be sequel to the high content of ascorbic acid in the leaves.[53] The extracts may owe their antioxidant protective effects to the presence in them of any 1 or a combination of 2 or more, of allicin, caffeic acid, ellagic acid, epicatechin, lycopene, naringenin, quercetin and silymarin; whose antioxidant and nephro-protective effects have been variously reported.

In this study, induction of oxidative stress by doxorubicin raised plasma urea and creatinine levels. This is in line with other reports of doxorubicin-induced elevation in plasma creatinine and urea levels.[2,7] Plasma biomarkers such as creatinine and urea concentrations are usually monitored to evaluate glomerular function, because of their inverse relationship with the latter.[69–73] Therefore, the reduction in the plasma creatinine and urea levels, produced by the extracts, is suggestive of their capacity to protect the nephrons from doxorubicin-induced damage,[8] and thus preserve the functional capacity of the glomerular filtration apparatus. The extracts' lowering of plasma creatinine, blood

Table 4

Per cent protection of the kidneys by the extracts

Parameter	Metformin	COLE-50mg	COLE-75mg	COLE-100mg	TPLE-50mg	TPLE-75mg	TPLE-100mg
Plasma							
Creatinine	141.2 ± 0.9^{a}	52.9 ± 9.3^{b}	82.4 ± 13.2^{b}	88.2 ± 21.6^{b}	76.5 ± 25.3^{b}	94.1 ± 15.0^{b}	152.9 ± 7.2^{a}
Urea	176.2 ± 1.8^{a}	32.0 ± 6.0^{c}	86.3 ± 3.1^{d}	21.0 ± 5.9^{c}	155.0 ± 2.4^{b}	85.5 ± 5.6^{d}	25.8 ± 4.3^{c}
Blood urea nitrogen	176.2 ± 1.8^{a}	32.0 ± 6.0^{c}	86.3 ± 3.1^{d}	21.0 ± 5.9^{c}	155.0 ± 2.4^{b}	85.5 ± 5.6^{d}	25.8 ± 4.3^{c}
Urea nitrogen/creatinine ratio	-758.1 ± 63.0^{a,d}	323.7 ± 128.1^{a,c}	62.2 ± 216.3^{a,d}	1588.7 ± 697.8^{c}	-1012.3 ± 274.9^{d}	373.7 ± 362.1^{a,c}	4476.1 ± 762.3^{b}
Renal							
Mg ATPase	90.5 ± 12.1^{a}	631.8 ± 53.0^{c}	130.5 ± 16.6^{a,b}	362.2 ± 27.4^{d}	205.0 ± 10.4^{b,e}	126.8 ± 23.3^{a,b}	221.4 ± 28.4^{e}
Na-K ATPase	258.3 ± 15.3^{a}	107.9 ± 24.4^{b}	147.4 ± 31.3^{b}	103.8 ± 25.7^{b}	118.8 ± 27.8^{b}	109.2 ± 32.6^{b}	171.3 ± 28.8^{b}
Ca ATPase	131.8 ± 8.8^{a,d}	57.9 ± 7.6^{c,e}	124.4 ± 11.0^{d}	40.9 ± 11.3^{e}	77.8 ± 6.7^{b,c}	157.1 ± 11.1^{a}	93.7 ± 9.5^{b}
Triglyceride	90.9 ± 15.9^{a}	117.6 ± 33.4^{a}	114.8 ± 17.2^{a}	147.4 ± 35.5^{a}	97.6 ± 28.1^{a}	129.4 ± 35.3^{a}	102.6 ± 34.7^{a}
Cholesterol	25.7 ± 9.6^{a}	105.3 ± 18.8^{b}	120.2 ± 19.9^{b}	91.1 ± 25.8^{a,b}	94.7 ± 21.3^{b}	125.1 ± 26.1^{b}	113.1 ± 30.9^{b}
Calcium	108.9 ± 10.7^{a}	89.1 ± 10.1^{b}	121.2 ± 11.8^{a}	132.3 ± 7.6^{a}	131.5 ± 11.1^{a}	132.0 ± 9.4^{a}	119.5 ± 6.1^{a}
Potassium	15.0 ± 2.5^{a}	37.9 ± 3.1^{c}	14.4 ± 4.2^{a}	49.5 ± 2.3^{c,d}	83.0 ± 7.6^{e}	70.1 ± 2.2^{b,e}	60.0 ± 7.2^{b,d}
Magnesium	341.4 ± 3.7^{a}	244.8 ± 5.0^{b}	189.2 ± 4.9^{c}	155.7 ± 2.9^{d}	265.2 ± 5.7^{e}	80.3 ± 3.1^{f}	241.6 ± 5.0^{b}
Chloride	46.0 ± 6.0^{a}	135.6 ± 8.7^{c}	38.8 ± 7.6^{a}	174.4 ± 7.3^{d}	142.5 ± 8.2^{b,c}	30.9 ± 1.6^{a}	161.7 ± 10.0^{b,d}
Sodium	99.1 ± 3.0^{a,c}	82.7 ± 4.1^{b}	92.0 ± 2.7^{a,b}	82.7 ± 7.1^{b}	81.0 ± 5.9^{b}	110.7 ± 3.5^{c}	80.0 ± 6.1^{b}
Ascorbic acid	44.8 ± 2.0^{a}	67.5 ± 2.1^{c}	69.9 ± 2.3^{c}	12.8 ± 1.6^{d}	13.5 ± 1.4^{d}	6.2 ± 0.3^{e}	25.2 ± 2.5^{b}
MDA	109.5 ± 14.3^{a,b}	109.2 ± 9.2^{a,b}	114.8 ± 15.6^{a,b}	132.4 ± 8.7^{a,b}	113.30 ± 7.0^{a,b}	96.4 ± 20.8^{a}	140.9 ± 12.7^{b}
SOD	213.0 ± 18.7^{a,c}	122.8 ± 16.2^{b}	164.7 ± 16.9^{b,c}	227.7 ± 13.2^{a}	182.9 ± 11.9^{a,c}	204.8 ± 30.6^{a,c}	197.0 ± 27.3^{a,c}
Catalase	101.7 ± 18.0^{a}	101.0 ± 16.2^{a}	58.7 ± 5.7^{b}	113.0 ± 6.0^{a,c}	78.0 ± 13.3^{a,b}	104.9 ± 5.0^{a}	141.5 ± 13.7^{c}
Glutathione peroxidise	133.5 ± 2.9^{a}	104.3 ± 12.9^{c,e}	61.7 ± 8.0^{b,c}	86.6 ± 12.6^{d}	57.8 ± 3.6^{b}	72.5 ± 5.8^{b,d}	92.4 ± 4.3^{d,e}

Values are mean ± SEM, n = 5. Values in the same row with different superscript letters differ significantly at $P < .05$.
MDA = malondialdehyde; SOD = superoxide dismutase.

urea nitrogen and urea may be due to their content of caffeic and chlorogenic acids, both of which had been reported to decrease plasma levels of kidney markers to near-normal levels.[74,75]

In this study, doxorubicin treatment caused significant increase in renal cholesterol and triglycerides. This is in conformity with reports of doxorubicin-induced increases in renal cholesterol and triglycerides levels in both humans and experimental animals.[76,77] However, pre-treatment with the extracts prevented this cholesterol and triglyceride accumulation. This effect may be sequel to the presence in the extracts, of chlorogenic acid, ellagic acid, naringenin and quercetin,[21,22,23,26,78] which are known to lower adiposity and triglyceride contents,[79–81] and modulate hepatic lipids.[82–85] The renal cholesterol lowering activity of the extracts has serious implications on the integrity and function of the renal cell membranes. This is because studies have shown that the level of cholesterol in membranes correlates inversely with the fluidity of membranes.[86–88] Cholesterol plays a major role in the control of the structure and dynamics of the lipid bilayer (fluidity); and therefore, can modulate the activities of various membrane transporters such as Ca^{2+}-ATPase, Mg^{2+}-ATPase, Na^+,K^+-ATPase and Ca^{2+} channels in various cells.[89–92]

In this study, the administration of doxorubicin produced significantly lowered Na^+,K^+-ATPase, Mg^{2+}-ATPase and Ca^{2+}-ATPase activities in the renal tissues. This corroborated earlier report of doxorubicin-induced significant reductions in renal Na^+,K^+-ATPase, Ca^{2+}-ATPase and Mg^{2+}-ATPase activities.[1,11] It however, negates the report by Ma et al,[93] of doxorubicin-induced significant activation of Na^+,K^+-ATPase activity. Pre-treatment with the extracts raised the renal activities of Na^+,K^+-, Mg^{2+}- and Ca^{2+}-ATPases. The extracts' ability to increase ATPase activities may be due to the presence of any one or both of ascorbic acid and epicatechin, earlier reported in them. According to Kumar et al[94] ascorbic acid or epicatechin prevents oxidative stress-induced lowering of Ca^{2+}-ATPase and Na^+,K^+-ATPase activities.

The extracts may have improved Na^+,K^+-ATPase, Ca^{2+}-ATPase and Mg^{2+}-ATPase activities by preventing doxorubicin-induced lipid peroxidation or oxidative stress and cholesterol loading, thereby preventing the subsequent oxidative modification of the enzymes, as well as the modification of the membrane lipid environment and fluidity of the plasma membrane. They may have accomplished the augmentation of ATPase activities via any one or a combination of 2 or more of the following. Firstly, the direct interaction between the phytoconstituents in the extracts and the enzymes may have resulted in changes in the enzymes' structure, and consequent changes in its activity, or protection of the enzymes' sulfhydryl groups,[95,96] from interacting with doxorubicin or its metabolites, or from oxidation and subsequent formation of disulphide bridges. Secondly, by reducing the concentration of free radicals and reactive oxygen species, and consequently, reducing oxidative stress and lipid peroxidation[94,97–101] Thirdly, by the impact of their phytoconstituents in preventing increased lipid order and lowered membrane fluidity;[90,91,97,101–104] which are products of increased cholesterol content and lipid peroxidation.[86,105] Lastly, by the impact of the phytoconstituents in the extracts, on the lipids of the plasma membrane which in turn may have led to changes in the surrounding lipid environment or protein-lipid interactions.[90,91,97,101,106–108]

Therefore, the reduction in lipid peroxidation (or oxidative stress) and/or tissue cholesterol, as well as protection of the enzymes' sulfhydryl groups may be responsible for the extracts ability to increase renal Na^+,K^+-ATPase, Ca^{2+}-ATPase and Mg^{2+}-ATPase activities. The significance of these modulations of renal ATPases cannot be overemphasized, given that Na^+,K^+-ATPase is important in controlling the reabsorption of Na^+ and water in the kidney[109,110]; while Ca^{2+}-ATPase is involved in renal active Ca^{2+} transport.[111,112]

Consequently, the elevated concentrations of renal calcium and sodium, as well as the lowered chloride, magnesium and potassium, induced by doxorubicin in this study, is reflective of compromised membranes of the renal tissues. However, pre-treatment with the extracts prevented the doxorubicin-induced electrolyte imbalance. This ability to modulate renal electrolytes may also be due to the presence of chlorogenic acid, which had been reported to improve mineral pool distribution in plasma, spleen and liver.[113] The reduction of renal cholesterol content may have resulted in the reduction in renal calcium content by the extracts. This is in view of the reports that decrease in cholesterol content of plasma membranes leads to decreased Ca^{2+} influx through the Ca^{2+} channel in plasma membranes, which results in decreases in intracellular calcium, and vice versa.[87,114–116] Reduction in membrane cholesterol has also been reported to stimulate the activities of Ca^{2+}-, Mg^{2+}- and Na^+,K^+-ATPases,[87,117,118] which modulates the transport of calcium, magnesium, potassium and sodium ions across plasma membranes,[90,119–127] and by extension, intracellular electrolyte balance. This suggests that modulation of renal calcium, sodium and magnesium levels by the extracts may have been achieved via an interplay of the modulation of renal cholesterol concentration and Ca^{2+}-, Na^+,K^+- and Mg^{2+}-ATPases.

Therefore, taken together, we can safely posit that the extracts acted by boosting endogenous antioxidant and modifying the micro-viscosity of renal membrane via lowering cholesterol levels, and reducing doxorubicin-induced oxidative stress (lipid peroxidation) and protein sulfhydryl modification; and that the ensuing increased fluidity caused improved Ca^{2+}-, Mg^{2+}- and Na^+,K^+-ATPase activities. The subsequent improvement in ion transport then led to improved electrolyte balance, especially, offsetting doxorubicin-induced calcium overload. This may be the mechanism of nephroprotective activities of the extracts. This therefore, is a suggestion of their prospect as resources for prevention or management of doxorubicin-induced renal toxicity.

Data accessibility statement

All relevant data are within the paper.

Competing interests

The authors have declared that no competing interests exist.

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