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Original article

Protective effect of lyophilized sapodilla (*Manilkara zapota*) fruit extract against CCl_4-induced liver damage in rats

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ABSTRACT

The tropical fruit sapodilla (*Manilkara zapota* syn. *Achras zapota*) is a rich source of nutrients, minerals and a myriad of bioactive phytochemicals such as flavonoids and catechins. Pharmacologically, sapodilla has been shown to exhibit anti-bacterial, anti-parasitic, anti-fungal, antiglycative, hypocholesterolemic and anti-cancer effects. However, its influence on hepatic tissue and serum lipids remains obscure. To address this, we used an *in vivo* model of liver damage to elucidate the effect of lyophilized sapodilla extract (LSE) treatment in carbon tetra chloride (CCl_4) intoxicated rats. Exposure of CCl_4 resulted in elevation of serum biomarkers of liver damage (aspartate transaminase, alanine aminotransferase, γ-glutamyl transferase and alkaline phosphatase), bilirubin and dysregulation of serum lipid profile (cholesterol and triglycerides). These effects were significantly and dose-dependently reversed by LSE treatment (250 and 500 mg/kg). Administration of LSE also reduced the structural damage caused by CCl_4 in the liver. Furthermore, determination of oxidative stress parameters (malondialdehyde and non-protein sulfhydryls) revealed that LSE treatment mitigated CCl_4-triggered modulation of both molecules. LSE also showed a strong antioxidant activity in 2,2-diphenyl-1-picrylhydrazyl (DPPH) and β-carotene-linoleic acid assays. In conclusion, the present study discloses the hepatoprotective and lipid-lowering effects of lyophilized sapodilla extract against CCl_4-induced liver damage, an effect, at least in part, mediated by its antioxidant activity.

1. Introduction

The liver plays a crucial biological role in metabolism and detoxification (Dutta et al., 2018). Hepatic injury may be triggered by alcohol consumption, viral infections and a myriad of xenobiotics. The chemical agent carbon tetrachloride (CCl_4) mediates redox-sensitive hepatotoxicity via generation of free radicals and subsequent lipid peroxidation (Lin et al., 2019; Suk and Kim 2012). Carbon tetrachloride has been used to simulate hepatic injury in laboratory animals and is a widely accepted *in vivo* model to study the pharmacological effects of hepatoprotective molecules (Liu et al., 2014; Zhang et al., 2013). Due to various side-effects of conventional drugs, there has been a renewed interest in exploiting the biological effects of nutraceuticals. Various bioactive molecules derived from natural sources such as flavonoids and catechins possess hepatoprotective effects (Okaiyeto et al., 2018). Consumption of fruits and vegetables may, thus, be therapeutically beneficial during the course of both acute and chronic liver injury.

The tropical fruit sapodilla (*Manilkara achras* (Mill) Fosb., syn *Achras sapota* L.) family: Sapotaceae is rich in nutrients and minerals, and is widely consumed fresh but its pulp is also incorporated into various culinary preparations (Lasekan and Abbas, 2012; Singh

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and Bothara 2014). Sapodilla is a good source of vitamins and minerals including vitamin A, vitamin B complex, vitamin C, carotene, calcium, potassium, magnesium and phosphorus (Srivastava et al., 2017). The leaves and fruits of sapodilla have been used in traditional medicine to treat several diseases (Kaneria et al., 2009; Lans et al., 2000; Ortiz et al., 2007). Sapodilla is enriched with phytochemicals such as catechin, epicatechin, leucocyanidin, leucodelphinidin, leucopelargonidin, and gallic acid (Ma et al., 2003; Wang et al., 2012). These bioactive constituents confer sapodilla with robust antioxidant properties (Mahattanatawee et al., 2006; Moo-Huchin et al., 2014; Shui et al., 2004). Sapodilla was shown to exhibit anti-bacterial (Rakholiya et al., 2014), anti-fungal (Nair and Chanda 2008), antiglycative (Shakthi Deve et al., 2014), hypocholesterolemic (Fayek et al., 2012), anti-cancer (Osman et al., 2011), and anti-parasitic (Rajakumar and Abdul, 2012) effects. Sapodilla was recently shown to trigger apoptosis in various cell lines by activating the mitochondrial pathway (Srivastava et al., 2014). In addition to these beneficial effects, sapodilla plums contain allergen proteins (Hegde et al., 2014), which may induce food-associated allergies (Hegde and Venkatesh 2002). Despite these pharmacological effects of sapodilla, the modulatory effects of sapodilla on liver injury remain elusive.

In the present study, we investigated the effect of lyophilized sapodilla extract (LSE) on various markers of liver damage and elucidated the role of redox mechanisms in LSE-mediated hepatoprotection in CCl_4-induced hepatic injury in rats.

2. Materials and methods

2.1. Plant material and dosage preparation

The fresh sapodilla fruits were purchased from a local vegetable and fruit vendor. The fruits were thoroughly washed with tap water, cut into small pieces, seeds removed and the juice obtained using an electric blender. The juice was lyophilized to get the dry powder using a lyophilizer. The freeze dried powder (LSE) was dissolved in distilled water and used in all the experiments, except for measuring the free radical scavenging and antioxidant activities, where methanolic extracts were used.

2.2. Animals

Wistar albino male rats, 180 ± 20 g were used in this study. The animals were caged individually in hygienic conditions and kept in a controlled environment with a 12 h light–dark cycle at 22 ± 3 °C for a week before starting the experiment. The animals had free access to purina chow diet and water *ad libitum*. The study protocol was approved by the Institutional Review Board (No. RAKMHSU-REC-08-2019-F-P).

2.3. Chemicals and reagents

All chemicals were procured from Sigma (Sigma-Aldrich, St. Louis, MO, USA). United and/or Roche Diagnostics kits (AST, ALT, ALP, Total Proteins, Cholesterol, Triglycerides, HDL, LDL, etc.) were used to determine the biochemical parameters on Reflotron Plus Analyzer (Roche Diagnostics GmbH, Mannheim, Germany) and on a Shimadzu UV mini 1240 spectrophotometer (Shimadzu Europe, Milano, Italy) for the measurement of MDA, NP-SH, and total proteins.

2.4. Carbon tetrachloride-induced hepatic toxicity and drug treatment

Five groups (I–V) of animals (N = 6) were used. Group I served as normal control and received all the vehicles via respective routes. Group II received only carbon tetrachloride (CCl_4) as intoxicated control. Groups III and IV were assigned as test groups. Group III and IV were pre-treated with LSE at doses of 250 and 500 mg/kg orally, daily for 17 days, whereas group V was pre-treated with silymarin (SIL) at a dose of 10 mg/kg b.w. orally, for 17 days; this group was used as a positive control. A dosage of 250 mg/kg for rat will be equivalent to 43 mg/kg for human according to dose conversion based on metabolically active mass of animals (Khan 2003). Silymarin is a mixture of flavonolignans extracted from the milk thistle [Silybum marianum (L.) Gaertneri] and is a potent hepatoprotective agent (Féher and Lengyel 2012). At the 16th day, groups II–V received CCl_4 in liquid paraffin (1:1) at a dose of 1.25 mL/kg/rat intraperitoneally. After 24 h, following CCl_4 challenge, rats were sacrificed and blood was collected by cardiac puncture, serum separated and stored at −80 °C until analysis. The liver was removed for biochemical and histological assessment.

2.5. Analysis of biomarkers for liver function

The serum biomarkers of liver function including alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl transferase (GGT), alkaline phosphatase (ALP), and bilirubin were analysed by commercial kits supplied by United Diagnostic Industry, KSA.

2.6. Determination of serum lipids

Serum total cholesterol (TC), high-density lipoprotein cholesterol (HDL), and triglycerides (TG) were determined by the Roche diagnostics kits (Roche Diagnostics GmbH, Mannheim, Germany) using the previously reported protocols. The levels of low-density lipoprotein cholesterol (LDL) and very low-density lipoprotein cholesterol (VLDL) were computed using the following equations: LDLC = TC-HDL-VLDL; VLDL = TG/5.27.

2.7. Determination of malondialdehyde (MDA)

A modified method of Utley et al. (1967) was used. The liver was homogenized (10% w/v, 1 mL) in 0.15 M KCl at 4 °C. After incubating (37 °C for 3 h) the homogenate, equal volume of trichloroacetic acid (TCA, 10%) was added and the mixture was centrifuged at 800*g* for 10 min. An aliquot (1 mL) was then mixed with equal volume of 0.67% thiobarbituric acid (TBA) and the tubes were kept in a boiling water bath for 10 min. After cooling at room temperature, the contents were diluted with 1 mL distilled water, and the absorbance was recorded at 535 nm. The level of MDA (nmol/g wet tissue) was calculated using the equation:

$$MDA(nmol/g) = \frac{Abs\ of\ sample \times dilution\ factor}{Slope\ of\ standard\ curve}$$

2.8. Analysis of nonprotein sulfhydryls (NP-SH)

The method of Sedlak and Lindsay (1968) was used for the estimation of NP-SH levels in rat liver. The tissue was homogenized in ice-cold ethylenediaminetetraacetic acid (EDTA, 0.02 M). The homogenate (1 mL) was mixed with equal volume of distilled water and 0.25 mL of trichloroacetic acid (50%), contents shaken for 10 min and centrifuged. An aliquot of supernatant (0.5 mL) was mixed with 1.0 mL ofTris buffer (0.4 M, pH 8.9) and 0.25 mL of 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB). After shaking the mixture, the absorbance was read at 412 nm against a reagent blank. The level of NP-SH in liver tissue was calculated according to the following equation:

$$NP-SH(nmol/g) = \frac{Abs\ of\ sample \times Conc\ of\ standard(nmol/g) \times dilution\ factor}{Abs\ of\ standard}$$

2.9. Analysis of total proteins (TP)

Serum TP was measured using a commercial kit (Crescent Diagnostics, Jeddah, Saudi Arabia) according to manufacturer's instructions. The Absorbance of coloured complex at 546 nm was directly proportional to total protein levels in the sample, which were calculated using the equation: Absorbance of sample/Absorbance of standard) × concentration of standard.

2.10. Free radical scavenging assay

The ability of LSE to scavenge the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was evaluated as reported earlier (Brand et al., 1995). The LSE was re-dissolved in methanol and serial concentrations (10–1000 μg/mL) of the extract, 125 μL DPPH (1.0 mM) and 375 μL solvent (methanol) were added. The contents were incubated at 25 °C for 30 min and the decrease in absorbance was measured at 517 nm. The free radical scavenging activity of LSE was calculated from the following equation:

$$\%\ of\ radical\ Scavenging\ activity = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

2.11. β-Carotene-linoleic acid assay

We used β-carotene bleaching method (Mothana et al., 2012) for measuring the antioxidant activity of LSE. One milliliter of a 0.2 mg/mL β-carotene solution was mixed with 0.02 mL of linoleic acid and 0.2 mL of Tween-20 followed by removal of organic solvent (chloroform) by heating at 40 °C in a rotary evaporator. The residual mixture was diluted with 10 mL of distilled water and briefly mixed to form an emulsion. The blank was prepared without using β-carotene. The control contained 0.2 mL of 80% methanol instead of extract. An aliquot (5 mL) of the emulsion was mixed with 0.2 mL of sample at the concentration of 1.0 mg/mL. Rutin (1.0 mg/mL) was used as a positive control. The tubes were kept in a water bath at 40 °C for 2 h and the absorbance was recorded at 470 nm at 15 min intervals, using a UV–visible spectrophotometer (UV mini-1240, Shimadzu, Japan). The following equation was used to calculate the percent antioxidant activity:

$$\%\ \text{antioxidant activity} = 1 - (Abs_0 - Abs_t)/(Abs^{\circ}_0 - Abs^{\circ}_t) \times 100$$

where Abs_0 and Abs°_0 are absorbance values recorded at zero time for sample and control, respectively. Abs_t and Abs°_t are the absorbance readings for sample and control, respectively, measured at 120 min.

2.12. Histopathology

The liver tissue samples were fixed in 10% neutral buffered formalin for 24 h and processed using a tissue processor. The processed tissue was then embedded in paraffin blocks and sections (5 μm thickness) were cut by a rotary microtome. Sections were stained with haematoxylin and eosin (H & E) and examined under a light microscope for histopathological changes.

2.13. Statistical analysis

The data were analysed by one-way analysis of variance (ANOVA) followed by post-hoc Dunnett's multiple comparison test, using the statistical package, SPSS version 10. P values less than 0.05 were considered significant.

3. Results

We explored the effect of LSE on serum biomarkers indicative of hepatic injury. Administration of CCl_4 (1.25 mL/kg) induced a significant elevation of the serum biomarkers AST, ALT, GGT, ALP and bilirubin (Table 1). This effect was significantly blunted in rats administered with LSE (250 and 500 mg/kg) in a dose-dependent manner (Table 1). The positive control silymarin-treated group similarly showed a significant reduction in CCl_4-triggered upregulation of serum biomarkers of liver injury (Table 1). These data suggest that LSE administration mediates hepatoprotective effects against chemically-induced liver damage.

Administration of CCl_4 significantly increased serum cholesterol, triglycerides, LDL and VLDL while reducing serum HDL (Table 2). These effects were significantly and dose-dependently mitigated by LSE. Similar to LSE, the positive control silymarin significantly curtailed CCl_4-induced changes in serum lipid profile (Table 2), thus, providing additional evidence of the hepatoprotective effects of LSE on CCl_4–triggered hepatocellular damage.

In view of the modulatory effect of LSE on CCl_4-induced hepatic injury, an additional series of experiments was performed to elucidate the underlying mechanism. We determined whether LSE treatment affected CCl_4-stimulated redox imbalance in the rat liver. We estimated hepatic MDA levels. As illustrated in Fig. 1, CCl_4 treatment significantly increased hepatic MDA levels, an effect that was significantly and dose-dependently curtailed by LSE and the positive control silymarin. These results suggest the participation of antioxidant effects of LSE in ameliorating CCl_4-induced liver damage.

To corroborate the aforementioned antioxidant effects of LSE on the hepatic tissue, we determined hepatic non-protein sulfhydryl levels. As depicted in Fig. 2, CCl_4 treatment significantly reduced NP-SH levels in rat liver, an effect significantly attenuated by both LSE and silymarin, suggesting that LSE-induced hepatoprotection in CCl_4-treated rats is mediated by its antioxidant effects. Further experiments addressed the effect of LSE on CCl_4–induced decrease in total hepatic protein. As shown in Fig. 3, CCl_4-triggered decreased hepatic protein levels were significantly and dose-dependently reversed by both LSE and silymarin treatment.

The potential antioxidant activity of the LSE was investigated on the basis of DPPH radical scavenging activity and of inhibition of linoleic acid oxidation. As demonstrated in Table 3, LSE was able to reduce the stable free radical DPPH (purple) to the yellow-colored DPPH-H in a concentration dependent manner. In

Table 1
Effect of lyophilized sapodilla extract (LSE) on serum marker enzymes in control and experimental rats.

Treatment	AST (U/L)	ALT (U/L)	GGT (U/L)	ALP (U/L)	Bilirubin (mg/dL)
Control	91.71 ± 4.20	33.86 ± 2.30	3.48 ± 0.34	332.1 ± 10.06	0.54 ± 0.01
CCl_4	296.0 ± 9.76**	236.33 ± 7.85**	11.71 ± 0.75**	560.1 ± 10.28**	2.42 ± 0.05**
LSE-250 + CCl_4	287.0 ± 8.00**	233.50 ± 2.88**	12.03 ± 0.41**	522.0 ± 7.04**#	2.11 ± 0.12**#
LSE-500 + CCl_4	291.8 ± 5.51**	202.16 ± 4.17**#	9.46 ± 0.24*	516.5 ± 8.92**#	2.06 ± 0.14**#
Silymarin-10 + CCl_4	196.6 ± 6.44*##	107.46 ± 4.79*##	5.10 ± 0.13##	413.8 ± 9.20*##	1.02 ± 0.06*##

All values represent mean ± SEM. *P < 0.01 and **P < 0.001 verses control; #P < 0.05 and ##P < 0.01 versus CCl_4 only group.

Table 2
Effect of lyophilized sapodilla extract (LSE) on serum lipoproteins in control and experimental rats.

Treatment	Cholesterol (mg/dL)	Triglycerides (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
Control	89.6 ± 3.03	65.9 ± 3.36	53.40 ± 2.44	23.00 ± 2.44	13.19 ± 0.67
CCl_4	200.3 ± 8.63**	157.1 ± 4.23**	26.80 ± 1.01*	142.10 ± 8.69**	31.43 ± 0.84*
LSE-250 + CCl_4	151.5 ± 5.59*##	129.8 ± 4.26*#	39.85 ± 2.22##	86.68 ± 6.81*##	25.96 ± 0.85*#
LSE-500 + CCl_4	123.0 ± 3.72##	101.4 ± 5.02*##	42.03 ± 1.45##	60.68 ± 5.01*##	20.28 ± 1.00##
Silymarin-10 + CCl_4	130.8 ± 6.17##	131.6 ± 5.05*##	36.91 ± 1.13##	67.58 ± 6.48*##	26.33 ± 1.01*#

All values represent mean ± SEM. *P < 0.01 and **P < 0.001 verses control; #P < 0.05 and ##P < 0.01 versus CCl_4 only group.

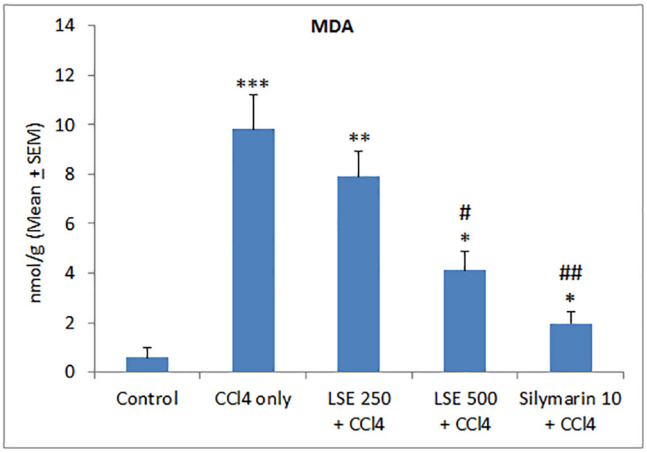

Fig. 1. Effect of lyophilized sapodilla extract (LSE) on liver tissue lipid peroxidation (MDA) levels in carbon tetrachloride (CCl_4) intoxicated rats. *P < 0.05, **P < 0.01 and ***P < 0.001 versus control; #P < 0.01 and ##P < 0.001 versus CCl_4 only group.

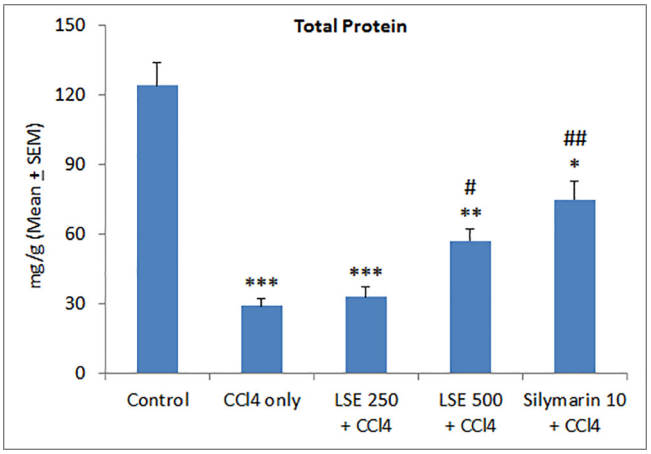

Fig. 3. Effect of lyophilized sapodilla extract (LSE) on liver tissue total protein levels in carbon tetrachloride (CCl_4) intoxicated rats. *P < 0.05, **P < 0.01 and ***P < 0.001 versus control; #P < 0.05 and ##P < 0.01 versus CCl_4 only group.

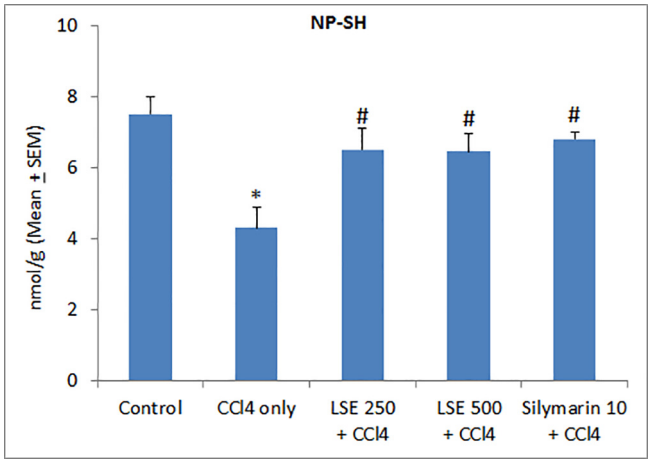

Fig. 2. Effect of lyophilized sapodilla extract (LSE) on liver tissue non-protein sulfhydryl (NP-SH) levels in carbon tetrachloride (CCl_4) intoxicated rats. *P < 0.01 versus control; #P < 0.05 versus CCl_4 only group.

β-carotene/linoleic acid antioxidant potential test, LSE was also able to inhibit the discoloration of β-carotene at a concentration of 1.0 mg/mL, showing the total antioxidant capacity as 74.4% as compared with the positive control, rutin (90.9%) (Table 3).

The results of histopathological assessment of hepatic tissues (Fig. 4) correlated with the above mentioned biochemical findings. Group A (control group) showed normal hepatic tissues. Group B (CCl_4-intoxicated rat) revealed severe fatty degeneration, ballooning, and inflammatory cell infiltration in addition to massive hemorrhages. Group C (250 mg/kg LSE) showed focal degeneration concomitant with vacuolization and patchy inflammatory cells infiltrations were observed. Group D (500 mg/kg LSE) showed insignificant change in the form of mild cellular degeneration. Group E (10 mg/kg silymarin) had normal appearance of hepatocytes population and portal triad architecture.

4. Discussion

Nonalcoholic fatty liver disease (NAFLD) refers to a wide spectrum of liver damage that spans from mild steatosis to nonalcoholic steatohepatitis (NASH), fibrosis, and cirrhosis (Lewis and Mohanty 2010). This disease is a major cause of elevation of liver enzymes. The global prevalence of NAFLD is about 25% and continues to raise worldwide (Mundi et al., 2019). A recent Swiss study has reported that the incidence of advanced liver disease is estimated to increase by approximately 40% by 2030 (Goossens et al., 2019). It is important to note that NAFLD is an independent risk factor for drug-related acute hepatitis; that means NAFLD patients are at higher risk for acute hepatotoxicity due to medications for other illnesses (Tarantino et al., 2007). An animal study also showed that nonalcoholic fatty liver sensitizes rats to CCl4 hepatotoxicity (Donthamsetty et al., 2007). Thus, searching for novel prophylactic and therapeutic hepato-protective agents with no side effects is of prime concern.

In this study we used CCl_4 treated rat model of liver damage. Besides elevating the levels of liver enzymes, blood glucose, non-esterified fatty acids in the serum and glycogen in liver, CCl_4 also significantly decreases phosphorylase activity in liver and increases carbohydrate intolerance and insulin resistance (Sadek et al., 2016). Administration of CCl_4 causes liver steatosis (Cui et al., 2017) and therefor it has been used to develop experimental models of NAFLD (Kanai et al., 2016; Chheda et al., 2014) and NASH (Tsuchida et al., 2018; Owada et al., 2018); the latter condition is a type of NAFLD with inflammation and liver cell damage along with

Table 3
Free radical scavenging activity and antioxidant activity of the methanolic extract of sapodilla as compared to other antioxidants.

	Radical scavenging activity (%)					Total antioxidant activity (%)
	10 (μg/mL)	50 (μg/mL)	100 (μg/mL)	500 (μg/mL)	1000 (μg/mL)	1000 (μg/mL)
Sapota	7.8	28.5	54.1	71.2	85.5	74.4
Ascorbic acid	16.8	80.2	90.1	92.8	94.9	–
Rutin*	–	–	–	–	–	90.9

*Rutin is a plant pigment (flavonoid) with potent antioxidant properties. It was used as a positive control.

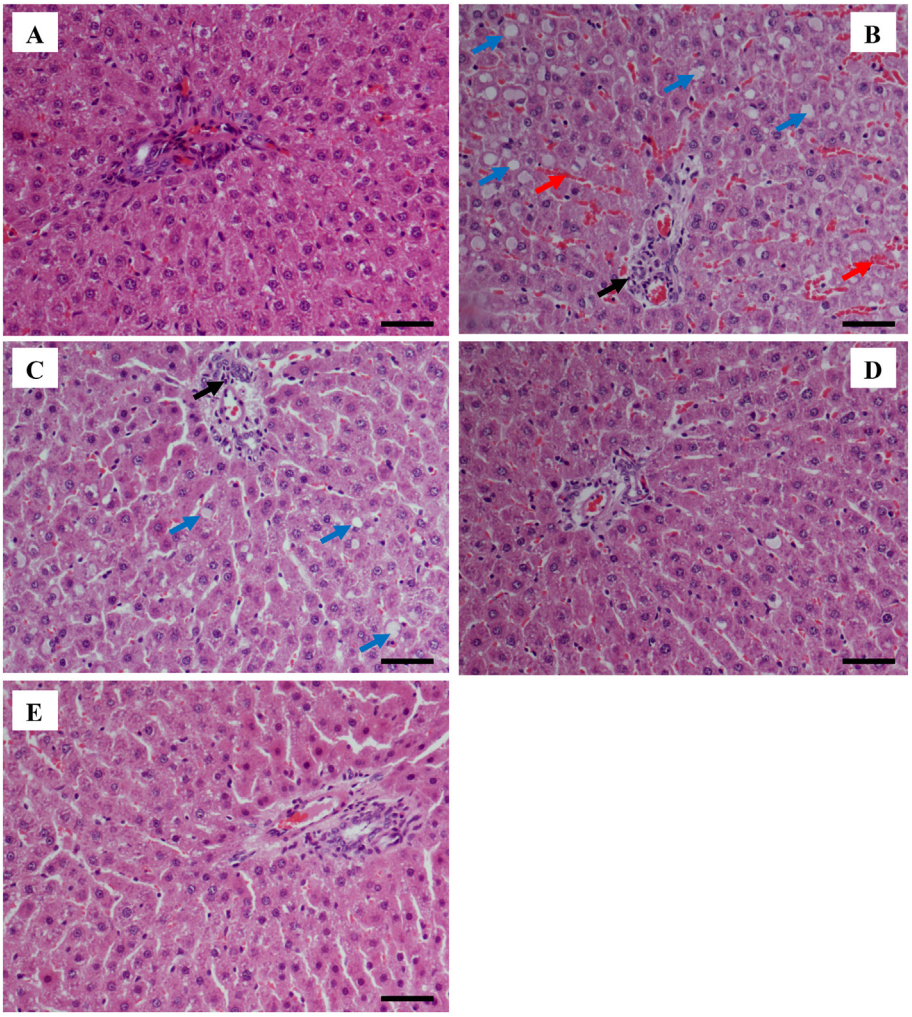

Fig. 4. Histopathology of livers of rats from different treatment groups. (A) Control group showing a normal structure; (B) CCl_4-intoxicated rat showing abundant fatty deposition and ballooning (blue arrow), inflammatory cells infiltration (black arrow) and massive hemorrhage (red arrow); (C) LSE (250 mg/kg) group showing mild steatosis, focal vacuolization (blue arrow) and inflammatory cells (black arrow); (D) LSE (500 mg/kg) group showing some mild pathological changes; (E) silymarin (10 mg/kg) showing normal appearance of hepatocytes. Scale bar = 100 μm.

fat accumulation. A mass spectrometry study showed that the damage to rat liver tissue by CCl_4 is accompanied with the alterations in diacylglycerols, cholesterol and fatty acids, such as linoleic and oleic acids (Otrubova et al., 2018).

This study showed that exposure of CCl_4 significantly elevated the levels of serum aminotransferases and other liver enzymes indicating the hepatic damage and severe liver dysfunction caused by CCl_4 (Table 1). Both AST and ALT are important biomarkers of liver function (Alwelaie et al., 2019; Al Asmari et al., 2018). The impairment of liver function in CCl_4 alone treated rats was accompanied with dyslipidemia (Table 2). Treatment with sapodilla significantly reversed the adverse effects of CCl_4 on liver function as well as serum lipid profile suggesting the pharmacological potential of sapodilla extract in liver disease. The flavonoids were previously shown to possess robust lipid-lowering effects (Assini et al., 2013). It is likely that the hypocholesterolmic effects of sapodilla are mediated by bioactive constituents such as flavonoids.

The hepatoprotective effect of sapodilla was also confirmed by histopathology. Administration of CCl_4 caused severe fatty degeneration, ballooning and inflammatory cell infiltration in rat liver (Fig. 4B). Fat accumulation is a hallmark of NAFLD that may lead to fibrosis, necrosis or even cirrhosis. Hepatocellular ballooning is considered as an important histological parameter in the diagnosis

of NASH and is indicative of a greater risk of disease progression (Caldwell et al., 2010). Observation of human liver biopsies has confirmed that accumulation of inflammatory cells such as macrophages is associated with progressive NAFLD (Wehr et al., 2014). Treatment of rats with sapodilla extract reversed the CCl_4-induced structural changes, indicating the regeneration of hepatic tissue.

Administration of CCl_4 alone significantly increased MDA (Fig. 1) and depleted NP-SH (Fig. 2) levels in rat liver. Both MDA and NP-SH are important indicators of oxidative stress (Reddy et al., 2015; Khan et al., 2012; Al Asmari et al., 2006; Tariq et al., 2002; Al Deeb et al., 2000). Treatment of rats with sapodilla extract as well as the antioxidant silymarin significantly reversed CCl_4-induced oxidative stress. Previous studies have shown that CCl_4 is capable of generating highly reactive free radicals that lead to oxidative stress (Jayesh et al., 2019) and inflammation (Torres et al., 2016). Several investigators have implicated oxidative stress (Chatterjee et al., 2013) and proinflammatory cytokines (Park et al., 2017; Mehta et al., 2013) in fatty liver disease. Whereas, compounds with antioxidant (Yang et al., 2015; Shah et al., 2015) and anti-inflammatory (Oró et al., 2016) properties have shown beneficial effects in protecting animals against chemical-induced liver damage. Treatment with ASB14780, a phospholipase A2 (PLA2) inhibitor, markedly ameliorated liver injury and hepatic fibrosis in CCl_4 treated mice (Kanai et al., 2016). Reduced production of eicosanoids in the liver suppressed the inflammation and oxidative stress resulting in the protection against chemical-induced liver injury in mice (Cao et al., 2013). In mouse models of acute CCl_4 and chronic methionine-choline-deficient (MCD) diet-induced hepatic injuries, treatment with anti-CXCL16 chemokine not only significantly decreased the infiltration of inflammatory macrophages into the liver but also reduced the levels of proinflammatory cytokines and reversed steatosis (Wehr et al., 2014).

Our results showed a dose-dependent free radical scavenging activity of sapodilla extract (Table 3). The anti-inflammatory activity of sapodilla has been reported earlier (Liu et al., 2019). Mechanistically, the reported biological effects of sapodilla are mediated by its bioactive constituents. Of the various polyphenols characterized from sapodilla extracts, methyl 4-O-galloylchlorogenate was shown to possess the highest antioxidant activity (Ma et al., 2003). Sapodilla is also rich in proanthocyanidins (Wang et al., 2012), which have been shown to possess a variety of beneficial health effects including hepatoprotection (Deng et al., 2012; Shin et al., 2010), an effect that could be attributed to its anti-apoptotic effect in hepatic cells (Ray et al., 1999). Ozturk et al. (2009) have shown the beneficial effects of apricot on CCl_4-induced liver steatosis and damage, due to its high radical-scavenging capacity antioxidant contents (beta-carotene and vitamin), suggesting that dietary intake of apricot can reduce the risk of liver steatosis and degeneration caused by free radicals. Exposure of CCl_4 caused significant decrease in total protein levels (Fig. 3) that may be attributed to CCl_4-induced decrease in several amino acids including the essential amino acids, leucine and isoleucine (Li et al., 2014). Supplementation of branched chain amino acids (BCAA) has been shown to modify CCl4-induced cirrhosis in rats (Jia et al., 2013).

In conclusion, the present study reveals hepatoprotective effect of lyophilized sapodilla extracts against CCl_4 induced hepatic damage, an effect, at least in part, mediated by its antioxidant activity. Since the liver injury caused by CCl_4 in rats mimics the NAFLD seen in humans, the dietary consumption of sapodilla fruit may have beneficial effects in prophylaxis or regeneration of hepatic tissue. This study was conducted in experimental animals and its relevance to humans requires additional clinical studies.

Acknowledgments

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