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In vivo protective effect of cinnamon aqueous extract in carbon tetrachloride-treated male albino rats

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ABSTRACT. The liver as an organ is important for the metabolism of drugs and toxins. However, it is not immune from environmental insults. Exposure of liver cells to carbon tetrachloride (CCl_4) results in the generation of tricholoromethyl radicals, which induce liver toxicity. This study aims at investigating the ameliorative effect of the cinnamon aqueous extract (CAE) against CCl_4-induced hepatotoxicity in male albino rats. Hepatotoxicity was induced in rats through the intraperitoneal administration of 0.5 mL kg^{-1} body weight of CCl_4. The analyses of the results obtained showed significant reduction in the levels of serum biochemical markers for 400 and 600 mg kg^{-1} bw of CAE protected rats as compared with CCl_4 group. In addition, CAE administration reversed liver tissue damaged via increased antioxidants markers. Histopathological examination of CAE treatment on rats showed improved changes to the liver damage caused by CCl_4 with no evidence of steatosis and inflammation. This result hence suggests that CAE has marked hepatoprotective and healing activities against CCl_4-induced liver damage and could serve as a suitable candidate in drug discovery for the treatment of liver toxicity.

Keywords: Hepatotoxicity; *Cinnamomum zeylanicum*; carbon tetrachloride; liver damage.

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Introduction

The liver, an important organ in the body that help sustain human life. It plays an important role to dominate numerous physiological processes which include different vital activities such as metabolism of drugs and toxins, secretion and storage (Shanmugasundaram & Venkataraman, 2006). Different stressors such as infectious agents, environmental pollutants, and hepatotoxins (carbon tetrachloride) are known to cause liver injuries (Kim et al., 2014). Carbon tetrachloride (CCl_4) is classified as one of the most harmful substances which is usually employed for the induction of liver injuries in experimental rats (Kim et al., 2014). The exposure to CCl_4 results in the generation of tricholoromethyl radicals which induce toxicity in rat liver (El-Sayed, Fouda, Mansour, & Elazab, 2015), raises the levels of hepatic lipid peroxidation and ultimately leading liver damage (Weber, Boll, & Stampfl, 2003). Reactive oxygen species in addition to the decrease in the body's inhibitory and scavenging mechanism are responsible for its increased oxidative stress. For this, the liver cells have developed a defense mechanism to combat oxidative stress such as catalase, superoxide dismutase and glutathione peroxidase as well as antioxidants substances such as vitamin E, ascorbic and GSH (Kaplowitz & Tsukamoto, 1996).

When the orally ingested chemicals and drugs inter into the body system, they firstly go to the liver organ which represent the target part for the toxicity of these substances (xenobiotics and drugs), in which they are metabolized into a substances with intermediate toxicity. Therefor a huge numbers of xenobiotics and drugs are probably described as a hepatotoxic substances (Ajith, Hema, & Aswathy, 2007).

When the liver is exposed to different kinds of stress, it becomes damaged and it is thus incapable of performing its routine functions. Due to that, an urgent medical care is essentially needed when the liver is damaged. This damage occurs gradually and accumulates over many years which leads to liver disease (Garcia-Tsao & Lim, 2009). Traditional plants are common medications employed by most of the population (Garcia-Tsao & Lim, 2009; Ghashghaii, Hashemnia, Nikousefat, Zangeneh, & Zangeneh, 2017; Goorani et al., 2018). The impression that traditional plants and their role in prevention and treatment cannot be dispensed (Najafi et al., 2017; Zangeneh, Zangeneh, Tahvilian, & Moradi, 2018).

Cinnamon (*Cinnamomum zeylanicum*) is a popular flavoring component, chiefly utilized in food manufacturing. Many studies have revealed that cinnamon has therapeutic and preventive effects against many diseases such as diabetes and glucose intolerance control (Khan, Safdar, Ali Khan, Khattak, & Anderson, 2003; Allen, Schwartzman, Baker, Coleman, & Phung, 2013), total cholesterol (Khan et al., 2003; Subash Babu, Prabuseenivasan, & Ignacimuthu, 2007), microbial diseases (Young & Oberg, 2000; Fabian, Sabol, Domaracka, & Bujnakova, 2006; Shahverdi, Monsef-Esfahani, Tavasoli, Zaheri, & Mirjani, 2007; Ranasinghe et al., 2013), anti-inflammatory (Yen, Lin, & Chang, 2010; Hong et al., 2012), the propagation of different cancer cell lines (Mancini-Filho, Van-Koiij, Mancini, Cozzolino, & Torres, 1998; Ka et al., 2003; Nishida et al., 2003), gastric lymphoma (Ozbayer et al., 2014) and antioxidant (Okawa, Kinjo, Nohara, & Ono, 2001; Murcia et al., 2004; Shen et al., 2012). Cinnamon extracts can affect β-carotene-linoleic acid through decreasing lipid peroxidation (Mancini-Filho et al., 1998). The effects of the antioxidant levels in cinnamon extract have previously being studied on carbon tetrachloride (CCl_4)-induced hepatotoxicity in animal (Fu, Zheng, Lin, Ryerse, & Chen, 2008).

This study investigates the ameliorative effect of the cinnamon aqueous extract (CAE) on CCl_4-induced hepatotoxicity in rats through the quantification of its potential antioxidant activities, the serum biochemical and histopathological analysis.

Material and methods

Plant materials

The barks of Cinnamon (*Cinnamomum zeylanicum*) were obtained from a local traditional market in Jeddah, Saudi Arabia and were identified by a Taxonomist. The barks were air dried at room temperature, washed with distilled water, dried again and grounded to fine powder. For the preparation of 10% decoction, 10 g of the Cinnamon bark powder was dissolved in 100 mL of distilled water and allowed to boil for about 30 minutes. The decoction was then cooled at room temperature, filtered and dispensed into a clean sterilized bottle; the bottles were then put into a refrigerator for storage until use.

Chromatographic analysis of Cinnamon extract (CE) by GC–MS

For the identification of bioactive compounds, GC–MS Chromatographic analysis of CE was performed using Agilent Technologies 7890B GC Systems equipped with 5977A Mass Selective Detector. Capillary column (HP-5MS Capillary; measuring 30.0m × 0.25 mm ID × 0.25 µm film) with helium as a carrier gas following a flow rate of 1.7 mL $min.^{-1}$ and 1 µl injection. Analysis of the sample was carried out with the column held for a period of 4 min. at 40˚C post injection, followed by elevation in temperature to 300°C (20°C $min.^{-1}$ heating ramp) along with a 3.0 min. hold. A split-less mode injection was applied at 300°C. MS scan range was (m z^{-1}): 50 - 550 atomic mass units (AMU) under electron impact (EI) ionization (70 eV). The bioactive compounds were identified by comparing the retention indexes of these compounds together with their spectra mass with the NIST library in the GC-MS system.

Animals and treatment

Male Albino rats weighing 203.30 ± 0.93 g were bought from the animal house of Department of Biology, King Abdulaziz University, Jeddah and housed to acclimatized in the laboratory for one week. They were kept under normal laboratory temperature (23 ± 2ºC) with daily 12 hours of light on/off cycle, fed with standard feeds and body weight gained was recorded weekly. All the experimental studies relating to the animals were conducted following the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH 1985); and in accordance with the World Medical Association Declaration of Helsinki regarding ethical conduct of research involving human subjects and/or animals.

The rats were separated into 8 groups with n = 6 has outlined below:

Group 1 (control): normal diet and water only.

Group 2 (CCl_4): subcutaneous injection of 0.5 mL Kg^{-1} bw (20% CCl_4 in paraffin oil) as previously described (Hesami et al., 2014) and commences from the start of the experiment.

Group 3 (CAE 400): 400 mg kg^{-1} bw of CAE orally for 4 weeks.

Group 4 (CCl_4 + CAE400): oral protective dose of 400 mg kg^{-1} bw of CAE daily for 4 weeks + a single dose of 0.5 mL Kg^{-1} bw (20% CCl_4 in paraffin oil) twice a day.

Group 5 (CCl_4 + CAE600): oral protective dose of 600 mg kg^{-1} bw of CAE daily for 4 weeks + a single dose of 0.5 mL Kg^{-1} bw (20% CCl_4 in paraffin oil) twice a day.

Group 6 (CCl_4 + PCAE400): This post-treatment group was subcutaneously injected with 0.5 mL Kg^{-1} bw of CCl_4 (20% CCl4 in paraffin oil) on the 14th day and then received oral dose of 400 mg kg bw CAE till experiment ends.

Group 7 (CCl_4 + PCAE600): This post-treatment group was subcutaneously injected with 0.5 mL Kg^{-1} bw of CCl_4 (20% CCl_4 in paraffin oil) on the 14th day and then received oral dose of 600 mg . kg bw CAE till experiment ends

Group 8 (Silm): Oral dose of 100 mg kg^{-1} bw silymarin daily for 4 weeks (positive control).

Serum biochemical assay

At the conclusion of the experiment, the rats were anesthetized with pentobarbitone sodium (60 mg kg^{-1}) after an overnight fast. Blood samples were dispensed in a centrifuge tube through cardiac puncture, kept to clot at 25°C for 15 minutes and centrifuged for 15 min. at 3000 rpm and 4°C. The serum levels of albumin (ALB) , alkaline phosphatase (ALP), HDL, Alanine Aminotransferase (Theocharis, Margeli, Skaltsas, Spiliopoulou, & Koutselinis, 2001), total protein (TP), total bilirubin (TBIL), Aspartate Aminotransferase (AST), LDL, triglyceride (TRIGL), Gamma-glutamyl transferase (GGT), cholesterol (Gäbele et al., 2009), glucose (GLUC), uric acid (UA) and urea were performed using a commercial assay kits and following to the manufacturer's instruction (EGY-CHEM for lab technology, Bader city, Egypt).

Oxidative stress markers

The liver homogenate was done by homogenizing the liver tissue in 10 volumes of ice-cold 100 mM phosphate buffer containing 1 mM EDTA pH 7.4 using a homogenizer and centrifuged for 15 min at 10,000 rpm and 4°C. The resulting supernatant was utilized to determine the activities of liver enzymes markers of oxidative stress (SOD, GST, CAT) and Malondialdehyde (MDA) levels of lipid peroxidation. These assays were performed using commercial kits (Bioassay Technology Laboratory, Shanghai Korian Biotech CO).

Histopathological analysis

Liver samples were fixed in 10% neutral buffered formalin solution once sacrificed, routinely processed, paraffin embedded, sectioned at a thickness of 4 µm using a microtome, and hematoxylin and eosin (H&E) stained. All morphological alterations were examined using light microcopy.

Statistical analysis

Statistical analysis of all data was done with SPSS version 20 software. Test of significance was achieved by employing Student t-test. Significance level p was set at $p < 0.05$.

Results

GC-MS investigation of cinnamon extract (CE)

Analysis of CE using GC-MS showed the phytochemical components as shown in Figure 1 and Table 1. The GC-MS record described that the occurrences of many bioactive compounds which are arranged alongside with retention time, relative area (%), molecular weight and chemical formula (Table 1). By matching the spectra mass of the components against the NIST library, 14 major compounds peaks were identified from the 32 phytochemicals in CE. The major bioactive compound was Cinnamaldehyde (17.22%) followed by alpha-Muurolene (15.81%), 9-Octadecenoic acid (Z)-, methyl ester (15.61%), Pentadecanoic acid (9.31%), 14-methyl-, methyl ester and gamma.-Muurolene (5.54%).

Body weight

As illustrated in Table 2 below, a significant increase in body weight was observed in all experimental groups except in rats treated with CCl_4, wherein a significant reduction in body weight (4.03 ± 1.39) was observed.

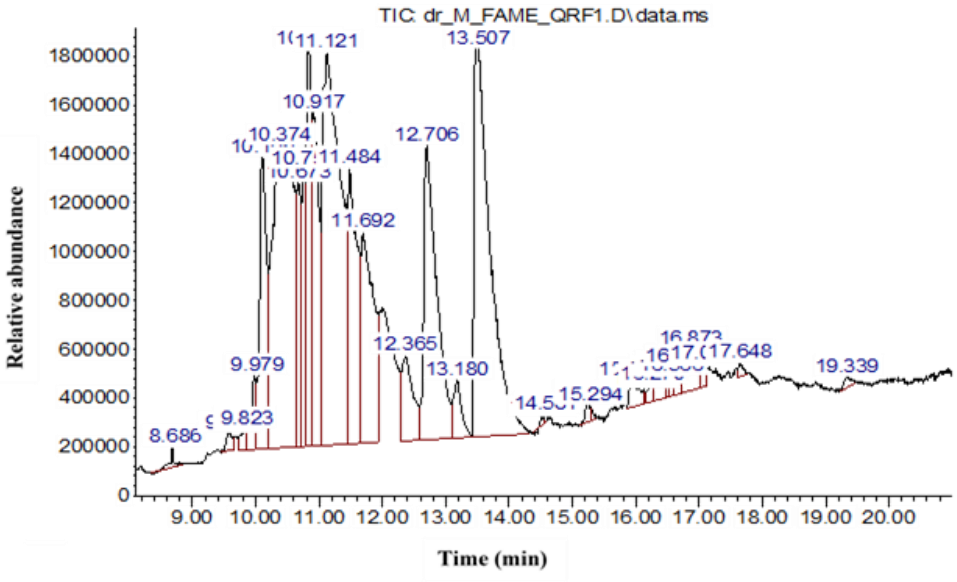

Figure 1. GCMS chromatogram of the n-hexane extract of *Cinnamomum zeylanicum*.

Table 1. Identification of bioactive ingredients in the hexane extract of Cinnamomum zeylanicum.

No	RT	Area Pct (%)	Compound	Mwt	M. formula
1	10.3721	15.8147	alpha.-Muurolene	204.3511	$C_{15}H_{24}$
2	10.6751	2.68	4-Methoxybenzyl alcohol, methyl ether	152.193	$C_9H_{12}O_2$
3	10.8325	4.9411	Epizonarene	204.3511	$C_{15}H_{24}$
4	10.9141	5.5402	gamma.-Muurolene	204.357	$C_{15}H_{24}$
5	11.1181	17.2188	Cinnamaldehyde (E)	132.16	C_9H_8O
6	11.4853	6.0556	Cinnamaldehyde (E)	132.16	C_9H_8O
7	11.6893	6.4927	Cinnamaldehyde (E)	132.16	C_9H_8O
8	12.3654	2.3238	Methyl 13-methyltetradecanoate	256.43	$C_{16}H_{32}O_2$
9	12.7035	9.3151	Pentadecanoic acid, 14-methyl-, methyl ester	270.45	$C_{17}H_{34}O_2$
10	13.5078	15.6086	9-Octadecenoic acid (Z)-, methyl ester	296.495	$C_{19}H_{36}O_2$
11	16.871	1.0262	Oleic Acid	282.47	$C_{18}H_{34}O_2$
12	17.0575	0.2562	13-Octadecenal, (Z)	266.469	$C_{18}H_{34}O$
13	17.6462	0.1646	1,2-Benzisothiazole, 3-(hexahydro-1H-azepin-1-yl)-, 1,1-dioxide	264.093	$C_{13}H_{16}N_2O_2S$
14	19.3365	0.1719	Oleic Acid	282.47	$C_{18}H_{34}O_2$

R.T. = retention time, Area Pct= peak area and Mwt= molecular weight.

Table 2. Effects of CAE on body of rat.

Group	Initial body weight (g)	Final body weight (g)
Control	200.11 ± 1.65	263.45 ± 3.18 *
CCl_4	203.22 ± 7.50	195 ± 5.50 *
CAE 400	204.33± 4.54	240.34 ± 4.85 **
CCl_4+CAE 400	202± 2.41	223 ± 2.17 **
CCl_4+CAE 600	201.83 ± 4.52	228 ± 2.48 **
CCl_4 + PCAE400	200.85 ± 3.76	231.66 ± 3.41 **
CCl_4 + PCAE600	206.97 ± 5.67	241.66 ± 4.41 *
Silymarin	206.97 ± 5.67	251.56 ± 6.41 *

Data were stated as mean ± SEM and test of significance was achieved using Student t-test with significant differences at p< 0.05 and p< 0.01 as shown for (*) and (**) with respect to initial body weight.

Effects of CAE on serum biochemical parameters

The serum level of rats in the CCl_4 treated group, showed a significant rise in the levels of ALP, ALT, AST, total bilirubin, cholesterol, GGT, glucose, LDL triglyceride, uric acid and urea whereas there were significant reduction in the HDL, TP and ALB levels in comparison with the control group ($p < 0.001$) (Table 3). However, rats pretreated with 400 and 600 mg kg^{-1} of CAE for four weeks as well as post-treatment with 400 and 600 mg kg^{-1} of CAE two weeks after the injection of CCl_4, showed a significant reduction in the serum levels of ALT, AST, ALP, TC, TG, LDL, and glucose and a rise in the levels albumin and HDL (Table 3).

Table 3. Effects of CAE on serum biochemical parameters.

Group	Control	CCl_4	CAE 400	CCl_4+CAE 400	CCl_4+CAE 600	CCl_4 + PCAE 400	CCl_4 + PCAE 600	Sily
ALB	4.57 ±	3.06 ±	4.46 ±	4.02 ±	4.22 ±	4.26 ±	3.95 ±	3.40 ±
(g dl^{-1})	0.31	0.34 ***	0.14	0.21	0.23###	0.52##	0.43##	0.51**
ALP	14.24 ±	32.83 ±	15.11 ±	29.27 ±	22.16 ±	32.20 ±	31.32 ±	16.65 ±
(U L^{-1})	1.24	0.73 ***	1.50	1.58###	2.51###	1.45	1.40##	0.86**
ALT	52.11 ±	104.01 ±	48.20 ±	126.86 ±	107.77 ±	119.49 ±	109.44 ±	61.47 ±
(U L^{-1})	9.68	9.78 ***	1.07	4.54###	10.16	4.63###	13.44#	2.63*
AST	152.66 ±	177.96 ±	174.36 ±	212.65 ±	230.44 ±	148.66 ±	213.70 ±	144.60 ±
(U L^{-1})	9.91	0.24***	36.41	7.11##	6.40##	96.57	3.08#	7.74
TBIL	0.76 ±	1.71 ±	0.71 ±	1.64 ±	1.55 ±	0.99 ±	0.79 ±	0.80 ±
(µmol L^{-1})	0.08	0.38***	0.05	0.23###	0.09	0.11##	0.12##	0.07
CHOL	65.20 ±	87.91 ±	66.79 ±	69.81 ±	68.16 ±	65.27 ±	62.67 ±	70.47 ±
(mmol L^{-1})	6.36	4.44***	2.48	6.04###	3.02###	3.73###	5.32###	4.30**
GGT	3.82 ±	7.56 ±	4.09 ±	3.96 ±	3.24 ±	4.20 ±	3.81 ±	4.97 ±
(U L^{-1})	0.16	0.36***	0.27 **	0.35###	0.22###	0.43###	0.27###	0.49**
GLUC	95.32 ±	134.16 ±	92.66 ±	112.73 ±	109.78 ±	122.57 ±	109.66 ±	90.50 ±
(mmol L^{-1})	4.56	9.10***	4.99 **	4.02###	7.53##	3.18##	9.98##	7.47
HDL	43.40 ±	21.67 ±	35.03 ±	36.48 ±	32.59 ±	27.84 ±	26.20 ±	28.92 ±
(mmol L^{-1})	5.99	0.59***	3.78**	3.61###	1.97###	1.03###	2.18##	2.11**
LDL	52.68 ±	77.34 ±	46.41 ±	66.04 ±	64.08 ±	48.46 ±	47.82 ±	47.58 ±
(mmol L^{-1})	1.63	1.95***	3.38**	2.79###	1.47###	1.16###	1.04##	2.01**
TP	7.20 ±	6.17 ±	7.40 ±	6.53 ±	6.77 ±	6.51 ±	6.47 ±	6.52 ±
(G dL^{-1})	0.76	0.32**	0.29	0.30##	1.70##	0.46#	0.17#	0.34*
TRIGL	56.53 ±	67.27 ±	54.84 ±	56.71 ±	63.17 ±	57.02 ±	54.69 ±	55.73 ±
(mmol L^{-1})	3.27	2.00***	8.17	1.90###	0.19##	7.29	4.26###	1.33
UA	2.66 ±	4.84 ±	2.89 ±	4.15 ±	3.97 ±	4.56 ±	4.14 ±	3.93 ±
(µmol L^{-1})	0.21	0.37***	0.38	0.80##	0.40##	0.31#	0.52#	0.70*
UREA	37.45 ±	125.57 ±	47.04 ±	119.33 ±	99.44 ±	120.38 ±	118.10 ±	104.85 ±
(mmol L^{-1})	3.67	6.49***	2.45**	5.09##	2.62###	0.72#	3.79#	8.51**

Data were stated as mean ± SEM and test of significance was achieved using Student t-test with significant differences at $p < 0.01$, $p < 0.05$, $p < 0.001$ as shown for (*),(**) and (***) with respect to normal control and by (#),(##),(###) with respect to CCl_4 treated group respectively.

Effect of CAE on CCl_4-induced oxidative stress

Animals treated with CCl_4 revealed a significant reduction ($p < 0.001$) in the hepatic homogenate levels of SOD, CAT and GSH accompanied by significant increase in lipid peroxidation ($p < 0.001$) has quantified in the MDA levels in the hepatic tissue homogenate in comparison with the animals in the normal control group (Figure 2). Moreover, pretreatment with 400 and 600 gm kg^{-1} bw CAE resulted in a significant rise ($p < 0.001$) in hepatic tissue homogenate levels of SOD, CAT and GSH in comparison with CCl_4 treated group. In addition, treatment with 400 and 600 gm kg^{-1} bw CAE significantly decreased ($p < 0.001$) the elevated hepatic tissue homogenate levels of MDA in comparison with CCl_4 group (Figure 2).

Histopathological analysis

The micrographs of the histopathological analyses using haematoxilin-eosin staining is shown in (Figure 3 A-H).

The micrograph of rats in the normal control group as well as the CAE treated groups showed a normal aspect, healthy, cellular construction and clear nucleus within its cytoplasm (Figure 3A and 3C). However, exposure of liver tissue to CCl_4 intoxication revealed deterioration in the feathery of the hepatic cells, steatosis, inflammation of the portal, sustained venous congestion and fibrosis (Figure 3B). Histopathological examination of liver tissue exposed to CCl_4 as well as daily oral dose of 400 mg/kgbw of CAE showed evident sustained venous congestion with feathery deterioration, absence of steatosis or small vesicular steatosis, minimal parenchymal and portal inflammation (Figure 3D). In addition, findings on liver sample from animals in CCl_4 group as well as daily oral dose of 600 mg kg^{-1} bw of CAE revealed an enhancement of the structural changes to the liver; with absence of sustained venous congestion, absence of steatosis, minimal parenchymal inflammation (Figure 3E).

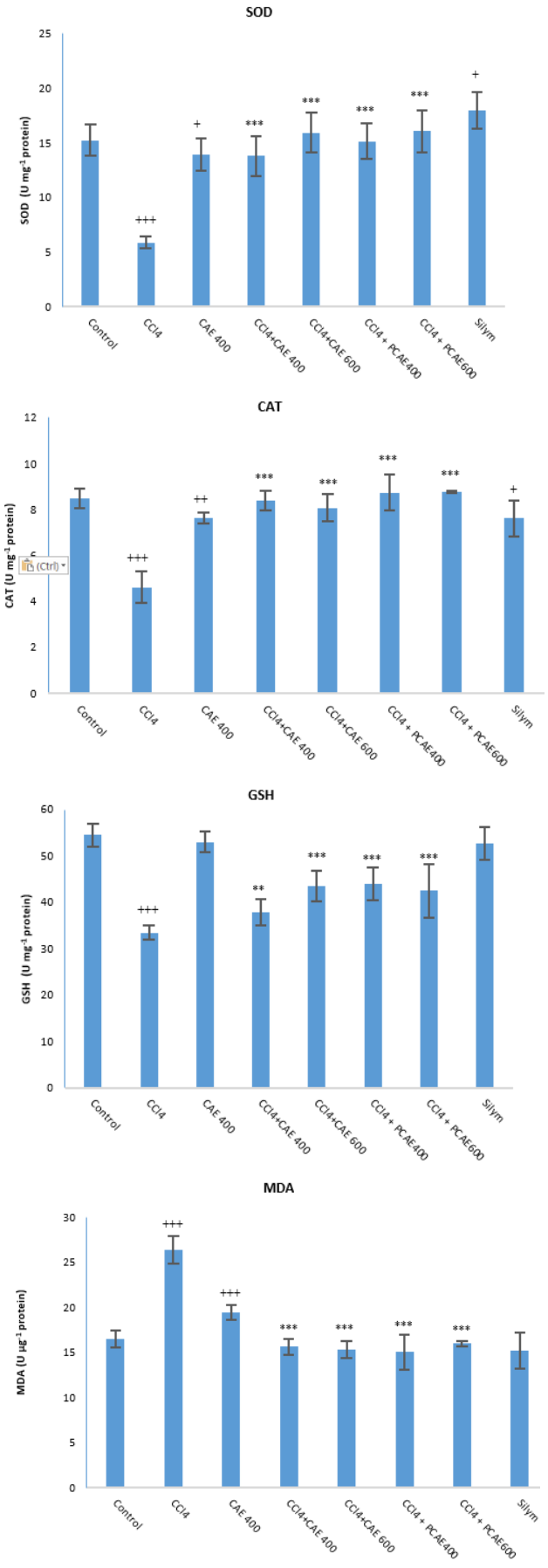

Figure 2. Effect of CAE on CCl4-induced oxidative stress. Data were stated as mean ± SEM and test of significance was achieved using Student t-test with significant differences at $p < 0.05$, $p < 0.01$ and $p < 0.001$ as shown for (+), (++) & (+++) in comparison with normal control and for (*), (**) & (***) in comparison with CCl_4 group, respectively.

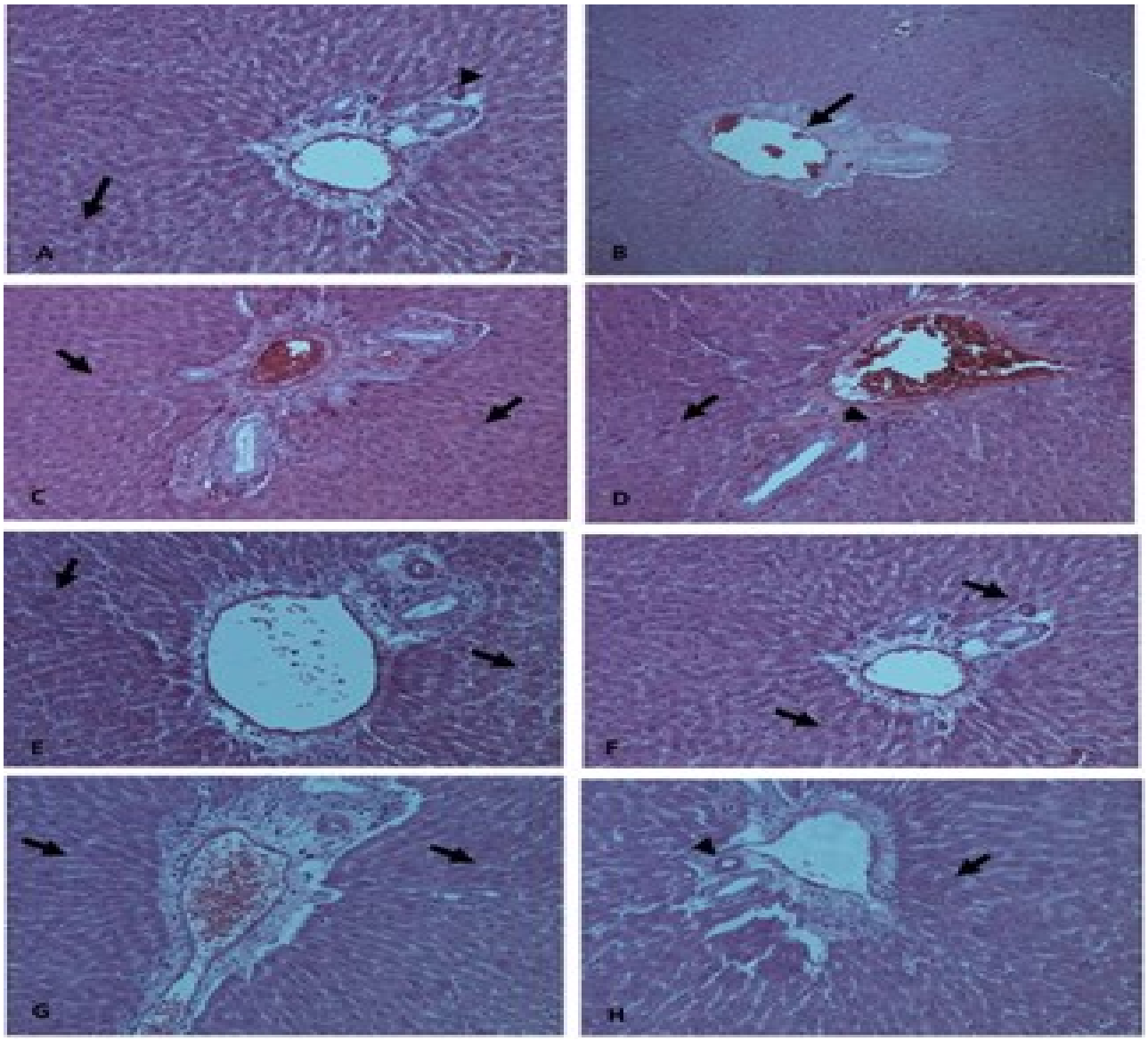

Figure 3. Effect of CAE on histopathological changes of liver tissues (20x). (A) Control group showed typical histological structure of hepatic lobule with central vein [arrowhead] bordering typical hepatic cells [arrows]. (B) CCl_4 exposed group showed sustained venous congestion and hepatocytes with feathery disintegration [arrows]. (C) CAE group (400 mg kg^{-1} bw) showed typical histological structure of hepatic lobule with central vein bordering typical hepatocytes [arrows]. (D) CCl_4 + CAE group (400 mg kg^{-1} bw) showed evident sustained venous congestion with feathery disintegration, absence of steatosis, minimal portal and parenchymal inflammation [arrows]. (E) CCl_4 + CAE group (600 mg kg^{-1} bw) showed hepatocytes structural enhancement with minimal vesicular steatosis [arrows]. (F) CCl_4 + post-treatment of CAE group 400 mg/kg bw and (G) 600 mg kg^{-1} bw showed less percentages of necrosis regenerating hepatocytes. (H) Silymarin group showed hepatoprotective activity with normal sinusoids [arrow] and intact portal veins [arrowhead].

Discussion

The current study revealed the hepatoprotective, antioxidant and restorative effects of aqueous extract of cinnamon on CCl_4-induced liver damage in rats. The liver as an important organ is responsible for drugs and xenobiotics metabolism. Various studies illustrated that CCl_4 is commonly utilized in the induction of liver injury since cytochrome P450 is responsible for its metabolism in hepatic cells, producing a strongly reactive trichloromethyl radical, that initiates a sequential events of lipid peroxidation, cellular impairment and thus leading to liver fibrosis (Shenoy, Somayaji, & Bairy, 2001; Weber et al., 2003; Fang, Lai, & Lin, 2008; Halliwell & Gutteridge, 2015). Apart of lipid peroxidation, CCl_4 is also a culprit in the depletion of tissue's CAT, SOD and GSH actions, and this could be due to oxidative alteration of those proteins (Augustyniak, Waszkiewicz, & Skrzydlewska, 2005). This damage was related to loss of the liver function stated as a substantial rise in the total bilirubin content in the serum and reduction in albumin, which are proposed as markers of hepatic function (Renugadevi & Prabu, 2010).

The results of this study revealed that oral administration of aqueous extract of cinnamon efficiently protected against the harmful loss of those antioxidant actions after CCl_4 injection, besides it is recognized to serve various biological functions, comprising cell protection from oxidative stress mediated by reactive oxygen species and free radicals (Nakamura, Torikai, & Ohigashi, 2001;Gäbele et al., 2009;). Bioactive compounds present in cinnamon were found to stimulate production of detoxifying agents and antioxidant enzymes in the transcriptional stage, through response elements of antioxidant (Masella, Di Benedetto, Varì, Filesi, & Giovannini, 2005) and to increase synthesis of γ-glutamylcysteine (Kim et al., 2007). Elevated serum levels of transaminases and ALP in CCl_4-treated rats reveal liver damage because those enzymes outflow from hepatocytes into the blood circulation at the event of tissue injury, which is permanently accompanied with hepatic necrosis (Naik & Panda, 2008). As a result of administration of cinnamon extract, the serum profile of the liver enzymes were almost restored to normal or a little above normal, demonstrating the protective effect against hepatic damage. The effectiveness of the hepatic cells in linked to the action of alkaline phosphatase. Hence, inhibition in the elevated serum ALP proposes the continuous biliary dysfunction in the liver of rat throughout prolonged hepatic intoxication with CCl_4. Depletion of albumin and total protein induced by CCl_4 injection is an additional indication of hepatic injury (Bies & Bonate, 2006). Cinnamon aqueous extract has ameliorated serum total protein level to near normal, which proves hepatoprotective activity of cinnamon. Enhancement of protein synthesis was progressive as a contributing hepatoprotective mechanism, enhancing the repairing phase and hepatocytes proliferation (Tadeusz, Teresa, & Krzysztof, 2001). Moreover, histopathological alterations were also observed demonstrating hepatic damage as a result of CCl_4 treatment. It has been reported earlier that CCl_4 injection leads to hepatic necrosis (Sun et al., 2003), fibrosis (Natsume et al., 1999) infiltration seen in mononuclear cell (Natsume et al., 1999), steatosis and disintegration of hepatocytes, increased mitotic activity (Theocharis et al., 2001) and liver cirrhosis (Zalatnai, Sarosi, Rot, & Lapsis, 1991). In addition, liver apoptosis has been linked to CCl_4 (Sun et al., 2001). Consequently, histological hepatic alterations owing to CCl_4 injection are consistent with former studies. Pre- and post- treatment involving cinnamon aqueous extract significantly ameliorated hepatocyte structure. The present findings thus suggest that treatment with cinnamon extract obviously reversed hepatic intoxication induced by CCl_4 injection. GC-MS screening of cinnamon revealed the occurrence of the most major compounds: cinnamaldehyde (17.22%), alpha-Muurolene (15.81%) and 9-Octadecenoic acid (Z), methyl ester (15.61%). Cinnamaldehyde is a major component in various food prepartions and the presence of this active compound could explain the effective role of cinnamon as an heaptoprotective, lipo-protective and reducing powers (Sharma et al., 2017; Sharma, Sharma, & Pandey, 2016). Further, different studies revealed that Cinnamaldehyde act as anti-inflammatory key *in vivo* and *in vitro* studies including neuro cells inflammation (Hwa et al., 2012) and cardiotoxicity (Pyo et al., 2013). The other bioactive compounds like octadecenoic acid (El-Raey, 2016), alpha-muurolene (Lubsandorzhieva, Boldanova, & Dashinamzhilov, 2013), flavonoids (Baek, Kim, Kyung, & Park, 1996) and alkaloids (Vijayan et al., 2003) were recognized to have hepatoprotective activity. In the current study, the existence of Octadecenoic acid in cinnamon extract could be the reason for its antioxidative efficacy and thus hepatic protection activity. The antioxidant capability of flavones is owing to the strong hydroxyl radical reactive potentials, and its ROS scavenging ability is a function of the number of the functional groups on its B-ring (Pannala, Chan, O'Brien, & Rice-Evans, 2001; Heim, Tagliaferro, & Bobilya, 2002).

Conclusion

The current study demonstrated that CAE is an important compound in the inhibition of the harmful effects arising from CCl_4. CAE treatment could considerably improve the level of lipid peroxidation largely while restoring the anti-oxidative enzymes and shifts the functioning of the liver to optimum level. Furthermore, CAE decreased hepatic lipid, vacuolar disintegration, reduced hepatic architectural damage. This finding thus demonstrated the hepatoprotective activity of CAE. Therefore, the active phytochemicals of this plant could possibly be used as a future candidate drug for the treatment of liver toxicity.

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