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Ameliorative Effect of *Costus speciosus* Extracts on Toxic Effects of Lead Acetate on Liver and Kidney of Male Rats

Abd El-Aziz A. Diab [1], Ebtisam M. Al-Mathal [2], Mansour H. Zahra[1], Mohamed F. Dowidar[3], Safaa A. El-Sherbeny[1], Mohammed Abu El-Magd*[4]

[1] *Zoology Department, Faculty of Science, Zagazig University, Zagazig, Egypt*
[2] *Department of Biology, College of Science, Imam Abdurrahman Bin Faisal University, 31441. Saudi Arabia.*
[3] *Biochemistry Department, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt*
[4] *Department of Anatomy & Embryology, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafrelsheikh, P.O. Box 33516, Egypt.*

Costus speciosus was succefully used to relieve much toxicity, however it is still unknown whether *C. speciosus* can ameliorate lead acetate (PbAc)-induced toxicity. This investigation set out to determine whether or not *C. speciosus* rhizome extracts in water, petroleum ether, and methanol could mitigate the hepatorenal toxicities caused by PbAc in rats. Animals were randomly assigned to five groups (n = 15 in each group), with each group further subcategorized into three subgroups (n = 5) according to exposure duration: normal control (Cnt), PbAc, and the remaining three groups treated with aqueous, petroleum ether, and methanolic *C. speciosus* rhizome extracts. The results showed that the harmful effects of PbAc were mitigated by all extracts as evidenced by 1) reduced blood glucose, 2) decreased liver (ALT, AST, and total bilirubin) and kidney (creatinine, urea, uric acid) function markers, 3) repressed lipid profile parameters (TG, TC, and LDL), 4) elevated serum levels of total protein, albumin, and HDL, 5) declined liver levels of lipid peroxidation marker (MDA), and 6) increased liver antioxidant enzymes (SOD, CAT, GPx) and GSH level. The most significant protection against PbAc poisoning was seen after one moth of treatment with the methanolic extract. Inhibition of oxidative stress and increase of antioxidant enzyme activity are two mechanisms by which *C. speciosus* extracts may ameliorate PbAc-induced hepato- and reno-toxicities. Since PbAc is toxic to livers and kidneys, these extracts, notably the methanolic extract, may serve as valuable ameliorative agents.

Keywords: Lead toxicity, *Costus speciosus,* Antioxidant activity, Oxidative Stress.

Introduction

Lead (Pb) is a heavy metal that is characterized by high atomic weight and density (5-fold change more than that of water). Pb is widely used in many applications and so the potential to expose to its toxicity (animals and human) and contamination (environment) is easier than other heavy metals**.** Previous studies reported the ability of lead acetate (PbAc) to disturb biochemical indices and induce oxidative stress damage to liver and kidney through elevation of reactive oxygen species (ROS) production, as evidenced by increase concentration of the lipid peroxidation biomarker, malondialdehyde (MDA), and inhibition of endogenous antioxidant enzyme activity, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) [1-3]. PbAc can also significantly reduce cholesterol, triglycerides and HDL and increase LDL in rats [2,4]. Several previous studies reported antioxidant, hypolipidemic, hepatoprotective, antidiabetic, anti-hemolytic effects for the traditional medicinal herb, *Costus speciosus* (Koen. Sm, family: Zingiberaceae) [5-8]. The phenolic compounds and flavonoids found in *C. speciosus* are responsible for its antioxidant action, which helps protect cells from free radicals and lipid peroxidation [6]. The hypolipidemic effect of *C. speciosus* was due to its methanolic extract components, sitosterol and diosgenin, which can reduce serum cholesterol by a

*Corresponding author: Mohammed A. El-Magd, mohamed.abouelmagd@vet.kfs.edu.eg, Tel: 00201068160301
ORCID: 0000-0002-3314-9202
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competitive inhibition mechanism with cholesterol absorption [5,7]. Serum aspartate transaminase (AST), alanine transaminase (ALT), and serum bilirubin levels all decreased significantly when methanolic extract of *C. speciosus* was administered [9]. Moreover, this extract also showed a protective effect against free radicals-induced hemolytic effect, probably through protecting RBCs membrane, which is rich in polyunsaturated fatty acids, from destruction by lipid peroxidation induced by ROS [10]. The anti-diabetic effect of *C. speciosus* was attributed to high content of diosgenin, which is used in the treatment of diabetes mellitus [8,11].

We conducted this study to investigate the impact of aqueous, petroleum ether, and methanolic extracts of *C. speciosus* on the adverse effect of PbAc toxicity on liver and kidney functions, lipid profile, blood glucose, lipid peroxidation and antioxidants activity.

Material and Methods

Preparation of C. speciosus Extracts

Clean *C. speciosus* rhizomes (1 kg) were air-dried for 10 days, was grinded using mixer grinder, powdered and packed into soxhlet apparatus to be extracted using following solvents in order based on their polarity methanol, petroleum ether and distilled water [12].

Experimental Design

Adult male *Sprague Dawley* albino rats (n = 75, each weight about 180–210 g, with average age of 6±0.5 w) fed standard pellet, had free access to drinking water free from Pb, and housed in plastic cages (also free from lead or other contaminations) under standard laboratory conditions (24 ± 2°C, good ventilation, 57 ± 4% humidity and 12-h light/dark cycle). We followed the rules and regulations for experimental animals use as approved by Animal Ethical Committee of Zagazig University, Egypt (ZU-IACUC/276/2021).

After lab acclimation for 1 week, animals were randomly assigned to five groups (n = 15 in each group), with each group further subcategorized into three subgroups (n = 5) according to exposure duration (10, 20, and 30 days). group I (normal control, Cnt) supplied with sodium chloride solution (50 mg/L), GII administrated PbAc (50 mg/kg body weight, bw, Oxford Lab. Co., India) [13], GIII co-administrated PbAc (as previously stated in GII) and aqueous extract of *C. speciosus* (200 mg/kg bw), GIV co-treated with PbAc and petroleum ether extract of *C. speciosus* (200 mg/kg bw), and group V co-administrated PbAc and methanolic extract of *C. speciosus* (200 mg/kg bw) [14]. All treatments were given orally by stomach gavage daily until the end of the experiment on the 30^{th} day.

Sampling

On the 10^{th}, 20^{th} and 30^{th} day of the experiment, blood samples were gathered from eye venous plexus on clean plain tubes for serum separation. After clotting, the serum was extracted by centrifuging the blood at 3500 rpm for 10 minutes. Rats were euthanized by decapitation at the three time points (10^{th}, 20^{th}, and 30^{th} day), and their livers and kidneys were immediately excised, and 0.5 g of each sample was homogenized in 5 ml of saline solution using electrical homogenizer, centrifuged at 3000 rpm for 15 min, and the obtained supernatants were collected for biochemical assays.

Biochemical Assay

The serum levels of aspartate transaminase (AST), alanine transaminase (ALT), total bilirubin, total protein and albumin were measured using commercially available diagnostic kits [15-17]. The serum levels creatinine, urea, uric acid, triglyceride (TG), total cholesterol (TC)**,** high (HDL) and low density lipoprotein (LDL) were determined using commercially available kits [16,18,19]. Liver homogenates were tested for malondialdehyde (MDA) and reduced glutathione (GSH) levels and antioxidant enzyme activity (SOD, CAT, and GPx) according to the manufacturer's instructions (Biodiagnostic, Egypt) [20,21]

Statistical Analysis

GraphPad Prism 8 was used to do a two-way analysis of variance (ANOVA) on the collected data, and Tukey's post hoc test was used to determine statistical significance across groups. Data were presented as means ± standard error (SE). When compared to the null hypothesis, $p<0.05$ values were judged significant.

Results

The activity the liver enzymes (AST and ALT), total bilirubin, and the kidney function parameters (creatinine, uric acid, and urea) were significantly ($p<0.05$) increased in the PbAc group (group II) as compared to the normal control group (Tables 1 and 2). These elevated levels were significantly ($p<0.05$) reduced in rats co-treated with PbAc and *C. speciosus* extracts (groups III-V), with lowest levels in methanolic extract-treated rats (group V), when compared with rats treated with PbAc alone. Treatment for long time (30 days) showed better results than 10 and 20 days treatment. In contrast, PbAc-intoxicated rats exhibited significant ($p<0.05$) decreases in the serum levels of total protein and albumin relative to the normal control (Table 1). *C. speciosus*-cotreated rats (groups III-V) had significant ($p<0.05$) increases in total protein and albumin, with highest levels in group V, in

TABLE 1. Effect of *C. speciosus* extracts on liver function parameters.

		Control (GI)	PbAc (GII)	Aqueous extract (GIII)	Petroleum ether extract (GIV)	Methanolic extract (GV)
AST (U/L)	10th day	32.00±1.7^{ca}	66.42±3.6^{aa}	60.00±1.33^{ab}	58.12±7.5^{bb}	54.21±6.3^{ca}
	20th day	31.00±1.1^{ca}	66.00±3.9^{aa}	55.21±1.20^{bb}	49.22±1.4^{bb}	44.14±5.2^{cb}
	30th day	31.00±1.1^{ca}	67.00±4.1^{aa}	39.00±1.10^{bc}	37.72±1.2^{cc}	30.21±6.5b^{cc}
ALT (U/L)	10th day	53.00±1.2^{ca}	97.32±2.6^{aa}	90.00±1.4^{bb}	86.33±1.1^{bb}	81.11±1.3^{ba}
	20th day	51.90±1.5^{ca}	98.15±2.8^{aa}	77.00±1.2^{bb}	70.33±1.1^{bb}	66.52± 2.1^{cb}
	30th day	50.02±1.8^{ca}	99.00±3.1^{aa}	62.00±1.1^{cc}	57.91±1.2^{cc}	54.00±3.1^{cc}
Total bilirubin (g/dl)	10th day	0.93±0.03^{ca}	3.14±0.04^{aa}	3.21±0.03^{aa}	3.00±1.01^{ba}	2.00±0.01^{ba}
	20th day	0.92±0.05^{ca}	3.45±0.02^{aa}	2.15±0.02^{bb}	2.00±0.02^{bb}	1.12±0.31^{cb}
	30th day	0.92±0.01^{ba}	3.72±0.01^{aa}	1.55±0.01^{bb}	1.49±0.02^{cb}	1.00±0.3^{cb}
Total protein (g/dl)	10th day	7.17±0.08^{aa}	2.29±0.25^{da}	2.55±0.11^{cb}	3.40±0.11^{bb}	3.72±0.02^{bc}
	20th day	7.30±0.06^{aa}	2.27±0.28^{da}	3.25±0.13^{cb}	4.82±0.12^{bb}	5.50±0.02^{abb}
	30th day	7.33±0.04^{aa}	2.25±0.30^{da}	5.33±0.15^{ca}	6.00±0.14^{ba}	7.00±0.02^{aa}
Albumin (g/dl)	10th day	3.80±0.06^{aa}	1.21±0.01^{da}	1.45±0.01^{cc}	1.88±0.01^{bb}	2.00±0.03^{bb}
	20th day	4.00±0.0^{aa}	1.21±0.02^{da}	2.20±0.02^{bb}	2.50±0.01^{bb}	3.34±0.07a^{bb}
	30th day	4.00±0.02^{aba}	1.20±0.01^{da}	3.50±0.04^{bb}	3.80±0.01^{ba}	4.20±0.11^{aa}

For each parameter, means within the table bearing different superscript letters are significantly different at p<0.05.

TABLE 2. Effect of *C. speciosus* extracts on kidney function parameters.

		Control (GI)	PbAc (GII)	Aqueous extract (GIII)	Petroleum ether extract (GIV)	Methanolic extract (GV)
Creatinine (mg/dl)	10th day	0.92±0.01^{ca}	3.98±1.03^{aa}	3.62±0.03^{aa}	3.55±0.04^{ba}	2.50±0.32b^{ca}
	20th day	0.90±0.03^{da}	4.00±1.02^{aa}	3.33±0.02^{ba}	3.02±0.05^{bb}	1.98±0.03^{cb}
	30th day	0.88±0.04^{da}	3.98±1.06^{aa}	2.00±0.01^{bb}	1.53±0.01^{cb}	1.02±0.01^{cb}
Uric acid (mg/dl)	10th day	1.60±0.01^{ca}	4.55±0.03^{aa}	3.55±0.03^{ba}	3.00±0.06^{ba}	3.02±0.02^{ba}
	20th day	1.40±0.05^{da}	4.80±0.06^{aa}	3.00±0.02^{bb}	2.40±0.03^{bb}	2.52±0.03^{cc}
	30th day	1.30±0.04^{ca}	5.15±0.09^{aa}	2.55±0.01^{cc}	2.00±0.02^{cc}	1.68±0.03^{cc}
Urea (mg/dl)	10th day	22.00±0.23^{ca}	34.00±0.39^{aa}	30.00±0.33^{ba}	28.00±0.51^{ba}	26.00±0.10^{ba}
	20th day	20.90±0.25^{da}	34.00±0.37^{aa}	25.00±0.21^{bb}	23.01±0.32^{bb}	21.30±0.50^{cb}
	30th day	19.60±0.27^{da}	35.20±0.35^{aa}	24.00±0.11^{cb}	21.00±0.33^{cc}	20.00±0.10^{cc}

For each parameter, means within the table bearing different superscript letters are significantly different at p<0.05.

comparison with PbAc-intoxicated rats. Again, long lasting treatment (for 30 days) caused notable improvement relative to the other two time points (10 and 20 days).

Fastening blood glucose levels and the serum levels of triglycerides (TG), total cholesterol (TC), and low density lipoprotein- (LDL) were significantly (p<0.05) increased in the PbAc group as compared to the normal control (Table 3). These biochemical parameters were significantly (p<0.05) reduced following co-administration of the three *C. speciosus* extracts, with lowest levels after treatment with the methanolic extract, compared to the PbAc group. In contrast, HDL was significantly (p<0.05) decreased in the PbAc group in comparison to the normal control group. Again, the HDL level was significantly (p<0.05) increased following the co-treatment with the three *C. speciosus* extracts, with highest level in methanolic extract-treated group, as compared to PbAc-treated rats. Again, the longer treatment (for 30 days) showed better improvement than the shorter treatment (10 and 20 days).

Rats treated with PbAc alone had significant (p<0.05) higher liver levels of the lipid peroxidation MDA marker compared to the normal control (Table 4). Treatment with different extracts of *C. speciosus* succeeded to significantly (p<0.05) decrease MDA hepatic levels in methanolic, petroleum ether and aqueous extract-treated groups. This decrease was clear especially in the methanolic extract-cotreated group. However, the liver activity of SOD, GPx, CAT and the contents of GSH were significantly (p<0.05) decreased in PbAc-intoxicated rats relative to the normal control but, co-treatment with *C. speciosus* extracts significantly (p<0.05) elevated the levels of these antioxidant parameters, with highest levels in the methanolic extract-cotreated group. Again, long lasting treatment (for 30 days) caused better effect than the other two time points (10 and 20 days).

Discussion

Our results and those reported by Suleman*, et al.* [22] exhibited that administration of PbAc led to hepatotoxicity which was marked by a significant elevation in serum levels of liver damage indicators (AST, ALT, and total bilirubin) that may be

attributed to disruption and destruction of hepatocytes cell membranes, leading to escape of liver enzymes and bilirubin to the circulation [15,16,23,24]. This adverse effect was relieved following co-treatment with *C. speciosus* methanolic extracts with best results for the methanolic extract, indicating a hepatoprotective effect for these extracts. In support, Verma and Khosa [9] also confirmed this hepatoprotective against CCL_4 toxicities as revealed by the reduction of ALT, AST and total bilirubin levels and restoration of liver function.

The present study also demonstrated that co-treatment with each of the three *C. speciosus* extracts and PbAc resulted in significantly increased serum levels of total protein and albumin. Similarly, a significant increase in total protein was also noticed following treatment with *C. speciosus* extracts in rats [25] and after treatment with other herb of the same family, ginger in broilers [26]. Increased level of albumin is a sign of liver tissue regeneration and a good indicator for restoring liver function [27]. In consistent with this notion, it was shown that the elevated serum albumin not only is associated with low recurrence rate of liver cancer but also can inhibit tumor cell proliferation [20].

The results supported those obtained by El-Ashmawy*, et al.* [28] and confirmed the reno-toxic effect of PbAc as evidenced by elevation in serum levels of kidney damage parameters (creatinine, uric acid and urea) following exposure to PbAc in rodents. This renotoxic effect was ameliorated by *C. speciosus* extracts which significantly decreased these parameters, indicating a possible reno-protective effect for these extracts. Similarly, Bavarva and Narasimhacharya [29] also reported a reno-protective effect for the *C. speciosus* methanolic extract in diabetic rats. These data suggest that *C. speciosus* extracts can restore the lost kidney function caused by PbAc and together with results obtained from liver function suggest *C. speciosus* extracts as potent protector against PbAc hepato- and reno-toxicities.

C. speciosus had been documented as an antidiabetic plant in India and this effect was attributed to its plenty content of diosgenin [11]. In agreement, we also found a similar hypoglycemic effect for *C. speciosus* extracts as revealed by significant fall in level of fastening blood glucose after co-treatment by *C. speciosus* extracts as compared to treatment by PbAc alone. For their hypoglycemic effect, *C. speciosus* extracts and their components could be used to reduce diabetic complications [25].

Moreover, we and Abdel-Moneim, et al. [4] found a significant elevation in serum levels of lipid profile parameters (TG, TC and LDL) and a significant reduction in HDL following treatment by PbAc. This deleterious effect was reversed after co-administration of *C. speciosus* extracts. In agreement with our results, Beattie*, et al.* [30] and ElRokh el*, et al.* [31] also showed hypolipidemic effect for *C. speciosus* when given to dietary or streptozocin-induced hypercholesterolaemic rats. This hypolipidemic effect was attribute to *C. speciosus* content of eremanthin, which possesses hypoglycemic and hypolipidemic activities and therefore could be used as antidiabetic drug [25].

The current results in agreement with other reports El-Magd, et al. [2] and Wang, et al. [3] confirmed the ability of PbAc to induce excessive production of free radicals which subsequently led to elevation of lipid peroxidation (as revelled by increased levels of MDA) and enhanced oxidative stress. Liver cells usually react by stimulating production of endogenous antioxidant enzymes (SOD, CAT, GPx) to get rid of these overproduced ROS [16,19,32]. However, these antioxidant system impaired by PbAc, leading to accumulation of ROS oxidative stress damage to proteins, DNA and lipids [2]. The latter are abundantly located in cell membranes and mitochondrial membranes and so can be easily targeted by free radicals, resulting in lipid peroxidation and loss of cell membrane integrity and subsequently release of liver enzymes (AST, ALT) and total bilirubin to circulation. Thus, it is likely that the loss of liver function and significant increase of AST and ALT activity, and the level of total bilirubin induced by PbAc may be due to lipid peroxidation of hepatocytes plasma membranes. This deteriorated effect was alleviated by *C. speciosus* extracts, suggesting anti-lipid peroxidative effect for these extracts. In consistent with our data, Krishnan*, et al.* [33] also reported high protective effect for *C. speciosus* against lipid peroxidation in rat liver.

The obtained results and those obtained by Wang, *et al.* [3] showed a significant decline in the activities of SOD, GPx and CAT and content of GSH in liver of PbAc-intoxicated rats. This declined antioxidant status may be due to the high affinity for PbAc to bind with SH group in the antioxidant enzymes. Furthermore, PbAc may compete with copper, zinc, selenium, and other critical trace elements required for the formation and function of antioxidant enzymes [2]. The antioxidant status disrupted by PbAc in the present study was improved by *C. speciosus* extracts. Phytoconstituents like flavonoids and phenolic compounds may be responsible for the antioxidant action of *C. speciosus* rhizome. The phenolic contents have redox characteristics that enable them to operate as

reducing agents, hydrogen donors, and singlet oxygen quenchers [34].

The majority of previous studies focus only on the role of *C. speciosus* methanolic extract and ignored the comparison with other extracts such as petroleum ether and aqueous. This may be the first research to compare between the effects of these three extracts and found that the methanolic extract had more potent hepato- and reno-protective, antioxidant, hypoglycemic, hypolipidemic, than petroleum ether and aqueous extracts. This superior effect for the methanolic extract may be attributed to presence of higher concentration of phenolic and flavonoid compounds [35]. Moreover, the best ameliorative effect for *C. speciosus* extracts was noticed in long term experiment (for 30 days) than the other two time points (10 and 20 days) indicating that the cumulative beneficial effect of these extracts.

TABLE 3. Effect of *C. speciosus* extracts on serum blood glucose and lipid profile.

		G1	GII	GIII	GIV	GV
Blood glucose (g/dl)	10th day	153.50±5.20^{ca}	309.75±19.76^{aa}	307.71±17.13^{aa}	270.55±18.51^{ba}	258.25±35.83^{ba}
	20th day	155.50±6.35^{da}	320.00 ±23.25^{aa}	255.33±37.10^{bb}	255.24±13.42^{bb}	172.93±31.33^{cb}
	30th day	158.00±6.00^{da}	325.15 ±8.10^{aa}	200.25±35.83^{bb}	160.00±30.80^{cc}	150.00±36.31^{cc}
TG (mg/dl)	10th day	66.75±4.40^{ca}	73.00±1.30^{aa}	73.03±2.60^{aa}	71.00±1.70^{ba}	70.63±2.40^{ba}
	20th day	66.00±4.00^{ba}	73.54±1.50^{aa}	69.61±1.20^{bb}	68.75±5.20^{bb}	67.33±1.70^{cb}
	30th day	65.82±3.50^{ca}	74.03±1.70^{aa}	68.31±1.30^{abc}	67.78±5.20^{cb}	65.00±0.80^{cb}
TC (mg/dl)	10th day	115.00±4.02^{ca}	210.50±25.94^{aa}	210.37±13.62^{aa}	166.25±29.91^{ba}	139.22±33.10^{ba}
	20th day	120.00±6.20^{ca}	220.00±20.00^{aa}	172.25±14.11^{ba}	135.31±11.46^{bb}	129.77±41.62^{bb}
	30th day	124.00±8.55^{ca}	226.15±16.25^{aa}	138.25±42.92^{ca}	122.60±24.94^{cb}	118.05±32.99^{cc}
LDL (mg/dl)	10th day	57.00±2.30^{ca}	149.90±1.33^{aa}	148.72±7.20^{aa}	91.41±2.31^{ba}	88.30±5.20^{ba}
	20th day	59.10±1.90^{da}	156.25±1.65^{aa}	122.30±4.40^{bb}	86.50±3.40^{bb}	76.32±2.47^{cb}
	30th day	60.15±1.75^{ca}	161.40±1.85^{aa}	92.00±4.60^{cc}	72.80±3.40^{bc}	70.01±3.33^{cc}
HDL (mg/dl)	10th day	44.02±1.20^{aa}	22.01±1.06^{cb}	23.01±1.43^{cc}	28.81±1.93^{bc}	30.01±1.55^{bc}
	20th day	42.50±1.40^{aa}	20.25±1.10^{cb}	28.29±1.30^{ba}	34.11±1.33^{bb}	37.51±1.70^{bb}
	30th day	44.00±1.65^{aa}	17.65±1.20^{cb}	35.42±1.03^{aa}	39.88±1.03^{aa}	41.31±1.00^{aa}

Means within the table bearing different superscripts letters are significantly different at p<0.05.

TABLE 4. Effect of *C. speciosus* extracts on lipid peroxidation marker and antioxidant parameters.

		G1	GII	GIII	GIV	GV
MDA (nmol/mg protein)	10th day	10.99±0.22^{ca}	16.41±0.52^{aa}	16.41±0.19^{aa}	13.00±0.19^{ba}	11.45±0.24^{da}
	20th day	10.55±0.24^{ea}	17.00±0.60^{aab}	14.00±0.12^{bb}	12.03±0.17^{cb}	10.01±0.19^{eb}
	30th day	10.10±0.25^{cda}	18.20±0.70^{ab}	12.30±0.30^{cb}	11.00±0.34^{db}	9.09±0.44^{ec}
SOD (U/mg protein)	10th day	2.81±0.05^{aa}	1.70±0.03^{cb}	1.99±0.03^{bb}	1.70±0.03^{cc}	2.00±0.17^{bb}
	20th day	3.00 ±0.07^{aa}	1.55±0.05^{db}	2.00±0.04^{cb}	2.00±0.02^{cb}	2.55±0.04^{ba}
	30th day	3.02±0.09^{aa}	1.32±0.08^{cb}	2.67±0.03^{ba}	2.65±0.06^{ba}	2.78±0.05^{aa}
GPx (U/mg protein)	10th day	7.48±0.11^{aa}	4.99±0.16^{eb}	5.79±0.19^{db}	6.25±0.13^{cc}	6.61±0.09^{bb}
	20th day	8.00±0.12^{aba}	4.5±0.15^{eb}	6.29±0.12^{bca}	7.06±0.09^{cb}	7.50±0.01^{aa}
	30th day	8.02±0.12^{aba}	4.3±0.13^{eb}	7.00±0.10^{ba}	7.38±0.12^{aa}	7.52±0.05^{aa}
GSH (U/mg protein)	10th day	8.38±0.02^{aa}	4.11±0.02^{da}	4.33±0.16^{dc}	6.00±0.04^{cc}	6.88±0.04^{bc}
	20th day	8.60±0.04^{aa}	3.75±0.04^{da}	6.01±0.23^{cb}	7.00±0.03^{bb}	7.65±0.04^{bb}
	30th day	8.95±0.08^{aa}	3.15±0.06^{da}	7.33±0.21^{ba}	8.00±0.08^{aa}	9.00±0.01^{aa}
CAT (H_2O_2 /gm tissue)	10th day	6.09±0.05^{aa}	2.55±0.02^{da}	2.77±0.01^{cc}	4.06±0.04^{bc}	5.06±0.01^{bb}
	20th day	6.35±0.07^{aa}	2.21±0.03^{da}	4.05±0.03^{cb}	5.00±0.03^{bb}	6.04±0.01^{aa}
	30th day	6.50±0.08^{aa}	2.00±0.05^{da}	5.30±0.01^{ba}	5.90±0.03^{aa}	6.05±0.02^{aa}

Means within the table bearing different superscripts letters are significantly different at p<0.5.

Conclusions

C. speciosus extracts, especially methanolic extract, can ameliorate the hepato- and reno-toxic effects induced by PbAc probably through reduction of ROS release and induction of antioxidant enzymes. Thus, *C. speciosus* extracts and their constituents could be used as protectants against the hepato- and reno-toxic effect of PbAc. Further investigations are required to determine the active constituents of these extracts and to unveil the actual molecular mechanism of their action.

Conflicts of interest

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Author`s contributions

"Authors contribute equally in this work"

References

1. Ibrahim, N.M., Eweis, E.A., El-Beltagi, H.S. and Abdel-Mobdy, Y.E. Effect of lead acetate toxicity on experimental male albino rat. *Asian Pacific Journal of Tropical Biomedicine*, **2**, 41-46 (2012).
2. El-Magd, M.A., Kahilo, K.A., Nasr, N.E., Kamal, T., Shukry, M. and Saleh, A.A. A potential mechanism associated with lead-induced testicular toxicity in rats. *Andrologia*, **49**, e12750 (2016).
3. Wang, J., Yang, Z., Lin, L., Zhao, Z., Liu, Z. and Liu, X. Protective effect of naringenin against lead-induced oxidative stress in rats. *Biological Trace Element Research*, **146**, 354-359 (2012).
4. Abdel-Moneim, A.M., El-Toweissy, M.Y., Ali, A.M., Awad Allah, A.A., Darwish, H.S. and Sadek, I.A. Curcumin Ameliorates Lead (Pb(2+))-Induced Hemato-Biochemical Alterations and Renal Oxidative Damage in a Rat Model. *Biological Trace Element Research*, **168**, 206-220(2015).
5. Carr, T.P., Cornelison, R.M. and Illston, B.J., Stuefer-Powell, C.L., Gallaher, D.D. Plant sterols alter bile acid metabolism and reduce cholesterol absorption in hamsters fed a beef-based diet. *Nutrition Research*, **22**, 745-754 (2002).
6. Sm Shoman Tmt, G. Potential Protective Effect of Costus speciosus or its Nanoparticles on Streptozotocin-induced Genotoxicity and Histopathological Alterations in Rats. *Nutr. Food Sci.*, **S3**, 2-7 (2015).
7. Subasinghe, S., Hettihewa, M., Gunawardena, S. and Thusharie, L. Methanol and water extracts of Costus speciosus (j.könig) sm. leaves reverse the high-fat-diet induced peripheral insulin resistance in experimental Wistar rats. *Int. Res. J. Pharm.*, **5**, 44-49 (2014).
8. Sulakshana, G. and Rani, A.S. HPLC analysis of diosgenin in three species of Costus. *Int. J. Pharma Sci. Res*, **5**, 747-749 (2014).
9. Verma, N. and Khosa, R.L. Evaluation of protective effect of ethanolic extract of Costus speciosus (Koen.) Sm. Rhizomes of carbon tetrachloride induced hepatotoxicity in rats. *Natural Product Radiance*, **8**, 123-126 (2009).
10. Saha, R. Comparative evaluation of the medicinal activities of methanolic extract of seeds, fruit pulps and fresh juice of Syzygium cumini in vitro. *Journal of Coastal Life Medicine* **1**, 300-308 (2013).
11. Choudhury, J. and Sarma, B. A review on antidiabetic property of Costus Speciosus (Kemuka). *Int. J. of Allied Med. Sci. and Clin. Research*, **4**, 601-606 (2016).
12. Alamoudi, E.F., Khalil, W., Ghaly, I.S., Hassan, N. and Ahmed, E. Nanoparticles from of costus speciosus extract improves the antidiabetic and antilipidemic effects against STZ-induced diabetes mellitus in albino rats. *Int. J. Pharm. Sci. Rev. Res.*, **29**, 279-288 (2014).
13. Batra, N., Nehru, B. and Bansal, M. The effect of zinc supplementation on the effects of lead on the rat testis. *Reprod Toxicol*, **12**, 535–540 (1998).
14. Remya, R. and Daniel, M. Phytochemical and pharmacognostic investigation of antidiabetic Costus pictus D. Don. *Int. J. Pharm. Biomed. Res*., **3**, 30-39(2012).
15. Abdelhadya, D.H., El-Magd, M.A., Elbialy, Z.I. and Saleh, A.A. Bromuconazole-induced hepatotoxicity is accompanied by upregulation of PXR/CYP3A1 and downregulation of CAR/CYP2B1 gene expression. *Toxicol Mech. Methods*, **27**, 544-550 (2017).
16. Abdelhady, D., El-Abasy, M., Abou-Asa, S., Elbialy, Z., Shukry, M., Hussein, A., Saleh, A. and El-Magd, M. The ameliorative effect of Aspergillus awamori on aflatoxin B1-induced hepatic damage in rabbits. *World Mycotoxin Journal*, **10**, 363-373 (2017).
17. Abdelhady, D.H., El-Magd, M.A., Elbialy, Z.I. and Saleh, A.A. Bromuconazole-induced hepatotoxicity is accompanied by upregulation of PXR/CYP3A1 and downregulation of CAR/CYP2B1 gene expression. *Toxicol. Mech. Methods*, **27**, 544-550 (2017).
18. Abu Gazia, M. and El-Magd, M.A. Effect of pristine and functionalized multiwalled carbon nanotubes on rat renal cortex. *Acta Histochemica*, **121(2),** 207-217 (2018).
19. Abu Khudir, R., El-Magd, M.A., Salama, A.F., Tousson, E.M. and El-Dsoki, S.M. Curcumin Attenuated Oxidative Stress and Inflammation on Hepatitis Induced by Fluvastatin in Female Albino Rats. *Alexandria Journal of Veterinary Sciences*, **62**, 102-115(2019).
20. Alzahrani, F.A., El-Magd, M.A., Abdelfattah-Hassan, A., Saleh, A.A., Saadeldin, I.M., El-Shetry, E.S., Badawy, A.A. and Alkarim, S. Potential Effect of Exosomes Derived from Cancer Stem Cells and MSCs on Progression of DEN-Induced HCC in Rats. *Stem Cells International*, **2018**, 17(2018). https://doi.org/10.1155/2018/8058979.
21. Elkeiy, M., Khamis, A., El-Gamal, M., Abo Gazia, M., Zalat, Z. and El-Magd, M. Chitosan nanoparticles from Artemia salina inhibit progression of hepatocellular carcinoma in vitro and in vivo. *Environ Sci. Pollut. Res. Int.*, **27**, 19016-19028 (2018).
22. Suleman, M., Ayaz, A.K., Zahid, H., Muhammad, A.Z., Sohaib, R., Farooq, R., Arshad, I. and Rafaqat, I. Effect of lead acetate administered orally at different dosage levels in broiler chicks. *African Journal of Environmental Science and Technology*, **5**, 1017-1026(2011).
23. Mohamed, Y., Basyony, M.A., El-Desouki, N.I., Abdo, W.S. and El-Magd, M.A. The potential therapeutic effect for melatonin and mesenchymal stem cells on hepatocellular carcinoma. *BioMedicine*, **9**, 23-29 (2019).
24. El-Magd, M.A., Zedan, A.M.G., Zidan, N.S., Sakran, M.I., Bahattab, O., Oyouni, A.A.A., Al-Amer, O.M., Alalawy, A.I. and Elmoslemany, A.M. Avocado Seeds-Mediated Alleviation of Cyclosporine A-Induced Hepatotoxicity Involves the Inhibition of Oxidative Stress and Proapoptotic Endoplasmic Reticulum Stress. *Molecules*, **27**, 7859 (2022).
25. Eliza, J., Daisy, P., Ignacimuthu, S. and Duraipandiyan, V. Antidiabetic and antilipidemic effect of eremanthin from Costus speciosus (Koen.)Sm., in STZ-induced

diabetic rats. *Chemico-biological Interactions*, **182**, 67-72 (2009).

26. Zhang, G.F., Yang, Z.B., Wang, Y., Yang, W.R., Jiang, S.Z. and Gai, G.S. Effects of ginger root (Zingiber officinale) processed to different particle sizes on growth performance, antioxidant status, and serum metabolites of broiler chickens. *Poultry Science*, **88**, 2159-2166 (2009).
27. Elmetwalli, A., Hashish, S.M., Hassan, M.G., El-Magd, M.A., El-Naggar, S.A., Tolba, A.M., Salama, A.F. Modulation of the oxidative damage, inflammation, and apoptosis-related genes by dicinnamoyl-L-tartaric acid in liver cancer. *Naunyn-Schmiedeberg's Archives of Pharmacology*, 396(11):3087-3099 (2023).
28. El-Ashmawy, I.M., El-Nahas, A.F. and Salama, O.M. Protective effect of volatile oil, alcoholic and aqueous extracts of Origanum majorana on lead acetate toxicity in mice. *Basic & Clinical Pharmacology & Toxicology*, **97**, 238-243 (2005).
29. Bavarva, J.H. and Narasimhacharya, A.V. Antihyperglycemic and hypolipidemic effects of Costus speciosus in alloxan induced diabetic rats. *Phytotherapy Research : PTR*, **22**, 620-626 (2008).
30. Beattie, J.H., Nicol, F., Gordon, M.J., Reid, M.D., Cantlay, L., Horgan, G.W., Kwun, I.S., Ahn, J.Y. and Ha, T.Y. Ginger phytochemicals mitigate the obesogenic effects of a high-fat diet in mice: a proteomic and biomarker network analysis. *Molecular Nutrition & Food Research*, **55** (Suppl 2), S203-213 (2011).
31. ElRokh el, S.M., Yassin, N.A., El-Shenawy, S.M. and Ibrahim, B.M. Antihypercholesterolaemic effect of ginger rhizome (Zingiber officinale) in rats. *Inflammopharmacology*, **18**, 309-315 (2010).
32. Badawy, A., Hassanean, H., Ibrahim, A.K., Habib, E.S., El- Magd, M.A. and Ahmed, S.A. Isolates From Thymelaea Hirsuta Inhibit Progression Of Hepatocellular Carcinoma In Vitro And In Vivo. *Natural Product Research*, **35**, 1799-1807 (2019).
33. Krishnan, K., Vijayalakshmi, N. and Helen, A. Beneficial effects of Costus igneus and dose response studies in streptozotocin induced diabetic rats. *Int. J. Curr. Pharm. Res*, **3**, 42-46(2011).
34. Baskar, A.A., Al Numair, K.S., Alsaif, M.A. and Ignacimuthu, S. In vitro antioxidant and antiproliferative potential of medicinal plants used in traditional Indian medicine to treat cancer. *Redox report : communications in Free Radical Research*, **17**, 145-156 (2012).
35. Selim, S. and Al Jaouni, S. Anti-inflammatory, antioxidant and antiangiogenic activities of diosgenin isolated from traditional medicinal plant, Costus speciosus (Koen ex.Retz.) Sm. *Natural Product Research*, **30**, 1830-1833 (2016).

تأثير تحسيني لمستخلصات نبات القسط على التأثيرات السامة لخلات الرصاص على الكبد والكلى للفئران الذكور

عبد العزيز دياب [1]، ابتسام المثال[2]، منصور زهرة[1] ، محمد دويدار[1] ، صفاء الشربينى[1] و محمد ابو المجد[3]

[1] قسم علم الحيوان - كلية العلوم - جامعة الزقازيق – مصر.

[2] قسم البيولوجى - كلية العلوم - جامعة عبد الرحمن بن فيصل- السعودية.

[3] قسم التشريح - كلية الطب البيطرى - جامعة كفر الشيخ- مصر.

تم استخدام نبات القسط بنجاح لتخفيف الكثير من حالات السمية ؛ ومع ذلك ، لا يزال من غير المعروف ما إذا كان نبات القسط يمكن أن يحسن من سمية خلات الرصاص .تهدف هذه الدراسة إلى تحديد ما إذا كان مستخلصات جذور نبات القسط باستخدام الماء وإيثر البترول والميثانول يمكن أن تخفف من السموم الكبدية والكلوية التي تسببها خلات الرصاص في الفئران. تم تقسيم الحيوانات عشوائيًا إلى خمس مجموعات) بكل مجموعة 15 فار، وتم تقسيم كل مجموعة إلى ثلاث فئات فرعية (بكل منه 5 فئران) وفقًا لمدة التعرض: وهذة المجموعات الخمس هى المجموعة الضابطة الطبيعية ، المجموعة المعالجة بخلات الرصاص ، والثلاث مجموعات المتبقية تتلقى مستخلصات جذور نبات القسط المائية وإيثر البترول والميثانول متزامنا مع خلات الرصاص. أظهرت النتائج أن جميع المستخلصات خففت من التأثيرات الضارة لـ خلات الرصاص كما يتضح من 1) انخفاض سكر الدم ؛ 2) انخفاض مؤشرات وظائف الكبد (ALTو AST والبيليروبين الإجمالي) وظائف الكلى (الكرياتينين واليوريا وحامض البوليا) ؛ 3) تقليل المعلمات الدهنية (TGو TC و LDL ؛ 4) ارتفاع مستوى البروتين الإجمالي والألبومين و HDL في المصل ؛ 5) انخفض مستوى MDA في الكبد ، وهو مؤشر على التأكسد الدهني ؛ و 6) زيادة إنزيمات الكبد المضادة للأكسدة SOD) و CAT و (GPx ومستوىGSH . كما شوهد أكبر حماية من التسمم بـ خلات الرصاص بعد شهر من المعالجة بالمستخلص الميثانولي. قد تكون تثبيط التوتر التأكسدي وزيادة نشاط إنزيم المضاد للأكسدة هما اثنان من الآليات التي يحسن بها مستخلصات نبات القسط من سمية خلات الرصاص المستحثة على الكبد والكلى. نظرًا لأن خلات الرصاص سام للكبد والكلى ، فقد تعمل هذه المستخلصات ، لا سيما المستخلص الميثانولي ، كعوامل تحسن قيّمة.

الكلمات الدالة: التسمم بالرصاص، نبات القسط، مضاد اكسدة ، الاجهاد المؤكسد.