Original Article

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DOI: 10.4103/jeca.jeca_21_18

Hepatoprotective effect of methanol fruit pulp extract of *Musa paradisiaca* on carbon tetrachloride-induced liver toxicity in Wistar rats

Mustapha Tosin Issa, Abel Nosereme Agbon, Sadiya Ufeli Balogun[1], Onesimus Mahdi[2], Khadijat Abubakar Bobbo[2], Felix Olu Ayegbusi[3]

Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, Ahmadu Bello University, Zaria, [1]Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, Kogi State University, Ayingba, [2]Department of Human Anatomy, College of Medical Sciences, Gombe State University, Gombe, [3]Department of Chemical Pathology, Faculty of Clinical Sciences, College of Health Sciences, Ahmadu Bello University Teaching Hospital, Shika, Nigeria

Address for correspondence:
Dr. Abel Nosereme Agbon, Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, Ahmadu Bello University, Zaria, Nigeria.
E-mail: bellulu09@gmail.com

Abstract:

CONTEXT: *Musa paradisiaca* (Banana) fruit pulp has been used in folk medicine to treat various kinds of ailments, such as dysentery, diarrhea, bronchitis, ulcer, fevers, and hemorrhages in different parts of the globe, including Nigeria, Western Africa.

AIM: This study was designed to histologically and biochemically assess the protective effect of methanol fruit pulp extract of *M. paradisiaca* (MFMP) on carbon tetrachloride (CCl_4)-induced hepatotoxicity in Wistar rats.

MATERIALS AND METHODS: Twenty-four Wistar rats were divided into six groups (I–VI; $n = 4$). Group I (control) was administered distilled H_2O (2 ml/kg), whereas Groups II, III, and IV were administered MFMP (500 mg/kg, 1000 mg/kg, and 1500 mg/kg, respectively) and Group V administered Silymarin (100 mg/kg), as the reference drug, for a period of 14 days. Hepatotoxicity was induced in rats by the administration of CCl_4 (1 ml, 1:1 solution: olive oil). On the 15th day, Groups II–VI were administered single dose of CCl_4. All administrations were through the oral route. After 12 h of CCl_4 administration, rats were euthanized and liver organs harvested for routine (H and E) histological tissue processing and blood samples collected for biochemical analysis of serum liver enzymes (alanine transaminase, aspartate transaminase, and alkaline phosphatase).

RESULTS: MFMP-treatment revealed remarkable histoarchitectural preservation of the liver parenchyma against CCl_4-induced liver damage and decreased ($P < 0.05$) serum liver enzyme levels elevated by CCl_4. Hepatoprotective activity was comparable with that of the reference drug, Silymarin.

CONCLUSION: Result suggests that MFMP possesses hepatoprotective potentials against chemically-induced acute hepatotoxicity in Wistar rats. Hepatoprotective potential of MFMP is possible as a result of its antioxidant properties.

Keywords:

Carbon tetrachloride, hepatoprotective, *Musa paradisiaca*, Wistar rat

Introduction

Man is exposed to various exogenous compounds, such as drugs and pollutants in their daily activities. The liver plays a critical role in the process of chemical alteration of most of these compounds (Chiang, 2014; Ullah *et al.*, 2016). This physiological activity of the liver results in the generation of highly reactive free radicals in the cell, which bind to membrane lipids causing lipid peroxidation and consequently damage the liver (Ali *et al.*, 2014; Ye *et al.*, 2018). CCl_4 is an established hepatotoxin, frequently used as a model of experimental hepatotoxicity (Cheng *et al.*, 2013).

How to cite this article: Issa MT, Agbon AN, Balogun SU, Mahdi O, Bobbo KA, Ayegbusi FO. Hepatoprotective effect of methanol fruit pulp extract of *Musa* paradisiaca on carbon tetrachloride-induced liver toxicity in Wistar rats. J Exp Clin Anat 2018;17:1-7.

Side effect manifestation is a major challenge with the administration of conventional or synthetic drugs for the treatment of liver-related diseases (Toori *et al*., 2015; Zarezade *et al*., 2018). Thus, it is imperative to seek for new medicines for liver disease, especially those of natural origin which are considered to be effective and safe alternative treatments, readily available and accessible (Yao *et al*., 2016; Gyawali *et al*., 2017). *Musa paradisiaca* (banana) is a tree-like herb and various parts of the plant used in folk medicine for a variety of ailments (Enechi *et al*., 2014; Abbas *et al*., 2016). This study histologically and biochemically assessed the hepatoprotective effect of methanol fruit pulp extract of *M. paradisiaca* (MFMP) on CCl_4-induced liver toxicity in Wistar rats. This study is imperative to scientifically demonstrate the pharmacological property of *M. paradisiaca in vivo* as potential therapeutics for inflammatory liver-related diseases.

Materials and Methods

Plant material collection and identification

M. paradisiaca (banana) fruit were obtained from a local market in Zaria, Kaduna State, Nigeria. Plant material was authenticated in the Herbarium Unit of the Department of Botany, Faculty of Life Sciences, Ahmadu Bello University (ABU), Zaria, with the Voucher Specimen Number: 173.

Extraction of plant materials

Preparation of MFMP was conducted at the Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, ABU, Zaria, employing the method of maceration. A brief description of the extraction protocol was as follows: shade-dried *M. paradisiaca* fruit pulp was pulverized, of which 250 g was macerated in 2.5 L of 100% methanol for 24 h. After which the solution was filtered using a Whatmann filter paper and the filtrate evaporated to dryness using H-H Digital Thermometer Water Bath (Mc Donald Scientific International-22050 Hz1.0A International Number) at 65°C. A yield of 60% of the extract was obtained.

Experimental animals

Twenty-four adult Wistar rats (male and female, 170–200 g) were obtained from Human Anatomy Animal House, Faculty of Basic Medical, College of Health Sciences, ABU, Zaria, housed in new wired cages in the same facility and allowed to acclimatized for 2 weeks before the commencement of experiments. The rats were housed under standard laboratory condition, light and dark cycles of 12 h, and were provided with standard rodent pellet diet and water *ad libitum*. The rats were categorized into control and treatment groups.

Drugs

- Olive oil (finest cold drawn) Bell, Sons and Co (DRUGGISTS) Ltd, Southport PR9 9AL, England
- Carbon tetrachloride (CCl_4) May and Baker limited Dagenham, England
- Silymarin (Micro Labs Limited 92, Sipcot, Hosur-635 126 India).

Experimental design

Twenty-four rats were divided into six Groups (I–VI) of four rats each. Group I was control, administered distilled H_2O (2 ml/kg) while Groups II-VI were treatment groups. Liver toxicity was induced in rats by the administration of CCl_4 (1 ml, 1:1 solution: olive oil) as reported by Ikyembe *et al.* (2014). Groups II, III, and IV were administered MFMP (500 mg/kg 1000 mg/kg and 1500 mg/kg, respectively) and Group V was administered Silymarin (100 mg/kg) as the reference drug, for a period of 14 days. After 12 h of the last MFMP or Silymarin administration; on the 15th day, all rats in the treatment groups (Groups II–VI) were administered a single dose of CCl_4. All administrations were via the oral route. After 12 h of CCl_4 administration, rats were humanely sacrificed under chloroform anesthesia and liver harvested for routine (hematoxylin and Eosin, H and E, staining) histological tissue processing for light microscopy and blood samples collected in 5 ml plain sample bottles from jugular vein for biochemical analysis of liver serum enzymes (aspartate aminotransferase, aspartate transaminase [AST]; alanine aminotransferase, alanine transaminase [ALT], and alkaline phosphatase [ALP]) analysis.

Phytochemical screening

Phytochemical analysis of MFMP was conducted in the Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, ABU, Zaria. The method of Trease and Evans (2002) for phytochemical screening was adopted.

Histological studies

The harvested liver organs of the rats were fixed in 10% buffered formalin. Tissues were processed routinely (H and E) for light microscopic examination in the Histology laboratory Unit of the Department of Human Anatomy, ABU, Zaria.

Biochemical studies

Biochemical analysis of serum liver enzymes (AST, ALT, and ALP) were assayed in the Department of Chemical Pathology, ABU Teaching Hospital, Shika using assay kits according to the manufacturer's instructions.

Data analysis

Results obtained were analyzed using the statistical software, Statistical Package for Social Scientist (SPSS version 18.0, SPSS Inc., 233 South Wacker Drive, 11

th Floor, Chicago, IL 60606-641, USA) and Microsoft Office Excel 2010 for charts. Results were expressed as mean ± standard error of the mean and the presence of significant differences among means of the groups was determined using one-way ANOVA with least significance difference *post hoc test* for significance. Values were considered significant when value of $P \leq 0.05$.

Results

Phytochemical screening

Qualitative phytochemical analysis of MFMP produced positive reaction for each of the following secondary metabolites: carboxylic acid, tannins, saponin, flavonoids, terpenoids, and steroid, whereas anthroquinone and glycosides were absent.

Histological studies

Light microscopic examination of liver sections of the rats in the control group revealed normal histoarchitecture of the liver parenchyma; the characteristic appearance of hepatic lobule unit: centrilobular venules (central veins), array of interconnected plates of hepatocytes (constituting two-thirds of the mass of the liver) radiating from the central vein, separated by vascular spaces (sinusoids) and portal tract/triad (hepatic portal vein, hepatic vein, and bile duct) [Figure 1a].

The liver sections of the rats treated with CCl_4 only (Group VI) revealed severe histoarchitectural distortion of the liver parenchyma which manifested as diffused necrosis of hepatocytes with pyknotic nuclei and hepatocellular vacuolation, infiltration of inflammatory cells and central vein congestion [Figure 1b]. The liver sections of Silymarin + CCl_4-treated rats showed normal cytoarchitecture of the liver with mild histoarchitectural distortion; infiltration of inflammatory cells and localized hepatocellular vacuolations, when compared with the severe distortions observed with CCl_4 only-treated group [Figure 1c]. The liver sections of MFMP (500 mg/kg) + CCl_4 and MFMP (1000 mg/kg) + CCl_4-treated rats, showed normal cytoarchitecture with mild histoarchitectural distortion, such as sinusoidal dilatation and infiltration of inflammatory cells [Figure 1d and e]. In MFMP (1500 mg/kg) + CCl_4-treated rats, the liver sections of rats revealed histoarchitectural distortion of liver parenchyma manifesting as hepatocellular necrosis, vacuolations, and sinusoidal dilations. However, observed distortions were not as severe when compared to CCl_4-treated group [Figure 1f].

Biochemical studies

Serum liver enzymes (AST, ALT, and ALP) were analyzed and revealed the following: relative to the control, elevated levels of AST were observed in all the treated groups, especially ($P < 0.05$) in CCl_4–treated group [Figure 2]. No remarkable ($P > 0.05$) difference

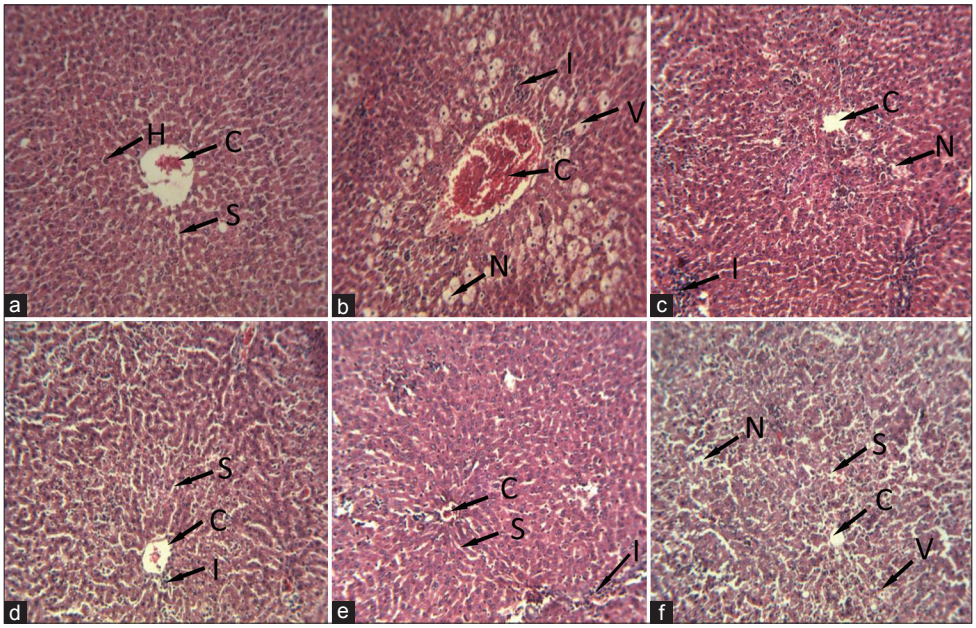

Figure 1: Micrograph of liver sections of Wistar rat. H and E, (Mag × 100). (a) Control (2 ml/kg distilled H_2O), showing normal histology. Central vein (c); Hepatocyte (h); Sinusoid (s). (b): CCl_4-treated, showing histoarchitectural distortion. Congested central vein (cC); Necrosis (n); Inflammatory cell infiltration (i); Hepatocellular vacuolation (v). (c): Silymarin (100 mg/kg) + CCl_4-treated, showing mild distortion of the histoarchitecture of the liver. Inflammatory cells infiltration (i); Necrosis (n). (d), Methanol fruit pulp extract of *Musa paradisiaca* (500 mg/kg) + CCl_4-treated, showing mild histoarchitectural distortion of the liver. Central vein (c); Inflammatory cells infiltration (i); Sinusoid (s). (e): Methanol fruit pulp extract of *Musa paradisiaca* (1000 mg/kg) + CCl_4-treated, showing mild distortion of the histoarchitecture of the liver. Central vein (c); Sinusoids (s); Inflammatory cells infiltration (i). (f) Methanol fruit pulp extract of *Musa paradisiaca* (1500 mg/kg) + CCl_4-treated, showing mild distortion of the histoarchitecture of the liver. Central vein (c); Necrosis (n); Sinusoidal dilation (s); Hepatocellular vacuolation (v)

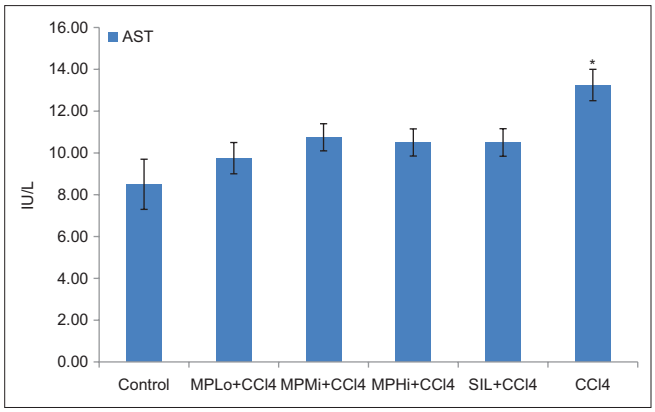

Figure 2: Effect of methanol fruit pulp extract of *Musa paradisiaca* on serum aspartate transaminase levels in Wistar rats. *n* = 4; means ± standard error of the mean. One-way ANOVA least significant difference *post hoc* test: * = *P* < 0.05 significant difference when compared with the control. Control: 2 ml/kg (distilled water); MPLo, MPMi and MPHi (methanol fruit pulp extract of *Musa paradisiaca* 500 mg/kg, 1000 mg/kg and 1500 mg/kg, respectively); SIL: Sylimarin (100 mg/kg); CCl_4: Carbon tetrachloride (1 ml)

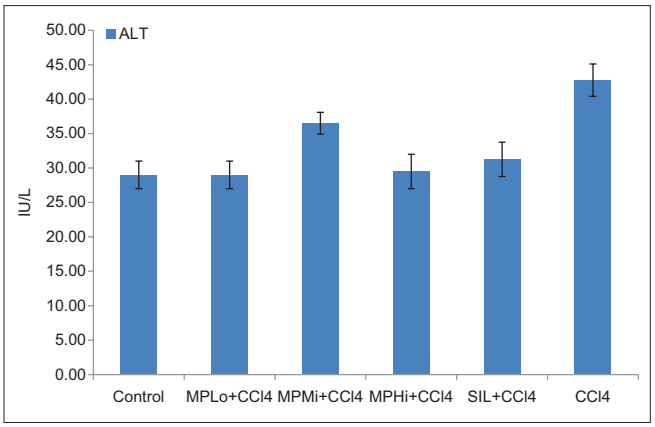

Figure 3: Effect of methanol fruit pulp extract of *Musa paradisiaca* on serum alanine transaminase levels in Wistar rats. *n* = 4; means ± standard error of the mean. One-way ANOVA least significant difference *post hoc* test: *P* >0.05 when compared with the control. Control: 2 ml/kg (distilled water); MPLo, MPMi, and MPHi (methanol fruit pulp extract of *Musa paradisiaca* 500 mg/kg, 1000 mg/kg, and 1500 mg/kg, respectively); SIL: Sylimarin (100 mg/kg); CCl_4: Carbon tetrachloride (1 ml)

in levels of ALT was observed in all the treated groups when compared to the control [Figure 3].

Remarkable ($P < 0.01$) up-regulation of ALP level was observed in all the treated groups, except MFMP (1,500 mg/kg) + CCl_4 and Silymarin (100 mg/kg) + CCl_4–treated groups when compared to the control. ALP levels of MFMP (1,500 mg/kg) + CCl_4 and Silymarin (100 mg/kg) + CCl_4–treated groups were remarkably ($P < 0.05$) decreased relative to CCl_4 only treated group [Figure 4].

Discussion

In this study, preliminary qualitative phytochemical screening of MFMP was carried out and hepatoprotective activity was evaluated employing histological examinations of liver sections and biochemical analysis of serum liver enzymes of CCl_4-induced hepatotoxicity model in Wistar rats.

In this study, phytochemical analysis of the plant extract, MFMP, revealed the presence of secondary metabolites such as, flavonoids, tannins terpenoids and steroid, and saponin, which is in accordance to reported constituents present in various parts of the plant (Abbas *et al*., 2016; Sundaram *et al*., 2018). Phytochemical constituents of natural agents with antioxidant properties have been reported to play pertinent roles in hepatoprotection from noxious exogenous substances (Swathi *et al*., 2011; Sarian *et al*., 2017). The presence of phenolic compound, such as flavonoid, has been established to have free radical scavenging and antioxidant activities (Heim *et al*., 2002; Seyoum *et al*., 2006).

CCl_4 is an established potent hepatotoxic compound, frequently used as a model of experimental hepatotoxicity (Ahsan *et al*., 2009; Cheng *et al*., 2013) which presents with features analogous to human acute hepatitis (Li *et al*., 2015) and a useful model system for assessing of hepatoprotective activity of plant agents (Adewusi and Afolayan, 2010; Huang *et al*., 2018). Several researches have demonstrated the critical role of oxidative stress in the pathophysiology of CCl_4-induced hepatotoxicity (Kepekci *et al*., 2013; Hafez *et al*., 2014). Within biological system, CCl_4 undergoes enzymatic activation, majorly $CYP2E_1$, into the trichloromethyl free radical (CCl_3) within the endoplastic reticulum membrane, which is followed by chloromethylation, saturation, peroxidation, and progressive destruction of the unsaturated fatty acid of the endoplasmic reticulum membrane phospholipids (Peng *et al*., 2009; Abbas *et al*., 2016). These processes are known as lipid peroxidation, leading to functional, and structural disruption of hepatocytes (Hogade *et al*., 2010; Ali *et al*., 2014).

In this study, liver parenchymal histoarchitectural distortion consequent to CCl_4 administration manifested as severe hepatocellular necrosis and vacuolation, infiltration of inflammatory cells and central vein congestion. These are an indication of oxidative damage (Padhy *et al*., 2007). Findings are in accordance with the reports of previous researches on histopathological changes following CCl_4 treatment in rodents (Mahli *et al*., 2015; Huda and Mosaddik, 2018). Necrosis is a pathological form of cell death sequential to exposure to abnormal stressors, such as chemical injury or toxin. Necrotic cells, unable to maintain membrane integrity, leak out their content which could elicit inflammation in the surrounding tissue (Kumar *et al*., 2009). Inflammation, infiltration of the inflammatory cells relative to CCl_4 intoxication as observed, is a protective response targeted to rid the biological system of cell

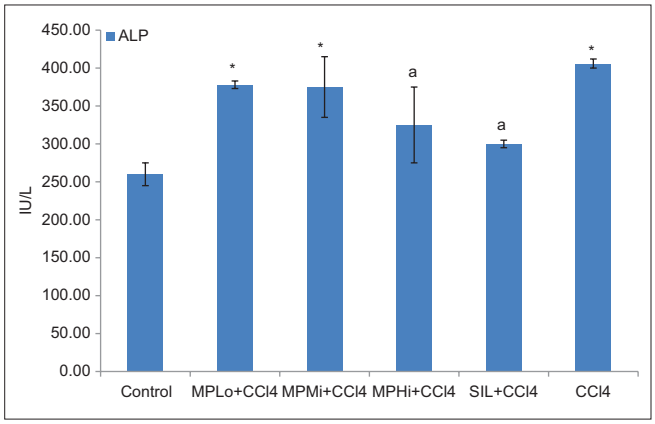

Figure 4: Effect of methanol fruit pulp extract of *Musa paradisiaca* on serum alkaline phosphatase levels in Wistar rats. $n = 4$; means ± standard error of the mean. One way ANOVA least significant difference *post hoc test:* *= $P < 0.05$ difference when compared with the control. a = $P < 0.05$ difference when compared with CCl_4. Control: 2ml/kg (distilled water); MPLo, MPMi and MPHi (methanol fruit pulp extract of *Musa paradisiaca* 500 mg/kg, 1,000 mg/kg and 1,500 mg/kg, respectively); SIL: Sylimarin (100 mg/kg); CCl_4: Carbon tetrachloride (1 ml)

injury inducing factors (e.g., toxin) and consequence of such injury (necrotic cells and tissues) (Kumar *et al*., 2009). Congestion of central vein relative to CCl_4 intoxication as observed, is a local increase in the volume of blood as a consequence of impaired outflow from the hepatic tissue (Cotran *et al*., 2005).

Silymarin is an established antioxidant used in the evaluation of hepatoprotective activity of potential nutraceuticals (Flora *et al*., 1998; Shalan *et al*., 2005; Pradhan and Girish, 2006; Shaker *et al*., 2010). Mild histoarchitectural changes in the liver sections observed in Silymarin-treated rats is indicative of the ameliorative effect of Silymarin against chemically-induced hepatotoxicity. Silymarin has anti-inflammatory potentials, act as a radical scavenger, thus, protecting membrane permeability and alter drug-induced histopathological changes (Song *et al*., 2006; Mahli *et al*., 2015).

The development and progression of liver-related diseases has been described in relation to free radicals generation and oxidative stress (Zhu *et al*., 2012; Jadeja *et al*., 2017). Thus, oxidative liver injury is commonly treated with the use of antioxidants (Sundaram *et al*., 2018). Mild histoarchitectural changes; histo-parenchymal preservation of the liver sections observed in MFMP-treated rats, especially with 1000 mg/kg dose, is suggestive of the protective effect of MFMP against chemically-induced hepatotoxicity. Consumption of antioxidants is an essential means of preventing or delaying the appearance of liver diseases (Ganesan *et al*., 2018). Mahmood *et al.* (2010) and Nirmala *et al.* (2012) reported the presence of antioxidants, tannins, and flavonoids, in *M. paradisiaca* to provide plant's hepatoprotective activity. Flavonoid have been reported as antioxidant in plants, having free radicals scavenging activity and is able to reduce the formation of free radical, chelate metal catalysts, down-regulate alpha-tocopherol radicals, inhibit oxidases, and up-regulate the levels of antioxidant enzymes in plasma (Pietta, 2000; Heim *et al*., 2002).

Plasma concentration levels of liver enzymes are important biochemical indicators of liver functionality (Zhao *et al*., 2018). During hepatic damage, cellular enzymes such as AST, ALT, and ALP and bilirubin leak into the serum resulting in up-regulation of their serum concentration. CCl_4 is known to induce hepatic alteration as, marked up-regulation in serum levels of ALP, AST and especially ALT which is considered the primary and specific marker of liver injury (Vozarova *et al*., 2002; Anand *et al*., 2011). In this study, remarkable up-regulation in the serum levels of AST and ALP indicated treatment-related hepatocellular damage in CCl_4 (only)-treated rat. Findings are consistent with reports on elevated hepatic biomarker levels following CCl_4 administration (Mosa and Khalil, 2015; Chen *et al*., 2018). Critical to the efficacy of any hepatoprotective agent is, its capacity to either ameliorate the harmful effect or restore the normal hepatic physiology altered by a hepatotoxin (Palanivel *et al*., 2008). Remarkable down-regulation of hepatic biomarker levels, especially ALP levels, in Silymarin-and MFMP-treated groups when compared to that of CCl_4 (only)-treated rat is suggestive of treatment-related hepatoprotective activity. Zarezade *et al.* (2018) reported oral administration of Silymarin (100 mg/kg) showed a significant decrease in the serum levels of hepatic biomarkers. Findings are in accordance with hepatoprotective activities of natural agents employed in folk medicine. Several reports on the potential benefits of medicinal plants have demonstrated the protective effects of these plants' extracts on experimentally-induced hepatotoxicity (Osman *et al*., 2011; Gong *et al*., 2012; Ikyembe *et al*., 2014). Mosa and Khalil (2015) reported a reduction of hepatic biomarkers levels in rats fed 10% peels of *M. paradisiaca*-supplemented diet. MFMP hepatoprotective effect could be mediated by its constituent phytochemical antioxidant properties targeted toward plasma membrane stabilization, thus preserving the structural integrity of hepatocytes; influence regeneration of damaged hepatocytes and repair of CCl_4-induced hepatic tissue damage (Osman *et al*., 2011). Natural agents' ability to attenuate toxin-induced hepatotoxicity is related to their intrinsic antioxidant properties, such as flavonoids and saponins (Yoshikawa *et al*., 2003; Prakash *et al*., 2008) which could have played a critical role in the hepatoprotective activity of MFMP.

Conclusion

MFMP possess potential hepatoprotective activity against CCl_4-induced hepatotoxicity that manifested as

histo-parenchymal preservation of liver sections and decrease in serum liver enzymes levels in Wistar rats. Hepatoprotective activity is probably consequent to the antioxidant activities of constituent phytochemicals.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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