Hautriwaic acid as one of the hepatoprotective constituent of Dodonaea viscosa

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A B S T R A C T
It is widely known that hepatitis and its complications such as cirrhosis or hepatocellular carcinoma are one of the major health problems of the world especially since no specific treatment is available. In the present study we investigated the hepatoprotective potential of the methanolic extract of the whole plant of Dodonaea viscosa and its ethyl acetate, aqueous, butanol and n-hexane fractions against carbon tetrachloride (CCL4) induced hepatotoxicity in rats. Hepatoprotection was assessed in terms of reduction in serum enzymes (ALT, AST, and ALP) that occur after CCL4 injury, and by histopathology and immune-histochemistry. The methanolic extract reduced the serum enzyme level (ALT, AST, and ALP) down to control levels despite CCL4 treatment. It also reduced the CCL4-induced damaged area to 0% as assessed by histopathology. The CD68+ macrophages were also reduced in number around the central vein area by the methanolic extract. These hepatoprotective effects were better than the positive control silymarin. Similar hepatoprotective activities were found with the ethyl acetate, and aqueous fractions of the methanolic extract. The butanol and n-hexane fractions showed elevated levels of ALT, AST and ALP as compared to the positive control silymarin. Histopathology showed ~30% damage to the liver cells with the butanol and n-hexane fractions which still showed some protective activity compared to the CCL4 treated control. HPLC fingerprinting suggested that hautriwaic acid present in the methanolic extract and its ethyl acetate, and aqueous fractions may be responsible for this hepatoprotective activity of Dodonaea viscosa which was confirmed by in vivo experiments.

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Introduction
Liver is the first organ to metabolize all foreign compounds and hence it is susceptible to injury that can result in different diseases such as hepatitis, cirrhosis or hepatocellular carcinoma. A major cause of these disorders is exposure to different environmental pollutants and chemicals e.g., paracetamol, carbon tetrachloride, thiocetamide, alcohol, etc. Worldwide, hepatitis is an important liver disease with a staggering incidence of 550 million (Alter 2006). It is more common in developing countries. In Pakistan almost 35 million people are believed to be infected with the virus (André 2000). Its complications such as cirrhosis of liver are formidable enemies since there is no specific treatment available for them. Many of the drug-induced liver injury cause morbidity and mortality around the world (Ghabril et al. 2010).

Conventional or synthetic drugs used in the treatment of liver diseases are inadequate and can have serious adverse effects. Many natural products of herbal origin are in use for the treatment of liver ailments. Polyphenolic compounds are widely distributed in plants and known to be excellent antioxidants in vitro and have the capacity to scavenge free radicals and protect antioxidant defenses in liver diseases (Sreeleatha et al. 2009).

Carbon tetrachloride (CCL4) is a widely used and well characterized animal model of chemical-induced, oxidative stress-mediated hepatotoxicity (Recknagel and Gende 1973). The symptoms of chronic liver injury in humans are similar to those of CCL4-induced chronic liver injury (Basu 2003). CCL4 induces the production of several types of reactive oxygen species (ROS) through cytochrome P450 thereby causing liver injury (Tada et al. 2003). These ROS can bind to polyunsaturated fatty acids, forming different radicals to produce lipid peroxide, which causes membrane damage and changes in enzyme activity (Weber et al. 2003), and consequently
induce hepatic injury, inflammation, necrosis and apoptosis, (Lin et al. 2009).

_Dodonaea viscosa_, is an evergreen shrub belongs to the family spindaceae, consists of approximately 2000 species and 150 genera. The genus _Dodonaea_ consists of 60 species. Previous work on _Dodonaea viscosa_ revealed the presence of major secondary metabolites isolated from _Dodonaea viscosa_ consist of diterpenoids, triterpenoid saponine organic acids, flavonoids, viscousine (Khan et al. 2013), methylenebissantin (Muhammad et al. 2012a,b) tannins, sterols (Wagner et al. 1987). _Dodonaea viscosa_ is widely used in folk medicine for treatment of skin diseases (Pirzada et al. 2010). The crude extracts of _Dodonaea viscosa_ have shown anti diabetic, antimalarial, antibacterial (Khurram et al. 2009) and gastroprotective activity (Arun and Asha 2008). Taking into account our interest in the medicinal plants of Pakistan (Riaz et al. 2002) and pharmacological significance of _Dodonaea viscosa_, the present hepato protective investigation was undertaken to identify natural compounds from _Dodonaea viscosa_ that may be used as potent hepato protective agents.

**Materials and methods**

**Plant materials**

The whole aerial parts of _Dodonaea viscosa_ were collected from the hills of Kurram agency, Khyber Pakhtoonkhwa province of Pakistan. The identification was done by Dr. Ijaz Khan, a plant taxonomist, Department of Botany, Post Graduate College, Kohat, Khyber Pakhtoonkhwa, Pakistan. A voucher specimen (DVPGCK-098) has been deposited in the herbarium of Department of Botany, Post Graduate College, Kohat.

**Extract preparation from _Dodonaea viscosa_**

The shade-dried plant (20 kg) material was ground into powder and extracted at room temperature with MeOH to yield the methanolic extract (2 kg). After removal of the solvent, the extract was suspended in H2O, and extracted with n-hexane, Ethyl acetate (EtOAc), and butanol to yield hexane (620 g), EtOAc (507 g), and butanol (750 g) fractions. The extract and fractions were kept at 4 °C for use in hepatoprotective activity. Hautriwaic acid was isolated and characterized as described earlier (Salinas-Sánchez et al. 2012).

**Animals**

Male wistar rats weighing 180–200 g were housed in individual cages kept at 22–26 °C under 12-h light/dark cycles, with free access to standard laboratory chow and tap water ad libitum. All animals received humane care and all protocols involving the animals were in compliance with the guidelines approved by the institutional ethics committee of ICCBS, Karachi University.

**CCL4-induced liver injury**

The animals were randomly divided into eight groups of six rats each as described below: Group 1 (normal control) were injected with vehicle only (1 ml/kg body weight olive oil); Group 2 (hepatitis model) were injected with intraperitoneal injection of CCL4 (1 ml/kg) with 1:1 olive oil; Group 3 (positive control) were injected with intraperitoneal injection of CCL4 (1 ml/kg) with 1:1 olive oil and also received silymarin 200 mg/kg (oral), per day 3 days before treatment and 2 days after treatment; Groups 4–8 were injected with intraperitoneal injection of CCL4 (1 ml/kg) with 1:1 olive oil but also received methanolic extract, aqueous, ethyl acetate, butanol, n-hexane fractions and hautriwaic acid respectively, at a dose of 100 mg/kg body weight per day for 3 days before treatment and 2 days after treatment.

**Blood biochemistry**

Wistar rats were sacrificed 48 hr after CCL4 administration under sodium pentothal anesthesia. Blood was collected by cardiac puncture. Serums were obtained for determination of liver damage by measuring the serum level of alanineaminotransferase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) using a dry chemistry analyzer (Roche Diagnostics, Mannheim, Germany).

**Histopathological examination of the liver**

Liver tissues were rapidly removed and fixed in 10% neutral buffered formalin, dehydrated through a graded series of alcohol, embedded in paraffin, and cut into 6 μm thick sections. The liver tissues were stained with Hematoxylin–Eosin (H&E). The tissues were then examined under bright field microscope at different magnification using a Nikon 90i microscope. Histopathological analysis of the liver under different conditions was carried out as follows. First the necrotic area was measured by in 30 different sections of the liver using the NIS-elements software from Nikon, Japan. Then the damaged area was expressed as percentage of the whole area of the section.

**Immunohistochemistry**

For immunohistochemistry, the liver sections were deparaffinized in xylene, rehydrated in graded alcohol, washed in water for 10–15 min. Each section was incubated with a blocking solution in phosphate buffered solution (PBS, Roti-immunoblock, Carl Roth, Karlsruhe, Germany) for 30 min at room temperature. Then, the sections were incubated with monoclonal CD68 (clone ED1, abcam) primary antibody (1:100) for 1 hour at 37 °C. After thoroughly washing with PBS, the sections were incubated with Texas Red-conjugated goat anti-mouse IgG (1:100) for 45 min. After washing extensively with PBS the nuclei were stained with DAPI (1:10,000 dilution of a 1 mg/ml stock solution) for 1 min, washed with PBS and mounted in mowiol 4-88 mounting media. Fluorescent images were acquired and analyzed using a Nikon 90i multichannel fluorescence microscope and a Nikon DXM 1200 C camera with NIS-Elements image analysis software AR 3.0 (Nikon, Japan). Image processing was performed with Adobe Photoshop software. CD68+ macrophage cells were counted from number of the DAPI stained nucleus that showed the CD68 antibody staining around it using the NIS-elements software. The CD68+ macrophages were counted around the central vein region in a round area of 104,025 mm².

**HPLC analysis**

HPLC analysis of MeOH extract of _Dodonaea viscosa_ and its various fractions were performed on Agilent 1200 Series, Rapid Resolution LC (RRLC) system, comprising Agilent binary pump SL with degasser, high performance autosampler SL with thermostat, thermostatted column compartment (TCC) and diode–array detector SL (DAD SL). Data acquisition and integration was controlled by Agilent Technologies ChemStation software. An Agilent Zorbax Eclipse XDB-C8 column (3.0 mm × 30 mm I.D., 1.8 μm) was used. The HPLC grade acetonitrile and methanol were purchased from Fisher Chemicals (USA). Water was purified using a Millipore® Milli-Q Plus system (Bedford, USA). The mobile phase was a binary gradient system composed of eluent A (95% water containing 0.01% HCOOH + 5% acetonitrile) and eluent B (0.01% formic acid in ACN) properly filtered and degassed for 15 min in ultra-sonic bath before use. The gradient program was: 10–60% B from 0 to 35 min, 60–35%
Fig. 1. Hepatoprotective activity of Dodonaea viscosa methanolic extract. Histopathology of liver showing normal (green arrows) central vein, hepatic cords and sinusoids in the untreated normal control (normal, A, B); pale necrotic areas (red arrows) after CCl₄ treatment in the control group (CCl₄, C, D); protection by the positive control 200 mg/kg silymarin (CCl₄ + silymarin E, F); protection by methanolic extract of Dodonaea viscosa (CCl₄ + MeOH, G, H). Note that the methanolic extract protection is better than that of silymarin. Scale bar for A, C, E and G is 250 μm and is shown in G. Scale bar for B, D, F and H is 25 μm and is shown in H. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

B from 35 to 38 min, 35–100% B from 38 to 41 min, flow rate was 0.3 ml min⁻¹ throughout the analysis. The samples and standard were injected through an auto sampler, the standard hauriwaic acid was injected 3 μl, the crude extract, ethyl acetate, water, butanol and hexane fractions were injected 1 μl each. Column temperature was maintained at 30 °C. The detection wavelength of DAD was 254 nm.

A solution of crude extract and its fractions were prepared in a 5 ml volumetric flasks with MeOH in the concentration of 2 mg/ml each. The stock solution of hauriwaic acid was prepared by accurately weighing 2 mg in a 5 ml volumetric flask and made up volume with methanol. Working solution was prepared by taking 200 μl from the stock and volume raised to 400 μl with methanol. All samples were filtered with 0.22 μm PTFE fluoropore syringe driven filter unit (Millipore, Bedford, USA) and preserved at 4 °C prior to LC analysis.

Results

Histopathology of the liver reveals better hepatoprotective activity of the MeOH extract of Dodonaea viscosa compared to silymarin

Histology of the normal control group showed central vein surrounded by hepatic cord of cells with clear sinusoidal spaces lined by endothelial cells (Fig. 1A and B). In contrast, 48 h after the animals were treated with CCl₄ the liver showed clear pale areas
of necrosis around the central vein (Fig. 1C and D, red arrow). These pale areas showed no hepatocytes but the presence of endothelial-like and mixed inflammatory cells. This indicated that the CCl₄ damages only the hepatocytes, an observation which is in line with the fact that the damaging free radical CCl₃⁻ is generated only in the hepatocytes. At times, cells surrounding this pale necrotic area showed hydropic degeneration. Healthy hepatocytes (Fig. 1C and D, green arrow) were also observed around the necrotic area which could easily be distinguished because of their darker staining characteristic with H/E staining. Further histopathological analysis (Fig. 3B) revealed ~49% damage in the treated group. Liver specimens obtained from the CCl₄ + silymarin treated group showed a big decrease (p < 0.001) in the size of the necrotic area around the central vein (Fig. 1E and F) compared to the CCl₄ control. However, a small amount of damage (~3%) was detected by histopathological analysis (Fig. 3B). Interestingly, the CCl₄ + MeOH extract showed no necrosis (Fig. 1G and H) and 0% damage (Fig. 3B). Therefore, the MeOH extract at a dose of 100 mg/kg showed better hepatoprotective activity (p < 0.001) compared to the positive control silymarin at a dose of 200 mg/kg.

The EtOAc and H₂O fractions of the MeOH extract of Dodonaea viscosa are responsible for its biological activity

Fig. 2 shows the effect of different fractions of the MeOH extract of Dodonaea viscosa on the histology of CCl₄ induced liver injury. The CCl₄ + EtOAc group showed protective effect similar to the MeOH extract with no sign of necrosis (Fig. 2A and B) and 0%
damage (Fig. 3B). Similarly the CCl₄ + H₂O group also showed no sign of necrosis (Fig. 2C and D) and 0% damage (Fig. 3B). In contrast, the CCl₄ + Butanol group showed more necrosis around the central vein region compared to the positive control silymarin with massive inflammatory infiltrate (Fig. 2E and F). Histopathological analysis showed ~29% damage (Fig. 3B) which is less than the normal CCl₄ control but more than the positive control. The CCl₄ + n-hexane group also showed damaged hepatocytes around the central vein (Fig. 2G and H). Interestingly, there was orange color in the damaged area indicating the presence of some orange colored substance in the n-hexane fraction. Histopathological analysis revealed ~28% damage (Fig. 3B) to the liver. These results indicate that the hepatoprotective compounds of the MeOH extract of Dodonaea viscosa are present in the EtOAc and H₂O fractions.

Dodonaea viscosa extracts attenuated hepatic enzyme release

As shown in Fig. 3A, CCl₄ treated rats showed significantly elevated level of serum ALT (1261 ± 133), AST (374 ± 36) and ALP (488 ± 5) compared with the normal control group ALT (92 ± 6), AST (127 ± 10) and ALP (139 ± 5). The positive control silymarin prevented (p < 0.001) this increase in the serum level of hepatic enzymes ALT (243 ± 14), AST (231 ± 7) and ALP (209 ± 5) to a great extent. However, the positive control silymarin did not bring the level of the enzymes down to the normal control group indicating a small amount of damage still remaining in the silymarin group. Interestingly, the MeOH extract of Dodonaea viscosa also prevented (p < 0.001) the increase in the serum level of hepatic enzymes ALT (78 ± 30), AST (163 ± 7) and ALP (164 ± 21). Similar to the histopathology results, the MeOH extract of Dodonaea viscosa was more effective (p < 0.001) in this activity compared to silymarin group. The MeOH extract of Dodonaea viscosa brought the levels of the enzymes down to normal control levels indicating no damage to the liver parenchyma despite the CCl₄ treatment. The EtOAc fraction inhibited (p < 0.001) the increase in serum enzymes ALT (105 ± 3), AST (142 ± 7) and ALP (141 ± 9). Similarly, the H₂O fraction also inhibited (p < 0.001) the increase in serum enzymes ALT (66 ± 6), AST (166 ± 10) and ALP (167 ± 36). However, the Butanol fraction with ALT (290 ± 5), AST (459 ± 7) and ALP (556 ± 20) and the n-hexane fraction with ALT (258 ± 6), AST (410 ± 5) and ALP (479 ± 4) did not appreciably reduce the hepatic enzymes. Interestingly, hautriwaic acid reduced the level of ALT (215 ± 16), AST (220 ± 7) and ALP (190 ± 5) than the positive control silymarin. These results prove that the hepatoprotective activity of the Dodonaea viscosa reside in the EtOAc and H₂O fraction due to the presence of hautriwaic acid.

**Dodonaea viscosa extract and fractions decrease the recruitment of CD68+ macrophages during CCl₄-induced acute injury**

Liver inflammation is associated with activation of CD68+ macrophages and trigger migration of macrophages into hepatic cords where these macrophages secrete proinflammatory cytokines such as TNF-α and IL-6 (Kiso et al. 2012). To elucidate the roles of CD68+ macrophages in CCl₄-induced liver injury, a specific Kupffer Cell (KC) marker, CD68, was used to monitor CD68+ macrophages activation. Normally, quiescent CD68+ macrophages are present in the hepatic sinusoids and around the central vein region (Fig. 4A, B and C) in a small number (71 ± 10) in the normal control liver (Fig. 6). After 48 h of CCl₄ administration, the number of CD68+ macrophages increased (p < 0.001) to (190 ± 8) around the central vein region (Fig. 4 D, E and F). A huge number of mixed inflammatory cells (Fig. 6) were also present around the damaged central vein region which was evident with DAPI staining. It was obvious from Fig. 6 that the positive control silymarin reduced (p < 0.001) the number of CD68+ macrophages (112 ± 10) compared to the CCl₄ control. However, the number of CD68+ macrophages present around the injured central vein region in the silymarin treated group was still higher (p < 0.001) compared to the normal control.
Interestingly, the MeOH extract of *Dodonaea viscosa*, significantly decreased \( p < 0.001 \) CD68+ macrophages \( (71 \pm 9) \) around the central vein (Fig. 6) to levels very similar to the normal control group. After CCl\textsubscript{4} treatment the activated CD68+ macrophages come out of the sinusoidal spaces where they normally reside. The MeOH extract treatment limited the CD68+ macrophages to the sinusoidal spaces despite the CCl\textsubscript{4} treatment as evidenced from the immunohistochemistry (Fig. 4J, K and L). Moreover, the EtOAc fraction significantly reduced \( p < 0.001 \) the number of CD68+ macrophages \( (74 \pm 8) \) as evident from immunohistochemistry (Fig. 5A, B and C). In EtOAc treated group the CD68+ macrophages were also localized (Fig. 6) to the sinusoidal spaces similar to the MeOH extract group. Likewise, \( H_2O \) fraction completely restricted \( p < 0.001 \) the CD68+ macrophages \( (75 \pm 9) \) (Fig. 6) to the sinusoidal spaces (Fig. 5D, E and F). Compared with normal control liver with Butanol treatment, the number of CD68+ macrophages \( (153 \pm 11) \) increased (Fig. 6) dramatically around the central vein region where pathology was evident (Fig. 5G, H and I). The number of CD68+ macrophages was also significantly elevated CD68+ macrophages (Fig. 6) \( (141 \pm 12) \) in n-hexane treated group (Fig. 5J, K and L).

**Hautriwai acid decrease CCl\textsubscript{4}-induced acute liver injury and recruitment of CD68+ macrophages**

Treatment with hautriwai acid (HA, Fig. 7G) at a dose of 100 mg/kg decreased the abnormality of liver architecture induced by CCl\textsubscript{4} (Fig. 7A and B) and showed hepatic cells with well-preserved cytoplasm, nucleus, nucleolus and central vein (Fig. 7B) as compared to silymarin treated group. The distribution of CD68+ macrophages in the hautriwai acid group (HA) was limited to the central vein region and sinusoidal spaces (Fig. 7C). After hautriwai acid administration, the number of CD68+ macrophages decreased \( p < 0.001 \) to \( (94 \pm 12, \text{Fig. 6}) \) around the central vein region (Fig. 7D, E and F). It is evident from Fig. 6 that hautriwai acid decreased the number of CD68+ macrophages compared to positive control silymarin.
HPLC fingerprinting of Dodonaea viscosa

MeOH extract of Dodonaea viscosa and its ethyl acetate, water, hexane, and butanol fractions were analyzed by HPLC. A comparative chromatogram of all fractions is presented in Fig. 8. MeOH extract showed the presence of several analytes; while major analytes were well resolved and eluted at various retention times, 4.85, 5.48, 13.04, 14.29, 16.28, 18.17, 19.46, 20.48, 22.94, and 30.61 min. Distribution of compounds from MeOH extract into various subfractions can be seen through comparison of their chromatograms with the MeOH extract (Fig. 8A). A detailed phytochemical investigation of Dodonaea viscosa has already been reported (Muhammad et al. 2012a,b; Shanmugavasan and Ramachandran 2011; Arun and Asha 2008) showing di- and triterpenes, saponins and flavonoids.

![Figure 5](image1.png)

**Fig. 5.** Effect of Dodonaea viscosa fractions on the number of CD68+ cells: Rats were treated with CCl4 + ethylacetate fraction (A, B, C), or with CCl4 + H2O fraction (D, E, F), or with CCl4 + Butanol fraction (G, H, I), or with CCl4 + n-hexane fraction (J, K, L), and the number of CD68+ cells (A, D, G, J) were analyzed by immunohistochemistry. The nucleus was stained with DAPI (B, E, H, K). C, F, I, L shows the merge of DAPI (green), CD68 (red) and DIC pictures. Note that the ethylacetate (A) and the H2O fraction (D) decreased the number of CD68+ cells around the central vein region in CCl4 induced liver injury; whereas Butanol (G) and n-hexane fraction (J) did not appreciably decrease the number. Scale bar is 50 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

![Figure 6](image2.png)

**Fig. 6.** Quantification of the CD68+ cells after Dodonaea viscosa extracts treatment on CCl4-induced liver injury: CCl4-induced liver injury showed increase in the number of CD68+ cells (p < 0.001) as compared to control in the central vein region (p < 0.001). Silymarin treatment (p < 0.001) somewhat reduced this number while methanolic extract (p < 0.001), ethyl acetate (p < 0.001), water fraction (p < 0.001) and hauwteraic acid (p < 0.001) reduced this number to control levels. Note that the methanolic extract and the ethyl acetate, water fractions and hauwteraic acid reduced the number of CD68+ cells around central vein region more than compared to silymarin (p < 0.001); whereas the Butanol and n-hexane fractions did not appreciably decrease the number compared to silymarin.
One of the compounds which was isolated in bulk amount from the same plant is hautriwaic acid. The peak of hautriwaic acid appeared at $R_t$ 16.23 in the chromatogram of the standard (Fig. 8F) and was also found at the same retention time in the chromatograms of samples.

**Discussion**

Carbon tetrachloride (CCL$_4$) is a well-known for producing chemical-induced hepatic injury. It is widely used for elucidating the mechanisms of various hepatotoxic effects such as fatty degeneration, necrosis, apoptosis and fibrosis (Manibusan et al. 2007; Weber et al. 2003) and to screen hepatoprotective activities of drugs (Lin et al. 2012; Ranawat et al. 2010). Currently, many natural products are being investigated as a source of antioxidants that can be used for hepatoprotective activity (Sreelatha et al. 2009). For instance, Silymarin from Milk Thistle (Silybum marinum) has been shown to have hepatoprotective activity in CCL$_4$-induced liver injury model. Silymarin is also approved by the FDA for treatment of liver diseases and we have used it as a positive control in our study. We have shown here that the MeOH extract and the EtOAc and H$_2$O fraction have better hepatoprotective activity compared to silymarin in a CCL$_4$-induced liver injury model.

A number of medicinal plants have been used in traditional folk medicine for the treatment of liver diseases (Dhiman and Chawla 2005). For the treatment of acute and chronic liver hepatitis, Abelmoschus manihot flower extracts has been used in traditional folk medicine. Total flavonoid isolated from A. manihot flower showed...
viscosa, MeOH extract with CCl₄ significantly maintained the structural integrity of hepatocytes, which may be attributed to the inhibition of CCl₄-induced free radical generation and in turn stabilizing the cell membrane. MeOH extract also normalized the ALT, AST and ALP values. Rats treated with MeOH extract completely prevented the hydropic degeneration and necrosis, which was evident from the histopathology of the CCl₄-induced injured liver.

EtoAc and H₂O fractions also preserved the hepatocyte structural integrity, and significantly decreased the levels of ALT, AST and ALP released from CCl₄ injured rat hepatocytes into the circulation.

Liver macrophages constitute resident and inflammatory macrophages both of which play an important role in liver inflammation. CCl₄ administration causes liver cell damage which is followed by inflammatory processes originating from the products of activated CD68+ macrophages (Edwards et al. 1993). Kiso et al. 2012 have shown that –induced acute hepatitis consists of two phases. Initial phase starts after 2 h of CCl₄ administration generating reactive oxygen species. In the second phase, after 8 h of CCl₄ administration oxidant induced activation of kupffer cells occur, with the release of proinflammatory cytokines like tumor TNF-α, TGF-β and IL-6. These cytokines in turn activate NF-κB and COX-2, leading to necrosis (Sun et al. 2001) and apoptosis (Shi et al. 1998) of liver hepatocytes. In our experiment rats treated with CCl₄ provoked CD68+ macrophage around the inflammatory central vein region. CCl₄-induced activation of CD68+ macrophage with subsequent hepatocellular injury was also shown by others (Edwards et al. 1993). Gadolinium chloride (GdCl₃), a specific inhibitor of CD68+ macrophage exhibited hepatoprotective effect via depletion of activated CD68+ macrophage in thiocetamide induced hepatotoxicity (Andrés et al. 2003). CD68+ liver macrophages and infiltrating neutrophils contribute to liver injury (Jaeschke and Smith 1997) in different experimental models of hepatotoxicity (Essani et al. 1995).

In our experiments, Dodonaea viscosa, MeOH extract significantly attenuated liver injury as well as reduced the increased number of CD68+ cells around the central vein region to normal level induced by CCl₄. The EtoAc and H₂O fractions also attenuated CD68+ cells activation and significantly reduced their number. Thus we suggest that the mechanism of the protection by Dodonaea viscosa is the result of a combination of diminished generation of ROS in the hepatocytes and lack of inflammatory and cytotoxic mediators released from the CD68+ macrophages.

We have identified a potent hepatoprotective compound from Dodonaea viscosa. Hepatoprotective activity in the methanolic crude extract and other fractions except butanol and hexane is due to the presence of hauitiwaic acid and may be due to some other compounds as well. However hauitiwaic acid from the EtoAc fraction of Dodonaea viscosa has been shown to have anti-inflammatory activity (Salinas-Sánchez et al. 2012). Hauitiwaic acid is soluble in moderate polar solvents like ethyl acetate and chloroform while hexane fraction showed a little activity because of hauitiwaic acid solubility in hexane is extremely poor. In ethyl acetate fraction, its solubility is moderate to good so its potential activity in that fraction is justified. Similarly butanol fraction showed poor activity because of absence of hauitiwaic acid. The higher activity of the water fraction was due to the ionized hauitiwaic acid left in the water fraction which all the other organic solvents failed to extract. So it can be concluded from the HPLC profile studies that hauitiwaic acid is one of the compounds responsible for the hepatoprotective activity of Dodonaea viscosa.

References


