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Restoration of plasma kidney and liver biomarkers in doxorubicin-treated Wistar rats by aqueous extracts of *Pleurotus tuberregium* sclerotia and *Cnidoscolus aconitifolius* leaves

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Abstract

The ability of aqueous extracts of sclerotia of *Pleurotus tuberregium* and leaves of *Cnidoscolus aconitifolius* to regulate plasma markers of kidney and liver function/integrity was investigated in doxorubicin-treated Wistar rats. Doxorubicin (dissolved in normal saline) was injected intraperitoneally (15 mg/kg body weight) into the rats; metformin was daily administered orally at 250 mg/kg, while the extracts were daily administered orally at doses of 50, 75, and 100 mg/kg. Compared to the test control, in both the doxorubicin pre-treatment (or ameliorative) study and the extract pre-treatment (protective) studies, the extracts and metformin-treated groups had significantly lower ($P < 0.05$) plasma levels of alkaline phosphatase, alanine transaminase and aspartate transaminase, and concentrations of creatinine, urea, and blood urea nitrogen. However, the plasma globulin, albumin, and total protein concentrations and the albumin/globulin ratio of the extract and metformin-treated groups were significantly higher ($P < 0.05$). The extracts prevented (in the protective study) or attenuated (in the ameliorative study) doxorubicin-induced increase in the levels of plasma markers of kidney and liver function/integrity, and afforded protection or recovery towards near-normal values.

Key words: albumin/globulin ratio, alkaline phosphatase, blood urea nitrogen, creatinine, doxorubicin, transaminases

Introduction

Doxorubicin is a well-established and highly effective anti-neoplastic agent that is used to treat several adult and paediatric cancers such as solid tumours, leukaemia, lymphomas, and breast cancer (Carvalho et al., 2009; Octavia et al., 2012). The successful use of doxorubicin has been hampered by its toxicity to numerous organs such as the heart (Yilmaz et al., 2006; Carvalho et al., 2009; Rashid et al., 2013; Indu et al., 2014; Afsar et al., 2017; Bordbar et al., 2019 ; Ikewuchi et al., 2021a), liver (Carvalho et al., 2009; Indu et al., 2014; Chen et al., 2016; Alghorabi et al., 2019; Ikewuchi et al., 2021b), kidneys (Yilmaz et al., 2006; Carvalho et al., 2009; Rashid et al., 2013; Ren et al., 2016; Bordbar et al., 2019; Sabapathy et al., 2019; Ikewuchi et al., 2021c), and lungs (Vapa et al., 2012; Jagetia and Lalrinpuii, 2018). Hepatonephrotoxicity induced by doxorubicin is accompanied by increased plasma levels of creatinine, urea, uric acid, gamma-glutamyl transferase, lactate dehydrogenase, alanine transaminase, aspartate transaminase, and alkaline phosphatase and decreased plasma albumin and total protein levels (Öz and İlhan, 2006; Yilmaz et al., 2006; Lee et al., 2013; Ahmed et al., 2019a).

Various bioactive compounds of plant origin have been reported to prevent or mitigate the nephrotoxicity and hepatotoxicity of doxorubicin (Surai, 2015). These compounds include allicin, caffeic acid, carotenoids (e.g. lycopene), catechin, ellagic acid, epicatechin, epigalloca-

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techin gallate, naringenin, quercetin, and silymarin, all of which have been reported to exert hepato-nephroprotective effects through attenuation of doxorubicin-induced oxidative stress in the liver and kidneys (Yagmurca et al., 2004; Kalender et al., 2005; Yilmaz et al., 2006; Gokcimen et al., 2007; El-Shitany et al., 2008; Patel et al., 2010; Rašković et al., 2011; Indu et al., 2014; Jambhulkar et al., 2014; Rudolfová et al., 2014; Surai, 2015; Omar et al., 2016; Ahmed et al., 2019a,b).

Numerous studies have shown that the sclerotia of *Pleurotus tuberregium* and leaves of *Cnidoscolus aconitifolius* contain the above mentioned antioxidants, in addition to ascorbic and chlorogenic acids, and other antioxidants belonging to the following family of phytochemicals: simple and polyphenols, glycosides, phytosterols and saponins (Ijeh et al., 2009; Ikewuchi and Ikewuchi, 2009, 2011; Azeez et al., 2010; Ikewuchi et al., 2013a,b, 2014, 2017; Otitolaiye and Asokan, 2016; Kuri-García et al., 2017; Ifeanacho et al., 2019a,b, 2020). Extracts of *P. tuberregium* sclerotia and *C. aconitifolius* leaves have been reported to protect the liver and kidney (Oyagbemi and Odetola, 2010; Saba et al., 2010; Adaramoye and Aluko, 2011; Adaramoye et al., 2011; Ikewuchi et al., 2017; Iwu et al., 2020). They have also been reported to be hypolipidaemic, anti-diabetic (Oladeinde et al., 2007; Ikewuchi et al., 2013a, 2021d; Achi et al., 2017; Ifeanacho et al., 2019a), anti-hypertensive (Ikewuchi et al., 2013a, 2014), and haematomodulatory (Azeez et al., 2010; Ifeanacho et al., 2020; Iwu et al., 2020; Onasanwo et al., 2020). In the present study, the ability of aqueous extracts of *P. tuberregium* sclerotia and *C. aconitifolius* leaves to regulate plasma markers of kidney and liver function/integrity was investigated in doxorubicin-treated rats.

Materials and methods

Procurement of materials

Fresh samples of the sclerotia of *P. tuberregium* were purchased from Mile 1 Market in Port Harcourt, Nigeria, while fresh leaves of *C. aconitifolius* (hospital too far) were collected from Farm Gardens in Aluu Community of Rivers State, Nigeria, and were duly identified as previously reported (Ikewuchi and Ikewuchi, 2009, 2011; Ikewuchi et al., 2013a,b, 2014, 2017; Ifeanacho et al., 2019a,b, 2020). Ninety Wistar rats (weight 80–130 g) were obtained from the Animal House of the Department of Physiology, University of Port Harcourt, Nigeria. The alanine transaminase, aspartate transaminase, alkaline phosphatase, creatinine, and urea kits were the products of Randox Laboratories Ltd., County Antrim, UK.

Preparation of extracts

The sclerotia and leaves were cleaned of dirt and dried before grinding into powder. The powders (5.3 kg of *P. tuberregium* sclerotia and 5 kg of *C. aconitifolius*) were separately soaked in 10 L of hot (boiled) water for 12 h. The resultant mixtures were filtered using a sieve cloth. The filtrates were then concentrated using a rotary evaporator prior to freeze-drying, yielding 145 g and 131 g of *P. tuberregium* sclerotia and *C. aconitifolius* leaves extracts, respectively. The resultant extracts of *P. tuberregium* sclerotia and *C. aconitifolius* leaves (hereafter termed PTSE and CALE, respectively) were weighed, reconstituted in distilled water, and administered to the experimental animals according to their individual weights and doses of their respective groups.

Experimental design

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 sorted into 18 groups of five animals each, with an average difference of ≤ 3 g in mean weight (FAO, 1991). Of these 18 groups, nine groups were used for the ameliorative study, while the remaining nine groups were used for the protective study. They were housed in cages at the Department of Physiology, University of Port Harcourt and allowed access to water and feed *ad libitum*. The treatment commenced after 1 week of acclimatization. The doxorubicin dose was adopted from Song et al. (2019). The doses of administration of the *P. tuberregium* sclerotia extract was adopted and modified from Ifeanacho et al. (2019a, 2020), that of *C. aconitifolius* extract was adopted and modified from Adaramoye and Aluko (2011), while that of metformin was adopted from Zilinyi et al. (2018).

Ameliorative (or doxorubicin pre-treatment) study

Doxorubicin was dissolved in normal saline and injected intraperitoneally (15 mg/kg body weight) into all

rats from all the groups, except for normal control which was administered normal saline instead of doxorubicin solution. After 1 week, the treatment was commenced and lasted for 14 days. Diabetmin™ (metformin HCl) was dissolved in distilled water and orally administered daily at the dose of 250 mg/kg to the metformin group. The extracts were administered through the same route at the dose of 50 mg/kg to PTSE–50 mg (*P. tuberregium* sclerotia extract) and CALE–50 mg (*C. aconitifolius* leaf-extract); 75 mg/kg to PTSE–75 mg (PTSE) and CALE–75 mg (CALE); and 100 mg/kg to PTSE–100 mg (PTSE) and CALE–100 mg (CALE). The normal and test control groups received distilled water instead of the extracts.

Protective (or extract pre-treatment) study

Diabetmin™ (dissolved in distilled water) was orally administered daily at the dose of 250 mg/kg to the metformin group. The extracts were administered through the same route at the dose of 50 mg/kg to PTSE–50 mg (PTSE) and CALE–50 mg (CALE); 75 mg/kg to PTSE–75 mg (PTSE) and CALE–75 mg (CALE); and 100 mg/kg to PTSE–100 mg (PTSE) and CALE–100 mg (CALE). The normal and test control groups received distilled water instead. On day 12, doxorubicin (in normal saline) was intraperitoneally injected (15 mg/kg) into all the groups, except for normal control which was administered normal saline instead.

Collection of plasma samples

On day 14th of the treatment in each study, the animals were sacrificed under chloroform anaesthesia, and blood samples were collected into heparin bottles. The blood samples were centrifuged at 1000 rpm for 10 min, and the plasma was collected into plain sample bottles and stored in a refrigerator for use in the assay.

Determination of plasma markers of kidney and liver function/integrity

The plasma levels of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total protein, albumin, creatinine, and urea were assayed according to the kit manufacturer's instructions. The plasma globulin level and the plasma albumin/globulin ratio were calculated using the following formulae (Paul, 2013):

1) Plasma globulin concentration =
= total protein concentration – albumin concentration

2) Plasma albumin to globulin ratio =
$$= \frac{\text{albumin concentration}}{\text{globulin concentration}}$$

The urea to creatinine ratio was calculated as follows (Manoeuvrier et al., 2017):

$$\text{Urea/creatinine ratio} = \frac{\text{Serum urea concentration [mmol/l]}}{\text{Serum creatinine concentration [mmol/l]}}$$

Determination of per cent recovery or protection

The level of restoration or safeguard of kidney and liver function/integrity, denoted as per cent recovery/protection, was calculated as follows (Ikewuchi et al., 2021b):

$$\text{Per cent recopvery (or protection)} = \frac{\text{Parameter}_{\text{test control}} - \text{Parameter}_{\text{treatment}}}{\text{Parameter}_{\text{test control}} - \text{Parameter}_{\text{normal control}}} \times 100$$

Statistical analysis of data

Statistical calculations were performed with the software Excel 2010 (Data Analysis Add-in). All data are expressed as mean ± standard error of the mean (SEM) and were analysed using one-way analysis of variance. Significant difference of the mean values was determined using the least significant difference test, and $P < 0.05$ was considered statistically significant.

Results

Regulation of plasma markers of kidney function

The influence of aqueous extracts of the sclerotia of *P. tuberregium* and the leaves of *C. aconitifolius* on plasma markers of kidney function in doxorubicin-treated rats is shown in Table 1. In both the doxorubicin pre-treatment (ameliorative) study and the extract pre-treatment (protective) study, the plasma creatinine levels (263.13 ± 3.11 and 172.00 ± 4.18 μmol/l, respectively) and urea levels (14.11 ± 0.41 and 13.67 ± 0.25 mmol/l, respectively) of the test control group were significantly higher ($P < 0.05$) than those of all the other groups. The CALE–100 mg group had the least plasma creatinine levels (19.85 ± 2.09 and 18.19 ± 1.28 μmol/l, respectively), while the least plasma urea concentrations were observed in the CALE–50 mg group (5.25 ± 0.15 mmol/l) of the ameliorative study and in the PTSE–75 mg group (5.15 ± 0.24 mmol/l) of the protective study. In both the ameliorative and protective studies, the blood urea

Table 1. Influence of aqueous extracts of *Pleurotus tuberregium* sclerotia and *Cnidoscolus aconitifolius* leaves on plasma markers of kidney function in doxorubicin-treated rats

Treatment	Creatinine [μmol/l]		Urea [mmol/l]		Blood urea nitrogen [mg/dl]		Urea nitrogen/creatinine ratio*	
	ameliorative	protective	ameliorative	protective	ameliorative	protective	ameliorative	protective
Normal control	$30.43 \pm 1.62^{a,g}$	30.43 ± 1.62^{a}	5.38 ± 0.14^{a}	5.38 ± 0.14^{a}	15.10 ± 0.40^{a}	15.10 ± 0.40^{a}	$183.01 \pm 10.63^{a,c,d}$	183.01 ± 10.63^{a}
Test control	263.13 ± 3.11^{c}	172.00 ± 4.18^{c}	14.11 ± 0.41^{c}	13.67 ± 0.25^{c}	39.63 ± 1.16^{c}	38.39 ± 0.69^{c}	53.63 ± 1.54^{c}	$79.74 \pm 2.67^{c,d}$
Metformin	50.72 ± 2.42^{d}	39.69 ± 2.09^{d}	7.83 ± 0.17^{d}	8.13 ± 0.19^{d}	21.99 ± 0.49^{d}	22.83 ± 0.55^{d}	$157.43 \pm 9.87^{a,c,d}$	206.37 ± 6.42^{a}
PTSE–50 mg	116.87 ± 1.21^{e}	96.59 ± 3.37^{e}	8.99 ± 0.46^{e}	9.71 ± 0.17^{b}	25.25 ± 1.29^{e}	27.28 ± 0.49^{b}	607.26 ± 325.73^{b}	100.86 ± 2.41^{d}
PTSE–75 mg	$24.81 \pm 1.28^{f,g}$	112.46 ± 2.09^{f}	11.22 ± 0.32^{f}	5.15 ± 0.24^{a}	31.52 ± 0.89^{b}	14.46 ± 0.67^{a}	$455.88 \pm 14.93^{b,d}$	45.80 ± 2.04^{c}
PTSE–100 mg	233.74 ± 4.83^{b}	100.89 ± 4.38^{e}	7.72 ± 0.16^{d}	10.31 ± 0.38^{b}	21.68 ± 0.46^{d}	28.97 ± 1.06^{b}	$33.14 \pm 1.11^{a,c}$	102.61 ± 3.12^{d}
CALE–50 mg	36.39 ± 1.48^{a}	124.04 ± 5.28^{g}	$5.25 \pm 0.15^{a,b}$	9.70 ± 0.30^{b}	14.75 ± 0.41^{a}	27.25 ± 0.83^{b}	$144.87 \pm 3.20^{a,c,d}$	$78.85 \pm 4.30^{c,d}$
CALE–75 mg	34.73 ± 2.45^{a}	62.85 ± 1.48^{h}	7.89 ± 0.25^{d}	5.56 ± 0.11^{a}	22.16 ± 0.69^{d}	15.60 ± 0.32^{a}	$234.90 \pm 20.10^{a,c,d}$	$88.68 \pm 3.04^{c,d}$
CALE–100 mg	19.85 ± 2.09^{f}	18.19 ± 1.28^{b}	$5.32 \pm 0.19^{a,b}$	8.21 ± 0.24^{d}	14.95 ± 0.53^{a}	23.06 ± 0.67^{d}	$290.12 \pm 42.59^{a,c,d}$	470.43 ± 54.86^{b}

Values are expressed as mean ± SEM, $n = 5$ animals per group; values in the same column with different superscript letters differ significantly at $P < 0.05$; * has no unit

Table 2. Effects of aqueous extracts of *Pleurotus tuberregium* sclerotia and *Cnidoscolus aconitifolius* leaves on the concentrations (g/l) of plasma non-enzyme markers of liver function in doxorubicin-treated rats

Treatment	Albumin		Total protein		Globulin		Albumin/globulin ratio*	
	ameliorative	protective	ameliorative	protective	ameliorative	protective	ameliorative	protective
Normal control	$12.354 \pm 0.495^{a,d}$	$12.354 \pm 0.495^{a,c}$	$142.937 \pm 5.988^{a,e}$	142.937 ± 2.344^{a}	$130.999 \pm 1.931^{a,e}$	130.999 ± 1.931^{a}	$0.094 \pm 0.003^{a,b}$	0.094 ± 0.003^{a}
Test control	7.172 ± 0.412^{c}	7.721 ± 0.322^{b}	101.443 ± 2.374^{c}	117.423 ± 0.794^{c}	90.922 ± 0.802^{c}	109.701 ± 0.891^{c}	$0.079 \pm 0.005^{a,e}$	0.070 ± 0.003^{b}
Metformin	10.410 ± 0.055^{b}	$12.631 \pm 0.912^{c,d}$	$134.335 \pm 2.123^{a,b}$	135.906 ± 2.980^{d}	$123.925 \pm 2.269^{d,e}$	123.856 ± 2.439^{d}	$0.084 \pm 0.002^{a,b,d}$	0.096 ± 0.004^{a}
PTSE–50 mg	$10.974 \pm 0.312^{a,b}$	$11.486 \pm 0.866^{a,d}$	129.986 ± 4.311^{b}	134.939 ± 0.995^{d}	$117.441 \pm 4.027^{b,d}$	123.453 ± 1.583^{d}	$0.094 \pm 0.004^{a,b}$	0.093 ± 0.008^{a}
PTSE–75 mg	$11.104 \pm 0.305^{a,b}$	10.272 ± 0.877^{a}	122.617 ± 6.496^{b}	128.376 ± 2.216^{b}	113.566 ± 6.305^{b}	$115.076 \pm 2.515^{b,c}$	$0.100 \pm 0.006^{b,c}$	$0.089 \pm 0.007^{a,d}$
PTSE–100 mg	13.418 ± 0.607^{d}	14.314 ± 0.260^{c}	129.986 ± 1.722^{b}	127.208 ± 1.861^{b}	$116.568 \pm 1.909^{b,d}$	$112.893 \pm 1.683^{b,c}$	0.116 ± 0.006^{c}	0.126 ± 0.002^{c}
CALE–50 mg	10.410 ± 0.239^{b}	$12.215 \pm 1.421^{a,c}$	$153.302 \pm 6.340^{d,e}$	144.676 ± 1.065^{a}	134.717 ± 0.525^{a}	132.461 ± 1.249^{a}	$0.077 \pm 0.002^{a,d}$	0.093 ± 0.011^{a}
CALE–75 mg	$11.972 \pm 1.660^{a,b,d}$	$10.688 \pm 0.531^{a,d}$	$133.369 \pm 4.835^{a,b}$	128.053 ± 2.607^{b}	$121.397 \pm 4.876^{b,d,e}$	$118.653 \pm 3.785^{b,d}$	$0.100 \pm 0.015^{b,c}$	0.088 ± 0.006^{a}
CALE–100 mg	$11.235 \pm 0.457^{a,b}$	$11.104 \pm 0.494^{a,d}$	161.718 ± 4.740^{d}	163.651 ± 2.086^{e}	150.483 ± 4.692^{f}	152.546 ± 1.960^{e}	$0.075 \pm 0.004^{d,e}$	$0.073 \pm 0.003^{b,d}$

Values are expressed as mean ± SEM, $n = 5$ animals per group; values in the same column with different superscript letters differ significantly at $P < 0.05$; * has no unit

Table 3. Ameliorative and protective effects of the extracts on the levels (U/l) of plasma enzyme markers of liver integrity

Treatment	Alkaline phosphatase		Aspartate transaminase		Alanine transaminase	
	ameliorative	protective	ameliorative	protective	ameliorative	protective
Normal control	72.3 ± 6.7 [a,f]	72.3 ± 6.7 [a]	328.4 ± 13.9 [a]	328.4 ± 13.9 [a]	151.0 ± 5.3 [a]	151.0 ± 5.3 [a]
Test control	134.6 ± 8.6 [c]	118.2 ± 4.6 [c]	504.3 ± 17.6 [c]	752.5 ± 15.0 [c]	306.0 ± 8.1 [c]	327.6 ± 12.6 [c]
Metformin	100.0 ± 3.5 [d]	87.4 ± 5.0 [d,e]	422.0 ± 16.6 [b]	305.0 ± 4.8 [a]	155.7 ± 3.8 [a]	151.0 ± 6.7 [a]
PTSE–50 mg	49.7 ± 7.6 [e]	95.5 ± 4.8 [e]	391.2 ± 7.0 [b]	434.4 ± 13.2 [d]	163.4 ± 18.0 [a]	136.3 ± 5.7 [a]
PTSE–75 mg	83.3 ± 3.9 [a,b]	33.9 ± 0.9 [f]	406.5 ± 8.9 [b]	334.4 ± 13.8 [a]	206.9 ± 6.4 [b]	216.0 ± 6.8 [d]
PTSE–100 mg	119.9 ± 5.3 [c]	69.2 ± 3.4 [a,g]	406.7 ± 10.2 [b]	385.0 ± 7.1 [b]	189.9 ± 4.8 [b]	145.6 ± 5.6 [a]
CALE–50 mg	87.6 ± 4.2 [b,d,f]	76.1 ± 3.7 [a,d]	323.0 ± 9.0 [a]	329.6 ± 7.7 [a]	204.8 ± 9.2 [b]	179.2 ± 7.8 [b]
CALE–75 mg	94.8 ± 4.4 [b,d]	50.4 ± 2.1 [b]	414.0 ± 3.0 [b]	406.7 ± 8.1 [d]	115.8 ± 4.7 [d]	142.1 ± 6.9 [a]
CALE–100 mg	71.1 ± 3.3 [a]	60.3 ± 1.9 [b,g]	478.0 ± 7.3 [c]	372.0 ± 7.6 b	206.4 ± 5.8 [b]	172.8 ± 4.1 [b]

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

nitrogen concentrations of the test control groups (39.63 ± 1.16 and 38.39 ± 0.69 mg/dl, respectively) were significantly higher ($P < 0.05$) than those of all the other groups, with the CALE–100 mg group having the least value (14.95 ± 0.53 mg/dl) in the ameliorative study and the PTSE–75 mg group having the least value (14.46 ± ± 0.67 mg/dl) in the protective study. In the ameliorative study, the urea nitrogen/ creatinine ratio of the test control group (53.63 ± 1.54) was significantly lower ($P < 0.05$) than those of the PTSE–50 mg group (607.26 ± 325.73) and the PTSE–75 mg group (455.88 ± ± 14.93), but not significantly different from those of all the other groups. In the protective study, the urea nitrogen/creatinine ratio of the test control group (79.74 ± 2.67) was significantly lower ($P < 0.05$) than those of the normal control (183.01 ± 10.63), metformin (206.37 ± 6.42), and CALE–100 mg (470.43 ± 54.86) groups, but not significantly different from those of all the other groups.

Regulation of plasma markers of liver function/integrity

As shown in Table 2, in the ameliorative and protective studies, the plasma albumin (7.172 ± 0.412 and 7.721 ± 0.322 g/l, respectively) and total protein (101.443 ± 2.374 and 117.423 ± 0.794 g/l, respectively) concentrations of the test control group were significantly ($P < 0.05$) lower than those of all the other groups. The lowest plasma albumin concentrations were observed in the PTSE–100 mg group (13.418 ± 0.607 and 14.314 ± ± 0.260 g/l, respectively), while the lowest total protein concentration was noted in the CALE–100 mg group (161.718 ± 4.740 and 163.651 ± 2.086 g/l, respectively). The plasma globulin concentration of the test control group (90.922 ± 0.802 and 109.701 ± 0.891 g/l, respectively) was significantly ($P < 0.05$) lower than those of all the other groups in the ameliorative study, while in the protective study, it was significantly ($P < 0.05$) lower than those of all the other groups, except PTSE–75 mg and PTSE–100 mg. The CALE–100 mg group had the highest globulin concentrations (150.483 ± 4.692 and 152.546 ± 1.960 g/l) in both studies. The plasma albumin/globulin ratio of the test control group (0.079 ± ± 0.005) in the ameliorative study was significantly lower ($P < 0.05$) than those of the PTSE–75 mg, PTSE–100 mg (0.116 ± 0.006, the highest), and CALE–75 mg groups, but not significantly different from those of all the other groups. In the protective study, the plasma albumin/ globulin ratio of the test control group (0.070 ± 0.003) was significantly lower ($P < 0.05$) than those of all the other groups, except for the CALE–100 mg group, with the PTSE–100 mg group showing the highest value of 0.126 ± 0.002.

The plasma ALP levels of the test control group in both the ameliorative (134.6 ± 8.6 U/l) and protective (118.2 ± 4.6 U/l) studies (Table 3) were significantly ($P < 0.05$) higher than those of the other groups, except for the PTSE–100 mg group (protective study only). The PTSE–50 mg group had the least plasma ALP level

Table 4. Per cent recovery of plasma markers of liver and kidney integrity/function

Parameter	Metformin	PTSE–50 mg	PTSE–75 mg	PTSE–100 mg	CALE–50 mg	CALE–75 mg	CALE–100 mg
Albumin	62.5 ± 1.1 [a]	73.4 ± 6.0 [a]	75.9 ± 5.9 [a]	120.5 ± 11.7 [b]	62.5 ± 4.6 [a]	92.6 ± 32.0 [a,b]	78.4 ± 8.8 [a]
Total protein	79.4 ± 5.1 [a]	69.0 ± 10.3 [a]	51.4 ± 15.5 [a]	69.0 ± 4.1 [a]	124.8 ± 15.2 [b]	77.1 ± 11.6 [a]	144.9 ± 11.3 [b]
Globulin	82.3 ± 5.4 [c,d]	66.2 ± 10.0 [a,d]	56.5 ± 15.9 [a]	64.0 ± 4.8 [a,d]	109.3 ± 1.3 [c]	76.0 ± 12.2 [a,d]	148.6 ± 11.7 [b]
Albumin/ globulin ratio	34.9 ± 12.5 [a]	102.9 ± 30.3 [a]	142.0 ± 40.2 [b]	252.3 ± 44.9 [a]	−12.8 ± 14.2 [a]	142.8 ± 103.6 [a]	−29.2 ± 24.3 [a]
ALP	55.6 ± 5.7 [a]	136.4 ± 12.2 [c]	82.4 ± 6.3 [b,e]	23.5 ± 8.5 [d]	75.4 ± 6.7 [a,b]	63.9 ± 7.1 [a,b]	102.0 ± 5.2 [e]
AST	46.8 ± 9.5 [a]	64.3 ± 4.0 [c]	55.6 ± 5.1 [a,c]	55.5 ± 5.8 [a,c]	103.1 ± 5.1 [b]	51.3 ± 1.7 [a,c]	14.9 ± 4.2 [d]
ALT	97.0 ± 2.5 [a]	92.0 ± 11.6 [a]	63.9 ± 4.1 [b]	74.9 ± 3. 1 [b]	65.3 ± 5.9 [b]	122.7 ± 3.0 [c]	64.3 ± 3.7 [b]
Creatinine	91.3 ± 1.0 [a]	62.9 ± 0.5 [c]	102.4 ± 0.6 [d]	12.6 ± 2.1 [e]	97.4 ± 0.6 [b]	98.2 ± 1.1 [b]	104.5 ± 0.9 [d]
Urea	71.9 ± 2.0 [a]	58.6 ± 5.3 [c]	33.1 ± 3.6 [d]	73.2 ± 1.9 [a]	101.4 ± 1.7 [b]	71.2 ± 2.8 [a]	100.6 ± 2.2 [b]
BUN	71.9 ± 2.0 [a]	58.6 ± 5.3 [c]	33.1 ± 3.6 [d]	73.2 ± 1.9 [a]	101.4 ± 1.7 [b]	71.2 ± 2.8 [a]	100.6 ± 2.2 [b]
Ur/Cr	80.2 ± 7.6 [a,b]	427.9 ± 251.8 [c]	310.9 ± 11.5 [b,c]	−15.8 ± 0.9 [a]	70.5 ± 2.5 [a,b]	140.1 ± 15.5 [a,b]	182.8 ± 32.9 [a,c]

Values are expressed as mean ± SEM, $n = 5$ animals per group; values in the same row with different superscript letters differ significantly at $P < 0.05$; BUN – blood urea nitrogen; Ur/Cr – urea nitrogen/creatinine ratio

Table 5. Per cent protection of plasma markers of liver and kidney function/integrity

Parameter	Metformin	PTSE–50 mg	PTSE–75 mg	PTSE–100 mg	CALE–50 mg	CALE–75 mg	CALE–100 mg
Albumin	106.0 ± 19.7 [a,b]	81.3 ± 18.7 [a]	55.1 ± 18.9 [a]	142.3 ± 5.6 [b]	97.0 ± 30.7 [a,b]	64.0 ± 11.5 [a]	73.0 ± 10.7 [a]
Total protein	72.4 ± 11.7 [a]	68.7 ± 3.9 [a]	42.9 ± 8.7 [b]	38.4 ± 7.3 [b]	106.8 ± 4.2 [c]	41.7 ± 10.2 [b]	181.2 ± 8.2 [e]
Globulin	66.5 ± 11.5 [a]	64.6 ± 7.4 [a]	25.2 ± 11.8 [b]	15.0 ± 7.9 [b]	106.9 ± 5.9 [c]	42.0 ± 17.7 [a,b]	201.2 ± 9.2 [d]
Albumin/ globulin ratio	111.1 ± 15.4 [a]	99.1 ± 34.4 [a]	80.6 ± 30.5 [a,c]	241.0 ± 7.4 [b]	95.3 ± 48.9 [a]	77.0 ± 26.3 [a]	10.3 ± 13.8 [c]
ALP	67.1 ± 10.8 [a]	49.6 ± 10.4 [a]	183.8 ± 2.0 [c]	106.7 ± 7.4 [d,e]	91.7 ± 8.0 [e]	147.7 ± 4.5 [b]	126.3 ± 4.0 [b,d]
AST	105.5 ± 1.1 [a]	75.0 ± 3.1 [c]	98.6 ± 3.2 [d]	86.7 ± 1.7 [b,f]	99.7 ± 1.8 [a,b,d]	81.5 ± 1.9 [e]	89.7 ± 1.8 [f]
ALT	100.0 ± 3.8 [a]	108.3 ± 3.2 [a]	63.2 ± 3.8 [c]	103.1 ± 3.2 [a]	84.1 ± 4.4 [b]	105.1 ± 3.9 [a]	87.7 ± 2.3 [b]
Creatinine	93.5 ± 1.5 [a]	53.3 ± 2.4 [c]	42.1 ± 1.5 [d]	50.2 ± 3.1 [c]	33.9 ± 3.7 [e]	77.1 ± 1.0 [f]	108.6 ± 0.9 [b]
Urea	66.8 ± 2.3 [a]	47.7 ± 2.1 [c]	102.8 ± 2.9 [b]	40.5 ± 4.5 [c]	47.8 ± 3.6 [c]	97.8 ± 1.4 [b]	65.8 ± 2.9 [a]
BUN	66.8 ± 2.3 [a]	47.7 ± 2.1 [c]	102.8 ± 2.9 [b]	40.5 ± 4.5 [c]	47.8 ± 3.6 [c]	97.8 ± 1.4 [b]	65.8 ± 2.9 [a]
Ur/Cr	122.6 ± 6.2 [a]	20.5 ± 2.3 [b]	−32.9 ± 2.0 [b]	22.1 ± 3.0 [b]	−0.9 ± 4.2 [b]	8.7 ± 2.9 [b]	378.3 ± 53.1 [c]

Values are expressed as mean ± SEM, $n = 5$ animals per group; values in the same row with different superscript letters differ significantly at $P < 0.05$; BUN – blood urea nitrogen; Ur/Cr – urea nitrogen/creatinine ratio

(49.7 ± 7.6 U/l) in the ameliorative study, while the PTSE–75 mg group had the least value (33.9 ± 0.9 U/l) in the protective study. The plasma AST levels of the test control group in both the ameliorative (504.3 ± ± 17.6 U/l) and protective (752.5 ± 15.0 U/l) studies were significantly higher ($P < 0.05$) than those of the other groups, except for the CALE–100 mg group (protective study). The CALE–50 mg group had the lowest plasma AST level (323.0 ± 9.0 U/l) in the ameliorative study, while the metformin group had the lowest level (305.0 ± ± 4.8 U/l) in the protective study. In both the ameliorative (306.0 ± 8.1 U/l) and protective (327.6 ± 12.6 U/l) studies, the plasma ALT levels of the test control were significantly ($P < 0.05$) higher than those of all the other groups. The CALE–75 mg group had the lowest plasma ALT level (115.8 ± 4.7 U/l) in the ameliorative study,

while the PTSE–50 mg group had the lowest value (136.3 ± 5.7 U/l) in the protective study.

Percentage recovery and protection

The administration of the extracts and metformin prevented (in the protective study) or attenuated (in the ameliorative study) doxorubicin-induced increase in plasma markers of kidney and liver function/integrity and afforded subsequent protection or recovery towards near-normal values. These findings are presented in Tables 4 and 5 as per cent recovery/protection of the parameters according to the different test treatments. The highest percentage recovery of 427.9 ± 251.8% was observed in the urea nitrogen/creatinine ratio of the PTSE–50 mg group, while the lowest recovery of 14.9 ± 4.2% was observed in the plasma AST level of the CALE–100 mg group. The highest percentage protection of 378.3 ± 53.1% was observed in the urea nitrogen/creatinine ratio of the CALE–100 mg group, while the lowest protection of 10.3 ± 13.8% was observed in the CALE–100 mg group.

Discussion

Plasma ALT, AST, ALP, and total bilirubin are the usual biomarkers for detecting liver damage and liver dysfunction in drug-induced liver injury (Panteghini and Bais, 2015; Ortega-Alonso et al., 2016; European Association for the Study of the Liver, 2019). In the present study, doxorubicin caused significant elevation in plasma levels of ALP, ALT, and AST and decreased plasma albumin, globulin, and total protein contents. This finding is in agreement with the results of other studies which reported doxorubicin-induced elevation in plasma levels of ALT, AST, and ALP and decrease in plasma albumin and total protein concentrations (Öz and İlhan, 2006; Lee et al., 2013; Ahmed et al., 2019a; Ikewuchi et al., 2021b). However, these adverse alterations in plasma levels were prevented or attenuated by treatment with the tested PTSE and CALE extracts to varying degrees, as shown by the values of their percentage recovery and protection in Tables 4 and 5. As shown in both tables, the extracts, especially at the doses of 75 mg/kg and 100 mg/kg compared favourably with metformin. The lowering of the plasma enzyme markers by the extracts is an indication of their hepatoprotective potential (Ikewuchi et al., 2017). The extracts may have protected the hepatic cell membrane from doxorubicin-induced damage, thereby restricting the leakage of the enzymes from the hepatocytes into the plasma (Ikewuchi et al., 2021b). This hepatoprotective effect is in corroboration with earlier reports of the hepatoprotective effects of the extracts of *P. tuberregium* sclerotia in alloxan-induced diabetic rabbits (Ikewuchi et al., 2017) and salt-induced hypertensive rats (Ikewuchi et al., 2013a) and of *C. aconitifolius* leaves on liver damage induced by ethanol (Adaramoye et al., 2011), paracetamol (Oyagbemi and Odetola, 2010), and carbon tetrachloride (Saba et al., 2010). This hepatoprotective effect of the extracts may be due to the presence of antioxidants (e.g. allicin, caffeic acid, lycopene, catechin, ellagic acid, epicatechin, epigallocatechin gallate, naringenin, quercetin, and silymarin), all of which possess hepatoprotective activities and are known to condition hepatocytes, resulting in enhanced regeneration of parenchymal cells and thereby protection against membrane fragility and leakage of the marker enzymes into the plasma (Awad et al., 2018; Ikewuchi et al., 2021b).

In the present study, doxorubicin increased plasma creatinine and urea levels. This finding agrees with other reports of doxorubicin-induced elevation of plasma creatinine and urea levels (Öz and İlhan, 2006; Yilmaz et al., 2006; Ikewuchi et al., 2021c). Because of their inverse relationship with glomerular function, plasma biomarkers such as creatinine and urea concentrations are usually monitored to evaluate glomerular function (Crook, 2012; Lamb and Price, 2015; Meisenberg and Simmons, 2017; Lieberman and Peet, 2018; Ikewuchi et al., 2021c). Therefore, the reduction in plasma creatinine and urea levels by the extracts is suggestive of their capacity to protect nephrons from doxorubicin-induced damage (Jambhulkar et al., 2014) and thus preserve the functional capacity of the glomerular filtration system (Ikewuchi et al., 2021c). The lowering of plasma creatinine, urea, and blood urea nitrogen levels by the extracts may be due to their content of caffeic and chlorogenic acids (Ikewuchi et al., 2017), both of which are reported to decrease plasma levels of kidney markers to near-normal levels (Pari and KarthiKesan, 2007; Lou et al., 2016). Similar nephroprotective effects were earlier reported for *P. tuberregium* extract on alloxan-induced diabetic rabbits (Ikewuchi et al., 2017) and on salt-induced hypertensive rats (Ikewuchi et al., 2013a) and for *C. aconitifolius* extract on carbon tetrachloride (Iwu

et al., 2020) and ethanol intoxicated (Adaramoye and Aluko, 2011) rats.

Conclusion

The results of the present study show that the tested extracts of *P. tuberregium* sclerotia and *C. aconitifolius* leaves showed a protective effect against doxorubicin-induced hepatorenal toxicity. The results also suggest that the sclerotia of *P. tuberregium* and leaves of *C. aconitifolius* may be potential candidates for the prevention and amelioration of doxorubicin-induced hepatorenal toxicity.

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