Effect of Some Medicinal Plant Seeds on CCl4-Induced Hepatotoxicity in Experimental Rats

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Abstract

There are many plants which are used as medicinal plants by many people such as purslane, chia and garden cress. The present study was carried out to investigate the effect of purslane, chia and garden cress seed powders on CCl4-induced hepatotoxicity in experimental rats. Rats were randomly assigned to 2 main groups including normal or negative control group (6 rats) which fed on basal diet only for 6 weeks, and injected group (36 rats) which injected subcutaneously with CCl4 in paraffin oil (50% v/v, 2ml/kg body weight) twice a week for two weeks to induce chronic damage in liver. After induction, rats were divided into 5 equal groups including positive control group which fed on basal diet only besides 4 herbs-treated groups which fed on basal diet supplemented with 5% of either dry purslane, chia, garden cress seed powders or their mixture, respectively. The curative period continued for 4 weeks. By its end, body weight gain and relative weights of some internal organs were calculated. In addition, serum and liver tissue homogenates were biochemically analyzed. The obtained results revealed that CCl4 exposure led to liver and kidney dysfunction and induced hyperlipidemia due to its oxidative effect. As a result of their antioxidant and phenolic compound contents, using purslane, chia and garden cress seed powders singly or as a mixture improved liver and kidney functions and induced hypolipidemic effect. So, patients who suffer from hepatotoxicity as result of CCl4 exposure can use these plant seeds to test their possible efficiency for their condition.

Key words: Hepatotoxicity, CCl4, purslane seeds, chia seeds, garden cress seeds, phenolic compounds, rats

Introduction

Liver is the largest organ of the human body weighing approximately 1500 g, and is located in the upper right corner of the abdomen on top of the stomach, right kidney and intestines and beneath the diaphragm. The liver performs more than 500 vital metabolic functions (Naruse et al., 2007), including the synthesis of glycogen, plasma proteins, clotting factors bile, urea, etc. (Saukkonen et al., 2006).

The liver disorders are one of the serious health problems throughout the world. More than 350 million people were affected with chronic hepatic infections worldwide (Salhab and Canelo, 2011). Hepatotoxicity refers to liver dysfunction or liver damage that is associated with an overload of drugs or xenobiotics (Navarro and Senior, 2006). Carbon tetrachloride (CCl4) is widely used for experimental induction of liver injury. The injury produced depends on CCl4 metabolism to a highly reactive of free radicals which initiate lipid peroxidation.
Antioxidant agents of natural origin have attracted special interest because they can protect from free radical. Numerous medicinal plants and their formulation are used for liver disorders in ethnomedical practices as well as in traditional medicine. (Parola et al., 1992)

Portulaca oleracea L. (POL), commonly known as purslane, is listed in the World Health Organization as one of the most used medicinal plants and it has been given the term ‘Global Panacea’ (Isnin et al., 2007). POL, as “vegetable for long life” in Chinese folklore, has a cosmopolitan distribution and widely used in many countries (Jin et al., 2013). Modern studies have shown that POL was a rich source of linolenic acid (LNA) and α-tocopherol (α-TCP) (Teixeira et al., 2010). POL exhibited a wide range of pharmacological effects such as anti-inflammatory (Chan et al., 2000), antibacterial (Zhang et al., 2002), skeletal muscle relaxant (Parry et al., 1993), stomach and mouth ulcers (Karimi et al., 2004), diabetic complications (Lee et al., 2012) and liver injuries (Liu et al., 2015).

Chia (Salvia hispanica L.) is an annual herbaceous plant which is native to southern Mexico and northern Guatemala (Ayerza and Coates, 2011; Capitanie et al., 2012). In recent years, chia seeds have been included in the human diet due to the health benefits associated with their composition (Ixtain et al., 2011). According to Marinelie et al. (2014), chia has been investigated and recommended for use due to its high nutritional value. It also contains a high number of antioxidants. Moreover, heavy metal analysis showed that chia seed contains them at safe levels, not exceeding the maximum metal levels for food safety, and the seed is also free from mycotoxins (Tetens, 2009). However, high percentage of fatty acids which are beneficial to health (mainly ω-3 PUFAs, especially α-linolenic acid) is the major cause behind the deep recommendations for use of chia seeds in supplementing commercial human diets (Coorey et al., 2012).

Lepidium sativum L. (Brassicaceae), a medicinal plant origin in Egypt and Middle East, is now cultivated in whole world and often referred to as garden cress. Lepidium sativum L. is used as remedy for inflammatory diseases, such as diabetes, arthritis, traumatic injuries, and hepatitis in traditional medicine (Bigoniya and Shukla, 2014). The extract of Lepidium sativum L. is reported to have various in vitro biological effects including antioxidant, anti-inflammatory, antidiarrheal, antimicrobial, antispasmodic and hepatoprotective action against oxidative damage and have a great potential for use as herbal hepatoprotective or dietary supplements (Del Valle Mendoza et al., 2014; Al-Sheddi et al., 2016). The present investigation was carried out to evaluate the effect of purslane, chia and garden cress seeds powder on hepatotoxicity induced by carbon tetra chloride (CCL₄) in experimental rats.

Materials and Methods

Materials:

Dry seeds of purslane (Portulaca oleracea L.), chia (Salvia hispanica L.) and garden cress (Lepidium sativum L.) were purchased from Arab company for Pharmaceutical and Medicinal plants, MEPACO, Egypt. A total of 42 adult male albino rats (Sprague-Dawley strain) were obtained from the animal colony, Helwan farm, Vaccine and Immunity Organization, Ministry of Health, Cairo Governorate, Egypt. All required chemicals were obtained from Elgomhouria Company for Trading Drugs, Chemicals and Medical Appliances, Cairo, Egypt. Casein, Vitamins mixture and salt mixture: Were purchased from El-Gomhoria Company, Cairo, Egypt.
**Methods:**

**Chemical analysis of seeds**

Phenolic compounds in seed powders were identified and determined by HPLC according to Goupy et al., (1999).

**Experimental animal**

Forty-two mature white Albino rats of an average body weight 150±10 g of Sprague Dawley Strain was used... Rats were fed on basal ration supplying the essential vitamins and trace elements and water supply was given ad-libitum.

**Preparation of Experimental diet**

Basal diet was prepared from fine ingredient per 100 g as follows: It had the following composition: Casein (≥ 80% protein) 14%, soybean oil 4%, cellulose 5%, mineral mixture 3.5%, vitamin mixture 1%, choline chloride 0.25%, DL-methionine 0.3% and corn starch up to 100g (Reeves et al., 1993).

**Experimental design**

Rats were divided into 2 main groups as follows:

- **The first main group (6 rats):** normal rats were fed on basal diet only as a negative control group for six weeks.
- **The second main group (36 rats):** normal rats were injected subcutaneously with CCl₄ in paraffin oil (50% v/v, 2 ml/kg body weight) twice a week for two weeks to induce chronic damage in the liver (Jayasekhar et al., 1997). Six rats were Slaughtered after injection (at the end of two weeks) to ensure of liver injury. AST and ALT were determined to ensure the induction, then the injected remaining rats divided into 5 equal groups as follows:
  - **The second injected group (6 rats):** were fed on basal diet only for 4 weeks as a positive control group.
  - **The third injected group (6 rats):** were fed on basal diet supplemented with 5% dry purslane seeds for 4 weeks.
  - **The fourth injected group (6 rats):** were fed on basal diet supplemented with 5% dry chia seeds for 4 weeks.
  - **The fifth injected group (6 rats):** were fed on basal diet supplemented with 5% dry garden cress seeds for 4 weeks.
  - **The sixth injected group (6 rats):** were fed on basal diet supplemented with the three dry seeds as a mixture (5%) for 4 weeks which were taken (5%) from each seeds and the mixture taken (5%) Body weight and feed intake were checked once a week. At the end, animals were weighed, fasted overnight, and then sacrificed under very light ether anesthesia.

**Biological evaluation**

At the end of the experiment, feed intake, body weight gain, relative organs weight and feed efficiency ratio were calculated according to Chapman et al., (1959).

**Biochemical analysis of serum:**

After sacrifice of rats, Blood samples were collected from hepatic portal vein of each rat into dry clean centrifuge tubes. Serum was carefully separated by centrifugation of blood samples at 3500 round per minute (rpm) for 15 minutes at room temperature, transferred into dry clean eendorf tubes, then kept frozen at - 20°C for later determinations.

Serum AST and ALT were determined in the serum according to the method described by Reitman and Frankel (1957). Serum albumin was determined according to Drupt (1974). Serum globulin was calculated according to the equation described by Chary and Sharma (2004). Malondialdehyde (MDA) was carried according to Sushmakumari et al., (1989).
Lipid peroxidation was estimated according to the method of (Ohkawa et al., 1979). Nitric oxide was followed as the method reported by Green et al.,(1982).Uric acid was determined in the serum according to the method described by Fossati et al., (1980).Urea nitrogen was determined in the serum according to the method described by Patton et al.,(1977).Creatinine forms colored complex when react with alkaline Picrate. This reaction described by Faulkner and King (1976).

Total cholesterol was determined in the serum according to the method described by Allain et al., (1974). Triglycerides were determined in the serum according to the method described by Trinder and Ann (1969). HDL-C was determined in the serum according to the method described by Lopes-Virella et al., (1977). Serum VLDL-C was calculated by the following equation: VLDL-C= Triglyceride/5 according to Friedwald et al., (1972).Serum LDL-C was calculated by the following equation: LDL-C = Total cholesterol – (HDL-C + VLDL-C) according to Friedwald et al., (1972).

Organ sampling:
Livers and kidneys were removed from rats by careful dissection, washed in saline solution (0.9%), dried using filter paper and independently weighed. A specimen from each organ was kept at (-80 0 C) for preparation of tissue homogenate for determination of antioxidant parameters. The homogenation was centrifuged at 1000 r.p.m for 10 minutes.

Statistical analysis:
Statistical analysis were carried out using one-way analysis of variance(ANOVA) test followed by Duncan test through the program of statistical packages for the social science (SPSS). Results were expressed as mean± SD. The differences among means at p < 0.05 were considered significant (Snedecor and Cochran, 1989)

Results

Phenolic compounds of seeds (µg/100g) by HPLC analysis
Purslane, chia,and garden cress seeds analyzed for their phenolic Compounds. The obtained results showed in table (1): purslane seeds recorded higher content of Protocatechuic, and catechol than garden cress and chia seeds. Purslane seeds recorded lower content of Gallic, 4-Amino benzoic, Caffeine, P-OH benzoic, Caffeic, Ellagic, P-Coumaric,3,4,4-Methoxy-cinnnamic and Coumarin than garden cress and chia seeds.

While Chia seeds recorded higher content of Caffeic, Vanillic,Ferulic, Salicylic, 3,4,5-methoxy-cinnamic, Coumarin, and Cinnamic than purslane and garden cress seeds. and recorded lower content of Chlorogenic than purslane and garden cress seeds.
Garden cress recorded higher content of Gallic, Pyrogallol, 4-Aminobenzoic, Catechin, Chlorogenic, Caffeine, P-Coumaric,Ellagic, Benzoic than purslane and chia seeds. and recorded lower content of Protocatechuic,Catechol, Salicylic and Cinnamic than purslane and chia seed.
Table (1)
Phenolic compounds of chia, purslane and garden cress seeds (µg /100g)

<table>
<thead>
<tr>
<th>Phenolic compounds</th>
<th>Test Methods</th>
<th>Chia seeds</th>
<th>Purslane seeds</th>
<th>Garden Cress seeds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gallic acid</td>
<td></td>
<td>34.20</td>
<td>15.54</td>
<td>56.94</td>
</tr>
<tr>
<td>Pyrogalol</td>
<td></td>
<td>478.88</td>
<td>332.11</td>
<td>732.43</td>
</tr>
<tr>
<td>4-Aminobenzoic</td>
<td></td>
<td>10.71</td>
<td>5.42</td>
<td>11.40</td>
</tr>
<tr>
<td>Protocatechuic</td>
<td></td>
<td>64.45</td>
<td>69.36</td>
<td>38.22</td>
</tr>
<tr>
<td>Catechin</td>
<td></td>
<td>104.34</td>
<td>124.28</td>
<td>312.05</td>
</tr>
<tr>
<td>Chlorogenic</td>
<td></td>
<td>29.52</td>
<td>33.37</td>
<td>76.37</td>
</tr>
<tr>
<td>Catechol</td>
<td></td>
<td>36.36</td>
<td>66.43</td>
<td>33.04</td>
</tr>
<tr>
<td>Caffeine</td>
<td></td>
<td>49.62</td>
<td>38.82</td>
<td>81.19</td>
</tr>
<tr>
<td>p-Hydroxybenzene</td>
<td></td>
<td>55.88</td>
<td>19.95</td>
<td>55.44</td>
</tr>
<tr>
<td>Caffeic</td>
<td></td>
<td>12.11</td>
<td>4.37</td>
<td>7.22</td>
</tr>
<tr>
<td>Vanillin</td>
<td></td>
<td>42.52</td>
<td>24.72</td>
<td>24.12</td>
</tr>
<tr>
<td>p-Coumaric</td>
<td></td>
<td>9.23</td>
<td>6.02</td>
<td>20.94</td>
</tr>
<tr>
<td>Ferulic</td>
<td></td>
<td>15.11</td>
<td>10.74</td>
<td>11.08</td>
</tr>
<tr>
<td>Iso-Ferulic</td>
<td></td>
<td>69.0</td>
<td>32.71</td>
<td></td>
</tr>
<tr>
<td>Eugenol</td>
<td></td>
<td>177.56</td>
<td>106.36</td>
<td>122.06</td>
</tr>
<tr>
<td>Alpha-Coumaric</td>
<td></td>
<td>1.35</td>
<td>0.97</td>
<td>1.33</td>
</tr>
<tr>
<td>Benzoic</td>
<td></td>
<td>94.77</td>
<td>70.23</td>
<td>211.88</td>
</tr>
<tr>
<td>Salicylic</td>
<td></td>
<td>128.14</td>
<td>85.47</td>
<td>77.69</td>
</tr>
<tr>
<td>3,4,5-Methoxy-Cinnamic</td>
<td></td>
<td>46.25</td>
<td>5.28</td>
<td>28.66</td>
</tr>
<tr>
<td>Coumarin</td>
<td></td>
<td>24.23</td>
<td>13.81</td>
<td>15.60</td>
</tr>
<tr>
<td>Cinnamic</td>
<td></td>
<td>18.85</td>
<td>3.81</td>
<td>9.94</td>
</tr>
</tbody>
</table>

Biological evaluation:
Nutrition evaluation:

Data presented in Table (2) showed the effect of feeding Purslane, Chia, Garden Cress, and their Mixture on feed intake, body weight gain and feed efficiency ratio in CCl₄-intoxicated rats. It could be noticed that the (+ve) control group recorded a significant decrease in feed intake, body weight gain and feed efficiency ratio compared with the (-ve) control group (p<0.05). All seed-treated groups recorded a significant increase compared with (+ve) control group, but they couldn't return three parameters toward its normal value recorded by (-ve) control group. Feeding mixed seeds improved the three parameters to value near the (-ve) control group.

Table (2)
Effect of feeding Purslane, Chia, Garden Cress seeds, and Seeds mixture on feed intake, body weight gain and feed efficiency ratio in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>FI (g/28 day) rat</th>
<th>BWG %</th>
<th>FER %</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-ve) control</td>
<td></td>
<td>505±11.80</td>
<td>36.40±2.30</td>
<td>.0981±.004</td>
</tr>
<tr>
<td>(+ve) control</td>
<td></td>
<td>351.60±8.03</td>
<td>16±1.30</td>
<td>.070±.006</td>
</tr>
<tr>
<td>Purslane Seeds (5%)</td>
<td></td>
<td>487.40±5.30</td>
<td>25.30±1.90</td>
<td>.081±.007</td>
</tr>
<tr>
<td>Chia Seeds (5%)</td>
<td></td>
<td>487.5±2.5</td>
<td>26.40±1.70</td>
<td>.078±.015</td>
</tr>
<tr>
<td>Garden Cress Seeds (5%)</td>
<td></td>
<td>484.5±7.10</td>
<td>24.90±2.05</td>
<td>.081±.006</td>
</tr>
<tr>
<td>Seed mixture</td>
<td></td>
<td>507.9±8.80</td>
<td>30.30±1.70</td>
<td>.094±.004</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.D.
Significance differences are expressed at p<0.05 using one-way ANOVA test and LSD test.

Relative organs weight

Data presented in Table (3) showed the effect of feeding Purslane, Chia, Garden Cress, and Seeds mixture on relative organs weight (liver and kidney) for hepatotoxicity rats. Relative liver and kidney weights...
kidney weight values showed significant increase in control (+ve) group as compared with negative control group (P < 0.05). Supplemented diets with Purslane, Chia and Garden cress (5%) showed significant decrease (P<0.05) when compared with control positive. The best result was found in rats fed on seeds mixture (5%) that showed significant decrease as compared to the control positive rats.

Table (3)
Effect of feeding Purslane, Chia, Garden Cress seeds and Seeds Mixture on relative organs weight (liver and kidney) in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>Liver %</th>
<th>Kidney %</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-Ve) control</td>
<td></td>
<td>6.90±2.44</td>
<td>1.30±21.17</td>
</tr>
<tr>
<td>(+Ve) control</td>
<td></td>
<td>13.30±1.06</td>
<td>1.90±14.14</td>
</tr>
<tr>
<td>Purslane Seeds</td>
<td></td>
<td>8.10±1.18</td>
<td>1.40±23.23</td>
</tr>
<tr>
<td>Chia Seeds</td>
<td></td>
<td>7.80±7.25</td>
<td>1.20±23.23</td>
</tr>
<tr>
<td>Garden Cress seeds</td>
<td></td>
<td>7.40±39.23</td>
<td>1.50±20.23</td>
</tr>
<tr>
<td>Seed mixture</td>
<td></td>
<td>7.08±52.24</td>
<td>1.30±22.23</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.D.
Significance difference is expressed at p<0.05 using one-way ANOVA test and LSD test.
Values which have different letters in each column differ significantly, while the difference among those with similar letters completely or partially not significant.

Biochemical Evaluations:
Liver functions:
Data presented in Table (4) showed the effect of feeding Purslane, Chia, Garden cress and their Mixture on serum Aspartate amino transferase (AST), Alanine amino transferase (ALT), Alkaline phosphatase (ALP), and Total bilirubin (Bil) in CCl4-intoxicated rats. The mean values of AST, ALT, ALP and Bil in the (+ve) control group showed significant increase as compared to the (-ve) group. The rats received purslane, chia and garden cress seeds as a powder (5%) showed significant decrease (P<0.05) of all liver enzyme compared to the (+ve) control group (Table 4). The best result was found in rats received mixture seeds (5%).

Table (4)
Effect of feeding Purslane, Chia, Garden Cress seeds and Seeds Mixture on serum (aspartate amino transferase and alanine amino transferase, Alkaline phosphatase, and Total bilirubin) in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>AST (IU/L)</th>
<th>ALT (IU/L)</th>
<th>ALP (IU/L)</th>
<th>Bil (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-Ve) Control</td>
<td></td>
<td>55.8±6.87</td>
<td>23.8±4.21</td>
<td>82.5±14.9</td>
<td>.39±1.2</td>
</tr>
<tr>
<td>(+Ve) Control</td>
<td></td>
<td>206.5±32.8</td>
<td>58±6.22</td>
<td>206.1±1.6</td>
<td>.87±0.9</td>
</tr>
<tr>
<td>Purslane Seeds</td>
<td></td>
<td>80.5±7.2</td>
<td>35.5±4.52</td>
<td>125.6±5.31</td>
<td>.57±0.09</td>
</tr>
<tr>
<td>Chia Seeds</td>
<td></td>
<td>83.1±7.1</td>
<td>38±5.33</td>
<td>106.8±8.8</td>
<td>.62±0.06</td>
</tr>
<tr>
<td>Garden cress Seeds</td>
<td></td>
<td>84.3±8.1</td>
<td>37.1±5.6</td>
<td>100.5±10.07</td>
<td>.59±0.06</td>
</tr>
<tr>
<td>Seed Mixture</td>
<td></td>
<td>66.3±7.41</td>
<td>31.1±4.8</td>
<td>82.6±6.81</td>
<td>.42±1.11</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.D.
Significance difference is expressed at p<0.05 using one-way ANOVA test and LSD test.
Values which have different letters in each column differ significantly, while the difference among those with similar letters completely or partially not significant.

Serum Albumin, Globulin and Total protein
Data presented in Table (5) showed the effect of feeding Purslane, Chia, Garden cress and their Mixture on serum Total protein, albumin and Globulin in CCl4-intoxicated rats. The mean values of total protein, albumin and globulin in the (+ve) control group showed significant decrease compared to the (-ve) control group. The rats that received purslane, chia and garden cress seeds as a
powder (5%) showed significant increase ($P<0.05$) of total protein, albumin and globulin as compared to the (+ve) control group as shown in Table (5). The best result was found in rats that received seeds mixture (5%).

Table (5)
Effect of feeding Purslane, Chia, Garden cress seeds and Seed Mixture on serum (Total protein, Albumin and Globulin) in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>Total protein (g/dl)</th>
<th>Albumin (g/dl)</th>
<th>Globulin (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-ve) Control</td>
<td></td>
<td>7.3±.40$^a$</td>
<td>4.2±.47$^a$</td>
<td>3.1±.54$^a$</td>
</tr>
<tr>
<td>(+ve) Control</td>
<td></td>
<td>3.0±.47$^a$</td>
<td>1.3±.25$^a$</td>
<td>1.7±.43$^a$</td>
</tr>
<tr>
<td>Purslane Seeds</td>
<td></td>
<td>4.2±.47$^a$</td>
<td>2.1±.27$^a$</td>
<td>2.1±.47$^a$</td>
</tr>
<tr>
<td>Chia Seeds</td>
<td></td>
<td>5.7±.30$^b$</td>
<td>3.5±.14$^b$</td>
<td>2.2±.69$^b$</td>
</tr>
<tr>
<td>Garden cress</td>
<td></td>
<td>5.8±.31$^b$</td>
<td>3.5±.35$^bc$</td>
<td>2.5±.91$^b$</td>
</tr>
<tr>
<td>Seed Mixture</td>
<td></td>
<td>6.2±.17$^b$</td>
<td>3.1±.24$^c$</td>
<td>3.1±.47$^b$</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.D.
Significance difference is expressed at $p<0.05$ using one-way ANOVA test and LSD test.
Values which have different letters in each column differ significantly, while the difference among those with similar letters completely or partially not significant.

**Serum lipid profile:**

Data presented in Table (6) showed the effect of feeding Purslane, Chia, Garden cress seeds and Seeds Mixture on serum Cholesterol, Triglyceride, high-density lipoprotein, low-density lipoprotein, Very low-density lipoprotein in CCl$_4$-intoxicated rats. The mean values of cholesterol and triglyceride were significantly increased in the (+ve) control rats compared with the (-ve) control group. All supplemented diets with purslane, chia and garden cress seeds powder (5%) showed significant decrease ($P<0.05$) comparing with the (+ve) control rats. The best result was obtained in rats fed on seeds mixture (5%).

The mean values of HDL were low in the (+ve) control group compared with the (-ve) control rats. All supplemented diets with purslane, chia and garden cress seeds powder (5%) showed significant increase ($P<0.05$) compared with the (+ve) control rats. The best result was obtained in rats fed on seeds mixture (5%). The mean values of LDL were significantly increased in the (+ve) control rats compared with the (-ve) control group. All supplemented diets with purslane, chia and garden cress seeds powder (5%) lowered the level of LDL ($P<0.05$) compared with the (+ve) control rats. The best result was found in rats fed on seeds mixture (5%). The mean value of VLDL in the (+ve) control was significantly higher than the (-ve) control group. The rats that received diets supplemented with purslane, chia and garden cress seeds powder (5%) showed significant decrease ($P<0.05$) compared with the (+ve) control rats. The best result was obtained in rats fed on seeds mixture (5%).
Table (6)

Effect of feeding Purslane, Chia, Garden cress seeds and Seeds mixture on serum (Cholesterol, Triglyceride, high-density lipoprotein, low-density lipoprotein and Very low-density lipoprotein) in rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Chol. (mg/dl)</th>
<th>T.G (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>LDL (mg/dl)</th>
<th>VLDL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(-ve) Control</td>
<td>88.1±7.7a</td>
<td>132±9.1b</td>
<td>49.1±4.7a</td>
<td>12.6±6.9a</td>
<td>26.5±1.8a</td>
</tr>
<tr>
<td></td>
<td>(+ve) Control</td>
<td>238±12.8a</td>
<td>238±12.8a</td>
<td>33.1±2.6a</td>
<td>157±11.2a</td>
<td>47.6±2.5a</td>
</tr>
<tr>
<td></td>
<td>Purslane Seeds</td>
<td>118.9±13.3b</td>
<td>174±34.5b</td>
<td>45.0±4.7b</td>
<td>39.1±14.7b</td>
<td>34.8±9.8b</td>
</tr>
<tr>
<td></td>
<td>Chia Seeds</td>
<td>123.7±12.1b</td>
<td>179.5±4.6b</td>
<td>47.0±4.8b</td>
<td>40.8±15.3b</td>
<td>36.0±8.9b</td>
</tr>
<tr>
<td></td>
<td>Garden cress seeds</td>
<td>131.3±6.5b</td>
<td>171.1±6.0ab</td>
<td>48.6±2.9b</td>
<td>48.5±5.1b</td>
<td>34.1±1.3b</td>
</tr>
<tr>
<td></td>
<td>Seed Mixture</td>
<td>98.5±5.4b</td>
<td>137.8±6.5b</td>
<td>52.0±4.8b</td>
<td>19.0±6.1b</td>
<td>27.5±1.3b</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.D.
Significance difference is expressed at p<0.05 using one-way ANOVA test and LSD test.
Values which have different letters in each column differ significantly, while the difference among those with similar letters completely or partially not significant.

Kidney functions:

Data presented in Table (7) showed the effect of feeding Purslane, Chia, Garden cress seeds and their Mixture on kidney functions in the intoxicated rats. The mean values of urea, creatinine and uric acid in the (+ve) control group revealed significant increase compared with the (-ve) control group. On supplementing diets with Purslane, Chia and Garden cress seeds induced (5%) significant decrease (P<0.05) compared with the (+ve) control rats. For all markers, the best results were noticed as a result of feeding seeds mixture (5%). For serum urea, chia seeds -fed group showed also normal value, while garden cress seeds-fed group showed normal uric acid value.

Table (7)

Effect of feeding Purslane, Chia, Garden cress seeds and Seed mixture on kidney functions in rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Urea (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
<th>U.A (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(-ve) Control</td>
<td>21.17±3.06a</td>
<td>.70±.56a</td>
<td>2.48±.27a</td>
</tr>
<tr>
<td></td>
<td>(+ve) Control</td>
<td>35.6±3.32a</td>
<td>1.45±.18a</td>
<td>4.23±.21a</td>
</tr>
<tr>
<td></td>
<td>Purslane Seeds</td>
<td>27.8±2.31a</td>
<td>.89±.65a</td>
<td>3.08±.40a</td>
</tr>
<tr>
<td></td>
<td>Chia Seeds</td>
<td>23.8±3.31a</td>
<td>.86±.70a</td>
<td>3.03±.60a</td>
</tr>
<tr>
<td></td>
<td>Garden cress Seeds</td>
<td>26±4.19a</td>
<td>.86±.73a</td>
<td>2.93±.33a</td>
</tr>
<tr>
<td></td>
<td>Seed Mixture</td>
<td>20.83±2.7a</td>
<td>.73±.937a</td>
<td>2.50±.25a</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.D.
Significance is expressed at p<0.05 using one-way ANOVA test and LSD test.
Values which have different letters in each column differ significantly, while the difference among those with similar letters completely or partially not significant.

Lipids peroxidation malondialdehyde (MDA), nitric oxide (NO) and tumor necrosis factor -α in liver tissues.

Data presented in Table (8) showed the effect of feeding Purslane, Chia, Garden cress seeds and Seeds Mixture on lipids peroxidation malondialdehyde (MDA), nitric oxide (NO) and tumor necrosis factor -α (TNF-α) in liver tissues of hepatotoxic rats. It could be noticed that positive control group recorded a significant increase (p<0.05) in the mean value of MDA, NO and TNF-α compared with the negative control group. On supplementing the rats diet with purslane, chia, garden cress seeds and seeds mixture as a powder (5%) the parameters showed significant decrease, (P<0.05) compared with the (+ve) control rats.
Table(8)
Effect of feeding Purslane, Chia, Garden cress seeds and Seed mixture on lipids peroxidation malondialdehyde, nitric oxide and tumor necrosis factor-α in liver tissues in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>MDA (mmol/ g )</th>
<th>NO (U/g)</th>
<th>TNF-α (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-ve) Control</td>
<td></td>
<td>0.15±0.010</td>
<td>0.136±0.009</td>
<td>0.14±0.018</td>
</tr>
<tr>
<td>(+ve) control</td>
<td></td>
<td>0.27±0.007</td>
<td>0.35±0.004</td>
<td>0.27±0.007</td>
</tr>
<tr>
<td>Purslane Seeds</td>
<td></td>
<td>0.15±0.006</td>
<td>0.17±0.007</td>
<td>0.15±0.006</td>
</tr>
<tr>
<td>Chia Seeds</td>
<td></td>
<td>0.17±0.002</td>
<td>0.17±0.006</td>
<td>0.17±0.002</td>
</tr>
<tr>
<td>Garden cress Seeds</td>
<td></td>
<td>0.17±0.002</td>
<td>0.17±0.011</td>
<td>0.17±0.006</td>
</tr>
<tr>
<td>Seed mixture</td>
<td></td>
<td>0.15±0.005</td>
<td>0.14±0.006</td>
<td>0.15±0.005</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.D.
Significance is expressed at p<0.05 using one way ANOVA test and LSD test.
Values which have different letters in each column differ significantly, while the difference among those with similar letters completely or partially not significant

Discussion

Purslane, Chia and Garden cress seeds have a different phenolic compounds in different proportions such as Gallic, Caffeine, Ellagic and coumarin. This agreed with the study Martinez and Paredes (2014) who showed that Caffeic acid is among the phenolic compounds already identified in chia products, and they play an important role in the prevention and management of different neurological disorders, such as epilepsy. Abd El aziz et al., (2014) showed that purslane contain of phenolic compound fractionated to catechein, chlorogenic, salicylic and pyrogallol. Elagic resulted in maximum concentration of phenolic, followed by salicylic, chlorogenic and catechein. In addition to these results in agreement with Abd El Salam et al., (2019) who showed The extract of (Garden Cress) GC seeds had high level of total phenols and total flavonoids. Gallic acid and hisperidin were the most abundant phenolic and flavonoid compounds in GC seed extract.

Injection by CCl₄ is associated with reduced nutrient digestion and absorption as a result of low bile secretion and associated with loss of appetite leading to, leading to weight loss. Behboodiet al., (2017) showed that CCl₄ caused significant reduction in feed intake and body weight gain. Feeding on Purslane, Chia and Garden cress seeds with the rats diet injected with ccl₄ which induced hepatotoxicity, led to significant increase in feed intake, body weight gain and efficiency ratio of rats. These results in agreement with Akrismet et al.,(2014) who studied the effect of purslane ethanolic extract administration on the body weights of CCl₄-treated rats. The results were the body weights of the rats significantly decreased in the CCl₄-treated group, but increased in the normal control and purslane extract-treated groups. Also results are in agreement with Poudyal et al., (2012) who showed the intake of chia seeds by the Wistar rats improve the food consumption and body weight. Also results are in agreement with ABEUGASIMET et al., (2008) who demonstrated the protective ability of seed extracts of Lepidium sativum on liver injury induced by CCl₄. The anorexic effect produced by CCl₄ due to its hepatotoxicity was masked by the use of Lepidium sativum.

The marked increase in relative liver and kidney weights in CCl₄–treated group which was reversed by supplementation with antioxidant sources, as in the present study, is in harmony with Akrismet et al., (2014) who noticed marked elevations in organ weights in CCl₄–treated groups. These elevations were reversed by administration with purslane extract. Also results agreed with Bárbara et al., (2016) who found that Chia seeds decreased the percentage of liver fat and liver weights due to lower accumulation of lipids in the body, which may have led to increased excretion of fat in the feces.
since animals fed with chia showed an increase in fecal weight compared to the control one. Shukla and Bigoniya (2013) showed hepatoprotective effect of Lepidium Sativum Linn (Cruciferae) by reducing the liver weight of ccl4 intoxicated rats.

CCl4-induced liver injury is the best-characterized model of xenobiotic-induced hepatotoxicity (Brautbar and Williams, 2002; Brent and Rumack, 1983). The bio-activation of CCl4, primarily through the activity of CYP2E1, generates the free radicals ‘CCl3 and CCl3OO‘, which results in hepatic damage. These free radicals initiate lipid peroxidation by abstracting a hydrogen atom from the polyunsaturated fatty acid of a phospholipid (Recknagel et al., 1989; Weber et al., 2003). The CCl4-induced lipid peroxidation in turn increases the permeability of plasma membrane to Ca2+, leading to severe disruption of calcium homeostasis and necrotic cell death (Weber et al., 2003). The extent of hepatic damage is assessed by the increase in serum levels of the cytoplasmic enzymes AST, ALT, ALP, Bil and by histopathological examination. The increased serum levels of AST and ALT have been attributed to damages in the structural integrity of the liver, as these cytoplasmic enzymes are released into circulation after cellular damage (Recknagel et al., 1989) Elevation of AST has been reported to be an index of hepatocellular injury in rats, whereas ALT elevation is more commonly associated with the necrotic state (Navarro and Senior, 2006). Serum ALP and GGT, which are important enzymes for assessing obstructive liver injury (Bulleet al., 1990; Kaplan, 1986), were also found to be significantly elevated in CCl4-treated rats. ALP activity is related to the functioning of hepatocytes. Suppression of increased ALP activity is indicative of the stabilization of biliary dysfunction in rat liver during chronic hepatic injury induced by CCl4 (Mukherjee, 2002). The increase in serum total bilirubin in ccl4 treated rats may be owing to blockage of bile ductules as a result of the inflammation and fibrosis in the portal triads and/or due to regurgitation of conjugated bilirubin from the necrotic hepatocytes to sinusoids (Ahmed, 2001).

All supplemented diet with purslane, chia and garden cress seeds in the present study decrease liver enzyme including transaminases (ALT and AST), alkaline phosphatase (ALP) and total bilirubin of rats injected with ccl4. These results are in agreement with Vogel (2002) who reported that treatment with purslane extract restored the liver enzymes to near normal level indicating protection against liver damage.

Elkhayatet al., (2008) found that 70% alcohol extract of P. oleracea significantly restored the hepatic marker enzymes and total bilirubin in hepatic injured rats to near-normal values. Ahmida (2010) reported that the hepatic protective effective effect of portulaca oleracea was due to the phytochemical present in it, including omega-3 fatty acids, β-carotene, flavonoids and alkaloids. Rafaela et al., (2015) confirmed that the consumption of white and black chia seeds was found to be effective for reducing liver damage and improvement in liver function. Sheisa et al., (2019) attributed these effects to its content of high level of omega-3 fatty acids. Additionally, another substance that has been found to improve both lipids and liver enzymes is α-linolenic acid. This substance is present at high levels in chia seeds, ranging from 14 to 20 g/100 g (55 to 65.8%) Heinze et al., (2012). The antioxidant in chia seeds such as Caffeic and Chlorogenic acid can improve both lipids and liver enzymes. Kweon et al., (2001). On the other hand, Abaelgasim et al., (2008) found that the use of seed extracts of Lepidium sativum protect the liver from damage and improved histologic picture and biochemical markers of liver damage. The CCl4 induced hepatotoxicity produced in rats leading to hepatic injury triggers the generation of toxic radicals which can be masked by using a correct antioxidant in adequate amount. The mechanism of the hepatoprotective action of the plant may be related to the ability of the plant to inhibit lipid peroxidation in the liver. The presence of flavonoids triterpens, alkaloid, tannin and coumarins in Lepidium sativum explain its role in hepatoprotection by
inhibiting the free radicals mediated damage. Moreover, the improvement in liver functions markers status in seeds–fed groups, in the present study, may be attributed to the presence of not only phenolic compounds, but also dietary fibers and polyunsaturated fatty acids.

Feeding on diet supplemented purslane, chia and garden cress seeds in the present study, led to significant increase in serum total protein, albumin and globulin of rats injected with CCl₄ that induced hepatotoxicity. These findings are in agreement with Anusha et al., (2011) showed the hepatoprotective effect of Portulaca oleracea (P. oleracea) by significantly restoring the levels of serum enzymes to normal and increase in albumin and total protein. CCl₄ impaired protein metabolism in the injured hepatocytes causing a release of non-protein and protein nitrogenous substances. This is associated with a decrease in the level of protein synthesis along with an increase in the activities of hepatic enzymes Anusha et al., (2011). The ability of purslane extract to restore the levels of TP, Alb might be due to its effect on the functional statuses of the poisoned liver and to protect against hepatotoxicity. Zamzami et al., (2019) evaluated the hepatoprotective efficacy of L. sativum seeds in white male New-Zealand rabbits. The results revealed that treatment of rabbits administered with CCl₄ with L. sativum seeds significantly repaired their liver injurious marker enzymes as well as bilirubin, total protein, and albumin, hence approving its hepatoprotective effect. Lepidium sativum improved the degree of structural damage and reduced inflammatory infiltration in hepatic cells. Balgoon (2019) assessed the protective role of Salvia officinalis essential oil (SO) against carbon tetrachloride (CCl₄)-induced liver and kidney damage in mice. The activity of SO was associated with increasing total protein, albumin, globulin, and prothrombin. SO is a potential candidate for counteracting hepato/renal injury associating CCl₄.

Feeding on purslane, chia and garden cress seeds in the present study, led to significant decrease in triglycerides, LDLc, VLDLc and significant increase in HDLc of rats injected with CCl₄ induced hepatotoxicity conducted by Gatreh et al., (2011), they found that The serum levels of LDL-C, and TG decreased significantly in purslane group. All the therapeutic values of purslane (P. oleracea) are attributed to the presence of many biologically active compounds including flavonoids, Alkaloids, Coumarins, and high content of ω-3 fatty acids were considerable beneficial in preventing heart attacks and strengthening the immune system Okafor and Ezejindu (2014). Predominantly containing polyunsaturated (omega-3) fatty acids, make purslane medicinal plant to have favorable effects on cholesterol and triglyceride levels Yokoyama et al., (2007).

Also results agree with Ricardo and Wayne (2005) who assessed the effect chia seed, which is the highest vegetative source of α–linolenic fatty acid, has on plasma total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triacylglycerol content (T.G) , and fatty acid profile when fed to rats. They found that the chia diets dramatically decreased triacylglycerol (T.G) levels, (LDL) level, cholesterol and increased HDL and omega-3 fatty acid contents in rat serum. These findings suggest that α-linolenic–rich chia may be an alternative to omega-3 sources for vegetarians and people allergic to fish and fish product. Amawi and Aljamal (2012) showed that the administration of Lepidium sativum improved lipid profile in hypercholesterolemic rats.

Supplementing rat diet with purslane, chia and garden cress seeds in the present study, led to a marked improvement in the kidney function abnormalized by CCl₄ injection evidenced better levels of urea, creatinine and uric acid. These results agreed with AbdAlah et al.,(2018) who investigated the effect of Purslane on kidney failure affected by copper toxicity in a rat model. They found that Purslane administration decreased the elevated level of creatinine and BUN in rats which received toxic levels of copper due to its antioxidant and anti-inflammatory properties. The results agreed with Fahmy et
Samah A. El-Hashash, et al. (2018) who ensured the protective role of Salvia officinalis essential oil (SO) against carbon tetrachloride (CCl₄)-induced liver and kidney damage in mice. The effect may occur via the antioxidant defense mechanism which in part related to the complexity of its chemical constituents. Also the result agreed with Loay and Wurood (2019) who reported that the ethanolic extract of garden cress has both renal protective and curative effects as it significantly reduced the blood levels of urea and creatinine which indicates increased glomerular filtration rate. In the present study rats diet supplemented with purslane, chia and garden cress seeds singly or as a mixture, led to significant decrease in lipids peroxidation and inflammation as manifested by decrease malondialdehyde (MDA), nitric oxide and TNF-α levels in liver tissues of rats. These findings were in line with Dkhil et al., (2011) who evaluated the anti-oxidative effects of (Portulaca oleracea) PO in adult male Waster albino rats. The results revealed that oral administration of PO improved liver and kidney functions. The improvement in all the indicators may be attributed to the presence of phenolic compound, which possess anti-oxidant, anti-diabetic and anti-inflammatory activities PO is the main source of anti-oxidant vitamins such as α-tocopherol, ascorbic acid, β-carotene and glutathione. Bárbara et al., (2016) investigated the influence of chia consumption on inflammation and oxidative stress in adult female ovariectomized rats fed a high-fat diet. Chia intake improved the antioxidant activity by increasing SOD expression, PPAR-α expression and catalase activity. In addition, chia consumption decreased the concentrations of the inflammatory markers IL-1β. The improvement in all the indicators may be attributed to the presence of phenolic compounds, vitamins, minerals, dietary fiber, and polyunsaturated lipids. (Silva et al., 2017).

Oliveira et al., (2017) found that Chia intake increased catalase activity in the standard diet probably due to compounds present in the seeds including phenolic compounds and antioxidants. The main phenolic compounds found in chia are rosmarinic acid, quercertin, myricetin, kaempferol, caffeic acid, and galic acid. These compounds provide benefits to the human body due to the presence of hydroxyl groups that are readily oxidized to produce the corresponding O-quinones, which are effective scavengers of reactive oxygen species. Phenolic compounds can also alter the recruitment of inflammatory cells, decreasing the production of pro-inflammatory mediators. (Fraga et al., 2010). Mazin et al., (2019) evaluated the hepatoprotective efficacy of L. sativum seeds in white male New Zealand rabbits. The results revealed that concurrent treatment of rabbits administrated with CCl₄ for 5 and 10 weeks with L. sativum seeds significantly reduction of lipid peroxidation and repaired the antioxidant enzymatic status and total protein restoring them to normal levels. The hepatoprotective action of L. sativum seeds could be due to down regulation of cytokines [TNF-α and interleukin-6 (IL-6)] and stress gene [inducible nitric oxide synthase (iNOS) and HO-1] messenger ribonucleic acid (mRNA) expression. Lepidium sativum ethanolic extract pre protection also improved the degree of structural damage and reduced inflammatory infiltration in hepatic cells. These outcomes established that Lepidium sativum ethanolic extract alleviates hepatic impairments and structural injury through the decay of oxidative stress, inflammation, and apoptosis in the liver (Raish et al., 2016).

**Conclusion**

According to the results obtained in the present study, it appears that supplementing diet with purslane, chia and garden cress seeds singly or as a mixture alleviated liver abnormalities and dysfunction associated with oxidative stress in liver tissues of CCl₄-intoxicated rats.
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تأثير بذور بعض النباتات الطبية على التسمم الكبدى المحدث بواسطة رابع كلوريد الكربون في جرزان التجارب

سامح أحمد الحشاش، أميرة مرسى المسلمى، امانتى على عبد المجيد

الملخص العربي

كثير من النباتات يتم استخدامها كنباتات طبية بواسطة كثير من الناس. تم إجراء هذه الدراسة لمعرفة تأثير بذور الرجراة والشيا وحب الرشاد على التسمم الكبدى المحدث بواسطة رابع كلوريد الكربون في جرزان التجارب. تم تقسيم جرزان إلى مجموعتين، المجموعة الأولى تتكون من 6 جرزان تغذت على الغذاء القياسى فقط لمدة 6 أسابيع، والمجموعة الثانية تتكون من 36 جرزان تم حقن كل جرزان بالطبخات الفينولوجية المذابة في زيت الزيتون (50% حجم/حجم 2 مل/كم من وزن الجسم) مرتين أسبوعيا لمدة أسبوعين لاحقلاً بتلوث كبدى مزمن. بعد التأكد من وجود التلف الكبدى المزمن، تم تقسيم 30 جرزان مصاب إلى 5 مجموعات متساوية. المجموعة الأولى تغذت بالغذاء القياسى فقط، أمراً المجموعات الأخرى (الثانية والثالثة والسادسة) فقد تم تغذيتهم بالغذاء القياسى مضافاً إليه مسحوق أي من بذور الرجراة، الشيا، حب الرشاد أو خليطهم على الترتيب (بسبة 5%). استمرت مدة العلاج 4 أسابيع، وفي نهاية كل جرزان حساب زيادة وزن الجسم والوزن النسبى لبعض الأعضاء الداخلية، كما تم تحليل عينات السيروم والكبد بيوكمائيا. أظهرت النتائج حدوث خلل في وظائف الكبد والكلى وارتفاع دهون الدم نتيجة التعرض لرابع كلوريد الكربون. أدت استخدام بذور الرجراة والشيا وحب الرشاد ككل أو خليطهم إلى تحسن في وظائف الكبد والكلى وانخفاض دهون الدم نتيجة التعرض لرابع كلوريد الكربون. وطبقاً لهذه النتائج يمكن للمريض الذين يعانون من تسمم الكبد نتيجة التعرض لرابع كلوريد الكربون استخدام هذه البذور ككل أو في صورة خليط (وهى الأفضل) لاختيار كفاءتها الممكنة في تحسين حالاتهم الصحية.

الكلمات المفتاحية: تسمم الكبد، رابع كلوريد الكربون، بذور الرجراة، بذور الشيا، بذور حب الرشاد، المركبات الفينولوجية، جرزان