Protective effect of aqueous leaf extracts of Chromolaena odorata and Tridax procumbens on doxorubicin-induced hepatotoxicity in Wistar rats

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Abstract
Background: The liver is one of the organs affected by doxorubicin toxicity. Therefore, in this study, the potential protective role of aqueous leaf extracts of Chromolaena odorata and Tridax procumbens against doxorubicin-induced hepatotoxicity was investigated.

Methods: In order to achieve this, their impact on hepatic biomarkers of oxidative stress, lipid and electrolytes’ profile, and plasma biomarkers of liver functions/integrity were monitored in doxorubicin treated rats. The animals were treated with either metformin (250 mg/kg body weight orally for 14 days) or the extracts (50, 75, and 100 mg/kg orally for 14 days) and/or doxorubicin (15 mg/kg, intraperitoneal, 48 h before sacrifice).

Results: The hepatic malondialdehyde, cholesterol, calcium, and sodium concentrations, and plasma activities of alanine and aspartate transaminases and alkaline phosphatase, as well as plasma albumin to globulin ratio of test control were significantly (P < .05) higher than those of the other groups. However, the plasma albumin, total protein, globulin, and total bilirubin concentrations; hepatic concentrations of ascorbic acid, chloride, magnesium, and potassium; and hepatic activities of catalase, glutathione peroxidase, and superoxide dismutase of test control were significantly (P < .05) lower than those of all the other groups.

Conclusions: Pretreatment with the extracts and metformin prevented to varying degrees, doxorubicin-induced hepatic damage, as indicated by the attenuation of doxorubicin-induced adverse alterations in hepatic biomarkers of oxidative stress, lipid and electrolyte profiles, and plasma biomarkers of hepatic function/integrity, and keeping them at near-normal values.

Keywords: cholesterol, Chromolaena odorata, doxorubicin, electrolyte profiles, hepatic oxidative stress, plasma liver biomarkers, Tridax procumbens

Introduction
The liver is one of the organs affected by doxorubicin toxicity.1-4 An increasing number of evidence supports the role of oxidative stress as a key mechanism of doxorubicin-induced hepatotoxicity,5-8 although many studies have also reported the involvement of apoptotic responses,9 as well as induction of the inflammatory cascade in the pathogenesis of doxorubicin-induced hepatotoxicity.3,4,9

The oxidative stress that results from doxorubicin’s toxicity in hepatic tissues is characterized by lipid peroxidation (often indicated by high malondialdehyde [MDA] levels),5,6,9,10 in addition to decreased levels of reduced glutathione5,6,9 and the antioxidant enzymes such as catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase, and glutathione-S-transferase.3,6,9,11 Doxorubicin-induced hepatic injury is accompanied by increased plasma levels of uric acid, gamma-glutamyl transferase, lactate dehydrogenase, alanine transaminase, aspartate transaminase, and alkaline phosphatase; and decreased plasma albumin and total protein levels.5,6,9

Metformin a biguanide, widely used in the treatment of type 2 diabetes,12 has been found to exert beneficial effects on various diseases including obesity, cancers (e.g., melanoma, breast, endometrial, bone, and colorectal cancers), and liver, cardiovascular, and renal diseases.12-17 In humans, metformin was also found to reduce the incidence of fatty liver diseases and to cause a histological response,12,18 whereas in animal trials, it prevented the development of high-fat diet-induced fatty liver disease in ob/ob mice, which displayed decreased liver triglyceride content.12,19 It has been reported to protect against doxorubicin-induced hepatic toxicity.10 Its hepatoprotective activity occurs via antioxidant, anti-inflammatory, and anti-apoptotic mechanisms10,20,21; as well as via activation of adenosine 50-monophosphate-activated protein kinase.12

Currently, there is a global propensity toward the use of plant products (herbal drugs), due to the belief that they are safer and more effective with fewer side effects than modern pharmaceutical drugs.22 In line with this, various bioactive compounds of plant origin have been reported to prevent or mitigate the
hepatotoxicity of doxorubicin via antioxidative activity. They include caffeic acid, carotenoids, catechin, epicatechin, epigallocatechin gallate, quercetin, and silymarin; all of which have been reported to exert hepatoprotective effects via attenuation of doxorubicin-induced oxidative stress in the liver.1,9,11,23–27 Others are alllic, apigenin, ascorbic acid, baicalin, chlorogenic acid, ellagic acid, gallic acid, genistein, kaempferol, lignans, lutein, myricetin, naringenin, nobiletin, and saponins; all of which have been reported to exert hepatoprotective effects via attenuation of carbon tetrachloride- or acetaminophen- or tamoxo-ifen- or lipopolysaccharide or D-galactosamine- or pyrogallol-induced oxidative stress in the liver.28–42

Previous studies have shown that the leaves of *Chromolaena odorata* and *Tridax procumbens*, and their extracts contain this aforementioned bioactive compounds43–51; all of which have been reported to be potent antioxidants.47,50,52

These antioxidants may account for the numerous pharmacological properties exhibited by these leaves and their extracts; such as hepatoprotective,51,56 antidiabetic,57,58 hematoprotective,59 nephroprotec- tive,55 antihypertensive,60 antihypolipidemic,48,63,66 as well as their anticancer activities.47,68 The leaf extracts of *C. odorata* and *T. procumbens* have also been reported to exhibit antioxidant activities.49,70 Therefore, this study is an attempt to harness the antioxidant properties of *C. odorata* and *T. procumbens* leaf extracts, for the prevention of doxorubicin-induced hepatotoxicity in Wistar rats.

Materials and methods

**Preparation of extracts**

Fresh samples of *C. odorata* and *T. procumbens* were harvested from within the University of Port Harcourt, and were duly identified as earlier reported.45–51,56,61–65 The leaves were rinsed in water and drained, to remove dirt, before macerating 6 kg of *C. odorata* and 5.5 kg of *T. procumbens*, respectively. 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 residues of *C. odorata* and *T. procumbens* (hereinafter referred to as *Chromolaena odorata* leaf extract (COLE) and *Tridax procumbens* leaf extract (TPLE), respectively, or extracts), were weighed, reconstituted in distilled water and administered to the experimental animals, according to their individual weights and dosages of their groups.

**Experimental design and sample collection**

Forty five Wistar rats (weight 120–190.6 g) were obtained from the Animal House of Department of Pharmacology, University of Port Harcourt. They were housed in cages therein, and allowed unfettered access to water and feed (product of Port Harcourt Flour Mills, Port Harcourt, Nigeria). All the experimental procedures used in this study were in agreement with the ethical guidelines for investigations using laboratory animals, and conformed to the guide for the care and use of laboratory animals.71 The animals were weighed and arranged into 9 groups of 5 animals each, with average differences in weight <2.95 g.72 The animals were acclimatized for 1 week, before commencing the treatment, which 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 via same route at 50 mg/kg to COLE-50 mg (COLE); 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.

On day 12, doxorubicin (in normal saline) was intra-peritoneally injected (15 mg/kg body weight), into all the groups, except the normal control which was administered normal saline instead. The doxorubicin dosage was adopted and modified from Song et al.4 The dosages of administration of the *C. odorata* extract was adopted and modified from Ikewuchi et al46,48,49; that of *T. procumbens* extract was adopted and modified from Ikewuchi et al.65,66 whereas that of metformin was adopted from Zilinyi et al.73

On day 14, the animals were sacrificed under chloroform anesthesia and blood samples were collected into heparin sample bottles, then their livers were collected, and their weights and sizes were recorded.48 The blood samples were centrifuged at 1000 rpm for 10 minutes, and the respective plasma samples were collected and stored for use in the assay (Table 1). The collected organs were homogenized in distilled water (at 0.4 g per 5 mL), and the resultant homogenates were stored and used for the assay (Table 1). The liver weights/sizes indices were determined according to the following the formula adopted from Ikewuchi et al.47

\[
\text{Liver weight or size index (\%)} = \frac{\text{Liver weight (g) or liver size (cm\textsuperscript{3})}}{\text{Body weight (g)}} \times 100
\]

**Assay of biochemical parameters**

All chemicals used in this study were of analytical grade and products of Sigma-Aldrich, St Louis, MO, USA. The triglyceride, cholesterol, calcium, alanine and aspartate transaminases and alkaline phosphatase, total protein, and albumin 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 and magnesium kits were products of Sigma-Aldrich, St Louis, MO, USA. The triglyceride, cholesterol, calcium, alanine and aspartate transaminases and alkaline phosphatase, total protein, and albumin kits were products of Sigma-Aldrich, St Louis, MO, USA. The triglyceride, cholesterol, calcium, alanine and aspartate transaminases and alkaline phosphatase, total protein, and albumin kits were products of Agappe Diagnostics Switzerland GmbH.

**Assay of hepatic biomarkers of oxidative stress and endogenous antioxidant status.** The MDA contents of the homogenates were analyzed in compliance with the method reported by Gutteridge and Wilkins.73 The assay mix was

| Table 1 |
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| **Samples collected and biomarkers evaluated** |
| **Sample type** | **Form used** | **Biomarkers evaluated** |
| Blood | Plasma | Plasma alanine and aspartate transaminases, alkaline phosphatase activities Total protein and albumin concentrations |
| Liver | Homogenates | Malondialdehyde, ascorbic acid, calcium, sodium, chloride, magnesium, potassium, cholesterol, and triglyceride concentrations Catalase, glutathione peroxidase, and superoxide dismutase activities |
prepared by combining 1 mL of glacial acetic acid, 1 mL of 1% thiobarbituric acid solution and 0.2 mL of sample. After zeroing the spectrophotometer with a blank containing 0.2 mL of distilled water in the place of the sample, they were read at 532 nm. The homogenates’ ascorbic acid contents were estimated by iodine titration, as adopted from Ikewuchi et al.74 One milliliter (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 a permanent blue color appeared.

The catalase activities of the homogenates were determined with the method reported by Beers and Sizer.76 The “sample tubes” consisted of 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” had 0.20 mL of distilled water instead of the sample. The assay of superoxide dismutase activities of the homogenates was carried with the method of Misra and Fridovich.77 The assay mix was prepared by combining 0.1 mL of sample and 1.25 mL of 0.05 M carbonate buffer. After equilibrating at room temperature, 1.5 mL of distilled water was used to zero the spectrophotometer, before reading absorbance at 520 nm, exactly 1 minute after adding 0.15 mL of 0.3 mM adrenaline. The “reference” had 0.1 mL of distilled water instead of the sample. Glutathione peroxidase activities of the homogenates were determined in compliance with the method of Rotruck et al.78 The assay mix prepared by combining 0.1 mL of 0.1M sodium phosphate buffer (pH 7.4), 0.1 mL of 10 mM sodium azide, 0.2 mL of 4M reduced glutathione, 0.1 mL of 25 M hydrogen peroxide, 0.5 mL sample, and 0.6 mL distilled water was incubated at 37°C for 3 minutes. The reaction was stopped by adding 0.5 mL 10% trichloroacetic acid. After centrifugation, the residual glutathione contents of the supernatants, was measured by combining 0.5 mL of the supernatants, 4.0 mL of 0.3 M disodium hydrogen phosphate solution, and 1 mL of 0.01 M 5,5-dithiobis-2-nitrobenzoic acid (DTNB) reagent, and reading at 412 nm, against a reagent blank containing only 4.5 mL phosphate solution and 1 mL DTNB reagent. Half milliliter of 4M glutathione solution (the standard) was treated in a similar way. The protein contents of the homogenates were estimated by the Lowry method.79

**Determination of percent protection**

The percent protection of the liver or the extent to which the extracts restored the measured biochemical parameters to normal values, in comparison to the test control (untreated or disease control) was calculated using the following formula.81

\[
\text{Percent protection} = \left(\frac{\text{Parameter}_{\text{test control}} - \text{Parameter}_{\text{treatment}}}{\text{Parameter}_{\text{test control}} - \text{Parameter}_{\text{normal control}}}\right) \times 100
\]

**Statistical analysis of data**

Excel 2010 (Data Analysis Add-in) software was used to carry out the statistical calculations. All data are expressed as mean ± standard error of the mean and were analyzed using 1-way analysis of variance. Significant difference of the means was determined by post-hoc analysis involving least significant difference test. In all, *P < .05* was considered statistically significant.

**Results**

**Hepatic biomarkers of oxidative stress and endogenous antioxidant status**

The effects of aqueous leaf extracts of *C. odorata* and *T. procumbens* on hepatic biomarkers of oxidative stress and endogenous antioxidants in doxorubicin treated rats is shown in Table 2. The hepatic MDA concentration of Test control was significantly (*P < .05*) higher, whereas the hepatic ascorbic acid concentration, and catalase, glutathione peroxidase, and superoxide dismutase activities of test control were significantly (*P < .05*) lower than those of all the others.

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**Table 2**

| Treatment          | 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     | 2.497 ± 0.440                     | 149.409 ± 2.740              | 1.895 ± 0.023                               | 1.090 ± 0.027                          | 2.128 ± 0.014                  |
| Test control       | 4.934 ± 0.653                     | 49.874 ± 1.111               | 0.984 ± 0.025                               | 0.615 ± 0.010                          | 1.884 ± 0.037                  |
| Metformin          | 2.186 ± 0.038                     | 126.461 ± 3.073              | 2.510 ± 0.025                               | 0.914 ± 0.040                          | 2.292 ± 0.020                  |
| COLE-50 mg         | 3.304 ± 0.338                     | 120.599 ± 3.217              | 2.110 ± 0.048                               | 0.890 ± 0.010                          | 2.157 ± 0.030                  |
| COLE-75 mg         | 2.734 ± 0.166                     | 353.624 ± 8.380              | 2.855 ± 0.046                               | 2.914 ± 0.031                          | 3.277 ± 0.028                  |
| COLE-100 mg        | 3.073 ± 0.197                     | 262.700 ± 9.664              | 3.000 ± 0.035                               | 3.269 ± 0.054                          | 2.682 ± 0.043                  |
| TPLE-50 mg         | 3.100 ± 0.217                     | 270.377 ± 5.800              | 2.727 ± 0.378                               | 3.549 ± 0.040                          | 2.640 ± 0.040                  |
| TPLE-75 mg         | 2.787 ± 0.246                     | 102.828 ± 7.851              | 1.775 ± 0.021                               | 1.212 ± 0.014                          | 2.705 ± 0.040                  |
| TPLE-100 mg        | 3.570 ± 0.137                     | 171.767 ± 6.435              | 1.927 ± 0.064                               | 1.399 ± 0.020                          | 2.766 ± 0.026                  |

*Values are mean ± standard error of the mean (SEM), *n* = 5 animals, per group. Values in the same column with different superscript symbols differ significantly at *P < .05*. COLE, Chromolaena odorata leaf extract; TPLE, Tridax procumbens leaf extract.*

**Assay of plasma biomarkers of liver function/integrity.** The assay procedures for the plasma alanine and aspartate transaminases, alkaline phosphatase, total protein, and albumin were compliant with the kits manufacturer’s instructions. The plasma globulin levels and plasma albumin/globulin ratios were calculated with the following formulae.80

i. Plasma globulin concentration = [total protein] − [albumin]

ii. Plasma albumin to globulin ratio = [Plasma albumin] / [Plasma globulin]
Hepatic lipids and electrolytes profiles
The hepatic cholesterol concentration of test control was significantly \((P < .05)\) higher than those of all the other groups (Fig. 1). However, the hepatic triglyceride concentration of the test control was only significantly \((P < .05)\) higher than those of normal control, COLE-50 mg, TPLE-75 mg, and TPLE-100 mg (Fig. 1). The hepatic calcium and sodium concentrations of test control were significantly \((P < .05)\) higher, whereas the chloride, magnesium, and potassium concentrations were significantly \((P < .05)\) lower than those of all the other groups (Table 3).

Plasma biomarkers of liver function/integrity
The plasma albumin, total protein, globulin, and total bilirubin concentrations of test control were significantly \((P < .05)\) lower, whereas the albumin to globulin ratio, and plasma activities of alanine and aspartate transaminases and alkaline phosphatase of test control were significantly \((P < .05)\) higher than those of all the other groups (Table 4).

Protection of hepatic biomarkers by the extracts
The administration of the extracts prevented doxorubicin-induced liver damage as signified by the attenuation of doxorubicin-induced adverse alterations in hepatic biomarkers of oxidative stress, lipid and electrolyte profiles, and plasma biomarkers of hepatic function/integrity; and caused a subsequent protection toward near-normal values. These protections are presented in Table 5 in the form of percent protection of the parameters.

Liver size and weight indices
The effects of aqueous leaf extracts of C. odorata and T. procumbens on the liver size and weight indices of doxorubicin treated rats is presented in Figure 2. The liver size of test control was only significantly \((P < .05)\) lower than those of the normal control, COLE-75 mg and TPLE-50 mg. However, the liver size index of test control was only significantly \((P < .05)\) lower than that of COLE-75 mg. The liver weight of test control was only significantly \((P < .05)\) lower than those of normal control and COLE-75 mg. Nevertheless, the liver weight index of test control was only significantly \((P < .05)\) lower than those of normal control, COLE-50 mg, COLE-75 mg, TPLE-50 mg, and TPLE-100 mg.

Discussion
Oxidative stress is one of the major contributors to doxorubicin toxicity, and one of the major causes of liver damage. In this study, doxorubicin treatment produced oxidative stress as

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**Table 3**

| Treatment   | Calcium (\(\mu\)g/mg protein) | Chloride (\(\mu\)Eq/mg protein) | Magnesium (\(\mu\)g/mg protein) | Potassium (\(\mu\)mol/mg protein) | Sodium (\(\mu\)Eq/mg protein) |
|-------------|-------------------------------|---------------------------------|---------------------------------|----------------------------------|-----------------------------|
| Normal      | 58.166 ± 1.668                | 7.225 ± 0.141                   | 3.106 ± 0.022                   | 0.676 ± 0.029                    | 42.987 ± 1.864              |
| Test Control| 98.057 ± 1.652                | 5.475 ± 0.142                   | 2.877 ± 0.015                   | 1.393 ± 0.012                    | 56.985 ± 0.848              |
| Metformin   | 69.225 ± 1.727                | 6.230 ± 0.190                   | 3.204 ± 0.009                   | 0.498 ± 0.013                    | 38.413 ± 0.721              |
| COLE-50 mg  | 63.727 ± 3.114\(x\)           | 6.565 ± 0.160                   | 3.234 ± 0.106                   | 0.452 ± 0.028                    | 37.630 ± 1.807              |
| COLE-75 mg  | 61.697 ± 2.294\(x\)           | 13.608 ± 0.223                  | 9.324 ± 0.042                   | 1.349 ± 0.049                    | 45.456 ± 1.637              |
| TPLE-50 mg  | 59.896 ± 1.185\(x\)           | 18.285 ± 0.345                  | 10.081 ± 0.040                  | 1.284 ± 0.023                    | 46.674 ± 1.517              |
| TPLE-75 mg  | 63.492 ± 1.875\(x\)           | 7.246 ± 0.209                   | 3.936 ± 0.021                   | 0.752 ± 0.018                    | 46.272 ± 0.862              |
| TPLE-100 mg | 60.480 ± 1.432\(x\)           | 7.229 ± 0.080                   | 5.083 ± 0.044                   | 0.631 ± 0.029                    | 45.246 ± 1.739              |

Values are mean ± standard error of the mean (SEM), \(n=5\) animals, per group. Values in the same column with different superscript symbols differ significantly at \(P < .05\). COLE, Chromolaena odorata leaf extract; TPLE, Tridax procumbens leaf extract.
indicated by the significant elevations in hepatic MDA level and reductions in ascorbic acid level and activities of superoxide dismutase, catalase, and glutathione peroxidase. This is in agreement with earlier reports of doxorubicin-induced increases in hepatic MDA levels, and decreases in superoxide dismutase, catalase, and glutathione peroxidase activities.5,6,9,10

However, pretreatment with the extracts and metformin attenuated the doxorubicin-induced oxidative stress by reducing the hepatic MDA and raising the levels of ascorbic acid and antioxidant enzymes. This antioxidant protective effect is in consonance with reports of concomitant antioxidant levels in alloxan-induced diabetic rats by *T. procumbens* extract,57 and improvement of antioxidant levels in the diaphragms of streptozotocin-induced diabetic rats by *C. odorata* extract.58 This result is in line with the suggestion by Lee et al52 that significant enhancements of endogenous enzymatic antioxidants by plant extracts might be a legitimate strategy for decreasing oxidative stress in the liver. So, these increases caused by the extracts, portends a consolidation of the endogenous antioxidant status of hepatic tissues, and their subsequent protection from free radical damage.57

The high content of ascorbic acid in the liver tissues may be the result of the high content of ascorbic acid in the leaves.44 This antioxidant protective effects of extracts may be sequel to their content of any one or a combination of some or all of: allicin, apigenin, ascorbic acid, baicalein, caffeic acid, carotenoids, catechin, chlorogenic acid, ellagic acid, epicatechin, epigallocatechin gallate, gallic acid, genistein, kaempferol, ligans, lutein, myricetin, naringenin, nobletin, quercetin, saponins, and silymarin,43-51 whose antioxidant and hepatoprotective activities have been variously reported.

Lipid peroxidation decreases membrane fluidity,83,84 and could compromise the integrity and function of the plasma membrane, thereby leading to leakages of materials from hepatocytes into the blood. Plasma aminotransferases (alanine and aspartate transaminases), alkaline phosphatase, and total bilirubin are the standard biomarkers for detecting and defining liver damage and liver dysfunction in drug-induced liver injury.85,86 In the present study, doxorubicin caused significant elevation in the plasma levels of alkaline phosphatase, alanine, and aspartate transaminases; as well as decreases in plasma albumin, globulin, total protein, and bilirubin. This is in consonance with other studies which reported

### Table 4

| Treatment | Albumin (g/L) | Total protein (g/L) | Globulin (g/L) | Albumin to globulin ratio | Total bilirubin (mg/dL) | Alanine transaminase (U/L) | Aspartate transaminase (U/L) | Alkaline phosphatase (U/L) |
|-----------|--------------|---------------------|---------------|--------------------------|------------------------|---------------------------|-----------------------------|--------------------------|
| Normal control | 26.48±0.304† | 46.72±0.483† | 14.95±0.555† | 1.164±0.038† | 1.853±0.019† | 136.903±4.111† | 125.438±5.051† | 25.944±0.077† |
| Test control | 17.79±0.268† | 25.160±0.531† | 7.363±0.591† | 2.482±0.205† | 1.674±0.025† | 201.559±4.444† | 176.325±2.136† | 41.952±1.899† |
| Metformin | 24.914±0.221† | 39.859±0.775† | 19.445±0.555† | 1.677±0.051† | 2.103±0.021† | 175.251±0.205† | 147.719±2.563† | 1.840±0.291† |
| COLE-50 mg | 21.899±0.220† | 46.482±0.538† | 24.182±0.669† | 0.990±0.032† | 1.922±0.023† | 151.824±1.783† | 153.080±2.525† | 12.696±2.657† |
| COLE-75 mg | 38.492±0.533† | 57.427±0.410† | 19.430±0.372† | 1.958±0.061† | 1.842±0.023† | 151.614±3.893† | 165.854±0.867† | 32.384±4.808† |
| COLE-100 mg | 21.486±0.326† | 67.439±0.971† | 45.952±1.250† | 1.470±0.020† | 1.872±0.097† | 133.919±8.752† | 149.101±2.037† | 23.368±2.579† |
| TPLE-50 mg | 36.172±0.436† | 67.711±0.711† | 30.939±0.038† | 1.176±0.050† | 2.099±0.016† | 128.265±7.006† | 132.453±0.573† | 11.960±2.909† |
| TPLE-75 mg | 31.023±0.495† | 59.618±0.631† | 28.595±0.250† | 1.085±0.016† | 1.812±0.010† | 146.065±8.489† | 162.085±1.486† | 27.784±5.930† |
| TPLE-100 mg | 23.534±0.432† | 64.599±0.960† | 40.925±1.047† | 0.577±0.022† | 1.909±0.013† | 132.348±1.965† | 156.745±3.567† | 32.936±5.519† |

Values are mean ± standard error of the mean (SEM), n=5 animals, per group. Values in the same column with different superscript symbols differ significantly at P<.05. COLE, Chromolaena odorata leaf extract; TPLE, *Tridax procumbens* leaf extract.

†Has no unit.
doxorubicin-induced elevations in plasma levels of alanine and aspartate transaminases and alkaline phosphatase activities, and decreases in plasma albumin and total protein concentrations. However, these adverse alterations were attenuated by pretreatment with the extracts and metformin. The lowering of these markers by the extracts is an indication of their hepatoprotective potential. The extracts may have protected the hepatic cell membranes from doxorubicin-induced damage, thereby restricting the leakage of these enzymes into the plasma. This hepatoprotective effect is in concord with earlier reports of hepatoprotective effects of leaf extracts of *Trichophragma* and *Codonopsis* against carbon tetrachloride-induced liver damage. This hepatoprotective effect of the extracts could be linked to the presence in them, of antioxidants (mentioned above), all of which have hepatoprotective activities, and are known to condition hepatocytes, so as to cause enhanced regeneration of parenchyma cells, and consequently protecting against membrane fragility and leakage of the marker enzymes into the bloodstream.

Whereas reduced glutathione primarily prevents the oxidation of water-soluble components, the lipophilic bilirubin protects lipids from oxidation. Therefore, sequel to the antioxidant property of bilirubin, and its ability to function as a cellular antioxidant, epidemiological studies have shown that levels of plasma bilirubin are inversely correlated with the risk for the development and progression of both chronic kidney disease and cardiovascular disease. Therefore, in the absence of liver disease, high levels of total bilirubin, as observed in this study, may confer some health benefits. In this study, the extracts prevented doxorubicin-induced increases in hepatic cholesterol and triglyceride levels. They extracts may owe this effect to the presence in them of any one or a combination of 2 or more of ellagic acid, quercetin, chlorogenic acid, naringenin, all of which modulates hepatic lipids (both triglyceride and cholesterol), and lower adiposity and triglyceride contents in adipose tissue. This tissue cholesterol-lowering activity of the extracts is quite significant, because studies have shown that the level of cholesterol in membranes is inversely correlated with the fluidity of membranes. The present results corroborated the reports of induction of increases in hepatic cholesterol and triglycerides levels in both humans and experimental animals by doxorubicin. It is also in conformity with the report by Ferrans that interaction of doxorubicin and its metabolites with membranes, results in interference with various functions of membranes, including Na+-, K+-dependent ATPase activity, calcium transport, and intracellular electrolyte balance. Therefore, the elevated hepatic concentrations of chloride, calcium, and sodium; and lowered magnesium and potassium, induced by doxorubicin in this study, are reflective of compromised membranes of hepatic tissues. However, pretreatment with the extracts prevented doxorubicin-induced electrolyte imbalance. This hepatic electrolytes’ modulating ability may be due to the presence of chlorogenic acid, which according to Rodriguez de Sotillo and Hadley improves mineral pool distribution in plasma, spleen, and liver.

The reduction of hepatic cholesterol content may have been responsible for the reduction in hepatic calcium content by the extracts. This is in view of the reports that decrease in cholesterol content of plasma membranes leads to decreased Ca²⁺ influx through the Ca²⁺ channel in plasma membranes, which results in

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**Figure 2.** Effects of the extracts on the liver weight and size indices of doxorubicin treated rats. Values are mean ± standard error in the mean, n=5 animals, per group. Bars in the same block, with different superscript letters differ significantly at P<.05.
decrease in intracellular calcium, and vice versa. 104, 110 Reduction in membrane cholesterol also stimulates the activities of Ca2+-ATPase, Mg2+-ATPase, and Na+-K+-ATPase, 104, 111, 112 which modulates transport of calcium, magnesium, potassium, and sodium ions across plasma membranes, and by extension, intracellular electrolyte balance.

Therefore, the above results suggest that the hepatoprotective activity of the extracts against doxorubicin-induced toxicity, may at least in part, be due to their ability to boost endogenous antioxidants, and modulate hepatic cholesterol and electrolyte profiles. This then, is an indication of their potential as resources for the management or prevention of doxorubicin-induced hepatic toxicity.

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Data accessibility statement
All relevant data are within the paper.

Conflicts of interest
None.

Competing interests
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