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Aqueous leafextracts of *Chromolaena odorata* and *Tridax procumbens* attenuated doxorubicin-induced pulmonary toxicity in Wistar rats

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Abstract

This study investigated the potential protective role of aqueous leafextracts of *Chromolaena odorata* and *Tridax procumbens* against pulmonary toxicity induced by doxorubicin. To this end, the effects of these extracts on the profiles of pulmonary biomarkers, lipids and electrolytes were monitored in doxorubicin-treated rats. Doxorubicin was intraperitoneally administered at 15 mg/kg body weight (48 h prior to sacrifice); metformin was orally administered daily at 250 mg/kg body weight (for 14 days); and both extracts were orally administered daily at 50, 75 and 100 mg/kg body weight (for 14 days).The concentrations of pulmonary malondialdehyde, cholesterol, triglyceride, calcium, chloride and sodium of Test control were significantly higher ($P < 0.05$) than those of the other groups. However, the concentrations of pulmonary ascorbic acid, reduced glutathione, magnesium and potassium as well as pulmonary catalase, glutathione peroxidase and superoxide dismutase activities of Test control were significantly lower ($P < 0.05$) than those of the other groups.The administration of the extracts prevented doxorubicin-induced adverse alterations in the profiles of pulmonary biomarkers of oxidative stress, cholesterol and electrolytes and maintained them within the normal ranges .Therefore, these herbal preparations from *C. odorata* and *T. procumbens* are promising candidates for the prevention/alleviation of doxorubicin-induced pulmonary toxicity.

Key words: *Chromolaena odorata*, doxorubicin, pulmonary lipids, electrolyte profiles, pulmonary oxidative stress, *Tridax procumbens*

Introduction

Doxorubicin induces toxicityin various organs, including lungs (Meadors et al., 2006; Injac et al., 2009; Srdjenovic et al., 2010; Vapa et al., 2012; Jagetia and Lalrinpuii, 2018).Pulmonary oedema, pneumonitis or lung fibrosis has been reported as one of the adverse side effects in cancer patients receiving doxorubicin alone or in combination with other chemotherapeutic drugs (Mazzotta et al., 2016; Irfan et al., 2017; Jagetia and Lalrinpuii, 2018). Oxidative stress is one of the mediators of pulmonary toxicity of doxorubicin (Öz and İlhan, 2006; Srdjenovic et al., 2010; Vapa et al., 2012). Oxidative stress caused by doxorubicin is characterised by significantly increased lipid peroxidation (high malondialdehyde), lowered reduced glutathione levels (Öz and İlhan, 2006; Injac et al., 2009; Srdjenovic et al., 2010; Vapa et al., 2012; Jagetia and Lalrinpuii, 2018), and lowered activities of antioxidant enzymes (such as catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase and glutathione transferase (Srdjenovic et al., 2010; Vapa et al., 2012; Jagetia and Lalrinpuii, 2018) and lactate dehydrogenase (Injac et al., 2009)).

Therefore, if pulmonary toxicity caused by doxorubicinis due to free radical formation and lipid peroxidation, then antioxidant therapy may protect against doxorubicin-induced toxicity in lungs (Kinnula et al., 2005; Vapa et al., 2012). Exogenous treatment with antioxidants has been shown to protect the lungs *in vivo* against doxo-

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rubicin-induced increased oxidant burden (Kinnula et al., 2005; Vapa et al., 2012). Thus, the use of antioxidants as protective agents could be a potential solution for doxorubicin-induced pulmonary toxicity.

Metformin, a drug widely used in the treatment of type 2 diabetes, exerts its effect through the activation of adenosine monophosphate-activated protein kinase (Park et al., 2012; Dean et al., 2016; Ismail Hassan et al., 2020). This drug has been reported to attenuate pulmonary injury by inhibiting the production of reactive oxygen species; by reducing inflammation, coagulation and fibrosis (Park et al., 2012; Garnett et al., 2013; Chen et al., 2015; Saisho, 2015; Forno, 2016; Chen et al., 2017; Yu et al., 2018; Ismail Hassan et al., 2020); and by maintaining mitochondrial membrane potential (Ismail Hassan et al., 2020). Metformin also reverses pulmonary hypertension through the inhibition of aromatase and oestrogen synthesis (Dean et al., 2016).

Studies have shown that allicin, chlorogenic acid and quercetin have pulmoprotective activities against cyclophosphamide- or lipopolysaccharide-induced toxicity (Zhang et al., 2010; Ashry et al., 2013; Şengül et al., 2017). The leaves of *Chromolaena odorata* and *Tridax procumbens* are rich in the above mentioned compounds, in addition to vitamin C; these leaves also contain an array of bioactive compounds belonging to the following families: allicins, benzoic acid derivatives, carotenoids, flavonoids, glycosides, hydroxycinnamic acid derivatives, lignans, phytosterols, saponins, tannins and terpenes (Phan et al., 2001; Ling et al., 2007; Igboh et al., 2009; Ikewuchi and Ikewuchi, 2009a; Ikewuchi et al., 2009, 2012, 2013, 2014a,b, 2015; Ikewuchi, 2012a,b; Onkaramurthy et al., 2013; Putri and Fatmawati, 2019; Cui et al., 2020). These antioxidant and antidyslipidemic (cholesterol and triglyceride lowering) agents (Dillard and German, 2000; Lawson, 2001; Francis et al., 2002; Prasad, 2005; Soetan, 2008; Zanwar et al., 2011; Ikewuchi et al., 2013, 2015, 2019; Ifeanacho et al., 2017) may account for the myriad pharmacological properties exhibited by these leaves and their extracts. Ikewuchi et al. reported the antidyslipidemic, antihypertensive, weight reducing, nephroprotective, cardioprotective, hepatoprotective and haematoprotective activities of leafextracts of *C. odorata* and *T. procumbens* (Ikewuchi and Ikewuchi, 2009b, 2011a, 2013; Ikewuchi et al., 2011a,b, 2012, 2014a,b, 2021a,b,c; Ifeanacho et al., 2020, 2021). The anticancer (Vishnu and Srinivasa, 2015; Adedapo et al., 2016), antioxidant (Putri and Fatmawati, 2019; Cui et al., 2020) of these extracts have also been reported in the present study, the effect of aqueous leafextracts of *C. odorata* and *T. procumbens* on doxorubicin-induced pulmonary toxicity 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's "Abuja park" campus and were identified as reported earlier (Ikewuchi and Ikewuchi, 2009b, 2011a, 2013; Ikewuchi, 2012a,b; Ikewuchi et al., 2009, 2011a,b, 2012, 2013, 2014a,b, 2015). Forty-five Wistar rats (weight 120–190 g) were obtained from and housed in cages at the Animal House of Department of Pharmacology, University of Port Harcourt, Nigeria. They were allowed uncontrolled access to water and feed (Port Harcourt Flour Mills, Port Harcourt, Nigeria). All chemicals used were of analytical grade and obtained from Sigma-Aldrich (St Louis, MO, USA). The cholesterol, triglyceride and calcium kits were obtained from Randox Laboratories Ltd, County Antrim, UK; the sodium and potassium kits were purchased from Atlas Medical, Cowley Rd, Cambridge, UK; and the chloride and magnesium kits were products of Agappe Diagnostics Switzerland GmbH.

Preparation of extracts

The leaves were cleaned to remove dirt. Next, 6 kg of *C. odorata* and 5.5 kg of *T. procumbens* leaves were macerated in distilled water and filtered through a sieve cloth. The resultant filtrates were dried in a water bath, and their residues (127 g and 116 g, respectively) were stored in the refrigerator for use in the assays. The resultant residues or leafextracts of *C. odorata* and *T. procumbens* (hereafter referred to as COLE and TPLE, respectively) were weighed, reconstituted in distilled water and administered to the experimental animals according to their individual weights and doses of their groups, such that the maximum volume of the reconstituted extracts received by each rat was 0.5 ml.

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 (National Research Council, 2011). The animals were weighed and arranged into nine groups of five animals each, with average differences in weight < 2.951 g (FAO, 1991). The treatment commenced after 1 week of acclimatisation and lasted for 14 days. Diabetmin™ (metformin HCl) (dissolved in distilled water) was orally administered daily at 250 mg/kg body weight to the Metformin group. The extracts were administered through the same route at 50 mg/kg to COLE-50 mg (COLE) and TPLE-50 mg (TPLE); 75 mg/kg to COLE-75 mg (COLE) and TPLE-75 mg (TPLE); and 100 mg/kg to COLE-100 mg (COLE) and TPLE-100 mg (TPLE). The Normal and Test control received distilled water instead of extract.

On day 12, doxorubicin was dissolved in normal saline and intraperitoneally injected (15 mg/kg body weight) into rats of all the groups, except the Normal control which was given normal saline instead of doxorubicin solution. The doxorubicin dose was adopted from Song et al. (2019). The doses of administration of the *C. odorata* extract was adopted and modified from Ikewuchi et al. (2014a,b); that of *T. procumbens* extract was adopted and modified from Ikewuchi et al. (2011a,b); and that of metformin was adopted from Zilinyi et al. (2018).

On day 14, the animals were sacrificed under chloroform anaesthesia; their lungs were collected, and their weights and sizes were recorded (Ikewuchi et al., 2014b). The collected organs were homogenised in distilled water (at 0.4 g per 5 ml), and the resultant homogenates were stored in the refrigerator and used for the assays. The weights/sizes indices of the lungs were determined according to the following formula (Ifeanacho et al., 2019).

Assay of pulmonary markers of oxidative stress, lipids and electrolyte concentrations

The malondialdehyde (MDA) contents of homogenates were determined according to the method of Gutteridge and Wilkins (1982). The ascorbic acid contents were determined by iodine titration (Ikewuchi and Ikewuchi, 2011b; Ikewuchi et al., 2021),and the reduced glutathione concentrations were determined according to the method of Sedlak and Lindsay (1968). The method of Beers and Sizer (1952) was adopted for the assay of catalase activities, while that of Misra and Fridovich (1989) was adopted for the assay of superoxide dismutase activities. The glutathione peroxidase activities were assayed according to the method reported by Rotruck et al. (1973). The Lowry method (Lowry et al., 1951) was used to estimate the protein concentrations of the homogenates. The cholesterol, triglyceride, calcium, sodium, potassium, chloride and magnesium contents of the homogenates were assayed according to the kit manufacturers' instructions, except that homogenates were used instead of plasma.

Determination of the percent of protection by the extracts

The percent of protection of the lungs by the extracts with respect to the various biochemical parameters determined was calculated as follows (Ikewuchi et al., 2017).

Statistical analysis

Statistical calculations were performed with Excel 2010 (Data Analysis Add-in) software. All data are expressed as mean ± standard error of the mean (SEM) with $n = 5$ animals per group, and the data were analysed by one-way analysis of variance. Significant difference of means was determined using the least significant difference test. A P value of < 0.05 was considered to be statistically significant.

Results

Effect of the extracts on pulmonary biomarkers of oxidative stress

The pulmonary malondialdehyde concentration (μmol/mg protein) of Test control (2.220 ± 0.078) was significantly higher ($P < 0.05$) than those of the other groups (Table 1), including the Normal control group (1.752 ± 0.072), and the COLE-100 mg group had the least value of 1.311 ± 0.053. The ascorbic acid (17.411 ± ± 0.446 μg/mg protein) and reduced glutathione (0.171 ± ± 0.003 μmol/mg protein) concentrations of the lungs of Test control were significantly lower ($P < 0.05$) than those of the other groups. The Normal control group had the highest ascorbic acid content (44.505 ± ± 1.417 μg/mg protein), while the COLE-50 mg group had the highest reduced glutathione content (0.425 ± ± 0.011 μmol/mg protein). The pulmonary catalase (2.434 ± 0.070 μmol/min/mg protein), glutathione per-

Table 1. Effects of aqueous leafextracts of *Chromolaena odorata* and *Tridax procumbens* on pulmonary biomarkers of oxidative stress in doxorubicin-treated rats

Treatments	Malondialdehyde [µmol/mg protein]	Ascorbic acid [µg/mg protein]	Reduced glutathione [µmol/mg protein]	Glutathione peroxidase [µmol/min/mg protein]	Superoxide dismutase [U/mg protein]	Catalase [µmol/min/mg protein]
Normal control	$1.752 \pm 0.072^{a,d}$	44.505 ± 1.417^{a}	0.247 ± 0.005^{a}	0.758 ± 0.014^{a}	0.888 ± 0.004^{a}	3.439 ± 0.007^{a}
Test control	2.220 ± 0.078^{c}	17.411 ± 0.446^{c}	0.171 ± 0.003^{c}	0.492 ± 0.015^{c}	0.641 ± 0.007^{c}	2.434 ± 0.070^{b}
Metformin	1.360 ± 0.088^{b}	20.612 ± 0.226^{d}	0.410 ± 0.013^{d}	0.823 ± 0.012^{d}	1.133 ± 0.011^{d}	4.053 ± 0.018^{c}
COLE-50 mg	1.727 ± 0.083^{d}	$21.928 \pm 0.547^{b,d}$	0.425 ± 0.011^{d}	0.647 ± 0.019^{e}	1.030 ± 0.014^{e}	4.458 ± 0.032^{d}
COLE-75 mg	1.433 ± 0.065^{b}	33.956 ± 0.856^{e}	0.279 ± 0.013^{e}	0.785 ± 0.015^{a}	0.984 ± 0.013^{f}	4.290 ± 0.024^{e}
COLE-100 mg	$1.311 \pm 0.053^{a,b}$	22.801 ± 0.940^{b}	0.209 ± 0.005^{f}	0.913 ± 0.016^{f}	0.767 ± 0.012^{b}	3.196 ± 0.043^{f}
TPLE-50 mg	1.696 ± 0.129^{d}	$21.672 \pm 0.541^{b,d}$	0.248 ± 0.008^{a}	0.681 ± 0.015^{e}	1.150 ± 0.009^{d}	4.108 ± 0.031^{c}
TPLE-75 mg	$1.541 \pm 0.139^{a,b,d}$	26.002 ± 0.337^{f}	0.336 ± 0.010^{b}	0.793 ± 0.008^{a}	1.127 ± 0.010^{d}	3.656 ± 0.028^{g}
TPLE-100 mg	$1.566 \pm 0.069^{a,b,d}$	$22.175 \pm 0.219^{b,d}$	$0.264 \pm 0.013^{a,e}$	0.994 ± 0.018^{b}	0.885 ± 0.003^{a}	3.286 ± 0.024^{h}

Values are expressed as mean ± SEM, $n = 5$; values in the same column with different superscript letters differ significantly at $P < 0.05$

Table 2. Effects of aqueous leafextracts of *Chromolaena odorata* and *Tridax procumbens* on the profiles of pulmonary electrolytes and lipids, and protein concentrations 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]	Cholesterol [mmol/mg protein]	Triglyceride [mmol/mg protein]	Protein [mg/g tissue]
Normal control	26.426 ± 2.095^{a}	6.957 ± 0.136^{a}	16.002 ± 0.114^{a}	$1.178 \pm 0.079^{a,c}$	$18.029 \pm 0.848^{a,c}$	$0.539 \pm 0.111^{a,c}$	$0.582 \pm 0.134^{a,d}$	43.337 ± 3.347^{a}
Test control	38.222 ± 2.584^{c}	13.466 ± 0.197^{c}	3.333 ± 0.239^{c}	0.600 ± 0.023^{b}	27.475 ± 0.733^{b}	0.701 ± 0.127^{c}	1.241 ± 0.041^{b}	39.732 ± 5.864^{a}
Metformin	20.571 ± 0.600^{b}	11.974 ± 0.086^{d}	6.234 ± 0.508^{d}	$1.082 \pm 0.023^{a,d}$	19.548 ± 0.406^{a}	$0.407 \pm 0.069^{a,b}$	0.856 ± 0.045^{c}	$46.643 \pm 8.021^{a,b}$
COLE-50 mg	19.654 ± 1.226^{b}	6.482 ± 0.179^{e}	15.797 ± 0.491^{a}	1.043 ± 0.030^{a}	$19.236 \pm 0.424^{a,d}$	0.317 ± 0.049^{b}	$0.738 \pm 0.062^{c,d}$	$51.042 \pm 3.658^{a,b}$
COLE-75 mg	20.508 ± 0.809^{b}	3.478 ± 0.057^{f}	13.211 ± 0.722^{e}	$1.103 \pm 0.037^{a,d}$	$18.903 \pm 0.891^{a,c}$	0.243 ± 0.038^{b}	0.509 ± 0.054^{a}	$51.754 \pm 4.591^{a,b}$
COLE-100 mg	20.758 ± 0.582^{b}	3.716 ± 0.080^{f}	$16.810 \pm 0.269^{a,f}$	1.044 ± 0.096^{a}	17.092 ± 0.653^{c}	$0.410 \pm 0.051^{a,b,d}$	$0.546 \pm 0.059^{a,d}$	$52.045 \pm 2.980^{a,b}$
TPLE-50 mg	20.836 ± 0.592^{b}	4.522 ± 0.140^{b}	$17.899 \pm 0.930^{b,f}$	1.349 ± 0.101^{c}	$18.159 \pm 0.477^{a,c}$	0.307 ± 0.043^{b}	0.433 ± 0.070^{a}	$49.628 \pm 1.569^{a,b}$
TPLE-75 mg	21.904 ± 0.410^{b}	6.126 ± 0.130^{e}	19.729 ± 1.077^{b}	$1.245 \pm 0.119^{a,c}$	$17.445 \pm 0.612^{c,d}$	$0.560 \pm 0.048^{c,d}$	$0.757 \pm 0.089^{c,d}$	40.926 ± 2.906^{a}
TPLE-100 mg	21.460 ± 0.759^{b}	5.292 ± 0.237^{g}	13.147 ± 0.739^{e}	$1.281 \pm 0.087^{c,d}$	19.884 ± 0.779^{a}	$0.343 \pm 0.052^{a,b}$	$0.551 \pm 0.085^{a,d}$	57.060 ± 5.289^{b}

Values are expressed as mean ± SEM, n = 5; values in the same column with different superscript letters differ significantly at $P < 0.05$

Table 3. Percent protection of the parameters following treatment with the extracts

Parameters	Metformin	COLE-50 mg	COLE-75 mg	COLE-100 mg	TPLE-50 mg	TPLE-75 mg	TPLE-100 mg
Cholesterol	181.7 ± 42.7 [a]	236.8 ± 30.3 [a,c]	282.6 ± 23.7 [c]	179.9 ± 31.7 [a]	243.2 ± 26.6 [a,c]	87.0 ± 29.5 [b]	221.2 ± 32.3 [a,c]
Triglyceride	59.1 ± 6.7 [a]	76.7 ± 9.3 [a,c]	111.0 ± 8.1 [b]	105.4 ± 8.8 [b,c,d]	122.3 ± 10.4 [b]	73.8 ± 13.3 [a,d]	104.7 ± 12.7 [b,c]
Calcium	149.6 ± 5.1 [a,b]	157.4 ± 10.4 [a]	150.2 ± 6.9 [a,b]	148.1 ± 4.9 [a,b]	147.4 ± 5.0 [a,b]	138.3 ± 3.5 [b]	142.1 ± 6.4 [a,b]
Potassium	83.5 ± 3.9 [a,c]	76.7 ± 5.2 [a]	87.1 ± 6.4 [a,c]	76.9 ± 16.6 [a]	129.6 ± 17.5 [b]	111.6 ± 20.6 [a,b]	117.9 ± 15.1 [b,c]
Magnesium	22.9 ± 4.0 [a]	98.4 ± 3.9 [c]	78.0 ± 5.7 [d]	106.4 ± 2.1 [e]	115.0 ± 7.3 [b,e]	129.4 ± 8.5 [b,c]	77.5 ± 5.8 [d]
Chloride	22.9 ± 1.3 [a]	107.3 ± 2.8 [c]	153.4 ± 0.9 [d]	149.8 ± 1.2 [d]	137.4 ± 2.2 [e]	112.8 ± 2.0 [c]	125.6 ± 3.6 [b]
Sodium	85.9 ± 4.9 [a]	89.7 ± 5.1 [a,c]	93.7 ± 10.8 [a,b]	115.6 ± 7.9 [b]	102.7 ± 5.8 [a,b]	111.3 ± 7.4 [b,c]	81.8 ± 9.4 [a]
Ascorbic acid	11.8 ± 0.8 [a]	16.7 ± 2.0 [a,b]	61.1 ± 3.2 [c]	19.9 ± 3.5 [b]	15.7 ± 2.0 [a,b]	31.7 ± 1.2 [d]	17.6 ± 0.8 [a,b]
Malondialdehyde	184.0 ± 18.8 [a]	105.5 ± 17.8 [b]	168.3 ± 14.0 [a,c]	194.5 ± 11.3 [a]	112.1 ± 27.7 [b,c]	145.3 ± 29.8 [a,b]	139.9 ± 14.7 [a,b]
Catalase	161.0 ± 1.8 [a]	201.3 ± 3.2 [b]	194.6 ± 2.4 [c]	75.8 ± 4.3 [d]	166.5 ± 3.1 [a]	121.6 ± 2.8 [e]	84.7 ± 2.4 [f]
Superoxide dismutase	199.0 ± 4.6 [a]	157.1 ± 5.8 [c]	138.5 ± 5.1 [d]	51.1 ± 4.9 [e]	205.6 ± 3.8 [a]	196.5 ± 4.2 [a]	98.7 ± 1.4 [b]
Glutathione peroxidase	124.5 ± 4.5 [a]	58.3 ± 7.2 [c]	110.3 ± 5.6 [a]	158.4 ± 6.1 [d]	71.2 ± 5.5 [c]	113.2 ± 2.9 [a]	188.7 ± 6.6 [b]
Reduced glutathione	315.0 ± 16.4 [a]	334.7 ± 14.7 [a]	143.2 ± 17.0 [c]	51.3 ± 6.2 [d]	101.9 ± 9.7 [b]	217.1 ± 13.5 [e]	123.4 ± 17.6 [b,c]

Values are expressed as mean ± SEM, n = 5; values in the same row with different superscript letters differ significantly at $P < 0.05$

Table 4. Effects of aqueous leafextracts of *Chromolaena odorata* and *Tridax procumbens* on the weight and size indices of the lungs of doxorubicin-treated rats

Treatments	Lung weight index		Lung size index	
	weight [g]	index [%]	size [cm^3]	index [%]
Normal control	1.453 ± 0.172 [a]	0.861 ± 0.086 [a]	2.040 ± 0.248 [a]	1.214 ± 0.138 [a]
Test control	1.376 ± 0.194 [a]	0.910 ± 0.117 [a]	1.900 ± 0.187 [a]	1.270 ± 0.138 [a]
Metformin	1.500 ± 0.311 [a]	0.899 ± 0.172 [a]	1.750 ± 0.250 [a]	1.069 ± 0.168 [a]
COLE-50 mg	1.177 ± 0.125 [a]	0.835 ± 0.122 [a]	1.992 ± 0.354 [a]	1.408 ± 0.266 [a]
COLE-75 mg	1.329 ± 0.151 [a]	0.869 ± 0.087 [a]	1.700 ± 0.200 [a]	1.130 ± 0.157 [a]
COLE-100 mg	1.579 ± 0.121 [a]	0.994 ± 0.084 [a]	2.020 ± 0.453 [a]	1.268 ± 0.290 [a]
TPLE-50 mg	1.201 ± 0.093 [a]	0.760 ± 0.063 [a]	1.800 ± 0.200 [a]	1.132 ± 0.111 [a]
TPLE-75 mg	1.376 ± 0.057 [a]	0.925 ± 0.066 [a]	2.300 ± 0.255 [a]	1.536 ± 0.176 [a]
TPLE-100 mg	1.281 ± 0.180 [a]	0.881 ± 0.125 [a]	2.000 ± 0.354 [a]	1.386 ± 0.250 [a]

Values are expressed as mean ± standard error in the mean, $n = 5$ animals per group; values in the same column with different superscript letters differ significantly at $P < 0.05$

oxidase (0.492 ± 0.015 μmol/min/mg protein) and superoxide dismutase (0.641 ± 0.007 Units/mg protein) activities of Test control were significantly lower ($P < 0.05$) than those of the other groups. The COLE-50 mg group had the highest catalase activity (4.458 ± 0.032 μmol/min/mg protein); the TPLE-100 mg group had the highest glutathione peroxidase activity (0.994 ± ± 0.018 μmol/min/mg protein); and the TPLE-50 mg group had the highest superoxide dismutase activity (1.150 ± 0.009 Units/mg protein).

Effect of the extracts on the profiles of pulmonary lipids and electrolytes

The pulmonary triglyceride concentration (mmol/mg protein) of Test control (1.241 ± 0.041) was significantly higher ($P < 0.05$) than those of the othergroups (Table 2); the pulmonary triglyceride concentration of the Normal control was 0.582 ± 0.134, while that of the TPLE-50 mg group was 0.433 ± 0.070. The cholesterol concentration (mmol/mg protein) of Test control (0.701 ± 0.127) was significantly higher ($P < 0.05$) than those of the Metformin, COLE-50 mg, COLE-75 mg, COLE-100 mg, TPLE-50 mg and TPLE-100 mg groups, but was not significantly different from that of the other groups (Table 2); the COLE-75 mg group showed the least value of 0.243 ± 0.038. The pulmonary calcium (38.222 ± 2.584 μg/mg protein), chloride (13.466 ± ± 0.197 μEq/mg protein) and sodium (27.475 ± ± 0.733 μEq/mg protein) levels of Test control were significantly higher ($P < 0.05$) than those of the other groups, while the pulmonary magnesium (3.333 ± ± 0.239 μg/mg protein) and potassium (0.600 ± 0.023 μmol/mg protein) levels of Test control were significantly lower ($P < 0.05$) (Table 2). The COLE-50 mg group had the lowest calcium content (19.654 ± ± 1.226 μg/mg protein), the COLE-75 mg group had the lowest chloride content (3.478 ± 0.057 μEq/mg protein), and the COLE-100 mg group had the lowest sodium content (17.092 ± 0.653 μEq/mg protein), The TPLE-75 mg group had the highest magnesium content (19.729 ± 1.077 μg/mg protein), while the TPLE-50 mg group had the highest potassium content (1.349 ± ± 0.101 μmol/mg protein). The pulmonary protein level of Test control (39.732 ± 5.864 mg/g tissue) was not significantly different from those of the other groups, except that of the TPLE-100 mg group (57.060 ± ± 5.289 mg/g tissue).

Protection of pulmonary biomarkers by the extracts and their effect on the weight index of the lungs

The administration of the extracts prevented doxorubicin-induced adverse alterations in the profiles of pulmonary biomarkers of oxidative stress, cholesterol and electrolytes and allowed them to be maintained at near-normal levels. These protection effects of the extracts are presented in Table 3 as the percent protection of the parameters. The highest protection of 282.6 ± ± 23.7% was recorded in the cholesterol content of the

COLE-75 mg group, while the least protection of 11.8 ± 0.8% was recorded in the ascorbic acid content of the Metformin group. The protective ability of the extracts compared favourably with that of the Metformin group. The weight, weight index, size, and size index of the lungs of Test control were not significantly different from those of the other groups (Table 4).

Discussion

Studies have shown thatoxidative stress is one of the major contributors to pulmonary toxicity induced by doxorubicin (Öz and İlhan, 2006; Srdjenovic et al., 2010; Vapa et al., 2012). In the present study, treatment with doxorubicin caused marked elevations in pulmonary MDA levels;reduction in ascorbic acid and reduced glutathione concentrations and reduction incatalase, glutathione peroxidase and superoxide dismutase activities (Table 1). This finding is in agreement with other studies (Öz and İlhan, 2006; Srdjenovic et al., 2010; Vapa et al., 2012; Jagetia and Lalrinpuii, 2018), which also reported that treatment with doxorubicin caused elevated MDA and lowered reduced glutathione concentrations as well as lowered pulmonary activities of catalase, glutathione peroxidase and superoxide dismutase. The high content of ascorbic acid in the leaves (Ikewuchi and Ikewuchi, 2009a) may have produced the high pulmonary ascorbic acid content. This antioxidant protective effect agrees with the report of Ikewuchi (2012a), wherein ocular antioxidant levels were found to be improved by *T. procumbens* extract in alloxan-induced diabetic rats, and with the report of Onkaramurthy et al. (2013), wherein the antioxidant levels of diaphragms were improved by *C. odorata* extract in streptozotocin-induced diabetic rats. Thus, this increased antioxidant level caused by the extracts signifies a boosting of endogenous antioxidant status of pulmonary tissues and consequent protection of these tissues from damage caused by free radicals (Ikewuchi, 2012a).

In the present study, doxorubicin caused a significant increase in the levels of pulmonary cholesterol and triglycerides (Table 2). This is in line with other reports of doxorubicin-induced increase in cardiac cholesterol and triglycerides (Subashini et al., 2007; Sharma et al., 2016). Nevertheless, pre-treatment with the extracts prevented this build-up of cholesterol and triglyceride. The reduction in cholesterol and triglyceride may be due to the effect of any one or a combination of two or more of ellagic acid, quercetin, chlorogenic acid and naringenin (Ikewuchi, 2012b; Ikewuchi et al., 2012, 2013, 2015; Pitakpawasutthi et al., 2016), which are known to cause marked decrease in intracellular/hepatic build-up of triglyceride and cholesterol (Wan et al., 2013; Snyder et al., 2016; Leng et al., 2018), and lowered adipogenesis (Cho et al., 2011; Alam et al., 2014; Okla et al., 2015). The importance of the lowered cholesterol content produced by the extracts cannot be overstated, given the role of cholesterol in membrane fluidity and function. Studies have shown that the higher the cholesterol content in a membrane, the lower is its fluidity, and vice versa (Le Grimellec et al., 1992; Bastiaanse et al., 1997). Thus, by virtue of its specific sterol-protein interactionsand the modification of the lateral distribution of components and internal properties of the lipid bilayer of the cell membrane (Yeagle, 2012), cholesterol plays a vital role in the control of the structure and dynamics of the lipid bilayer (especially with regard to fluidity), and therefore, it can moderate the activities of various membrane transporters such as Ca^{2+} channels, Ca^{2+}-ATPase, Mg^{2+}-ATPase and Na^{+}, K^{+}-ATPase (Balut et al., 2006; Grebowski et al., 2013; Krokosz and Grebowski, 2016; Garcia et al., 2019).

Reactive oxygen species initiate free radical-mediated chain reactions, resulting in the conversion of membrane unsaturated fatty acids into lipid peroxides, which disrupts integrity of the cell membrane and causes compromise of membrane ion transporters, consequently leading to compromised ion transport (Zaidi and Michaelis, 1999; Kumar et al., 2002; Torlińska and Grochowalska, 2004; Conrard and Tyteca, 2019). Therefore, the elevated pulmonary chloride, calcium and sodium levels and lowered magnesium and potassium concentrations observed in the Test control rats are reflective of the damaged membranes of the pulmonary tissues resulting from doxorubicin toxicity. Reactive oxygen species may affect intracellular calcium signalling by directly inducing extracellular Ca^{2+} inflow or activating inositol triphosphate, leading to Ca^{2+} release from the sarcoplasmic reticulum and a subsequent extracellular Ca^{2+} inflow (Cai and Hu, 2014; Penniston et al., 2014). However, in the present study, the extracts countered doxorubicin-induced adverse alterations in pulmonary electrolyte balance. This ability of the extracts to modulate the profile of pulmonary electrolytes may be due to the presence of

chlorogenic acid, a compound reported to improve mineral pool distribution in plasma, liver and spleen (Rodriguez de Sotillo and Hadley, 2002). This effect by the extracts may have been a sequel to their reduction of pulmonary oxidative stress and/or modulation of ATPases. This modulation of electrolyte balance is noteworthy because in airway smooth muscle cells, an increase in intracellular Ca^{2+}concentration acts as a major contributing factor of force generation, cell proliferation, contraction, migration, cytokine production and other cellular responses (Ito, 2014; Xiao et al., 2014). Likewise, alterations in intracellular Mg^{2+}concentration can control the activity of Mg^{2+}-dependent enzymes, energy production, nucleic acid and protein synthesis, nerve transmission and stabilisation of lipid membranes and nucleic acids (Sanui and Rubin, 1982; Payandeh et al., 2013; Gröber et al., 2015).

The positive modulation of pulmonary electrolyte profiles by the extracts may also have been a sequel to their reduction of pulmonary cholesterol and/or modulation of ATPases. Reduction in membrane cholesterol has been reported to stimulate the activities of Ca^{2+}-ATPase, Mg^{2+}-ATPase and Na^{+}, K^{+}-ATPase (Kutryk and Pierce, 1988; Bastiaanse et al., 1997), which controls the passage of calcium, magnesium, potassium and sodium ions through plasma membranes (Doneen, 1993; Vasic et al., 2009; Strehler, 2013; Penniston et al., 2014;Clausen et al., 2017; Obradovic et al., 2018) and thus moderates intracellular electrolyte balance. Several studies have also reported that the decrease in the cholesterol content of plasma membranes leads to decreased Ca^{2+} inflow through the Ca^{2+} channel in plasma membranes, with the resultant decrease in intracellular Ca^{2+} and vice versa (Gleason et al., 1991; Bastiaanse et al., 1997).

On the basis of the above findings, it could be concluded that the extracts acted by modifying the microviscosity of the pulmonary membrane by lowering cholesterol levels and reducing doxorubicin-induced oxidative stress (lipid peroxidation) and protein sulfhydryl modification; the resultant increased fluidity and enhanced ion transport led to improved electrolyte balance (especially, by attenuating doxorubicin-induced calcium overload). This may be the mechanism of pulmoprotective activities of the extracts. These findings thus indicate the potential of these extracts as a resource for the management/prevention of doxorubicin-induced pulmonary toxicity.

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