Original Article

TIPS Trends in Pharmaceutical Sciences 2017: 3(4): 255-266.

Hepatoprotective Effects of *Avicennia marina* (Forssk.) Vierh.

Omid Farshad[1,2], Reza Heidari[1,*], Hamidreza Mohammadi[2], Amin Reza Akbarizadeh[2], Mohammad M. Zarshenas[3,4,*]

[1]Pharmaceutical Sciences Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.

[2]Department of Pharmacology and Toxicology, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran.

[3]Department of Phytopharmaceuticals (Traditional Pharmacy), School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran.

[4]Medicinal Plants Processing Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.

Abstract

The xenobiotics-induced liver injury is a major clinical complication. Hence, finding hepatoprotective agents could have clinical value. Herbal medicines are a major source of biologically active chemicals which could be applied as hepatoprotective agents. The current study was designed to assess the hepatoprotective properties of *Avicennia marina* (AM) extract and its different fractions. *In vivo*, the hepatoprotective effect of AM total extract against CCl_4-induced acute liver injury was evaluated in rats, and a series of histopathological, biochemical, and oxidative stress parameters were monitored. *In vitro*, the protective effect of AM extract fractions (Petroleum ether, Chloroform, Ethyl acetate, and Ethanol) was evaluated on human liver hepatoma cells (HepG2). Severe elevation in serum level of liver injury biomarkers, along with liver tissue histopathological changes, lipid peroxidation, and liver tissue glutathione depletion were detected in CCl_4-treated rats. On the other hand, CCl_4-induced toxicity was evident *in vitro* by significant cell death. It was found that AM extract provided significant protection against CCl_4 toxicity *in vivo* by decreasing serum biomarkers of liver injury and tissue markers of oxidative stress. *In vitro*, the protective effect of AM extract fractions (Chloroform, Ethyl acetate, and Ethanol) was evident as these fractions significantly decreased CCl_4 cytotoxicity. As AM extract exhibited significant suppression of oxidative stress markers, its antioxidant effect could play a significant role in its hepatoprotective properties.

Keywords: Hepatoprotection, Natural product, Oxidative Stress.

1. Introduction

The liver is prone to xenobiotics-induced injury due to its central role in the metabolism of foreign compounds (1,2). Liver injury is a significant clinical problem induced by different xenobiotics including drugs and environmental pollutants (2-7). The xenobiotics-induced liver injury could lead to patient death if not appropriately managed. Hence, finding new pharmacological interventions to protect liver has clinical value. Many hepatoprotective chemicals have been studied to find a way to protect the liver from toxic insults (6, 8-15). Recently, interest in the discovery of natural hepatoprotective agents has increased. Principal candidates in this discovery process are medicinal plants (16-18). Due to the presence of a wide range of biologically active chemicals, different herbal medicine could be applied as the

Corresponding Author: Reza Heidari and Mohammad M. Zarshenas, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran. Email: rheidari@sums.ac.ir; zarm@sums.ac.ir

Recieved: 15/08/2017; Accepted: 26/11/2017

source of hepatoprotective molecules.

Carbon tetrachloride (CCl_4)-induced liver injury is extensively applied as an animal model of hepatic failure and liver fibrosis (19). Severe reactive oxygen species (ROS) formation and its deleterious adverse events which finally lead to hepatic failure, are attributed to CCl_4-induced liver failure animal model (19). The hepatoprotective properties of many herbal remedies have been evaluated in CCl_4-induced hepatotoxicity model to find hepatoprotective molecules with potential therapeutic capabilities (20-23).

Several pharmacological effects have been attributed to *Avicennia marina* (Forssk.) Vierh. (Avicinnaceae; AM). It has been found that AM extract possesses antimicrobial, anti-inflammatory, antidiabetic, antiviral, and antitumor activity (24-29). It has been found that the extract of Avicinnaceae species also showed protective effects in different experimental models (30-32). Some investigations also mentioned the antioxidant effects of Avicinnaceae species (33-35). Hence, AM extract might provide hepatoprotection against CCl_4-induced hepatotoxicity and oxidative stress.

The current investigation was designed to evaluate the protective effects of AM leave extract and respective fractions against CCl_4-induced toxicity *in vitro* and *in vivo*.

2. Materials and methods

2.1. Plant Material and Chemicals

Fresh leaves of AM were collected during March 2016 from the Persian Gulf, Nayband, Bushehr, Iran. Samples were authenticated by a botanist (Botany Department, Shiraz University, Shiraz, Iran). The plant leaves were dried at the room temperature. HepG2 cells were obtained from Pasteur Institute (Tehran, Iran). RPMI-1640 and FBS were from GibCo (United States). Trypan blue, methyl thiazolyl diphenyl-tetrazolium bromide (MTT), Dithio-bis-nitrobenzoic acid (DTNB), CCl_4 and Na_2HPO_4 were purchased from Merck (Darmstadt, Germany). Thiobarbituric acid (TBA) and Trichloroacetic acid (TCA) were obtained from Sigma Chemical Company (St. Louis, USA).

2.2. Extract preparation

Approximately, 500 g of fine powder leave samples were extracted with 1 L 80% V/V ethanol/water via maceration method for 24 hrs. Subsequently, the extract was concentrated by a rotary evaporator and gently dried via speed vacuum. The total solid yield of hydroalcoholic extract was 56 g/kg dry crude sample. The solid extract was subsequently dissolved in a mixture of water and ethanol and traditionally fractionated to the ether, chloroform, ethyl acetate and ethanol fractions, using a separation funnel. Fractions were orderly concentrated by rotary evaporator and subsequently dried in a speed vacuum system.

2.3. Phenolic determination of total extract

Total polyphenol content was determined by a modification in the folin-ciocalteu method using ascorbic acid as standard (36). For preparation of a calibration curve, 0.5 ml aliquots of 0.024, 0.075, 0.105 and 0.3 mg/ml ethanol-gallic acid solutions were mixed with 2.5 ml folin- ciocalteu reagent (diluted ten-fold) and 2 ml (75 g/l) sodium carbonate. The contents were mixed and allowed to stand for 30 min. The absorption at λ=765 nm was measured in a UV-Vis spectrophotometer (T80 plus, PG Instrument, UK). Half of one ml total extract was mixed with the same reagents as described above. One hour later, the absorption was measured for the determination of plant phenolics. All assessments were performed in triplicate, and the total phenolic content in plant total extract was calculated in ascorbic acid equivalents.

2.4. Antioxidant assays of total extract

The antioxidant activities of total extract were measured in term of radical scavenging power by using stable free radical; 2, 2 –Diphenyl- 1-picrylhydrazyl (DPPH)(37). A 20 µL of the sample at various concentrations (50-800 µg/ml) were added to 200 µL of a methanol solution of DPPH (100 mM) in a microplate (7). Negative controls were prepared with 20 µL methanol and 200 µL DPPH in triplicate. Each well contained 200 µL solution of DPPH (100 mM) and 20 µL of extract. The microplates were incubated at 25 °C for 30 min, and the absorbance was measured at 492 nm using a microplate reader (Stat

Fax® 2100, Awareness Technology, Inc., USA). Ascorbic acid was used as an antioxidant standard. The obtained data were used to determine the concentration of the samples required to scavenge 50% of the DPPH free radicals (IC_{50}). The percent inhibition was plotted against the concentrations of the extract, and the IC_{50} was obtained from the fitted linear curve.

2.5. Ferric Reducing Antioxidant Power (FRAP)

FRAP assay was carried out according to the method described by Benzie using $FeSO_4.7H_2O$ as the standard (38). Reagent A:300 mM acetate buffer (pH=3.6), reagent B: 0 mM TPTZ in 40 mM HCl and reagent C:20 mM $FeCl_3.6H_2O$ was prepared. The FRAP reagent was prepared by mixing reagents A:B:C in 10:1:1 just before use. About 30 µL of FRAP reagent was introduced into 10 µL of samples and standard. The mixture was incubated at 37 °C for 10 minutes. The absorbance reading was set at 593 nm within 1 min interval for 6 min (38).

2.6. In vitro hepatoprotective assay

HepG2 were cultured and maintained in an RPMI-1640 medium (pH=7.3, containing 0.37% $NaHCO_3$ supplemented with 10% fetal bovine serum albumin, 100 IU/ml penicillin, and 100 IU/ml streptomycin) and a humidified 5% CO_2-95% O_2 mixture at 37 °C (39). Cells were seeded in 96-well microplates (30000 cells/well/90 µl) and routinely cultured in a humidified incubator for 24 hrs. The cells were then treated with concentrations 10 and 50 mg/ml of the extracts (Ether, Chloroform, Ethyl acetate, and Ethanol 10 µl/well.) 1 hr. before CCl_4 100 mM (CCl_4/ethanol; 1/10).

After 24 hrs. of incubation, 10 µl of the MTT solution was added to every well. Cells were re-incubated for an additional 4 hrs. The cell culture media and MTT solution were removed, and the cells remained in the bottom of the wells. Then, 100 µl of DMSO was added to each well to dissolve the formazan crystals formed. The absorbance of the purple dye was measured at a λ=570 nm.

2.7. In vivo hepatoprotective activity studies

2.7.1. Animals

Male Sprague-Dawley rats (200-250 g) were purchased from the Laboratory Animals Research Center of Shiraz University of Medical Sciences. Animals were maintained under controlled conditions of 12 h light/12 h dark, ≈40% humidity, and ambient temperature of 23±2 ºC. Rats had free access to a standard laboratory chow diet (Behparvar®, Tehran, Iran) and tap water. All the experiments were performed in conformity with the guidelines for care and use of experimental animals approved by a local ethics committee in Shiraz University of Medical Sciences, Shiraz, Iran.

Rats were randomly divided into four groups (n=6 in each). Group I received olive oil (1 ml/kg, i.p); group II received CCl_4/olive oil (1:1, 1 ml/kg, i.p) as treated control; groups III and IV received the extract with doses of 20 and 40 mg/kg respectively, by i.p. injection for 3 days and CCl_4/olive oil (1:1, 1 ml/kg, i.p.) on the third day. Twenty four hours after CCl_4 injection animals' blood and liver samples were collected.

2.7.2. Serum biochemical analysis and liver histopathology

Blood was collected from the abdominal inferior vena cava under thiopental anesthesia (70 mg/kg, i.p) and the liver was removed. The blood samples were allowed to clot at 25 °C, and serum was prepared by centrifugation (3,000 g, for 20 minutes, 4 ºC). Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH) activities were measured with commercial kits (Pars Azmun®, Tehran, Iran) (17, 40, 41). For histopathological evaluation, samples of liver were fixed in buffered formalin solution (10% formaldehyde, 0.4% sodium phosphate monobasic, NaH_2PO_4, and 0.64% sodium phosphate dibasic, Na_2HPO_4, in double distilled water) (42). Paraffin-embedded sections of liver were prepared and stained with hematoxylin and eosin (H&E) before light microscope viewing (17, 43). Histological damages were scored as follows: 0: absent; +: mild; ++: moderate; and +++: severe (44).

2.7.3. Lipid peroxidation in liver tissue

Thiobarbituric acid reactive substances (TBARS) assay was employed to measure the amount of lipid peroxidation in the liver tissue. Briefly, the reaction mixture consisted of 0.5 mL of 10% liver tissue homogenate, 1 mL of 1% thiobarbituric acid, and 3 mL of 1% phosphoric acid. The mixture was thoroughly mixed and then heated in boiling water (100 °C) for 45 minutes (40, 41, 45). Samples were centrifuged (3,000 g for 5 minutes) and the absorbance of developed pink color was read at λ=532 nm (Ultrospec 2000® UV spectrophotometer) (45).

2.7.4. Hepatic glutathione content

Liver glutathione contents were measured by determining non-protein sulphydryls with the Ellman reagent (46). Liver tissue samples (200 mg) were homogenized in 8 ml of EDTA (20 mM). Then, 5 mL of the tissue homogenate was mixed with 4 mL of double distilled water and 1 mL of trichloroacetic acid (TCA) (50 % w/v) (47). The mixture was vortexed and centrifuged (20 minutes, 700 g, 4 ºC). Then, 2 mL of the samples supernatant was mixed with 0.1 ml of the Ellman reagent (DTNB, 10 mM in methanol), and the absorbance of developed yellow color was measured at 412 nm with an Ultrospec 2000® UV spectrophotometer (41, 48, 49).

2.8. Statistical analysis

Results are shown as Mean±SEM for at least six animals in each group. Comparisons between multiple groups were made by a one-way analysis of variance (ANOVA) followed by Turkey's post hoc test. Differences between groups were considered significant when $P<0.05$

3. Results

The results of DPPH radical-scavenging activity, total phenolic content, and FRAP assay of total extract of AM are presented in Table 1. The Phenolic content (mg/g dry weight) of methanolic extract was measured as ascorbic acid equivalent. The level of phenolic content was 0.9±0.06 mg/g (Table 1). The percent deterrence of DPPH radical by AM leaves extract (10.7±3.3) was compared to a known antioxidant, quercetin (Table 1). AM extract demonstrated significant antioxidant capacities with FRAP test (Table 1). This extract showed 42±9 mM Fe^{2+}/mg sample values somehow similar to diminishing power of ascorbic acid (60.75 mM Fe^{2+}/mg sample) (Table1).

CCl_4 administration to rats caused hepatotoxicity as judged by elevated serum transaminases level (Figure 1), serum lactate dehydrogenase (LDH) activity (Figure 1). The serum biochemical changes after CCl_4 administration was accompanied with significant alterations in liver tissue histopathology (Table 2). Severe liver fatty changes, centrilobular necrosis, and inflammatory cells infiltration were detected in CCl_4-treated animals (Table 2). It was found that total extract of AM (20 and 40 mg/kg, i.p, three consecutive days before CCl_4) effectively mitigated CCl_4-induced hepatotoxicity as revealed by lower serum transaminase activity and LDH level (Figure 1). Administration of the AM extract (20 and 40 mg/kg, i.p, three consecutive days before CCl_4) also attenuated liver histopathological changes in CCl_4-treated rats (Table 2).

CCl_4 caused a significant amount of thiobarbituric reactive substances (TBARs) formation in rat liver tissue (Figure 2). On the other hand, it was found that AM extract significantly decreased CCl_4-induced lipid peroxidation in rat liver (Figure 2). Significant depletion of liver glutathione reservoirs was also detected in CCl_4-intoxicated rats (Figure 2). It was found that administration of a total extract of AM (20 and 40 mg/kg, i.p, three consecutive days before CCl_4) preserved liver glutathione stores at a higher level (Figure 2).

Table 1. Total phenolic content and antioxidant activity as IC_{50} (µg/mL) and Fe (µg/mL) DPPH for FRAP assays of *Avicennia marina* total extract, respectively (Mean±SD).

Total phenolic contents (mg ascorbic acid/g dry weight)	DPPH IC_{50} (mg/g)	FRAP
0.9±0.06	10.7±3.3	42±9

Table 2. Effect of *Avicennia marina* extract on histopathological damages induced by CCl_4 injection in rats.

Liver tissue histopathological changes				
Treatment	**Necrosis**	**Fatty changes**	**Hepatocytes Deformation**	**Lymphocyte Infiltration**
Control	-	-	-	-
CCl_4 (3 ml/kg)	+++	+++	++	+++
CCl_4 + AM 20 mg/kg	++	-	+	+
CCl_4 + AM 40 mg/kg	+	+	+	+

0: absent +: mild++: moderate +++: severe.

Rats received the total extract of Avicennia marina (i.p for three consecutive days; AM) before CCl_4.

In another part of this research on HepG2 cells, different AM extract fractions (Petroleum ether, Chloroform, Ethyl acetate, and Ethanol) were tested on HepG2 cells to assess their cyto-protective effects.

CCl_4 administration caused more than 50% loss of cell viability in the concentration of 100 mM in 24 hrs. of incubation. When cells were pre-incubated with AM extract fractions (Chloroform, Ethyl acetate, and Ethanol, 10 and 50 mg/ml), CCl_4-induced cytotoxicity was significantly attenuated (Figure 3). Petroleum ether fraction of AM extract did not have protective effects against CCl_4-induced cell death (Figure 3). On the other

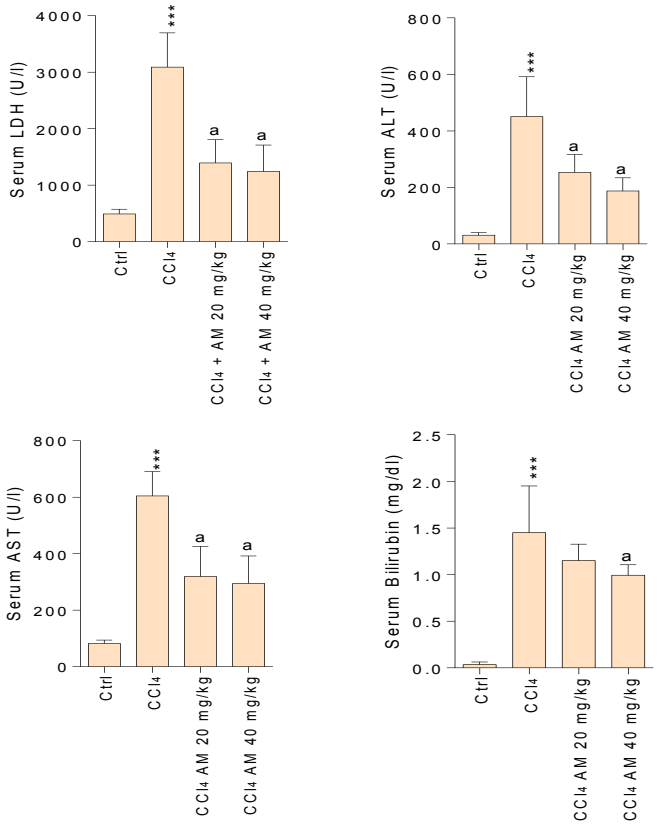

Figure 1. Effect of different doses of the *Avicennia marina* extract on biomarkers of liver injury in CCl_4-intoxicated rats. AM.: Total Extract of *Avicennia marina*.
Data are given as Mean±SD (n=6).
*** Indicates significantly different from control group (P<0.05).
[a] Indicates significantly different from CCl_4-treated group (P<0.001).

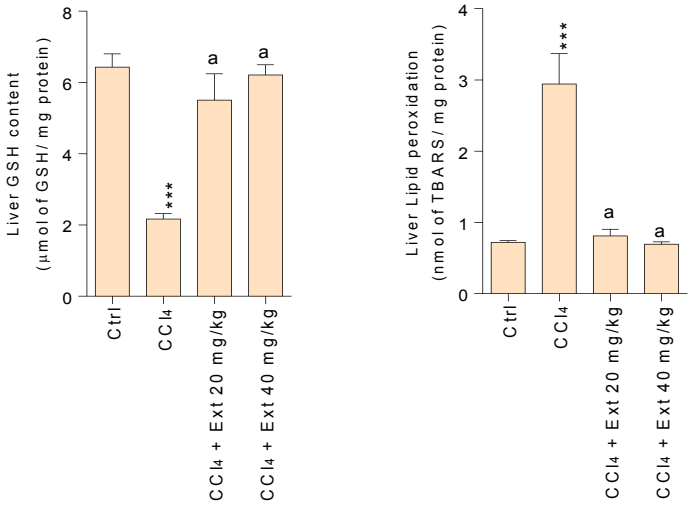

Figure 2. Effect of *Avicennia marina* extract on markers of oxidative stress in CCl_4-treated rats.
Data are given as Mean±SD (n=6). AM.: Total Extract of *Avicennia marina*.
*** Indicates significantly different from control group (P<0.05).
[a]Indicates significantly different from the CCl_4-treated group (P<0.001).

hand, the AM extract petroleum ether fraction itself caused cytotoxicity towards HepG2 cells (10 and 50 mg/ml) (Figure 3). Other fractions of AM extract were not cytotoxic when administered alone (10 and 50 mg/ml) (Figure 3).

4. Discussion

CCl_4¬-induced liver injury is widely used as an experimental model to study the effects of hepatoprotective agents (50-52). CCl_4¬ is metabolized by liver cytochrome P4502E1 (CYP2E1) system to produce a highly reactive metabolite

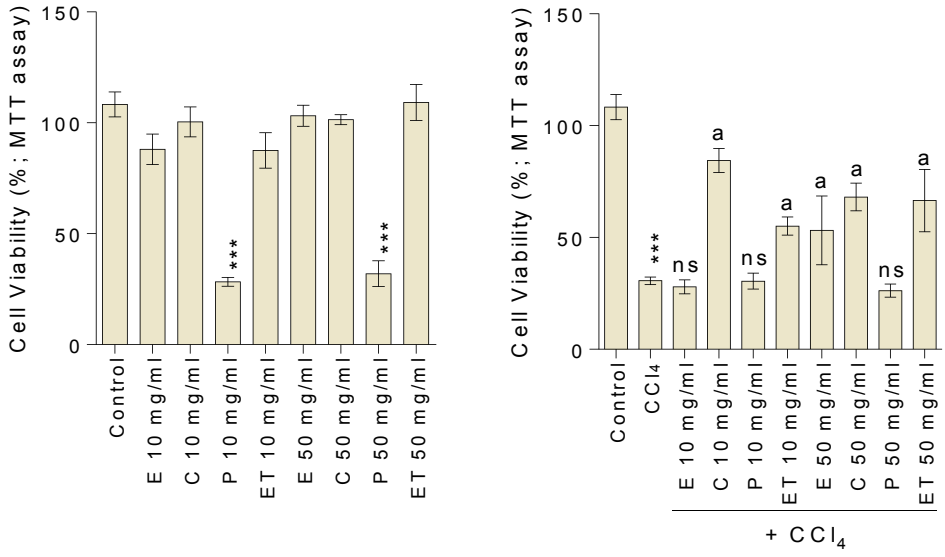

Figure 3. The effect of different extracts of *Avicennia marina* on CCl_4–induced toxicity in HepG2 cells. P: Petroleum ether, C: Chloroform, E: Ethyl acetate, and ET: Ethanol fractions of AM extract.
Data are given as Mean±SD (n=4).
*** Significant difference as compared with control cells (P<0.05).
[a]Significant difference as compared with CCl_4-treated group (P<0.05).
ns: not significant as compared with CCl_4-treated group

which starts a chain of free radical-mediated reactions resulting in peroxidation of biomembrane lipids and damage to other components such as intracellular proteins (53). Hence, the dominant mechanism involved in CCl_4 hepatotoxicity is believed to be associated with oxidative stress and its consequences including GSH depletion and lipid peroxidation (54). On the other hand, herbal medicines have been widely studied for their hepatoprotective activity (55). The current study was designed to assess the antioxidant and hepatoprotective effect of a total extract of AM as well as its fractions against CCl_4¬-induced toxicity.

In this study, CCl_4 caused a significant elevation in serum biomarkers of liver injury and histopathological changes in rat liver. Moreover, CCl_4 also decrease the hepatic glutathione reservoirs and increased lipid peroxidation in the liver tissue. These changes specify the occurrence of oxidative stress following CCl_4 administration to rats. On the other hand, the phytochemical screening of AM revealed that the extract contains alkaloids, flavonoids, triterpenoids, glycosides, and steroids (24, 26, 34, 56, 57). Several studies mentioned the antioxidant potential of Avicinnaceae species in different experimental models (33-35). As oxidative stress and its deleterious consequences play a significant role in CCl_4-induced liver injury (58), a part of the hepatoprotective mechanism of AM extract might be related to its antioxidant properties (Figure 4).

Different chemicals are characterized in AM (57). As mentioned, flavonoids are essential constituents of AM extract (57). There is a sizeable circumstantial evidence implicating the hepatoprotective properties of flavonoids in different experimental models (59, 60). Polyphenol compounds such as quercetin, gallic acid, and curcumin efficiently prevented CCl_4 induced liver injury in previous investigations (61-64). On the other hand, chemicals such as sterols present in AM extract might act as growth factors which enhance liver tissue regeneration (56). Hence, these chemicals could be responsible for hepatoprotective effects of AM extract (Figure 4).

Indeed the polarity of AM extract constituents is different. Hence, these chemicals might distribute in different fractions of AM extract. Therefore, the different cytoprotective profile of AM extract fractions was observed in this study. The hepatoprotective fractions (Polar fractions; Figure 4) of AM extract in the current study, preserved cell viability against CCl_4-induced toxicity. Hence, we might be able to propose that more polar chemicals of AM extract are responsible for its hepatoprotective effects (Figure 4).

Several studies proposed the role of natural products in inhibiting CYP2E1 and blocking CCl_4 bioactivation (65). Although not investigated in the current study, such mechanism might also be involved in the hepatoprotective properties of the AM extract and its constituents.

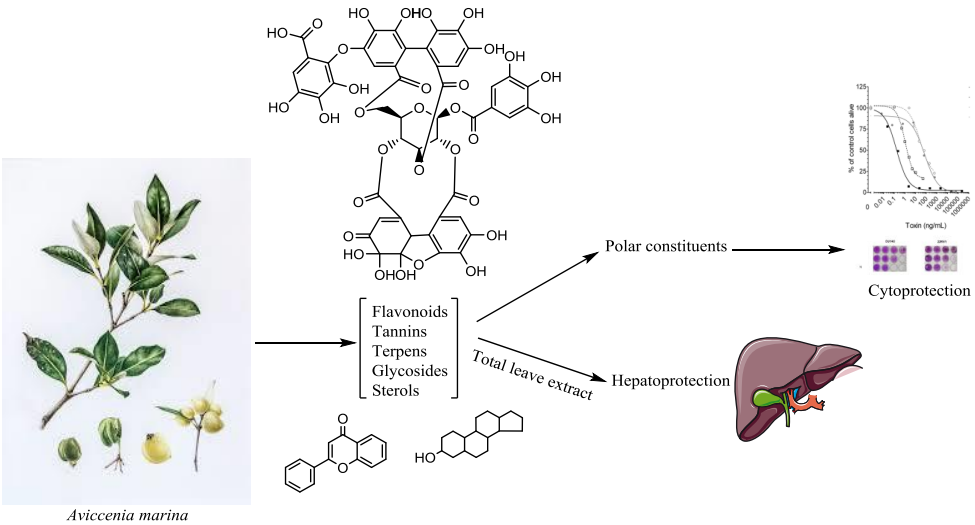

Figure 4. Different chemicals such as antioxidant flavonoids present in *Avicennia marina* extract might counteract CCl_4-induced oxidative stress and its deleterious consequences.

Collectively, it is proposed that the hepatoprotective effects of AM extract might be attributed to the presence of protective compounds such as flavonoids and sterols (Figure 4). These chemicals provide antioxidant and tissue regenerating properties. On the other hand, these compounds have different physicochemical properties and might distribute in different extract fractions due to their polarity. As polar fractions of AM extract alleviated CCl_4-induced toxicity, we might be able to propose that the hepatoprotective chemicals of AM extract are dissolved in these fractions. The exact chemical(s) responsible for the hepatoprotective properties of AM and the mechanism(s) of hepatoprotection deserves more investigations to be cleared.

Acknowledgments

The authors gratefully thank the Pharmaceutical Sciences Research Center of Shiraz University of Medical Sciences for providing technical support of the current study.

Conflict of Interest

None declared.

5. References

1. Abboud G, Kaplowitz N. Drug-induced liver injury. *Drug Saf.* 2007;30;277-94.
2. Heidari R, Niknahad H, Jamshidzadeh A, Abdoli N. Factors affecting drug-induced liver injury: antithyroid drugs as instances. *Clin Mol Hepatol.* 2014;20;237-48.
3. Lee WM. Drug-induced hepatotoxicity. *New Eng J Med.* 2003;349;474-85.
4. Abdoli N, Heidari R, Azarmi Y, Eghbal MA. Mechanisms of the Statins Cytotoxicity in Freshly Isolated Rat Hepatocytes. *J Biochem Mol Toxicol.* 2013;27;287-94.
5. Heidari R, Niknahad H, Jamshidzadeh A, Eghbal MA, Abdoli N. An Overview on the Proposed Mechanisms of Antithyroid Drugs-Induced Liver Injury. *Adv Pharm Bull.* 2015;5;1-11.
6. Heidari R, Babaei H, Eghbal M. Mechanisms of methimazole cytotoxicity in isolated rat hepatocytes. *Drug Chem Toxicol.* 2013;36;403-11.
7. Niknahad H, Jamshidzadeh A, Heidari R, Zarei M, Ommati MM. Ammonia-induced mitochondrial dysfunction and energy metabolism disturbances in isolated brain and liver mitochondria, and the effect of taurine administration: relevance to hepatic encephalopathy treatment. *Clin Exp Hepatol.* 2017;3;141-51.
8. Yilmaz O, Ersan Y, Ozsahin AD, Ozturk AI, Ozkan Y. Consequences of the Combined α-tocopherol, Ascorbic Acid and α-lipoic Acid on the Glutathione, Cholesterol and Fatty Acid Composition in Muscle and Liver of Diabetic Rats. *Iran J Bas Med Sci.* 2013;16;165-72.
9. Heidari R, Babaei H, Eghbal MA. Amodiaquine-induced toxicity in isolated rat hepatocytes and the cytoprotective effects of taurine and/or N-acetyl cysteine. *Res Pharm Sci.* 2014;9;97-105.
10. Heidari R, Moezi L, Asadi B, Ommati MM, Azarpira N. Hepatoprotective effect of boldine in a bile duct ligated rat model of cholestasis/cirrhosis. *Pharma Nutrition.* 2017;5;109-17.
11. Najafi N, Jamshidzadeh A, Fallahzadeh H, Omidi M, Abdoli N, Najibi A, Azarpira N, Heidari R, Niknahad H. Valproic Acid-Induced Hepatotoxicity and the Protective Role of Thiol Reductants. *Trend Pharm Sci.* 2017;3;63-70.
12. Niknahad H, Hosseini H, Gozashtegan F, Ebrahimi F, Azarpira N, Abdoli N, Heidari R. The Hepatoprotective Role of Thiol Reductants against Mitoxantrone-Induced Liver Injury. *Trend Pharm Sci.* 2017;3;113-22.
13. Jamshidzadeh A, Heidari R, Latifpour Z, Ommati MM, Abdoli N, Mousavi S, Azarpira N, Zarei A, Zarei M, Asadi B, Abasvali M, Yeganeh Y, Jafari F, Saeedi A, Najibi A, Mardani E. Carnosine ameliorates liver fibrosis and hyperammonemia in cirrhotic rats. *Clin Res Hepatol Gastroenterol.* 2017;41;424-34.
14. Heidari R, Babaei H, Eghbal MA. Cytoprotective Effects of Taurine Against Toxicity Induced by Isoniazid and Hydrazine in Isolated Rat Hepatocytes. *Arh Hig Rada Toksikol.* 2013;64;201-10.
15. Mobasher MA, Jamshidzadeh A, Heidari R, Ghahiri G, Mobasher N. Hepatoprotective effects of Artemia salina L. extract against carbon tetrachloride-induced toxicity. *Trend Pharm Sci.* 2016;2;259-64.
16. Sharma V, Singh M. Attenuation of N-

nitrosodimethylamine induced hepatotoxicity by Operculina turpethum in Swiss Albino mice. *Iran J Basic Med Sci.* 2014;17;73-80.
17. Jamshidzadeh A, Heidari R, Razmjou M, Karimi F, Moein MR, Farshad O, Akbarizadeh AR, Shayesteh MRH. An in vivo and in vitro investigation on hepatoprotective effects of Pimpinella anisum seed essential oil and extracts against carbon tetrachloride-induced toxicity. *Iran J Bas Med Sci.* 2015;18;205-11.
18. Niknahad H, Heidari R, Mokhtebaz T, Mansouri S, Dehshahri S, Abdoli N, Najibi A. Evaluating the effects of different fractions obtained from Gundelia tournefortii extract against carbon tetrachloride-induced liver injury in rats. *Trend Pharm Sci.* 2016;2;25-34.
19. Weber LWD, Boll M, Stampfl A. Hepatotoxicity and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model. *Crit Rev Toxicol.* 2003;33;105-36.
20. Tong J, Yao X, Zeng H, Zhou G, Chen Y, Ma B, Wang Y. Hepatoprotective activity of flavonoids from Cichorium glandulosum seeds in vitro and in vivo carbon tetrachloride-induced hepatotoxicity. *J Ethnopharmacol*. 2015;174;355-63.
21. Farida T, Salawu OA, Tijani AY, Ejiofor JI. Pharmacological evaluation of Ipomoea asarifolia (Desr.) against carbon tetrachloride-induced hepatotoxicity in rats. *J Ethnopharmacol.* 2012;142;642-6.
22. Niranjana Murthy H, Dandin VS, Yoeup Paek K. Hepatoprotective activity of ginsenosides from Panax ginseng adventitious roots against carbon tetrachloride treated hepatic injury in rats. *J Ethnopharmacol*. 2014;158, Part A;442-6.
23. Jadon A, Bhadauria M, Shukla S. Protective effect of Terminalia belerica Roxb. and gallic acid against carbon tetrachloride induced damage in albino rats. *J Ethnopharmacol.* 2007;109;214-8.
24. Itoigawa M, Ito C, Tan HTW, Okuda M, Tokuda H, Nishino H, Furukawa H. Cancer chemopreventive activity of naphthoquinones and their analogs from Avicennia plants. *Cancer Lett.* 2001;174;135-9.
25. El-Komy MM, Mouafi FE. Mitigating effect of Avicenna marina on indomethacin induced gastric ulcer in male albino rats. *Egyp J Basic Appl Sci.* 2016;3;155-63.
26. Mouafi FE, Abdel-Aziz SM, Bashir AA, Fyiad AA. Phytochemical analysis and antimicrobial activity of mangrove leaves (Avicenna marina and Rhizophora stylosa) against some pathogens. *W Appl Sci J.* 2014;29;547-54.
27. Ravikumar S, Gnanadesigan M, Suganthi P, Ramalakshmi A. Antibacterial potential of chosen mangrove plants against isolated urinary tract infectious bacterial pathogens. *Int J Med Med Sci.* 2010;2;94-9.
28. Gurudeeban S, Satyavani K, Ramanathan T, Balasubramanian T. Antidiabetic effect of a black mangrove species Aegiceras corniculatum in alloxan-induced diabetic rats. *J Adv Pharm Technol Res.* 2012;3;52.
29. Keivan Z, Taherzadeh M, Yaghoubi R, Tajbakhsh S, Rastian Z, Fouladv M, Sartavi K. Antiviral activity of Avicennia marina against herpes simplex virus type 1 and vaccine strain of poliovirus (An in vitro study). *J Med Plant Res.* 2009;3;771-75.
30. V VP, C G. Protective effect of marine mangrove Rhizophora apiculata on acetic acid induced experimental colitis by regulating anti-oxidant enzymes, inflammatory mediators and nuclear factor-kappa B subunits. *Int Immunopharmacol.* 2014;18;124-34.
31. Al-Attar AM. Protective effect of Avicennia alba leaves extract on gastric mucosal damage induced by ethanol. *Res J Med Plant*. 2011;5;477-90.
32. Kar DR, Farhad MS, Sahu PK. A review on pharmacological profiles of ethno-medicinal plant: Avicennia alba Blume. *Int J PharmTech Res*. 2015;7;370-3.
33. Banerjee D, Chakrabarti S, Hazra AK, Banerjee S, Ray J, Mukherjee B. Antioxidant activity and total phenolics of some mangroves in Sundarbans. *African J Biotechnol.* 2008;7:805-10.
34. Ramadan MF, Amer MMA, Mansour HT, Wahdan KM, El-Sayed RM, El-Sanhoty S, El-Gleel WA. Bioactive lipids and antioxidant properties of wild Egyptian Pulicaria incise, Diplotaxis harra, and Avicennia marina. *J Verbrauch Lebensm.* 2009;4;239.
35. Thirunavukkarasu P, Ramanathan T, Ramkumar L, Shanmugapriya R, Renugadevi G. The antioxidant and free radical scavenging effect of Avicennia officinalis. *J Med Plant Res.* 2011;5;4754-8.
36. Alia M, Ramos S, Mateos R, Bravo L, Goya L. Response of the antioxidant defense sys-

tem to tert-butyl hydroperoxide and hydrogen peroxide in a human hepatoma cell line (HepG2). *J Biochem Mol Toxicol.* 2005;19;119-28.
37. Sattayasai J, Chaonapan P, Arkaravichie T, Soi-ampornkul R, Junnu S, Charoensilp P, Samer J, Jantaravinid J, Masaratana P, Suktitipat B. Protective effects of mangosteen extract on H 2 O 2-induced cytotoxicity in SK-N-SH cells and scopolamine-induced memory impairment in mice. *PLoS One.* 2013;8;e85053.
38. Benzie IF, Strain J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal Biochem.* 1996;239;70-6.
39. Niknahad H, Heidari R, Alzuhairi AM, Najibi A. Mitochondrial dysfunction as a mechanism for pioglitazone-induced injury toward HepG2 cell line. *Pharm Sci.* 2015;20;169-74.
40. Heidari R, Jamshidzadeh A, Keshavarz N, Azarpira N. Mitigation of Methimazole-Induced Hepatic Injury by Taurine in Mice. *Sci Pharm*. 2014;In Press.
41. Heidari R, Niknahad H, Jamshidzadeh A, Azarpira N, Bazyari M, Najibi A. Carbonyl Traps as Potential Protective Agents against Methimazole-Induced Liver Injury. *J Biochem Mol Toxicol*. 2015;29;173-81.
42. Heidari R, Jamshidzadeh A, Niknahad H, Mardani E, Ommati MM, Azarpira N, Khodaei F, Zarei A, Ayarzadeh M, Mousavi S, others. Effect of taurine on chronic and acute liver injury: Focus on blood and brain ammonia. *Toxicol Report.* 2016;3;870-9.
43. Moezi L, Heidari R, Amirghofran Z, Nekooeian AA, Monabati A, Dehpour AR. Enhanced anti-ulcer effect of pioglitazone on gastric ulcers in cirrhotic rats: The role of nitric oxide and IL-1b. *Pharmacol Report.* 2013;65;134-43.
44. Jamshidzadeh A, Heidari R, Golzar T, Derakhshanfar A. Effect of Eisenia foetida Extract against Cisplatin-Induced Kidney Injury in Rats. *J Diet Suppl.* 2016;13;551-9.
45. Uchiyama M, Mihara M. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. *Anal Biochem.* 1978;86;271-8.
46. Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Anal Biochem.* 1968;25;192-205.
47. Heidari R, Taheri V, Rahimi HR, Shirazi Yeganeh B, Niknahad H, Najibi A. Sulfasalazine-induced renal injury in rats and the protective role of thiol-reductants. *Ren Fail.* 2016;38;137-41.
48. Heidari R, Babaei H, Roshangar L, Eghbal MA. Effects of enzyme induction and/or glutathione depletion on methimazole-induced hepatotoxicity in mice and the protective role of N-acetylcysteine. *Advanc Pharm Bull.* 2013;4;21-8.
49. Heidari R, Jamshidzadeh A, Keshavarz N, Azarpira N. Mitigation of Methimazole-Induced Hepatic Injury by Taurine in Mice. *Sci Pharm.* 2015;83;143-58.
50. Gaudio E, Onori P, Franchitto A, Sferra R, Riggio O. Liver metabolic zonation and hepatic microcirculation in carbon tetrachloride-induced experimental cirrhosis. *Digest Dise Sci.* 1997;42;167-77.
51. Ritter C, Reinke A, Andrades M, Martins MR, Rocha J, Menna-Barreto S, Quevedo J, Moreira JCF, Dal-Pizzol F. Protective effect of N-acetylcysteine and deferoxamine on carbon tetrachloride-induced acute hepatic failure in rats. *Crit Care Med.* 2004;32;2079-83.
52. Vargas-Mendoza N, Madrigal-Santillán E, Morales-González A, Esquivel-Soto J, Esquivel-Chirino C, García-Luna Y González-Rubio M, Gayosso-de-Lucio JA, Morales-González JA. Hepatoprotective effect of silymarin. *World J Hepatol*. 2014;6;144-9.
53. Czaja MJ, Xu J, Alt E. Prevention of carbon tetrachloride-induced rat liver injury by soluble tumor necrosis factor receptor. *Gastroenterology.* 1995;108;1849-54.
54. Weber LWD, Boll M, Stampfl A. Hepatotoxicity and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model. *Crit Rev Toxicol.* 2003;33;105-36.
55. Ghosh N, Ghosh R, Mandal V, Mandal SC. Recent advances in herbal medicine for treatment of liver diseases. *Pharm Biol.* 2011;49;970-88.
56. W. Hogg R, T. Gillan F. Fatty acids, sterols and hydrocarbons in the leaves from eleven species of mangrove. Phytochemistry. 1984;23;93-97.
57. Sharaf M, El-Ansari MA, Saleh NAM. New flavonoids from Avicenniamarina. *Fitoterapia*. 2000;71;274-7.
58. Kim S-H, Cheon HJ, Yun N, Oh S-T, Shin E, Shim KS, Lee S-M. Protective effect of a mixture of Aloe vera and Silybum marianum

against carbon tetrachloride-induced acute hepatotoxicity and liver fibrosis. *J Pharmacol Sci.* 2009;109;119-27.
59. Tirkey N, Pilkhwal S, Kuhad A, Chopra K. Hesperidin, a citrus bioflavonoid, decreases the oxidative stress produced by carbon tetrachloride in rat liver and kidney. *BMC Pharmacol.* 2005;5.
60. Tipoe GL, Leung TM, Liong EC, Lau TYH, Fung ML, Nanji AA. Epigallocatechin-3-gallate (EGCG) reduces liver inflammation, oxidative stress and fibrosis in carbon tetrachloride (CCl 4)-induced liver injury in mice. *Toxicology.* 2010;273;45-52.
61. Jadon A, Bhadauria M, Shukla S. Protective effect of Terminalia belerica Roxb. and gallic acid against carbon tetrachloride induced damage in albino rats. *J Ethnopharmacol.* 2007;109;214-8.
62. Tung Y-T, Wu J-H, Huang C-C, Peng H-C, Chen Y-L, Yang S-C, Chang S-T. Protective effect of Acacia confusa bark extract and its active compound gallic acid against carbon tetrachloride-induced chronic liver injury in rats. *Food Chem Toxicol.* 2009;47;1385-92.
63. Pavanato A, Tuñón MJ, Sánchez-Campos S, Marroni CA, Llesuy S, González-Gallego J, Marroni N. Effects of quercetin on liver damage in rats with carbon tetrachloride-induced cirrhosis. *Dig Dis Sci.* 2003;48;824-9.
64. Cui Y, Han Y, Yang X, Sun Y, Zhao Y. Protective effects of quercetin and quercetin-5',8-disulfonate against carbon tetrachloride-caused oxidative liver injury in mice. *Molecules.* 2013;19;291-305.
65. Bhadauria M, Nirala SK, Shukla S. Propolis protects CYP 2E1 enzymatic activity and oxidative stress induced by carbon tetrachloride. *Mol Cell Biochem*. 2007;302;215-24.