



Musa paradisiaca leaves extract ameliorates cyclophosphamide-induced hepato-renal toxicity in mice

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ABSTRACT

Chemotherapeutic agents, cyclophosphamide (CTX) is accompanied by severe adverse effects on multi-vital organs. Leaves and peels of *Musa* trees showed promising biomedical applications. The study aims to assess the ameliorative effects of *Musa* leaves (MLE) and *Musa* peels (MPE) extracts on the hepato-renal dysfunctions caused by CTX in mice. The acute median lethal dose (LD₅₀) was determined using forty mice (CD-1). Other forty male CD-1 mice were divided into four groups (n=10) as follows: Group1 (Gp1) had injected intraperitoneally (i.p.) with 200 µl of sterile saline. Gp2 had injected i.p. with a single dose of CTX as 200 mg/kg b.wt. Gp3 and Gp4 were injected with CTX as in Gp2 then injected with 1/20 LD₅₀ of MLE (175 mg/kg) or MPE (170 mg/kg) daily, respectively. Fifty percent (50%) of mice in all groups were sacrificed after 15 days of treatments, and the rest of mice were sacrificed by day 30. All groups were bled at days 3, 9, 15, 23 and 30 to determine the hematological changes. Biochemical parameters, liver, and kidney histological changes were assessed at days 15 and 30 of treatment. The LD₅₀ of MLE and MPE were 3500 and 3400 mg/kg, respectively. CTX injection led to a significant decrease in white blood cells (WBCs) count starting from day 3 to day 12 (leucopenic phase), increased liver enzymes (AST and ALT) activities, induced liver, and kidney histological alterations. Treatment with MLE post CTX partially restored the total WBCs count in the leucopenic phase. Further, MLE treatment improved the biochemical and histopathological changes as evidenced by restoring liver, kidney functions and improving their histological architectures in CTX-injected mice. Collectively, MLE treatment could protect the hepato-renal tissues from toxicities which were induced by CTX.

Keywords:

Antioxidants, Apoptosis, Cyclophosphamide, Extract, Leaves, *Musa* sp., Necrosis, Peels.

1. Introduction

Banana (*Musa sp*) is an elongated herbaceous flowering plant produced edible fruits, belongs to the genus *Musa* and family *Musaceae* (Bailey, 2017). Bananas are distributed in most tropical and sub-tropical nations. Phytochemical analysis of banana fruit showed that there are several alkaloids, flavonoids, saponins, tannins, glycosides and terpenoids

(Kumar et al., 2012). Banana pectin have been extracted and characterized to be used as pharmaceutical excipients in tablet formulation (Bansal et al., 2014). Tannins and glycosides were abundant in fresh stem juice phytochemical screening, with moderate quantities of alkaloids, saponins, polyphenols, flavonoids, and reducing sugars (Siddique et al.,

2018). Banana peels can be good sources of carbs, minerals, and fiber and they may be a useful supply of minerals for cattle feed, it contains a lot of phytochemicals and phytonutrient components, the majority of which are antioxidant. Ripe banana peel includes a variety of chemicals, including delphinidin, anthocyanins, cyaniding, and catecholamines. Carotenoids such as beta-carotene and alpha-carotene have been discovered (Goel and Sairam, 2002). It has been reported that the banana leaves had no toxic effect or death up to 2000 mg/kbw. *M. sapientum* seed methanolic extract showed antidiarrheal, antioxidant, and antibacterial potential (Hossain et al., 2011). Hydro-ethanolic extracts of different parts of banana trees products showed antidiabetic activity and gastro-protective effects in STZ-induced diabetic in rats (Abdel Aziz et al., 2020). It has been reported that the different parts of banana (*Musa sp.*) had antiviral and cytotoxic activity (Panda et al., 2020). Furthermore, *M. paradisiaca* has been found to improve kidney functions due to their phytochemical constituents in mice (Abbas et al., 2017). Mature green fruits of banana improved semen quality of adult male Wistar rats, banana stem aqueous extract showed hepatoprotective activity and antihyperlipidemic effects in rats (Dikshit et al., 2012; Alabi et al., 2013). *M. sapientum* ethanolic leaves extract displayed antioxidant and antibacterial action against multidrug resistant (Karuppiyah and Mustaffa, 2013). Effects of *M. sapientum* banana stem extract as anti-hypercholesterolemic and antihyperlipidemic in rats have been documented (Dikshit et al., 2016). Finding medication for cancer patients that is less harmful is proving to be a huge issue. By reducing the negative effects of chemotherapy After 3 days of treatment, a single injection of 200 mg/kg of CTX in mice caused significant lymphopenia and reduced the total W.B.Cs in the peripheral blood at days 3–12, as well as in the spleen and bone marrow at days 3–6. The total number of W.B.Cs returned to normal level during the recovery phase starting from day 12 to 16 after CTX treatment in naïve mice. Natural products have been used for ameliorating chemotherapy-induced side

effects (Zhang et al., 2018). For instance, administration of thymoquinone can be able to lower CTX-induced toxicity through the up-regulation of antioxidant mechanisms (Kamarzaman et al., 2014). Furthermore, *Persea americana* phytochemicals can also be used to reduce the side effects of CTX in cancer therapy. In addition, it has been reported that propolis is considered as an effective agent to ameliorate CTX toxicity in mice (El-Naggar et al., 2015). *M. paradisiaca* stem extract had hematological and immunomodulatory properties, which could be owing to its phytochemicals stimulating and forming erythropoietin. The goal of this investigation was to see if *Musa* leaves and peel extracts might protect mice from CTX-induced hepatorenal damage.

2. Materials and Methods

Chemicals

Cyclophosphamide (CTX) was purchased from Merck company (Germany). Kits of aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, creatinine, superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) were purchased from Bio-diagnostic company (Egypt). The rest of the chemicals were bought from local chemical trade companies.

Preparation of MLE and MPE

Leaves and peels of *Musa paradisiaca* were collected from banana farms in the governorate of El-Gharbiah and transported to Tanta University's Animal Research Laboratory, Zoology Department, Faculty of Science. After washing twice to eliminate any chemicals or dust, the leaves and peels were cut into extremely small pieces and dried in the shade. Using a mechanical mortar, these compartments were ground and 50 g of each were mixed with 500 ml of 70% ethanol and stored for three days. Finally, the supernatants were filtered and left to dry to get the hydro-alcohol extracts of MLE and MPE, then stored at 4 °C for further use.

Mice

Eighty male CD-1 albino mice (20 ± 2 g) were procured from the National Research Center (NRC, Cairo, Egypt) in order to establish the

MLE and MPE LD₅₀ and their potential therapeutic efficacy against CTX induced toxicity. Under laboratory temperature and humidity settings, animals were housed six to a cage in a 12 hour/12-hour dark/light cycle. Mice were maintained for a week before the experiment to allow them to adapt. The animals were experimented on, transported, and cared for according to the ethical rules established by the animal care and use committee, which were based on the National Institutes of Health's handbook for the care and use of laboratory animals (NIH Publications No. 8023, revised 1996).

Determination the median lethal dose (LD₅₀) of MLE and MPE

A total of 40 male CD-1 albino mice (20 ± 2 g) were divided into 10 groups (n=4) to estimate the LD₅₀ following intraperitoneal (i.p.) injection of MLE or MPE. These groups were injected with a single dose of either MLE or MPE (1 - 5 g/kg) i.p. The mice were then observed for 24 hours to see if there was any acute toxicity. The LD₅₀ value was determined by probit analysis (Finney, 1971).

Experimental design

Forty male (CD-1) mice were divided into four groups (n=10). The 1st group (Gp1) was a control, injected with 200 µl of sterile saline i.p. Gp2 had injected with a single dose of CTX (200 mg/kg b.wt) i.p. Gp3 had injected with CTX as in Gp2, then injected with 1/20 LD₅₀ of MLE (175 mg/kg) daily for a month. Gp4 had injected with CTX as in Gp2, and then injected with 1/20 LD₅₀ of MPE (170 mg/kg) daily for a month. Fifty percent (50%) of mice in all groups (Gp1- Gp4) were sacrificed on day-15, and the rest of mice were sacrificed by day-30. Then, mice from each group bled at days- 3, 9, 15, 23 and 30 to determine the kinetic hematological changes. Blood samples from each group at day 15 and 30 were collected to assess the biochemical parameters. Liver and kidney tissues were collected at the same time points for histopathological investigations.

Determination the percentages of the total body weight changes

All mice groups were weighted at the start of the experiment (I.B.wt) and at the end of the

experiment (F.B.wt). The percentage of the total body weight change (% B.wt) was calculated as follows: $(F.B.wt - I.B.wt / I.B.wt) \times 100$.

Determination of hematological and biochemical parameters

The electronic blood counter (Mendary, China) was used to determine red blood cells (RBCs), hemoglobin content (Hb g/dl), platelets, white blood cell (W.B.Cs), and their differential counts in blood samples from all groups. Colorimetric methods were used to determine ALT, AST activities, urea, creatinine levels, the antioxidant activities of SOD, CAT, and MDA level.

Histopathological investigation of liver and kidney tissues

The liver and kidney tissue samples were taken and fixed in 10% formalin. After finishing tissue processing in various series of alcohol and xylene, paraffin blocks were prepared. 5 µm sections from paraffin blocks were cut and then stained with hematoxylin and eosin before being examined using a light microscope (Optika light microscope (B- 350) to look for significant cellular damage (Bancroft and Gamble, 2008).

Statistical analysis

The significance of differences among treatment groups was determined using one-way analysis of variance (ANOVA). The significant effect of treatment was demonstrated using the Dunnet test comparing all groups to the control group. The statistical significance criteria were established at $p < 0.05$. All data is presented as mean ± SD.

3. Results

Acute LD₅₀ of MLE and MPE in mice

The LD₅₀ was determined after 24 hrs of injection using different doses of MLE and MPE. Different groups (n=4) were injected i.p. with different doses varied from 1 to 5 g/kg. LD₅₀ of MLE and MPE were 3500 and 3400 mg/kg, respectively (Fig. 1).

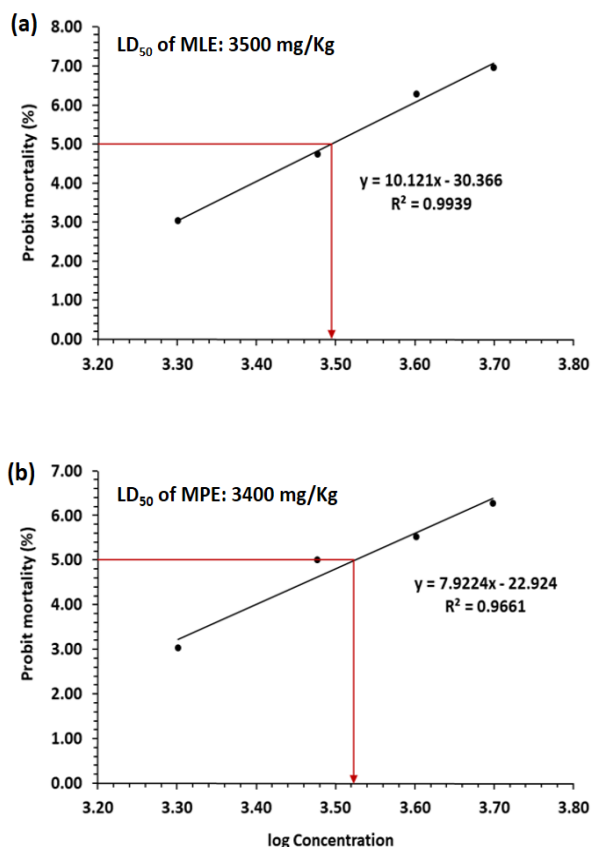


Fig. 1. The median lethal dose (LD₅₀) of MLE (a) and MPE (b). MLE: Musa leaves extract; MPE: Musa peels extract

Administration of MLE post CTX injection restored body weight

The initial (I.B.wt) and final body weights (F.B.wt) of different groups were determined at days 0, 15 and 30 post CTX injection. A significant decrease in the F.B.wt post 15 or 30 days of CTX injection, the % of B. wt changes was 13.70 post 30 days of injection. Treatment with MLE post CTX injection led to a significant increase in the F.B.wt, the % of B. wt changes was 36.50 post 30 days of injection. MPE treatment after CTX injection led to a substantial increase in the F.B.wt when compared to CTX treated group; the % of b. wt changes was 27.27 post 30 days of injection (Fig. 2a) and (Table 1).

MLE treatment post CTX-injection partially restored W.B.Cs count in the leucopenic phase

As compared to the CTX-injected group, the R.B.Cs number and Hb % did not show any significant changes in other different groups (Table 2a). However, as compared to control

group (Gp1), group of mice which had treated with CTX (Gp2), CTX/MLE (Gp3), CTX/MPE (Gp4) showed decrease in their platelets count (Table 2a). In Gp2, by day 3 post CTX-injection, the total number of W.B.Cs count was decreased from 8×10^3 to $0.5 \times 10^3/\mu\text{l}$. MLE treatment after CTX injection (Gp3), restored the total number of W.B.Cs partially but significantly when compared with CTX injected group (Gp2). MPE treatment post CTX injection (Gp3) did not show any significant change in the total W.B.Cs count when compared with CTX injected mice (Gp2) (Fig. 2b). CTX injection caused marked reduction in absolute numbers and % of both neutrophils and lymphocytes comparing to their values in control group (Gp1). In contrast, CTX-injection resulted in a considerable increase in the absolute number and % of monocytes when compared to Gp1. MLE treatment after CTX injection led to an increase in the absolute number and % of neutrophils and decreased the absolute number and % of lymphocytes. However, treatment with MPE after CTX injection (Gp4) decreased the absolute number and % of neutrophils, lymphocytes partially and monocytes as compared to mice injected with CTX (Gp2) (Table 2).

Treatment with MLE post CTX injection ameliorates the hepato-renal toxicity induced by CTX

CTX injection increased the activities of liver transaminases significantly in comparison to values of control group (Gp1) post 15 and 30 day of treatment. MLE treatment post CTX led to a partial decrease in AST and ALT activities on day 15 (Fig. 3a and b). MLE treatment for 30 days after CTX injection resulted in significant reductions in ALT and AST activities (Table 3). At days 15 and 30, treatment with MPE after a CTX injection resulted in a partial improvement in AST and ALT (Fig. 3a and b). As compared to control group (Gp1), mice groups which had treated with CTX (Gp2), CTX/MLE (Gp3) and CTX/MPE (Gp4) showed decrease in their total protein and albumin concentrations (Table 3). Total bilirubin values decrease in CTX/MLE and CTX/MPE while increase in CTX injected mice comparing to control group

(Table 3). When compared to control mice, CTX caused a considerable increase in urea and creatinine levels after 15 and 30 days of injection. When mice were given MLE on days 15 or 30 after receiving CTX, their levels of urea and creatinine were significantly lower than when they were given CTX alone. MPE treatment post CTX injection didn't show any significant change in urea and creatinine levels at 15 or 30 days when compared to mice injected with CTX alone (Fig. 3c and d) and (Table 3).

Treatment with MLE ameliorated CTX-induced oxidative stress in mice

When compared to the values in the normal control group (Gp1), CTX injection resulted in a significant decrease in activities of SOD and CAT accompanied with a large increase in MDA levels in sera. MLE treatment resulted in a significant rise in SOD, CAT, and a significant decrease in MDA levels on days 15 and 30 compared to the CTX-injected group. MPE treatment after CTX injection didn't show any marked change in SOD, CAT and MDA compared to CTX injected group at days 15 and 30 (Fig. 4) and (Table 4).

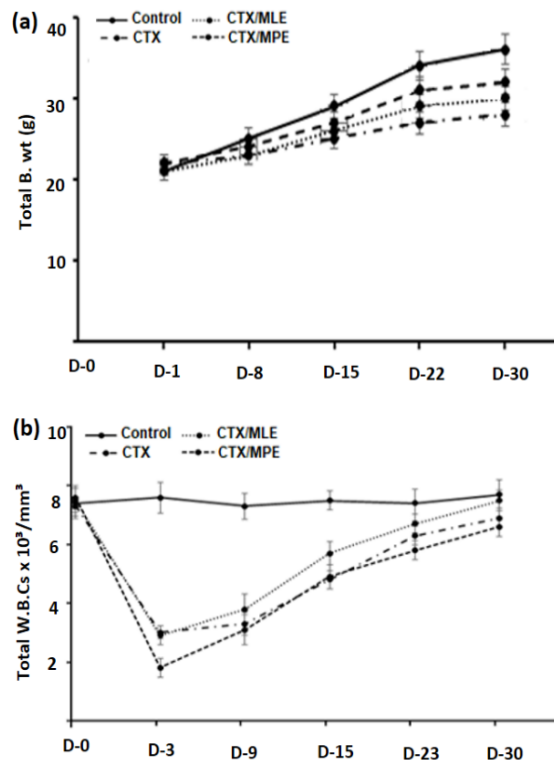


Fig. 2. The kinetic of total body weight changes (a). The kinetic of W.B.Cs count changes post treatment with CTX, CTX/MLE, CTX/MPE (b). CTX: Cyclophosphamide, MLE: Musa leaves extract; MPE: Musa peels extract; W.B.Cs :White blood cells.

Table 1. Initial, final body weights and % change in body weight after- 30 days of CTX injection

Groups	I. B. wt. (g.)	F. B. wt. (g.)	% change in body wt.
Control	23.5 ± 3.2	35.50 ± 2.28	51.06
CTX alone	22.2 ± 2.2	25.07 ± 0.84	13.70*
CTX/MLE	22.45 ± 3.3	30.37 ± 3.22	36.50**
CTX/MPE	22.58 ± 3.9	28.43 ± 1.16	27.27**

The values represented mean ± SD; CTX: Cyclophosphamide, MLE: Musa leaves extract; MPE: Musa peels extract; I.B.wt: Initial body weight; F.B.wt: Final body weight. The values represented means ± S.D.; **p* < 0.05, significantly different from control group, ***p* < 0.05, significantly different from CTX group.

Table 2a. The total RBCs count, Hb level and Plat count in different groups of mice after 30 days of CTX injection.

Groups	RBCs (x10 ⁶ /μL)	Hb (g/dL)	Plat. (x10 ³ /μL)
Control	6.67 ± 1.71	12.97 ± 0.97	968.88 ± 76.7
CTX alone	7.25 ± 1.62	13.3 ± 1.12	695.67 ± 64.28*
CTX/MLE	8.0 ± 0.4	12.9 ± 1.0	771.3 ± 62.2
CTX/MPE	7.46 ± 0.89	12.43 ± 1.46	743.33 ± 58.33

The values represented means ± S.D.; CTX: Cyclophosphamide, MLE: Musa leaves extract; MPE: Musa peels extract; R.B.Cs: Red blood cells; Hb: Hemoglobin; Plat.: Platelets. **p* < 0.05, significantly different from control group, ***p* < 0.05, significantly different from CTX group.

Table 2b. Total W.B.Cs and their differential percentages in different groups of mice post 30 days of CTX injection.

Groups	WBCs ($\times 10^3/\mu\text{l}$)	Differential count		
		Neut. (%)	Lymph. (%)	Mono. (%)
Control	7.43 \pm 1.39	17.0 \pm 5.0	80.33 \pm 6.51	2.67 \pm 0.58
CTX alone	4.2 \pm 0.4*	8.67 \pm 5.13*	69.0 \pm 9.54	8.67 \pm 5.2*
CTX/MLE	5.5 \pm 0.8**	11.7 \pm 2.1**	54.3 \pm 11.8	11.7 \pm 2.1
CTX/MPE	3.6 \pm 2.46	7.33 \pm 2.08	58.67 \pm 11.5	7.33 \pm 2.1

The values represented means \pm S.D.; CTX: Cyclophosphamide, MLE: Musa leaves extract; MPE: Musa peels extract; W.B.Cs: White blood cells. * $p < 0.05$, significantly different from control group, ** $p < 0.05$, significantly different from CTX group.

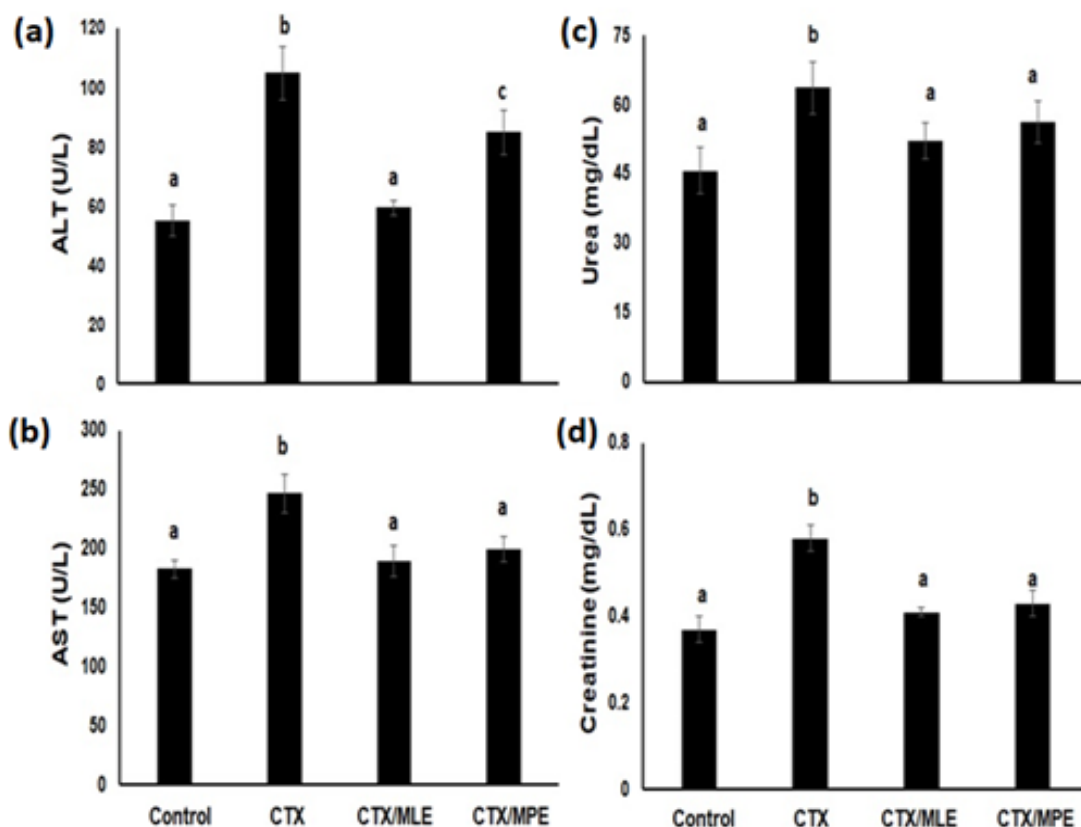


Fig. 3 (a-d). ALT, AST, urea, and creatinine levels post 15 days of treatments with CTX, CTX/MLE, CTX/MPE. CTX: Cyclophosphamide, MLE: Musa leaves extract; MPE: Musa peels extract. Means that don't share a letter showed significant difference.

Table 3. Activities of ALT, AST, urea, and creatinine levels in different groups post 30 days of CTX injection.

Groups	ALT (U/L)	AST (U/L)	Urea (mg/dL)	Creatinine (mg/dL)
Control	59.3 \pm 3.5	189.3 \pm 2.5	40.67 \pm 7.42	0.37 \pm 0.06
CTX alone	95.0 \pm 5.9 *	275.7 \pm 4.0*	60.05 \pm 2.01*	0.55 \pm 0.01*
CTX/MLE	53.0 \pm 5.6**	204.3 \pm 2.1**	47.67 \pm 2.52 **	0.47 \pm 0.05
CTX/MPE	77.3 \pm 10.1	255.0 \pm 2.6	46.00 \pm 5.03**	0.47 \pm 0.06

The values represented means \pm S.D.; CTX: Cyclophosphamide, MLE: Musa leaves extract; MPE: Musa peels extract; ALT: Alanine amino transaminase; AST: Aspartate aminotransferase. * $p < 0.05$, significantly different from control group, ** $p < 0.05$, significantly different from CTX group.

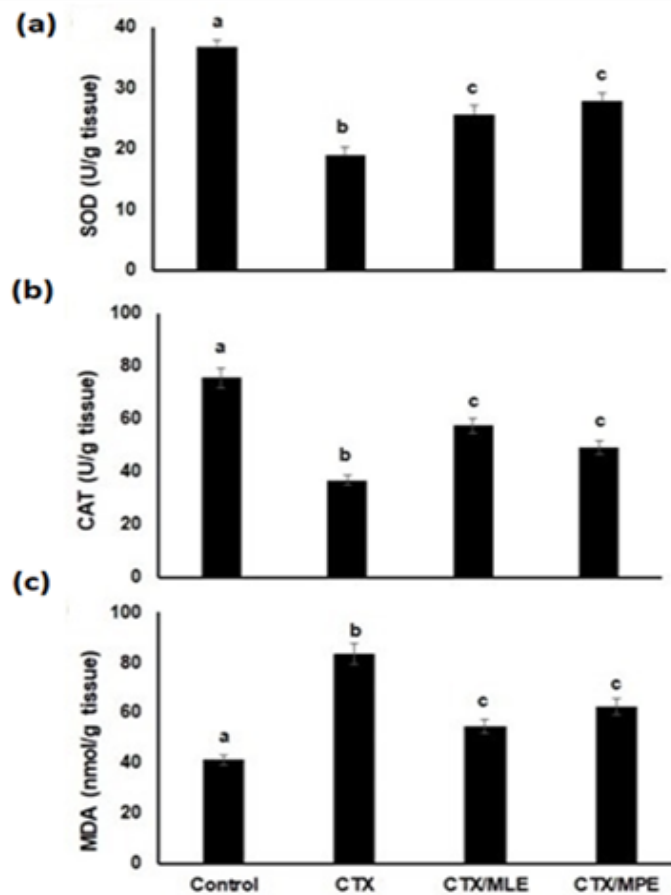


Fig. 4. SOD, CAT activities, and MDA levels post 15 days of treatment with CTX, CTX/MLE, CTX/MPE. a) SOD activity, b) CAT activity; C) MDA level. CTX: Cyclophosphamide, MLE: Musa leaves extract; MPE: Musa peels extract. Means that don't share a letter showed significant difference.

Table 4. Activities of SOD, CAT and MDA in different groups post 30 days of CTX injection.

Groups	Oxidative stress parameters		
	SOD (IU/mg tissue)	CAT ($\mu\text{M}/\text{min}/\text{mg}$ tissue)	MDA (nmol/g tissue)
Control	35.71 ± 1.2	72.45 ± 3.9	38.43 ± 1.98
CTX alone	$21.23 \pm 1.6^*$	$43.45 \pm 1.65^*$	$69.21 \pm 2.32^*$
CTX/MLE	$29.43 \pm 1.5^{**}$	$60.65 \pm 2.34^{**}$	50.14 ± 2.54
CTX/MPE	26.26 ± 1.7	55.34 ± 2.56	55.87 ± 2.65

The values represented means \pm S.D.; CTX: Cyclophosphamide, MLE: Musa leaves extract; MPE: Musa peels extract; SOD: Superoxide dismutase; CAT: Catalase; MDA: Malondialdehyde. * $p < 0.05$, significantly different from control group, ** $p < 0.05$, significantly different from CTX group.

Administration of MLE improved CTX-induced liver histopathological changes

Liver sections of control mice showed a normal hepatic structure in which polygonal hepatocytes with prominent nuclei are arranged in a radial pattern. The hepatic strands alternate with narrow blood sinusoids lined by an endothelial cell layer containing Kupffer cells (Figs. 5a and 7a). CTX-

injected mice's liver sections showed marked disorganization, congested central veins, mostly of hepatocytes with vacuolated cytoplasm. Others had pyknotic nuclei, also necrotic areas, this hepatic disorganization increased in day 30 more than day 15 of CTX injection (Figs. 5b and 7b). Liver sections of mice treated with MLE post CTX-injection showed partial improvement

of the hepatic architecture with minimal tissue degeneration but there is cellular infiltration still appear around the wide central vein at day 15 of the treatment (Fig. 5c). After 30 days of treatment with MLE post CTX-injection showed improvement of the more normal hepatic architecture, the hepatocytes with slight widening of blood sinusoids and central vein, pyknotic, vesicular nuclei and megakaryocytic ones, were seen (Fig. 7c). The liver section of mice that treated with MPE post CTX injection for 15 days showed vacuolated hepatocytes that have either pyknotic or enlarged nuclei; others show eosinophilia, dilated congested sinusoidal spaces and central veins (Fig. 5d). Mice treated with MPE for 30 days after CTX injection had a centrilobular pattern of degeneration, necrosis, and vacuolated hepatocytes, branched and congested central vein, enlargement of blood sinusoids, pyknotic, vesicular nuclei, and megakaryocytic nuclei in their liver (Fig. 7d).

Administration of MLE improved CTX-induced kidney histopathological changes

In the control section of the kidney, the renal cortex appeared normal, with a normal glomerulus, proximal and distal convoluted tubules, and Bowman's capsule, there are no signs of inflammation (Figs. 6a and 8a). The kidney sections of CTX-injected mice (Gp2) showed glomeruli appeared congested, shrunken and destructed with irregular bowman's space, the majority of the renal tubules were injured with degenerated epithelial lining cells make it lose their characteristic appearance and intertubular hemorrhage. In Gp2, the renal damage and intertubular hemorrhage in the kidney increased at 30 days of CTX injection (Figs. 6b and 8b). Kidney sections of mice that were treated with MLE post CTX injection (Gp3) for 15 days showed partially improvement in glomeruli structure but accompanied with glomerular shrinkage and intertubular hemorrhage were still appearing (Fig. 6c). However, after 30 days of treatment with MLE post CTX-injection, an improvement in glomeruli with glomerular shrinkage disappeared. Further investigations of kidney

sections of Gp3 showed that most of the renal tubules were improved with normal appearance and renal blood vessels were normal (Fig. 8c). Kidney sections of mice treated with MPE post CTX injection for 15 days showed disorganized glomeruli with narrow Bowman's space, damaged renal tubules with undistinguished lining epithelium which lost their distinctive structure (Fig. 6d). Kidney sections of mice that were treated with MPE post CTX injection for 30 days showed destroyed renal tubules that lost their typical appearance (Fig. 8d).

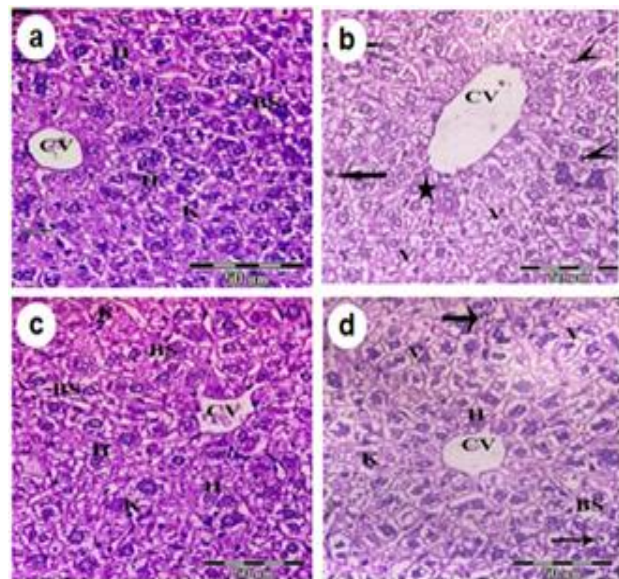


Fig. 5. Photomicrograph of liver sections (high magnification; magnification power = 400) stained with hematoxylin and eosin from the different groups post 15 days of CTX injection. (a) Control group showing normal hepatic structure with radial arrangement of polygonal hepatocyte containing pronounced nuclei (H), central vein (Cv), blood sinusoids (Bs) lined by endothelial cells and distinct phagocytic Kupffer cells (K). (b) CTX-injected mice showing marked disorganization, congested central veins (Cv) with degeneration of its epithelial lining, mostly of the hepatocytes are highly degenerated with vacuolated cytoplasm (V), others show eosinophilia (arrows), area around central vein associated with cellular infiltration (arrowhead). (c) MLE/CTX-treated mice showing improvement of hepatocytes that regained their normal architecture. Slight cytoplasmic degeneration surrounding the nuclei may appear (thin arrows). (d) MPE/CTX-treated mice showing wide central veins (Cv) and radiating hepatic strands (H). Most hepatocytes are vacuolated; others show eosinophilia (arrows) and a

small number of pyknotic nuclei (thick arrows) and Kupffer cells (K).

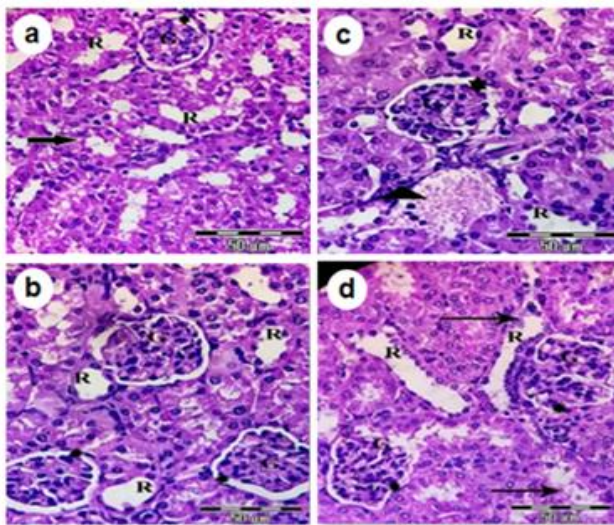


Fig. 6. (a) Kidney section of control group showing normal structure of the cortex, normal glomeruli (G) with normal bowman's space (*), and normal renal tubules (R) lined by cuboidal epithelium (thick arrow). (b) Cyclophosphamide (CTX)-injected mice showing destructed, shrunken, and congested glomeruli (G) with irregular bowman's space (*), most of the renal tubules (R) were damaged; cellular hemorrhage at the intertubular spaces was observed (arrowhead). (c) MLE/CTX-treated mice showing more or less normal like architecture of glomeruli (G) after disappearance of glomerular shrinkage and mild degeneration of renal epithelial cells of renal tubules (R). (d) MPE/CTX-treated mice showing disorganized glomeruli (G) with narrow Bowman's space (*), the renal tubules (R) were damaged, loss their typical appearances, and their lining epithelium were undistinguished with cellular degeneration and widening of renal lumen (arrows).

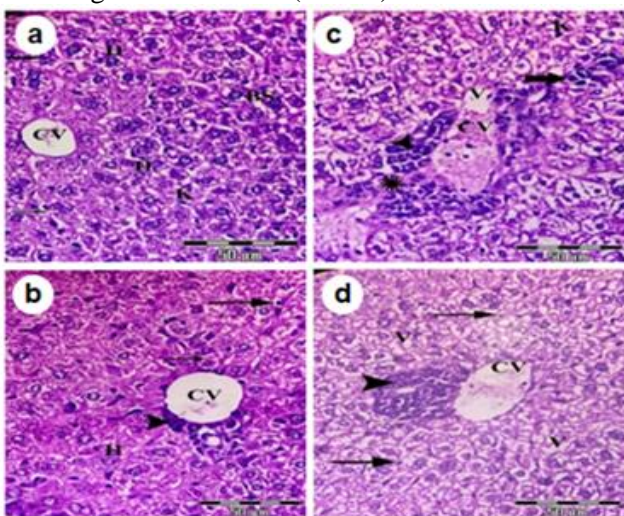


Fig. 7. Photomicrograph of liver sections (high magnification; magnification power = 400) stained with hematoxylin and eosin from the different groups

post 30 days of CTX injection. (a) Control group. (b) Cyclophosphamide (CTX)-injected mice showing marked disorganization, congested central veins (Cv) with intensive degeneration of its epithelial lining, mostly of the hepatocytes are highly degenerated with vacuolated cytoplasm (V), others with pyknotic nuclei (thick arrows), also necrotic areas were seen (*), area around central vein associated with cellular infiltration (arrowhead). (c) MLE/CTX-treated mice showing improvement of hepatocytes that regained their normal architecture. Slight cytoplasmic degeneration surrounding the nuclei may appear (thin arrows), slight cellular infiltration around central vein (arrowhead). (d) MPE/CTX-treated mice showing hepatic degeneration with irregular congested (thick arrows) and mega karyocytic ones, area of infiltration around central vein (arrowhead).

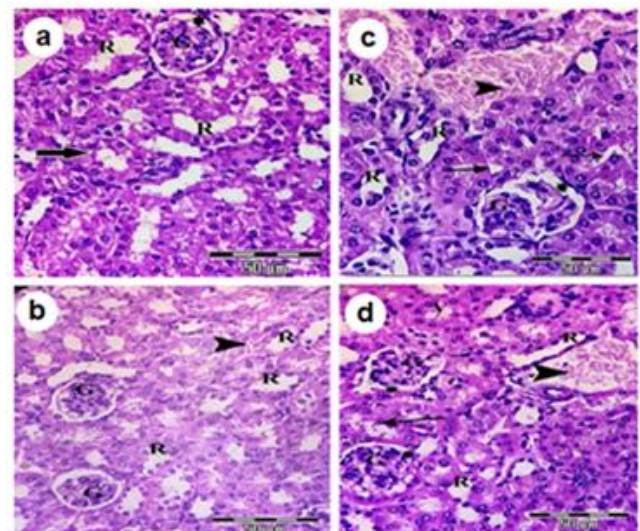


Fig. 8. (a) Kidney section of control group. (b) Cyclophosphamide (CTX)-injected mice showing destructed, shrunken and congested glomeruli (G) with irregular bowman's space (*), damaged renal tubules (R) and lost their characteristic appearance with distinct vacuolated and highly degenerated lining epithelium; cellular infiltration at the intertubular spaces was observed (arrows), appearance of eosinophilic hyaline substance (**) and hemorrhage (arrowhead). (c) MLE/CTX-treated mice showing more or less normal like architecture of glomeruli with slight shrinkage (G) and mild degeneration of renal epithelial cells of renal tubules (R), hemorrhage area may appear (arrowhead). (d) MPE/CTX-treated mice showing disorganized glomeruli (G) with irregular Bowman's space (*), widening of some renal tubules (R), occluded with hyaline casts (arrows) and degeneration of some epithelial lining, others with vacuolated cytoplasm (V), damaged and their contents intermixed with each other.

4. Discussion

Musa sp. is one of the well-known plants with nutritive, as well as medicinal value that have been used in traditional medicine. Different parts of *Musa* trees including fruit, flower, root, and stem are used to treat a variety of human disorders (Bhaskar et al., 2011). *Musa sp* has been reported to have many advantageous effects in several diseased conditions, including atherosclerosis, hyperlipidemia, hypertension, and thyroid dysfunctions. The chemotherapeutic agent CTX is well-known in treating various types of cancer; however, exert multiple adverse effects on multi-vital organs (Vinaykumar et al., 2010). In the present study, LD₅₀ of MLE and MPE that killed 50% of mice were 3500 and 3400 mg/kg, respectively. Previous study reported that the acute toxicity study of *M. paradisiaca* peels methanolic extract gave an LD₅₀ of 2849.56 mg/kg (Akpanyung et al., 2019). It has been reported that there was no toxic effect or death up to 2000 mg/kgbw of banana leaves extract (Abagale et al., 2018). In this study, the results revealed that CTX injection causes a considerable reduction in total body weight in mice; this reduction in body weight could be related to the drug's side effects on various body organs and tissues. These findings were in line with previous study that found CTX injection resulted in a considerable reduction in the percent in body weight change in mice (El-Naggar et al., 2015). Treatment with MLE post CTX injection for 15 or 30 days led to a significant increase in the percent in the body weight change. These results were in accordance with previous study showed the effects of herbal extracts of herbaceous plants on toxicity caused by CTX in mice (Kim et al., 2018). While MPE treatment after CTX injection led to a substantial increase in the body weight at 15- and 30-day post CTX injection. The number of WBCs and neutrophils count in mice given CTX exhibited a considerable decrease. This result was in accordance with a previous study which found that after three days of CTX injection, the total WBC count decreased (Salem et al., 2012). They

showed that the leucopenic phase post CTX-injection extended from day 3 to day 12. In the current study, MLE treatment after CTX injection partially restored the hematological alterations in mice. The obtained result agreed with previous study showed that *M. paradisiaca* stem extracts had hematological and immunomodulatory properties, which could be owing to its phytochemicals stimulating and forming erythropoietin. The major limitation of using CTX is normal tissues injury or damage, which leads to numerous side effects. CTX-injected mice had considerably higher activities of AST and ALT, according to our findings. Treatment with MLE and MPE post 15 or 30 days of CTX injection attenuated the CTX toxic effect on the liver function by decreasing the activities of AST and ALT. This finding was agreed with previous study reported some natural products were able to attenuate the toxic effect of CTX (Habibi et al., 2015). Previous studies showed that banana stem aqueous extract had hepatoprotective activity and antihyperlipidemic effects in rats (Dikshit et al., 2012). Urea and creatinine analysis showed that levels were increased post 15 or 30 days of CTX-injection. Treatment with MLE post CTX injection for 15 or 30-days caused significant reduction in urea and creatinine levels. There was no significant change in the levels of urea and creatinine after MPE treatment post CTX injection at 15 or 30 days. This data was in line with previous studies showing the effect of herbal plants on renal injury induced by CTX (Ayza et al., 2020). Furthermore, *M. paradisiaca* improves kidney functions due to their phytochemical constituents in mice (Abbas et al., 2017). The present study showed that MLE and MPE have significant antioxidant and ameliorative effects against CTX-induced hepatorenal toxicities that is evidenced by significant increase in the CAT and SOD activities and significant reduction in MDA level. Previous study showed that *M. sapientum* ethanolic leaves extract had antioxidant (Karuppiah and Mustaffa, 2013). Antioxidant activity was discovered using the β -

carotene. bleaching method, DPPH free radical scavenging method and the linoleic acid emulsion method in an aqueous acetone extract of banana peel. Previous study reported that the antioxidant activity of *M. paradisiaca* extracted flavonoids in rats, they found that the banana flavonoids increased the activities of superoxide dismutase SOD and CAT, possibly explaining the lower levels of peroxidation products such as hydroperoxides, MDA and conjugated dienes (Vijayakumar et al., 2008). Significant alterations in histological appearance characterized by necrosis and vacuolation of liver and kidney were observed in CTX- injected mice (El-Naggar et al., 2017). In the current work, the liver tissue of CTX injected mice showed loss of cellular organization, many hepatocytes have degeneration and necrosis, as well as cytoplasmic vacuolation. Pyknotic nuclei and blood vessel congestion are two prominent nuclear alterations. Treatment of mice injected with CTX with MLE resulted in an improvement of the hepatic architecture, branched central vein, slight widening of blood sinusoids, pyknotic nuclei and vesicular ones were seen. This finding agreed with previous study that showed MLE was effective for the prevention of

CTX-induced hepatic damage in mice (Habibi et al., 2015). On contrast to the effect of MLE on CTX induced toxicity, MPE did not show any significant improvement. Consistent with previous studies, this study showed that CTX – injection induced histological alterations in kidney tissue. Kidney of the mice treated with MLE post CTX injection showed an improvement in glomeruli and renal tubules for 15 or 30 days. These findings were in line with previous finding who studied the effect of herbal plants on renal injury induced by CTX. However, the kidney of the mice that treated with MPE post CTX injection showed no obvious improvement in the renal architecture (Ayza et al., 2020). This study concluded that CTX treatment led to leucopenic phase started from day 3 to 12 which led to alterations in the biochemical parameters of both liver and kidney and induced histological destruction. However, treatment with MLE post CTX for 15 or 30 days potentially restored the vitality of the liver and kidney organs, improved the hepato-renal architectures, and partially restored the number of W.B.Cs count in the leucopenic phase post CTX-injection

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Conflicts of interest

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