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Comparative Study of The Protective Role of Black Berry Juice and Silymarin Against Liver Damage Induced By Carbon Tetrachloride In Rats

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ABSTRACT

Liver diseases are amongst the most serious health problems in the world today and hepatocellular carcinoma is one of the world’s deadliest cancers. Black berry is closely linked to its protective properties against free radical attack. Therefore, the aim of this study was to evaluate the role of black berry juice (BBJ) or silymarin in ameliorating the hepatotoxicity and oxidative stress induced by carbon tetrachloride (CCl4) in male rats. Male Sprague-Dawley rats were injected intraperitoneally with CCl4 at a dose of 0.5 ml/kg daily for 7 days to induce hepatic damage. The rats were orally administered with black berry juice (1.6 g/kg b.wt) or silymarin (200 mg/kg b.wt) prior to CCl4 challenge. The effect of BBJ and silymarin on serum transaminase, alkaline phosphatase and total bilirubin were demonstrated in rats intoxicated by CCl4. Furthermore, the effects of BBJ and silymarin on lipid peroxidation and hepatic antioxidants (SOD, catalase, and GPx) as well as serum GST were estimated. The protective effect was confirmed by the investigation of tissue slides stained with hematoxylin-eosin and Masson’s trichrome reagent. The BBJ and silymarin produced significant hepatoprotective effect as indicated by a significant inhibition of the activity of serum enzymes, bilirubin and lipid peroxidation levels accompanied by significant elevation in the activity of hepatic SOD, catalase, and GPx as well as serum GST activity. Liver sections of animals treated with CCl4 showed centrilobular congestion, ballooning degeneration, vacuolization, necrosis with loss of hepatocytes, leading to disintegration of hepatic cords, fibrosis accompanied with microvesicular steatosis and mononuclear cellular infiltration. Masson’s trichrome stain showed congestion and thickening of the portal tract with dense fibrous tissue. The hepatic lesions induced by CCl4 were significantly attenuated by BBJ or silymarin pretreatment. These results suggest that black berry juice as well as silymarin possess antioxidant properties could prevent liver dysfunction induced by CCl4, and black berry juice and silymarin might be potential candidates for prevention of hepatic disorders.

Key words: Black berry; silymarin; hepatotoxicity; carbon tetrachloride; rats.

Introduction

Liver disease is a worldwide serious health problem. Liver is an organ of paramount important as it plays an essential role in maintaining the biological equilibrium of vertebrates. The spectrum of its functions include, metabolism of carbohydrates, lipids and proteins, blood coagulation and immuno-modulation. Liver also has an important role in the metabolism and disposition of chemicals (xenobiotics) to which the organ is exposed directly or indirectly such as different kinds of undesirable contaminants in food and air such as food additives, industrial chemicals, pesticides and others (Rajesh and Latha, 2004).

Carbon tetrachloride is a xenobiotic hazardous air pollutant. It is an organic compound most often found in the air as a colorless gas. CCl4 is widespread in the environment due to its extensive past use and persistence. It was used in the production of refrigeration fluid and propellants for aerosol cans, as a pesticide, as a cleaning fluid and degreasing agent, in fire extinguishers, and in spot removers. It stays in air for a long time, with a half-life of 30 to 100 years (Ohura et al., 2006a,b). High exposure to CCl4 has been identified as a potent hepatotoxin, induces free radicals-mediated lipid peroxidation of the cytoplasmic membrane phospholipids (Jeon et al., 2003) leading to accumulation of lipid-derived cytotoxic oxidants and alteration in the antioxidant status causing liver injury (Kanter et al., 2005). It also induces hydrobic degeneration, centrilobular necrosis, fatty liver (steatosis), fibrosis and hepatoma (Shih et al., 2005).
As oxidative stress plays a central role in liver pathologies and their progression, the use of antioxidants have been proposed as therapeutic agents, as well as drug co-adjuvants, to counteract liver damage. So there is a worldwide trend to go back to traditional medicinal plants.

One of the most important natural diets with anti-oxidant properties is black berries. Berries are among one of the most widely consumed fruits in the human diet. Berry fruits, wild or cultivated, are proved as a traditional and rich source of bioactive compounds, possessing important biological activities such as flavonoids (anthocyanin), some minerals (Na, K, Ca, Se, Zn and P), vitamins (vitamin A, B complex, C and E) phenolic acids (galic, p-coumaric, caffeic, ferulic) and phenolic polymers (ellagic acids) (Facchini et al., 2004). The anti-oxidant capacity of black berries was related to their constituents’ particularly total phenolics and anthocyanins (Stoner, 2009). Moreover, it was found that, the contents of vitamin C, vitamin E, selenium and zinc in fresh fruit of blackberry has a good effect on human body through protection the integrity of cells and internal structure of cells, avoiding some enzymes and internal components of cells from being destructed. These contents have anti-oxidant activity and they can improve immunity, play an antagonistic role as protective agents from toxic substances (Dunn and Ellis, 2005).

Silymarin (SMN), an extract of Silybum marianum, has a well established hepatoprotective properties. It is a standardized mixture of flavonolignans including silybinin, isosilybinin, silydianin and silychristin. SMN effectively scavenges free radicals, antagonizes lipid peroxidation, and stabilizes cell membranes (Letteron et al., 1990). At the molecular level, SMN stimulates RNA and protein synthesis leading to faster regeneration, repair, and renovation after liver injury. SMN also modulates inflammation and TNF-α production in vitro and in vivo (Katiyar et al., 2005) and binds to hepatocellular receptors, preventing toxins from binding to those sites (Letteron et al., 1990). Therefore, it seems of interest to evaluate the anti-oxidant activity and hepatoprotection effect of black berry juice in comparison with silymarin against CCl4 - induced hepatic injury in rats.

Materials and Methods

Chemicals:

Carbon tetrachloride (CCl4), was obtained from Sigma Chemical Co. (St. Louis, MO, USA) and silymarin was purchased from SEDICO Co. Cairo, Egypt. Fresh black berry fruits were obtained from local market (Cario, Egypt), then washed, homogenized in distilled water and the juice was freshly prepared daily. The dose of black berry (1.6 g/kg b.wt) was used in this study according to the previous studies of Sauebin et al. (2004). Siriwoharn et al. (2004) reported that 100 g black berry contains 317 mg anthocyanin. So 1.6 g black berry contains 5 mg anthocyanin. All other chemicals and solvents were of the highest grade commercially available.

Experimental animals:

Forty - eight of adult Sprague Dawley rats of either sex weighing 120–130 g obtained from Animal House Colony of Helwan farm, Egypt. The animals bred in the Animal House Colony of Faculty of Medicine for Girls, Al- Azhar University, Cairo, Egypt and were housed under standard laboratory conditions (12 h light and 12 h dark) in a room with controlled temperature (24.3°C) during the experimental period. The rats were provided ad libitum with tap water and fed with standard commercial rat chow. Animal procedures were performed in accordance with Guidelines for Ethical Conduct in the Care and Use of Animals.

Experimental design:

After one week of acclimation, animals were divided into six groups (each group consisting of four males and four females). Group (1) served as untreated control, received distilled water intraperitoneally (i.p) once daily for 7 days. Group (2) received black berry juice (BBJ) in a dose of 1.6 g/kg b.wt orally by gavages once daily for 7 days (Sauebin et al., 2004). Group (3) received silymarin in a dose of 200 mg/kg b.wt orally by gavages once daily for 7 days (Morazzoni and Bombardelli, 1995). Group (4) received CCl4 in a dose of 0.5 ml/kg b.wt i.p once daily for 7 days (Ashokshenoy et al., 2001). Group (5) received black berry juice (BBJ) in a dose of 1.6 g/kg b.wt orally by gavages followed by CCl4 in a dose of 0.5 ml/kg b.wt, i.p once daily for 7 days. Group (6) received silymarin in a dose of 200 mg/kg, b.wt orally by gavages followed by CCl4 in a dose of 0.5 ml/kg b.wt i.p once daily for 7 days.

Blood collection and tissue homogenate:

At the end of the treatment period, blood samples were collected from the retro-orbital vein plexus and direct cardiac puncture, under ether anesthesia and the serum was used for the assay of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), glutathione – S
transf erase (GST) activity and, total bilirubin. The rats were sacrificed by cervical dislocation and the liver of each animal was dissected and weighted, then immediately homogenized in 50 mM ice-cold phosphate buffer (pH 7.4) to give 20% homogenate (w/v) (Lin et al., 1998). The homogenate was centrifuged at 1700 rpm and 4°C and the supernatant (20%) was used for the determination of hepatic lipid peroxidation and it was further diluted with phosphate buffer solution to give 2% and 0.5% dilutions for the determination of hepatic catalase (0.5%), superoxide dismutase (0.5%) and glutathione peroxidase (2%) activities.

Biochemical analyses:

Serum ALT and AST activities were measured by the dinitophenylhydrazene (DNPH) method according to Reitman and Frankel (1957) using commercial kits (Biodiagnostic CO., Egypt). Alkaline phosphatase activity in serum was estimated by the method of King (1965) using commercially available kit obtained from Biodiagnostic, Egypt. Hepatic lipid peroxidation was assayed by the measurement of malondialdehyde (MDA) by spectrophotometric method (Satoh, 1978) using commercial kits (Biodiagnostic reagent kits, Egypt). The level of lipid peroxidation was expressed as nmol/g liver tissue. Hepatic catalase (CAT) activity was carried out spectrophotometrically by the modified method of Aebi, (1984) using 50 μl diluted liver homogenate using kit purchased from Biodiagnostic CO., Egypt. Hepatic(SOD) activity was determined spectrophotometrically as the ability of the enzyme to inhibit the phenazine methosulphate- mediated reduction of nitroblue tetrazolium dye by the method of Nishikimi et al. (1972) using 50 μl diluted liver homogenate using kit from (Biodiagnostic CO., Egypt). Hepatic(GPX) activity was assayed by spectrophotometric method using reduced glutathione and cumene hydroperoxide as substrate using 20μl diluted liver homogenate by the modified method of Paglia and Valentine (1967) using Ransel kit obtained from Randox Laboratories Co., UK. The specific activity of hepatic catalase, superoxide dismutase and glutathione peroxidase was expressed as units/g liver tissue. Serum GST activity was estimated by ELISA according to Platz et al. (1997) method using kit produced by Biotin international LTD. Colorimetric determination of serum bilirubin was done by the method described by Sherlock (1951) using kit purchased from Randox Laboratories Co., UK.

Assessment of liver damage:

Samples of the liver from all animals were fixed in 10% neutral formalin and embedded in paraffin wax. Sections (4 μm thickness) were stained with hematoxylin and eosin (H&E) for histological examination. Other sections from liver were stained with Masson’s trichrome for the determination of fibrosis (Drury et al., 1980).

Statistical analysis:

Data were expressed as mean ±S.E. The data was analyzed by an analysis of variance (ANOVA) and the level of significance was determined by Duncan's multiple range tests (Duncan, 1955), to clarify the significant between the individual groups. P values less than 0.05 were considered significant. Results were processed by the computer programs.

Results:

The results of the current study revealed that the activity of AST, ALT, ALP and the level of total bilirubin were significantly (p < 0.01, p < 0.001) increased by (94.4, 90.8, 45.6 and 157.3 % respectively) in CCl4 intoxicated group as compared to the control group indicating the severity of hepatic injury caused by CCl4. However, there were no changes in the activity of the various liver enzymes and in the level of total bilirubin in rats treated with BBJ and silymarin alone as compared to the control group. The activity of the above enzymes and the level of the total bilirubin were significantly reversed in the groups pretreated with BBJ or silymarin prior to CCL4. Noteworthy, the effect of BBJ at the dose of 1.6 g/kg was comparable to that of the reference drug silymarin Table 1 , 2.

Intraperitoneal administration of CCL4 resulted in significant decrease (p < 0.05) in serum GST activity by 44.7% as compared to the control group. Treatment with BBJ and silymarin alone resulted in no change in the activity of serum GST as compared to the control group. There was significant increase (p < 0.01) in GST activity in the groups pretreated with BBJ or silymarin prior to CCL4 as compared to the CCl4 intoxicated group. The two pre-treatment agents could restore its value toward the normal value of the control Table 2.
Table 1: Effect of black berry juice (BBJ) and silymarin pretreatment on CCL4 - induced alteration in liver enzymes.

<table>
<thead>
<tr>
<th>Groups</th>
<th>AST (U/ml)</th>
<th>ALT (U/ml)</th>
<th>ALP (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>40.11 ± 5.45</td>
<td>45.15 ± 6.15</td>
<td>364.12 ± 12.60</td>
</tr>
<tr>
<td>BBJ</td>
<td>38.20 ± 11.45</td>
<td>42.53 ± 9.30</td>
<td>359.24 ± 11.00</td>
</tr>
<tr>
<td>Silymarin</td>
<td>39.19 ± 12.56</td>
<td>41.31 ± 11.27</td>
<td>360.10 ± 10.23</td>
</tr>
<tr>
<td>CCL4</td>
<td>77.98 ± 5.18***</td>
<td>86.14 ± 5.10***</td>
<td>530.05 ± 13.45***</td>
</tr>
<tr>
<td>BBJ + CCL4</td>
<td>47.60 ± 3.48b</td>
<td>52.92 ± 4.6b</td>
<td>386.26 ± 14.02b***</td>
</tr>
<tr>
<td>Silymarin + CCL4</td>
<td>46.58 ± 4.16b</td>
<td>50.61 ± 5.3b</td>
<td>374.34 ± 10.01b***</td>
</tr>
</tbody>
</table>

Within each column, means superscript with the same letter are not significantly different. **P<0.01 and ***P<0.001.

(a) Significantly different from control group. (b) Significantly different from CCL4 - intoxicated group.

Table 2: Effect of black berry juice (BBJ) and silymarin pretreatment on serum glutathione-S- trasferase (GST) and total bilirubin level in rats subjected to CCL4 toxicity.

<table>
<thead>
<tr>
<th>Groups</th>
<th>GST (μg / L )</th>
<th>Bilirubin (μg / dl )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>29.11 ± 3.42</td>
<td>0.89 ± 0.08</td>
</tr>
<tr>
<td>BBJ</td>
<td>23.65 ± 1.35</td>
<td>0.75 ± 0.11</td>
</tr>
<tr>
<td>Silymarin</td>
<td>25.00 ± 2.71</td>
<td>0.80 ± 0.04</td>
</tr>
<tr>
<td>CCL4</td>
<td>16.09 ± 1.21*</td>
<td>2.29 ± 0.02***</td>
</tr>
<tr>
<td>BBJ + CCL4</td>
<td>26.71 ± 2.31**</td>
<td>1.60 ± 0.03***</td>
</tr>
<tr>
<td>Silymarin + CCL4</td>
<td>27.90 ± 2.04b</td>
<td>1.28 ± 0.06b***</td>
</tr>
</tbody>
</table>

Within each column, means superscript with the same letter are not significantly different. *P<0.05, ** P<0.01 and ***P<0.001.

(a) Significantly different from control group. (b) Significantly different from CCL4 - intoxicated group.

SOD, CAT and GPx were measured as an index of antioxidant status of hepatic tissues. Significant decrease in hepatic SOD, catalase, and GPx activity by 51.5, 41.4 and 37.5% respectively were observed in rats intoxicated with CCl4 as compared to the control group. Treatment with BBJ and silymarin alone resulted in insignificant increase in SOD, CAT and GPx activity as compared to the control group. There was significant increase (p < 0.01, p < 0.05) in SOD, CAT and GPx activity in the groups pretreated by BBJ or silymarin prior to CCL4 as compared to the CCL4 intoxicated group. Table 3.

Malondialdehyde (MDA) level is widely used as a marker of free radical mediated lipid peroxidation injury. We measured MDA levels in the liver of the different studied groups and the results are shown in Table 3. MDA levels in CCL4 intoxicated group were significantly higher by (49.4%) than those in the control group (52.61 ± 1.91 vs 35.22 ± 1.43 nmol/g liver, p < 0.01). Treatment with BBJ and silymarin alone resulted in no changes in the levels of MDA as compared to the control group. MDA levels in the groups pretreated by BBJ or silymarin prior to CCL4 were significantly lower (p < 0.01, p < 0.05) than those in the CCL4 intoxicated group. These findings indicated that the free radicals being released in the liver were effectively scavenged when treated with the BBJ and silymarin.

Table 3: Effect of CCL4 exposure on indicators of oxidative stress and oxidative damage in hepatic tissue of rats (mean ± SE).

<table>
<thead>
<tr>
<th>Groups</th>
<th>MDA(nmol/g liver)</th>
<th>CAT(U/g liver)</th>
<th>SOD(U/g liver)</th>
<th>GPX(U/g liver)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>35.22 ± 1.43</td>
<td>2164 ± 98.3</td>
<td>302 ± 31.2</td>
<td>72 ± 4.01</td>
</tr>
<tr>
<td>BBJ</td>
<td>33.44 ± 2.74</td>
<td>2198 ± 86.2</td>
<td>310 ± 24.6</td>
<td>85 ± 7.77</td>
</tr>
<tr>
<td>Silymarin</td>
<td>36.31 ± 1.50</td>
<td>2200 ± 95.1</td>
<td>317 ± 25.4</td>
<td>87 ± 8.71</td>
</tr>
<tr>
<td>CCL4</td>
<td>52.61 ± 1.91***</td>
<td>1049 ± 80.7***</td>
<td>177 ± 15.6*</td>
<td>45 ± 2.14***</td>
</tr>
<tr>
<td>BBJ + CCL4</td>
<td>41.70 ± 2.25***</td>
<td>1501 ± 82.4***</td>
<td>266 ± 18.5***</td>
<td>51 ± 0.12*</td>
</tr>
<tr>
<td>Silymarin + CCL4</td>
<td>44.66 ± 2.19*</td>
<td>1519 ± 85.3***</td>
<td>271 ± 19.2***</td>
<td>52 ± 1.12*</td>
</tr>
</tbody>
</table>

Within each column, means superscript with the same letter are not significantly different. *P<0.05, ** P<0.01 and ***P<0.001.

(a) Significantly different from control group. (b) Significantly different from CCL4 - intoxicated group.

Histopathological examination:

The histological examination of liver sections in the control rat or those treated with BBJ and silymarin showed the normal hepatocytes architecture and the central vein (Fig. 1A). Liver sections of animals intoxicated with CCl4 showed centrilobular congestion, ballooning degeneration, vacuolization, necrosis with loss of hepatocytes, leading to disintegration of hepatic cords, fibrosis accompanied with microvesicular steatosis and mononuclear cellular infiltration (Fig1B,C&D). Pretreatments with BBJ and silymarin alone resulted in no changes in the levels of MDA as compared to the control group. MDA levels in the groups pretreated by BBJ or silymarin prior to CCL4 were significantly lower (p < 0.01, p < 0.05) than those in the CCL4 intoxicated group. These findings indicated that the free radicals being released in the liver were effectively scavenged when treated with the BBJ and silymarin.
Masson’s trichrome stain of liver section of rats pretreated with BBJ or silymarin prior CCl₄ showed mild to moderate accumulation of collagen fibers around the central vein (Fig. 2D & E).

**Fig. 1:** Photomicrograph of the liver section of (A) control rat showing the normal hepatocytes architecture and the central vein, (B-D) rats liver intoxicated with CCl₄ showing centrilobular congestion, ballooning degeneration, vacuolization, necrosis with loss of hepatocytes, leading to disintegration of hepatic cords, fibrosis accompanied with microvesicular steatosis and mononuclear cellular infiltration, (E) rat pre-treated with silymarin prior CCl₄, (F) rat pre-treated with BBJ prior CCl₄, showing mild hydropic degeneration of hepatocytes, and a normal lobular appearance of the liver. (H&E×200)
**Fig. 2:** Photomicrograph of the liver section of (A) control rat showing minimal features of connective tissue around central vein, (B,C) rat intoxicated with CCl₄ showing congestion and thickening of the portal tract with dense fibrous tissue, (D) rat pre-treated with silymarin prior CCl₄ showing a mild accumulation in connective tissue around central vein and (E) rat pre-treated with BBJ prior CCl₄ showing moderate accumulation of collagen fibers around the central vein. (H&E×200)

**Discussion:**

In the present study, CCl₄ intoxicated group showed significant increase in serum liver enzyme activities AST, ALT, ALP and TB level indicating liver injury, meanwhile there was significant decrease in serum GST activity. These results are in-agreement with Liu et al. (2011) who reported that the administration of CCl₄ increased serum hepatic enzyme levels AST, ALT, ALP and TB due to increased production of free radicals, which initiate lipid peroxidation leading to cellular damage. CCl₄ is biotransformed by cytochrome P-450 in the liver to produce highly reactive trichloromethyl free radical. This radical, in the presence of oxygen generated by metabolic leakage from mitochondria, causes lipid peroxidation of membrane lipid. This leads to loss of...
In animals pretreated with BBJ and silymarin there was significant decrease in serum hepatic enzymes and total bilirubin while, there was significant increase in serum GST activity as compared to CCl4 intoxicated group indicating that both silymarin and BBJ have hepatoprotective activity.

Valcheva-Kuzmanova et al. (2004) observed that, the fruits of Aronia melanocarpa are rich in anthocyanins which are plant pigments that inhibit the increase of plasma AST and ALT activities induced by CCl4. Also, Khalil (2009) stated that, the black mulberry juice significantly decrease the levels of ALT, AST in diabetic group compared to the control group. Moreover, the study of Hassan and Yousef (2009) revealed that BBJ reduced AST and ALT and has a protective effect against NaF-induced hepatotoxicity by antagonizing the free radicals generation and enhancement of the antioxidant defence mechanisms. Thus, anthocyanin could be a potential candidate for the prevention of hepatic disorders. Also, (Singh, 2008) reported that pretreatment with potato peel (PPE) are rich in polyphenols and posses strong antioxidant activity significantly normalized the altered activity of GST (Pantelidis et al., 2007).

Ahmed et al. (2003) reported that, silymarin has an antihepatotoxic activity against carbon tetrachloride-induced hepatotoxicity in rats. Silymarin protects liver against the increase in serum ALT, AST, and ALP. Silymarin is known to have hepatoprotective effect (Kang et al., 2004). Also silymarin can inhibit lipid peroxidation by reacting with peroxyl radicals. This ability of silymarin leads to significant increase in cellular antioxidant defense machinery by ameliorating the deleterious effects of free radical reaction and the increase in GSH content (Jensen et al., 2003).

Silymarin can prevent the absorption of toxins into the hepatocytes by occupying the binding sites as well as inhibiting many transport proteins at the membrane. These actions along with anti-peroxidative property make silymarin a suitable candidate for the treatment of iatrogenic and toxic liver diseases (Pradhan and Girish, 2006).

In the present study, administration of CCl4 in rats caused significant decrease in the activity of hepatic CAT, SOD, GPx. Also, administration of CCl4 induced lipid peroxidation (as measured by malondialdehyde (MDA) content in rat liver). These results are in accordance with Khan et al. (2010) and Srivastava and Shivanandapba.2010 who reported that hepatic antioxidant enzyme activities were decreased in the liver of rats administered with CCl4. Also, Wang et al. (2010) observed increased level of hepatic lipid peroxidation (MDA) in rats exposed to CCl4.

Our data also revealed that pre-treatment of rats with BBJ and silymarin leads to significant increase in hepatic CAT, SOD and GPx activities accompanied with significant decrease in hepatic MDA level.

Wang et al. (2010) observed that, the level of SOD in liver homogenate was significantly higher and MDA was significantly lower in blueberry treated group than those in CCl4-induced hepatic fibrosis group. Also, Yang et al. (2010) revealed that, administration of powder of mulberry fruit to rats fed on high-fat diet resulted in significant decline in levels of serum and liver content of thiobarbituric acid related substances, a lipid peroxidation product. While SOD of red blood cell and liver, as well blood GPx activities were significantly increased. The fruits of Aronia melanocarpa are rich in anthocyanins- that could prevent elevation of MDA formation in rat liver (Valcheva-Kuzmanova et al., 2004). Silymarin produced significant increase in the levels of SOD and CAT which decreased by CCl4 expouser (Kumar et al., 2005). Also, silymarin pretreatment treatment reduced the Doxorubicin -induced increase in MDA to 4-fold (Patel et al., 2010).

Also, the histological results in the present study showed that, liver tissue more or less restore the normal histological picture when the animals pretreated by BBJ or silymarin prior to CCl4. These results were in agreement with Domitrovic and Jakovac. (2011) who observed that bilberry induces the resolution of liver fibrosis induced by CCl4 via decreasing oxidative stress. Wang et al. (2010) observed that blueberry alleviated the hepatic fibrosis induced by CCl4. Blueberry exert protective effects on acute liver injury induced by d-galactosamine and lipopolysaccharide. It reduced the injury to hepatocytes (including inflammation and secretion of pro-inflammatory cytokines), improves barrier functions, and have antioxidant activity (Osman et al., 2007). Domitrovic and Jakovac (2010) found that, the livers of mice receiving anthocyanidin delphinidin 10 mg/kg showed only sporadic hepatic lesions, hepatocyte ballooning and fatty degeneration, most of necrotic cells have been replaced by hepatocytes, with the livers showing maintained histoarchitecture, similar to
controls, the therapeutic effects of delphinidin in CCL₄-induced liver fibrosis promoting extracellular matrix degradation, hepatic stellate cells (HSC) inactivation and down-regulation of fibrogenic stimuli, with strong enhancement of hepatic regenerative capability. Anthocyanin delphinidin has been shown to possesses antioxidant, anti-inflammatory, antimutagenic and anti-angiogenic properties (Azevedo et al., 2007). Valcheva-Kuzmanova et al. (2004) reported that, the fruits of *Aronia melanocarpa* are rich in anthocyanins with anti-inflammatory and antioxidant activity dose-dependently reduced the necrotic changes in rat liver induced by CCL₄.

Pal et al., (2010) found that, the animals treated with ethanol extract of *Beta vulgaris* root which contains anthocyanin pigments exhibited significant liver protection against CCl₄ as evident by the presence of normal hepatic cords, absence of necrosis and lesser fatty infiltration. Hou et al. (2010) reported that, anthocyanin-rich extract from black rice reducing the histological adverse effect of alcohol on liver of male Wistar rats. Bao et al. (2008) revealed that, bilberry extract alleviated stress-induced liver damage. Sautebin et al., (2004) indicated that, the anthocyanins contained in the blackberry extract attenuated the hepatic and pancreatic injury induced by endotoxic shock.

Sreelatha et al., (2009) and Abdel Salam et al., (2009) found that, the silymarin treated group showed almost normalization of fatty accumulation and necrosis induced by CCL₄. Tsai et al., (2008) demonstrated that silymarin had beneficial effects in reversing hepatic fibrosis induced by CCl₄ exposure in rats may beat least through promoting the apoptosis of the activated hepatic stellate cells. Flora et al. (1998) suggested that, silymarin may have a significant therapeutic advantage in liver fibrosis. It could be concluded from the present results that, black berry juice could be as well as the reference drug silymarin possessed a protective role against CCl₄-induced hepatic injury in rats through inhibiting liver inflammation and lipid peroxidation and enhancing hepatic antioxidant status.

References


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