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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 all 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,[3–8] although many studies have also reported the involvement of apoptotic responses,[3,9] 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 glutathione[5,6,9] 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 mechanisms[10,20,21]; 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

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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 allicin, apigenin, ascorbic acid, baicalein, 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 tamoxifen- 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 compounds[43–51]; all of which have been reported to be potent antioxidants.[47,50,52–55] These antioxidants may account for the numerous pharmacological properties exhibited by these leaves and their extracts; such as hepatopro- tective,[51,56] antidiabetic,[57,58] hematoprotective,[59] nephroprotec- tive,[60] antihypertensive,[46,49,61,62] hypolipidemic and weight reducing,[48,63–66] as well as their anticancer[67,68] activities. The leaf extracts of *C odorata* and *T procumbens* have also been reported to exhibit antioxidant activities.[69,70] 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) 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.

On day 12, doxorubicin (in normal saline) was intra- peritioneally 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 al[46,48,49]; 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.[74]

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

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 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.[75] The assay mix was

Table 1

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.[60] 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 0.5 mL of 0.1 M sodium phosphate buffer (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 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 4 mM 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 hepatic lipids and electrolytes profiles. The cholesterol, triglyceride, calcium, sodium, potassium, chloride, and magnesium contents of the homogenates were assayed according to the kits manufacturer's instructions; except that homogenates were used instead of plasma.

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]

$$\text{i. Plasma globulin concentration} = [\text{total protein}] - [\text{albumin}]$$

$$\text{ii. Plasma albumin to globulin } r\text{atio} = \frac{[\text{Plasma albumin}]}{[\text{Plasma globulin}]}$$

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]

$$\text{Percent protection} = \frac{\text{Parameter}_{\text{test control}} - \text{Parameter}_{\text{treatment}}}{\text{Parameter}_{\text{test control}} - \text{Parameter}_{\text{normal control}}} \times 100$$

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 bya 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.

Table 2

Effects of the extracts on hepatic biomarkers of oxidative stress

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	$2.497 \pm 0.440^{*,\dagger}$	$149.409 \pm 2.749^{*}$	$1.895 \pm 0.023^{*}$	$1.009 \pm 0.027^{*}$	$2.128 \pm 0.014^{*}$
Test control	$4.934 \pm 0.653^{\ddagger}$	$49.874 \pm 1.111^{\ddagger}$	$0.984 \pm 0.025^{\ddagger}$	$0.615 \pm 0.010^{\ddagger}$	$1.984 \pm 0.037^{\ddagger}$
Metformin	$2.186 \pm 0.038^{\dagger}$	$126.461 \pm 3.073^{\dagger}$	$2.510 \pm 0.025^{\dagger}$	$0.914 \pm 0.040^{\dagger}$	$2.292 \pm 0.020^{\dagger}$
COLE-50 mg	$3.304 \pm 0.338^{*,\S}$	$120.559 \pm 3.217^{\dagger}$	$2.110 \pm 0.048^{*}$	$0.896 \pm 0.010^{\dagger}$	$2.157 \pm 0.030^{*}$
COLE-75 mg	$2.734 \pm 0.166^{*,\dagger,\S}$	$353.624 \pm 8.380^{\|}$	$2.855 \pm 0.046^{\dagger,\S}$	$2.914 \pm 0.031^{\|}$	$3.277 \pm 0.028^{\|}$
COLE-100 mg	$3.073 \pm 0.197^{*,\dagger,\S}$	$262.700 \pm 9.664^{\P}$	$3.000 \pm 0.035^{\S}$	$3.269 \pm 0.054^{\P}$	$2.682 \pm 0.043^{\S,\P}$
TPLE-50 mg	$3.100 \pm 0.217^{*,\dagger,\S}$	$270.377 \pm 5.800^{\P}$	$2.727 \pm 0.378^{\dagger,\S}$	$3.549 \pm 0.040^{\#}$	$2.640 \pm 0.040^{\P}$
TPLE-75 mg	$2.787 \pm 0.246^{*,\dagger,\S}$	$102.828 \pm 7.851^{\#}$	$1.775 \pm 0.021^{*}$	$1.212 \pm 0.014^{**}$	$2.705 \pm 0.040^{\S,\P}$
TPLE-100 mg	$3.570 \pm 0.137^{\S}$	$171.767 \pm 6.433^{\S}$	$1.927 \pm 0.054^{*}$	$1.399 \pm 0.020^{\S}$	$2.766 \pm 0.026^{\S}$

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.

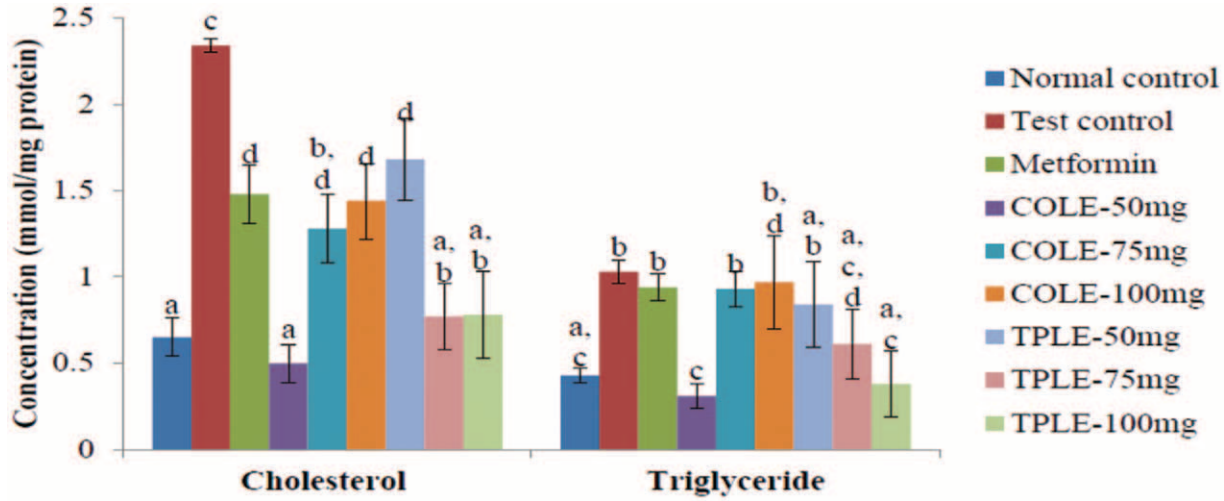

Figure 1. Effects of the extracts on hepatic cholesterol and triglyceride concentrations of doxorubicin treated rats. Values are mean±standard error in the mean, n=5 animals, per group. Bars in the same block, with different superscript letters differ significantly at $P<.05$.

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.[3,4,7,8] In this study, doxorubicin treatment produced oxidative stress as

Table 3

Effects of the extracts on the hepatic electrolytes profiles of doxorubicin treated rats

Treatment	Calcium (μg/mg protein)	Chloride (μEq/mg protein)	Magnesium (μg/mg protein)	Potassium (μmol/mg protein)	Sodium (μEq/mg protein)
Normal control	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‡	0.139±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$^{||}$	6.656±0.160*,§	3.234±0.168*	0.452±0.028§	37.630±1.807§
COLE-75 mg	61.697±2.294$^{*,||}$	13.608±0.223¶	9.324±0.042§	1.349±0.049¶	45.456±1.637*
COLE-100 mg	62.389±0.554$^{*,||}$	18.285±0.345†	10.081±0.040¶	1.284±0.023¶	46.674±1.517$^{*,||}$
TPLE-50 mg	59.896±1.185$^{*,||}$	12.548±0.335$^{||}$	10.549±0.049†	1.969±0.042$^{||}$	50.311±0.708$^{||}$
TPLE-75 mg	63.492±1.875$^{||}$	7.246±0.209*	3.936±0.021$^{\#}$	0.752±0.018†	46.272±0.862*
TPLE-100 mg	60.480±1.432$^{*,||}$	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.

Table 4

Effects of the extracts on plasma biomarkers of liver function/integrity

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	26.48q ± 0.304†	49.718 ± 0.483†	23.238 ± 0.620†	1.144 ± 0.038†	1.853 ± 0.019†	136.903 ± 4.811†,‡	125.438 ± 4.051†	25.944 ± 4.077†
Test control	17.797 ± 0.266§	25.160 ± 0.531§	7.363 ± 0.591§	2.482 ± 0.205§	1.674 ± 0.025§	201.559 ± 4.844§	176.325 ± 2.136§	41.952 ± 1.899§
Metformin	24.914 ± 0.221$^{\|\|}$	39.859 ± 0.775$^{\|\|}$	14.945 ± 0.555$^{\|\|}$	1.677 ± 0.051$^{\|\|}$	2.103 ± 0.018$^{\|\|}$	175.251 ± 2.057$^{\|\|}$	146.719 ± 2.630$^{\|\|}$	1.840 ± 0.291$^{\|\|}$
COLE-50 mg	21.899 ± 0.220¶	46.081 ± 0.538¶	24.182 ± 0.669†	0.909 ± 0.032¶	1.922 ± 0.025¶	151.824 ± 1.783¶	153.080 ± 2.525$^{\|\|,\P}$	12.696 ± 2.657$^{\P,\#}$
COLE-75 mg	38.492 ± 0.533‡	57.922 ± 0.416‡	19.430 ± 0.372¶	1.985 ± 0.061‡	1.842 ± 0.023†	151.614 ± 3.892¶	165.854 ± 0.867‡	32.384 ± 4.898†,§
COLE-100 mg	21.488 ± 0.326¶	67.439 ± 0.972**	45.952 ± 1.250‡	0.470 ± 0.020$^{\#}$	1.827 ± 0.008†	133.919 ± 8.752$^{\dagger,\#}$	149.101 ± 2.037$^{\|\|}$	23.368 ± 2.579†,¶
TPLE-50 mg	36.172 ± 0.436**	67.111 ± 0.711**	30.939 ± 1.038**	1.176 ± 0.050†	2.009 ± 0.016$^{\#}$	128.265 ± 7.006$^{\ddagger,\#}$	132.453 ± 0.573†	11.960 ± 2.909$^{\dagger,\#}$
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 ± 4.688†,¶	162.085 ± 1.486$^{\#,\ddagger}$	27.784 ± 5.930†
TPLE-100 mg	23.534 ± 0.432$^{\#}$	64.459 ± 0.960$^{\#}$	40.925 ± 1.047$^{\#}$	0.577 ± 0.022$^{\#}$	1.909 ± 0.013$^{\#}$	132.348 ± 1.955$^{\P,\#}$	156.745 ± 3.667$^{\P,\#}$	32.936 ± 5.191†,§

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.

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 the improvement of ocular 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 al[82] 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, lignans, lutein, myricetin, naringenin, nobiletin, 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 5

Percent protection by the extracts

Parameter	Metformin	COLE-50 mg	COLE-75 mg	COLE-100 mg	TPLE-50 mg	TPLE-75 mg	TPLE-100 mg
Albumin	82.0 ± 2.5*	47.2 ± 2.5†	238.3 ± 6.1 ‡	42.5 ± 3.8†	211.6 ± 5.0§	152.3 ± 5.7$^{\|\|}$	66.1 ± 5.0¶
Total protein	59.9 ± 3.2*	85.2 ± 2.2‡	133.4 ± 1.7§	172.2 ± 4.0$^{\|\|}$	170.8 ± 2.9$^{\|\|}$	140.3 ± 2.6§	160.0 ± 3.9†
Globulin	47.8 ± 3.5*	105.9 ± 4.2†	76.0 ± 2.3‡	243.1 ± 7.9§	148.5 ± 6.5$^{\|\|}$	133.7 ± 1.6$^{\|\|}$	211.4 ± 6.6¶
Albumin/globulin ratio	60.2 ± 3.8*	117.5 ± 2.4‡	37.1 ± 4.6§	150.3 ± 1.5†	97.6 ± 3.8$^{\|\|}$	104.4 ± 1.2$^{\|\|}$	142.3 ± 1.6†
Total bilirubin	239.5 ± 9.8*	138.6 ± 13.9‡	94.0 ± 12.6†	85.5 ± 4.4†	186.7 ± 9.1§	77.1 ± 5.8†	131.3 ± 7.2‡
Alkaline phosphatase	250.6 ± 1.8*	182.8 ± 16.6*,‡	59.8 ± 30.6†	116.1 ± 16.1†,‡	187.4 ± 18.2*	88.5 ± 37.0†	56.3 ± 32.4†
Aspartate transaminase	58.2 ± 5.2*	45.7 ± 5.0*,‡	20.6 ± 1.7§	53.5 ± 4.0*	86.2 ± 1.1$^{\|\|}$	28.0 ± 2.9†,§	38.5 ± 7.2†,‡
Alanine transaminase	40.7 ± 3.2*	76.9 ± 2.8‡	77.2 ± 6.0‡	104.6 ± 13.5†,§	113.4 ± 10.8§	85.8 ± 7.3†,‡	107.0 ± 3.0†,§
Hepatic triglyceride	27.4 ± 11.2*	117.2 ± 10.2†	29.9 ± 14.3*	24.0 ± 37.8*	41.8 ± 35.1*,‡	73.8 ± 28.1*,†	106.7 ± 27.1†,‡
Hepatic cholesterol	50.4 ± 10.3*	108.6 ± 6.4‡	62.6 ± 12.1*,†	52.9 ± 12.7*	38.8 ± 14.0*	92.5 ± 11.4†,‡	91.9 ± 14.5†,‡
Hepatic calcium	72.3 ± 4.3*	86.1 ± 7.8†	91.1 ± 5.8†	89.4 ± 1.4†	95.7 ± 3.0†	86.6 ± 4.7†	94.2 ± 3.6†
Hepatic potassium	66.9 ± 2.5*	58.3 ± 5.2*	225.4 ± 9.1‡	213.4 ± 4.2‡	341.0 ± 7.8§	114.3 ± 3.4$^{\|\|}$	91.7 ± 5.4†
Hepatic magnesium	143.2 ± 3.7*	156.0 ± 73.7*	2824.1 ± 18.4†	3155.6 ± 17.6‡	3360.7 ± 21.3§	463.8 ± 9.0$^{\|\|}$	966.4 ± 19.3¶
Hepatic chloride	43.1 ± 10.9*	67.5 ± 9.1*,‡	464.7 ± 12.7§	732.0 ± 19.7$^{\|\|}$	404.2 ± 19.2†	101.2 ± 11.9	100.2 ± 4.6‡
Hepatic sodium	132.7 ± 5.2*	138.3 ± 12.9*	82.4 ± 11.7†	73.7 ± 10.8†,‡	47.7 ± 5.1‡	76.5 ± 6.2†	83.9 ± 12.4†
Hepatic ascorbic acid	76.9 ± 3.1*	71.0 ± 3.2*,‡	305.2 ± 8.4§	213.8 ± 9.7¶	221.5 ± 5.8¶	53.2 ± 7.9‡	122.5 ± 6.5†
Hepatic malondialdehyde	112.8 ± 1.6*	66.9 ± 13.9†,‡	90.3 ± 6.8*,†	76.4 ± 8.1†,‡	75.3 ± 8.9†,‡	88.1 ± 10.1*,†	56.0 ± 5.6‡
Hepatic superoxide dismutase	75.7 ± 10.2*	71.3 ± 2.6*	583.4 ± 8.0†	673.5 ± 13.7‡	744.5 ± 10.2§	151.5 ± 3.5$^{\|\|}$	198.9 ± 5.0
Hepatic catalase	213.4 ± 13.7*	119.6 ± 20.8‡	894.2 ± 19.3§	482.8 ± 29.7$^{\dagger,\|\|}$	453.9 ± 27.8$^{\|\|}$	499.0 ± 28.0$^{\dagger,\|\|}$	541.0 ± 17.7†
Hepatic glutathione peroxidase	167.4 ± 2.8*,§	123.6 ± 5.3‡,§	205.3 ± 5.0*,†	221.2 ± 3.8†	191.3 ± 41.5*,†	86.8 ± 2.3‡	103.4 ± 5.9‡

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

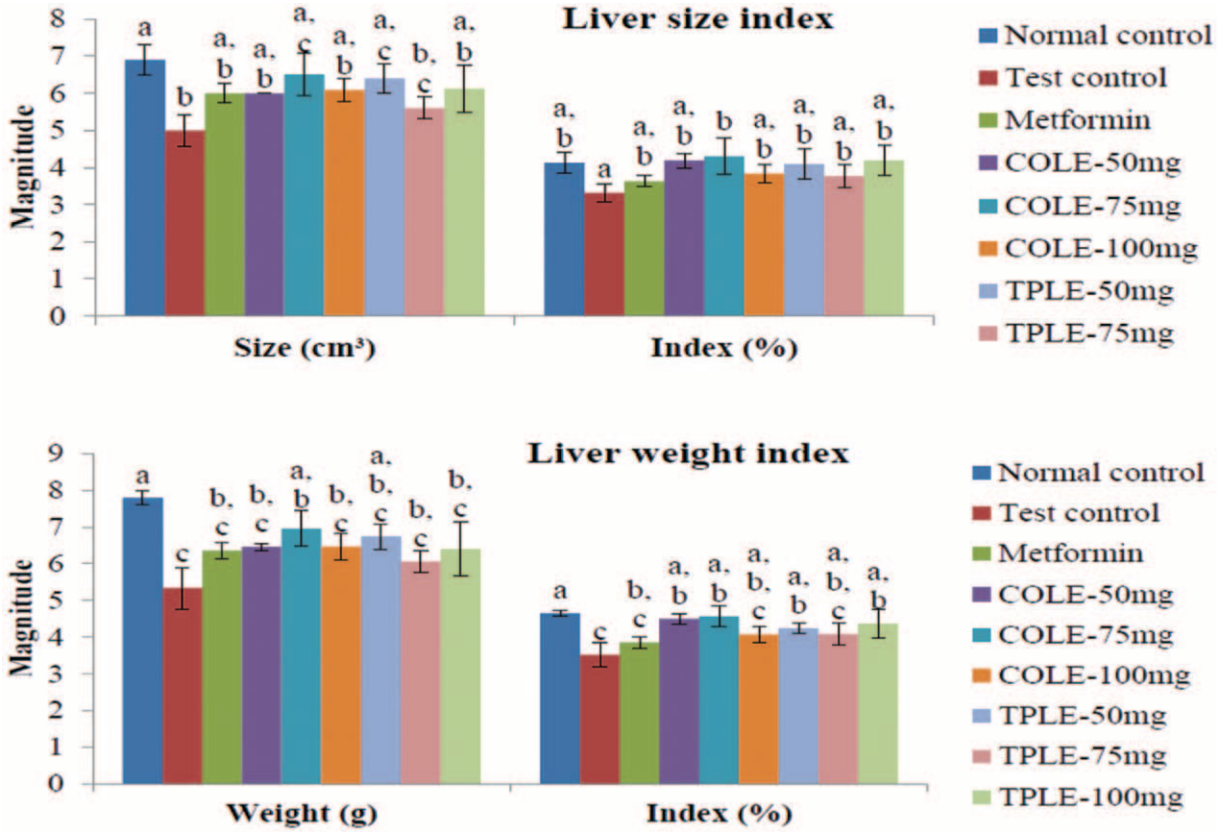

Figure 2. Effects of the extracts on the liver weight and size indices of doxorubicin treated rats. Values are mean ± standard error in the mean, n = 5 animals, per group. Bars in the same block, with different superscript letters differ significantly at $P < .05$.

doxorubicin- induced elevations in plasma levels of alanine and aspartate transaminases and alkaline phosphatase activities, and decreases in plasma albumin and total protein concentrations.[5,6,9] 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.[81] 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 *T procumbens*[51] and *C odorata*[56] 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.[87] Therefore, sequel to the antioxidant property of bilirubin,[88–90] and its ability to function as a cellular antioxidant,[86,91] 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.[88,89,90,92–96] Therefore, in the absence of liver disease, high levels of total bilirubin, as observed in this study, may confer some health benefits.[92,95,97]

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,[46,47,50,51] all of which modulates hepatic lipids (both triglyceride and cholesterol),[98–100] and lower adiposity and triglyceride contents in adipose tissue.[101–103] 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.[83,104,105] The present results corroborated the reports of induction of increases in hepatic cholesterol and triglycerides levels in both humans and experimental animals by doxorubicin.[106,107] It is also in conformity with the report by Ferrans[108] 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[109] 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 Ca^{2+} influx through the Ca^{2+} channel in plasma membranes, which results in

decrease in intracellular calcium, and vice versa.[104,110] Reduction in membrane cholesterol also stimulates the activities of Ca^{2+}-ATPase, Mg^{2+}-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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References

[1] Indu R, Azhar TS, Nair A, Nair CKK. Amelioration of doxorubicin induced cardio- and hepato-toxicity by carotenoids. J Cancer Res Therapeut. 2014;10:62–67.
[2] Chen X, Zhang Y, Zhu Z, et al. Protective effect of berberine on doxorubicin-induced acute hepatorenal toxicity in rats. Mol Med Rep. 2016;13:3953–3960.
[3] Alghorabi AA, Kabel AM, Abd Elmaaboud MA. Doxorubic Insights into dynamics clinical uses adverse effects. Cancer Res J Treat. 2019;7:17–20.
[4] Song S, Chu L, Liang H, et al. Protective effects of dioscin against doxorubicin-induced hepatotoxicity via regulation of Sirt1/FOXO1/NF-kb signal. Front Pharmacol. 2019;10:1030.
[5] Oz E, ilhan MN. Effects of melatonin in reducing the toxic effects of doxorubicin. Mol Cell Biochem. 2006;286:11–15.
[6] Lee IC, Kim SH, Baek HS, et al. Melatonin improves adriamycin-induced hepatic oxidative damage in rats. Mol Cell Toxicol. 2013;9:257–265.
[7] Cichoz-Lach H, Michalak A. Oxidative stress as a crucial factor in liver disease. World J Gastroenterol. 2014;20:8082–8091.
[8] Mansouri E, Jangaran A, Ashtari A. Protective effect of pravastatin on doxorubicin-induced hepatotoxicity. Bratisl Lek Listy. 2017;118:273–277.
[9] Ahmed OM, Abdul-Hamid MM, El-Bakry AM, et al. Camellia sinensis and epicatechin abate doxorubicin-induced hepatotoxicity in male Wistar rats via their modulatory effects on oxidative stress, inflammation, and apoptosis. J Applied Pharmaceut Sci. 2019;9:30–44.
[10] Aleisa AM, A1-Rejaie SS, Bakheet SA, et al. Protective effect of metformin on cardiac and hepatic toxicity induced by adriamycin in Swiss albino mice. Asian J Biochem. 2008;3:99–108.
[11] Jambhulkar S, Deshireddy S, Jestadi DB, Periyasamy L. Quercetin attenuating doxorubicin induced hepatic, cardiac and renal toxicity in male albino Wistar rats. Am J Phytomed Clin Therapeut. 2014;2:985–1004.
[12] Lv Z, Guo Y. Metformin and its benefits for various diseases. Front Endocrinol. 2020;11:191.
[13] Kim EK, Lee SH, Jhun JY, et al. Metformin prevents fatty liver and improves balance of white/brown adipose in an obesity mouse model by inducing FGF21. Mediators Inflamm. 2016;2016:5813030.
[14] Bell S, Farran B, Mcgurnaghan S, et al. Risk of acute kidney injury and survival in patients treated with metformin: an observational cohort study. BMC Nephrol. 2017;18:163.
[15] Podhorecka M, Ibanez B, Dmoszynska A. Metformin-its potential anticancer and anti-aging effects. Postepy Hig Med Dosw. 2017;71:170–175.
[16] Lee M, Katerelos M, Gleich K, et al. Phosphorylation of acetyl-CoA carboxylase by AMPK reduces renal fibrosis and is essential for the anti-fibrotic effect of metformin. J Am Soc Nephrol. 2018;29:2326–2336.
[17] Kheniser KG, Kashyap SR, Kasumov T. A systematic review: the appraisal of the effects of metformin on lipoprotein modification and function. Obes Sci Pract. 2019;5:36–45.
[18] Lavine JE, Schwimmer JB, Van Natta ML, et al. Effect of vitamin E or metformin for treatment of nonalcoholic fatty liver disease in children and adolescents: the TONIC randomized controlled trial. JAMA. 2011;305:1659–1668.
[19] Lin HZ, Yang SQ, Chuckaree C, Kuhajda F, Ronnet G, Diehl AM. Metformin reverses fatty liver disease in obese, leptin-deficient mice. Nat Med. 2000;6:998–1003.
[20] Pandey A, Kumar VL. Protective effect of metformin against acute inflammation and oxidative stress in rat. Drug Dev Res. 2016;77:278–284.
[21] Rizk FH, El Saadany AA, Dawood L, et al. Metformin ameliorated methotrexate-induced hepatorenal toxicity in rats in addition to its antitumor activity: two birds with one stone. J Inflamm Res. 2018;11:421–429.
[22] Alamgir ANM. Therapeutic Use of Medicinal Plants and Their Extracts: Volume 1, Progress in Drug Research 73. Switzerland: Springer International Publishing AG; 2017.
[23] Kalender Y, Yel M, Kalender S. Doxorubicin hepatotoxicity and hepatic free radical metabolism in rats. The effects of vitamin E and catechin. Toxicology. 2005;209:39–45.
[24] Gokcimen A, Cim A, Tola HT, et al. Protective effect of N-acetylcysteine, caffeic acid and vitamin E on doxorubicin hepatotoxicity. Hum Exp Toxicol. 2007;26 6:519–525.
[25] Patel N, Joseph C, Corcoran GB, Ray SD. Silymarin modulates doxorubicin-induced oxidative stress, Bcl-xL and p53 expression while preventing apoptotic and necrotic cell death in the liver. Toxicol Applied Pharmacol. 2010;245:143–152.
[26] Raskovic A, Stilinovic N, Kolarovic J, Vasovic V, Vukmirovic S, Mikov M. The protective effects of silymarin against doxorubicin-induced cardiotoxicity and hepatotoxicity in rats. Molecules. 2011;16:8601–8613.
[27] Rudolfová P, Hanusová V, Skálová L, Bártíková H, Matousková P, Bousová I. Effect of selected catechins on doxorubicin antiproliferative efficacy and hepatotoxicity *in vitro*. Acta Pharm. 2014;64:199–209.
[28] Girish C, Koner BC, Jayanthi S, Ramachandra Rao K, Rajesh B, Pradhan SC. Hepatoprotective activity of picroliv, curcumin and ellagic acid compared to silymarin on paracetamol induced liver toxicity in mice. Fundam Clin Pharmacol. 2009;23:735–745.
[29] Ozturk IC, Ozturk F, Gul M, Ates B, Cetin A. Protective effects of ascorbic acid on hepatotoxicity and oxidative stress caused by carbon tetrachloride in the liver of Wistar rats. Cell Biochem Function. 2009;27:309–315.
[30] Rasool MK, Sabina EP, Ramya SR, et al. Hepatoprotective and antioxidant effects of gallic acid in paracetamol-induced liver damage in mice. J Pharm Pharmacol. 2010;62:638–643.
[31] Sindhu ER, Firdous AP, Preethi KC, Kuttan R. Carotenoid lutein protects rats from paracetamol-, carbon tetrachloride- and ethanol- induced hepatic damage. J Pharm Pharmacol. 2010;62:1054–1060.
[32] Bigoniya P, Singh CS, Shrivastava B. *In vivo* and *in vitro* hepatoprotective potential of kaempferol, a flavone glycoside from *Capparis spinosa*. Int J Pharm Biol Sci. 2013;3:139–152.
[33] Dong D, Zhang S, Yin L, et al. Protective effects of the total saponins from *Rosa laevigata* Michx fruit against carbon tetrachloride-induced acute liver injury in mice. Food Chem Toxicol. 2013;62:120–130.
[34] Fan YJ, Rong Y, Li PF, et al. Genistein protection against acetaminophen-induced liver injury via its potential impact on the

activation of UDP-glucuronosyltransferase and antioxidant enzymes. Food Chem Toxicol. 2013;55:172–181.
[35] Matić S, Stanić S, Bogojević D, et al. Methanol extract from the stem of Cotinus coggygria Scop., and its major bioactive phytochemical constituent myricetin modulate pyrogallol-induced DNA damage and liver injury. Mutat Res. 2013;755:81–89.
[36] Yang J, Wang XY, Xue J, Gu ZL, Xie ML. Protective effect of apigenin on mouse acute liver injury induced by acetaminophen is associated with increment of hepatic glutathione reductase activity. Food Funct. 2013;4:939–943.
[37] Hermenean A, Ardelean A, Stan M, et al. Antioxidant and hepatoprotective effects of naringenin and its β-cyclodextrin formulation in mice intoxicated with carbon tetrachloride: a comparative study. J Med Food. 2014;17:670–677.
[38] Suddek GM. Allicin enhances chemotherapeutic response and ameliorates tamoxifen-induced liver injury in experimental animals. Pharmaceut Biol. 2014;52:1009–1014.
[39] Pang C, Sheng YC, Jiang P, Wei H, Ji LL. Chlorogenic acid prevents acetaminophen-induced liver injury: the involvement of CYP450 metabolic enzymes and some antioxidant signals. J Zhejiang Univ Sci B. 2015;16:602–610.
[40] Shen B, Chen H, Shen C, et al. Hepatoprotective effects of lignans extract from Herpetospermum caudigerum against CCl_4-induced acute liver injury in mice. J Ethnopharmacol. 2015;164:46–52.
[41] He Z, Li X, Chen H, et al. Nobiletin attenuates lipopolysaccharide/D—galactosamine—induced liver injury in mice by activating the Nrf2 antioxidant pathway and subsequently inhibiting NF—κB-mediated cytokine production. Mol Med Rep. 2016;14:5595–5600.
[42] Zhou HC, Wang H, Shi K, Li JM, Zong Y, Du R. Hepatoprotective effect of baicalein against acetaminophen-induced acute liver injury in mice. Molecules. 2019;24:131.
[43] Igboh MN, Ikewuchi JC, Ikewuchi CC. Chemical profile of *Chromolaena odorata* L. (King and Robinson) leaves. Pak J Nutr. 2009;8:521–524.
[44] Ikewuchi CC, Ikewuchi JC. Comparative study on the vitamin composition of some common Nigerian medicinal plants. Pac J Sci Technol. 2009;10:367–371.
[45] Ikewuchi JC, Ikewuchi CC, Igboh MN. Chemical profile of *Tridax procumbens* Linn. Pak J Nutr. 2009;8:548–550.
[46] Ikewuchi JC, Ikewuchi CC, Enuneku EC, et al. Alteration of blood pressure indices and pulse rates by an aqueous extract of the leaves of *Chromolaena odorata* (L) King and Robinson (Asteraceae). Pac J Sci Technol. 2012;13:348–358.
[47] Ikewuchi JC, Ikewuchi CC, Ifeanacho MO. Analysis of the phytochemical composition of the leaves of *Chromolaena odorata* King and Robinson by gas chromatography-flame ionization detector. Pac J Sci Technol. 2013;14:360–378.
[48] Ikewuchi JC, Ikewuchi CC, Ifeanacho MO. Attenuation of salt-loading induced cardiomegaly and dyslipidemia in Wistar rats by aqueous leaf extract of *Chromolaena odorata*. Pharmacol Pharm. 2014;5:160–170.
[49] Ikewuchi JC, Ikewuchi CC, Ifeanacho MO. An aqueous extract of the leaves of *Chromolaena odorata* moderated plasma biochemical and hematological indices of sub-chronic salt-loaded rats. Asian J Pharm Res. 2014;4:24–35.
[50] Ikewuchi CC, Ikewuchi JC, Ifeanacho MO. Phytochemical composition of *Tridax procumbens* Linn leaves: potential as a functional food. Food Nutr Sci. 2015;6:992–1004.
[51] Ikewuchi JC. An aqueous extract of the leaves of *Tridax procumbens* Linn (Asteraceae) protected against carbon tetrachloride induced liver injury in Wistar rats. Pac J Sci Technol. 2012;13:519–527.
[52] Dillard CJ, German JB. Phytochemicals: nutraceuticals and human health. J Sci Food Agric. 2000;80:1744–1756.
[53] Francis G, Kerem Z, Makkar HPS, Becker K. The biological action of saponins in animal systems: a review. British J Nutr. 2002;88:587–605.
[54] Ikewuchi JC, Ikewuchi CC, Ifeanacho MO. Nutrient and bioactive compounds composition of the leaves and stems of *Pandiaka heudelotii*: a wild vegetable. Heliyon. 2019;5:e01501.
[55] Ifeanacho MO, Ikewuchi CC, Ikewuchi JC. Investigation of the profile of phenolic compounds in the leaves and stems of *Pandiaka heudelotii* using gas chromatography coupled with flame ionization detector. Food Sci Nutr. 2017;5:646–652.
[56] Palanisamy P, Chandra RM, Jaykar B, Venkateshwarlu BS, Pasupathi A. Evaluation of hepatoprotective activity of whole plant extract of *Chromolaena odorata* King and H. Rob in carbon tetrachloride and rifampicin induced rats. IntJ Pharm Teach Practices. 2014;5:1574–1581.
[57] Ikewuchi JC. Alteration of plasma biochemical, haematological and ocular oxidative indices of alloxan induced diabetic rats by aqueous extract of *Tridax procumbens* Linn (Asteraceae). EXCLI J. 2012;11:291–308.
[58] Onkaramurthy M, Veerapur VP, Thippeswamy BS, Reddy TN, Rayappa H, Badami S. Anti-diabetic and anti-cataract effects of *Chromolaena odorata* Linn., in streptozotocin-induced diabetic rats. J Ethnopharmacol. 2013;145:363–372.
[59] Ifeanacho MO, Ikewuchi JC, Ikewuchi CC. Effects of aqueous leafextracts of *Chromolaena odorata* and *Tridax procumbens* on doxorubicin-induced hematologic toxicities in Wistar rats. Pol J Natur Sci. 2020;35:493–505.
[60] Ikewuchi CC, Ifeanacho MO, Ikewuchi JC. Moderation of doxorubicin-induced nephrotoxicity in Wistar rats by aqueous leaf-extracts of *Chromolaena odorata* and *Tridax procumbens*. Porto Biomed J. 2021;6:e129.
[61] Ikewuchi JC, Onyeike EN, Uwakwe AA, et al. Effect of aqueous extract of the leaves of *Tridax procumbens* Linn on blood pressure components and pulse rates of sub chronic salt-loaded rats. Pac J Sci Technol. 2011;12:381–389.
[62] Ikewuchi JC, Ikewuchi CC. Moderation of haematological indices, plasma electrolytes and markers of hepato-renal function in subchronic salt-loaded rats by an aqueous leaf extract of *Tridax procumbens* Linn (Asteraceae). Pac J Sci Technol. 2013; 14:362–369.
[63] Ikewuchi JC, Ikewuchi CC. Alteration of plasma lipid profile and atherogenic indices of cholesterol loaded rats by *Tridax procumbens* Linn: Implications for the management of obesity and cardiovascular diseases. Biokemistri. 2009;21:95–99.
[64] Ikewuchi JC, Ikewuchi CC. Anti-cholesterolemic effect of aqueous extract of the leaves of *Chromolaena odorata* (L) King and Robinson (Asteraceae): Potential for the reduction of cardiovascular risk. Pac J Sci Technol. 2011;12:385–391.
[65] Ikewuchi JC, Onyeike EN, Uwakwe AA, Ikewuchi C. Weight reducing and hypocholesterolemic effect of aqueous extract of the leaves of *Tridax procumbens* Linn on sub-chronic salt-loaded rats. Int J Biol Chem Sci. 2011;5:680–687.
[66] Ifeanacho MO, Ikewuchi JC, Ikewuchi CC, Nweke PC, Okere R, Nwate TLB. Prevention of doxorubicin-induced dyslipidaemia, plasma oxidative stress and electrolytes imbalance in Wistar rats by aqueous leaf-extracts of *Chromolaena odorata* and *Tridax procumbens*. Scientific African. 2020;11:e00636.
[67] Vishnu PP, Srinivasa RA. Evaluation of anticancer activity of *Tridax procumbens* leaf extracts on A549 and Hep G2 cell lines. Asian J Pharm Clin Res. 2015;8:129–132.
[68] Adedapo AA, Oyagbemi AA, Fagbohun OA, Omobowale TO, Yakubu MA. Evaluation of the anticancer properties of the methanol leaf extract of *Chromolaena odorata* on HT-29 cell line. J Pharmacogn Phytochem. 2016;5:52–57.
[69] Andriana Y, Xuan TD, Quy TN, Minh TN, Van TM, Viet TD. Antihyperuricemia, antioxidant, and antibacterial activities of *Tridax procumbens* L. Foods. 2019;8:21.
[70] Putri DA, Fatmawati S. A new flavanone as a potent antioxidant isolated from *Chromolaena odorata* L. leaves. Evid Based Compl Alt Med. 2019;2019:1453612.
[71] National Research CouncilGuide for the Care and Use of Laboratory Animals. 8th edWashington, DC: The National Academies Press; 2011.
[72] FAOProtein quality evaluation: report of Joint FAO/WHO Expert ConsultationFAO Food and Nutrition Paper 51. Rome, Italy: Food and Agriculture Organization of the United Nations; 1991.
[73] Zilinyi R, Czompa A, Czegledi A, et al. The cardioprotective effect of metformin in doxorubicin-induced cardiotoxicity: the role of autophagy. Molecules. 2018;23:1184.
[74] Ifeanacho MO, Ikewuchi CC, Ikewuchi JC. Anti-diabetic effect of a flavonoid and sitosterol - rich aqueous extract of *Pleurotus tuber-regium* sclerotia in alloxan-induced diabetic rabbits. Endocr Metab Immune Disord Drug Targets. 2019;19:1148–1156.
[75] Gutteridge J, Wilkins S. Copper-dependent hydroxyl radical damage to ascorbic acid. FEBS Letters. 1982;137:327–330.
[76] Beers RF, Sizer IW. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J Biol Chem. 1952;195:133–140.
[77] Misra HP, Fridovich I. The role of superoxide anion in the autooxidation of epinephrine and a single assay for superoxide dismutase. J Biol Chem. 1972;247:3170–3175.

[78] Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. Selenium: biochemical role as a component of glutathione peroxidase. Science. 1973;179:588–590.
[79] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951;193:265–275.
[80] Paul S, Mukherjee KL, Gosh S. Biochemical test profiles.Medical Laboratory Technology, Volume III. A Procedure Manual for Routine Diagnostic Tests. 2nd ed, Chapter 34. 2013;Tata McGraw- Hill Education, New Delhi, India:952-970.
[81] Ikewuchi CC, Ikewuchi JC, Ifeanacho MO. Restoration of plasma markers of liver and kidney functions/integrity in alloxan-induced diabetic rabbits by aqueous extract of *Pleurotus tuberregium* sclerotia. Biomed Pharmacother. 2017;95:1809–1814.
[82] Lee CH, Park JH, Cho JH, et al. Effect of Oenanthe javanica extract on antioxidant enzyme in the rat liver. Chinese Med J. 2015;128:1649–1654.
[83] Le Grimellec C, Friedlander G, El Yandouzi EH, Zlatkine P, Giocondi MC. Membrane fluidity and transport properties in epithelia. Kidney Int. 1992;42:825–836.
[84] Wong-Ekkabut J, Xu Z, Triampo W, Tang IM, Tieleman DP, Monticelli L. Effect of lipid peroxidation on the properties of lipid bilayers: a molecular dynamics study. Biophys J. 2007;93:4225–4236.
[85] Ortega-Alonso A, Stephens C, Lucena MI, Andrade RJ. Case characterisation, clinical features and risk factors in drug-induced liver injury. Int J Mol Sci. 2016;17:e714.
[86] European Association for the Study of the LiverEASL clinical practice guidelines: drug-induced liver injury. J Hepatol. 2019;70:1222–1261.
[87] Sedlak TW, Saleh M, Higginson DS, Paul BD, Juluri KR, Snyder SH. Bilirubin and glutathione have complementary antioxidant and cytoprotective roles. Proc Natl Acad Sci U S A. 2009;106:5171–5176.
[88] McArdle PF, Whitcomb BW, Tanner K, Mitchell BD, Shuldiner AR, Parsa A. Association between bilirubin and cardiovascular disease risk factors: using Mendelian randomization to assess causal inference. BMC Cardiovasc Dis. 2012;12:16.
[89] Cure E, Yuce S, Cure MC. Gilbert's syndrome. Arch Med Rev J. 2014;23:220–236.
[90] Ziberna L, Martelanc M, Franko M, Passamonti S. Bilirubin is an endogenous antioxidant in human vascular endothelial cells. Sci Rep. 2016;6:29240.
[91] Baranano DE, Rao M, Ferris CD, Snyde SH. Biliverdin reductase: a major physiologic cytoprotectant. Proc Natl Acad Sci U S A. 2002;99:16093–16098.
[92] Schwertner HA, Vitek L. Gilbert syndrome, UGT1A1*28 allelel, and cardiovascular disease risk: possible protective effects and therapeutic applications of bilirubin. Atherosclerosis. 2008;198:1–11.
[93] Horsfall LJ, Nazareth I, Petersen I. Cardiovascular events as a function of serum bilirubin levels in a large, statin-treated cohort. Circulation. 2012;126:2556–2564.
[94] Vitek L. Bilirubin and atherosclerotic diseases. Physiol Res. 2017;66 (suppl 1):S11–S20.
[95] DiNicolantonio JJ, McCarty MF, O'Keefe JH. Antioxidant bilirubin works in multiple ways to reduce risk for obesity and its health complications. Open Heart. 2018;5:e000914.
[96] Tsai MT, Tarng DC. Beyond a measure of liver function—bilirubin acts as a potential cardiovascular protector in chronic kidney disease patients. Int J Mol Sci. 2019;20:117.
[97] Sedlak TW, Synder SH. Bilirubin benefits: cellular protection by a biliverdin reductase antioxidant cycle. Pediatrics. 2004;113:1776–1782.
[98] Padma VV, Lalitha G, Shirony NP, Baskaran R. Effect of quercetin against lindane induced alterations in the serum and hepatic tissue lipids in Wistar rats. Asian Pac J Trop Biomed. 2012;2:910–915.
[99] Wan CW, Wong CN, Pin WK, et al. Chlorogenic acid exhibits cholesterol lowering and fatty liver attenuating properties by up-regulating the gene expression of PPAR-a in hypercholesterolemic rats induced with a high-cholesterol diet. Phytother Res. 2013;27:545–551.
[100] Leng L, Xiao Y, Mo Z, et al. Synergistic effect of phytochemicals on cholesterol metabolism and lipid accumulation in HepG2 cells. BMC Complement Altern Med. 2018;18:122.
[101] Cho KW, Kim YO, Andrade JE, Burgess JR, Kim Y-C. Dietary naringenin increases hepatic peroxisome proliferators-activated receptor alpha protein expression and decreases plasma triglyceride and adiposity in rats. Eur J Nutr. 2011;50:81–88.
[102] Alam MA, Subhan N, Rahman MM, Uddin SJ, Reza HM, Sarker SD. Effect of citrus flavonoids, naringin and naringenin, on metabolic syndrome and their mechanisms of action. Adv Nutr. 2014;5:404–417.
[103] Okla M, Kang I, Kim DM, et al. Ellagic acid modulates lipid accumulation in primary human adipocytes and human hepatoma Huh7 cells via discrete mechanisms. J Nutr Biochem. 2015;26:82–90.
[104] Bastiaanse EML, Hold KM, Van der Laarse A. The effect of membrane cholesterol content on ion transport processes in plasma membranes. Cardiovasc Res. 1997;33:272–283.
[105] Yeagle PL. The roles of cholesterol in the biology of cells. The Structure of Biological Membranes. 3rd ed. 2012;CRC Press, Boca Raton, FL:119-129.
[106] Subashini R, Ragavendran B, Gnanapragasam A, Yogeeta SK, Devaki T. Biochemical study on the protective potential of *Nardostachys jatamansi* extract on lipid profile and lipid metabolizing enzymes in doxorubicin intoxicated rats. Pharmazie. 2007;62:382–387.
[107] Sharma M, Tuaine J, McLaren B, et al. Chemotherapy agents alter plasma lipids in breast cancer patients and show differential effects on lipid metabolism genes in liver cells. PLoS One. 2016;11: e0148049.
[108] Ferrans VJ. Morphologic assessment of cardiac lesions caused by anthracyclines. Anthracycline Antibiotics in Cancer Therapy. Developments in Oncology, vol. 10. 1982;Springer, Dordrecht, The Netherlands:331-347.
[109] Rodriguez de Sotillo DV, Hadley M. Chlorogenic acid modifies plasma and liver concentrations of: cholesterol, triacylglycerol, and minerals in (fa/fa) Zucker rats. J Nutr Biochem. 2002;13:717–726.
[110] Gleason MM, Medow MS, Tulenko TN. Excess membrane cholesterol alters calcium movements, cytosolic calcium levels, and membrane fluidity in arterial smooth muscle cells. Circ Res. 1991;69:216–227.
[111] Ortega A, Mas-Oliva J. Cholesterol effect on enzyme activity of the sarcolemmal Ca^{2+}, Mg^{2+}-ATPase from cardiac muscle. Biochim Biophys Acta. 1984;773:231–236.
[112] Kutryk MJ, Pierce GN. Stimulation of sodium-calcium exchange by cholesterol incorporation into isolated cardiac sarcolemmal vesicles. J Biol Chem. 1988;263:13167–13172.