Effect of Apricot kernel on Some Hematological, Histological and Biochemical Parameters in CCl4-induced Liver Injury in rats

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Abstract
The purpose of the present study was to find out the effect of apricot kernel on carbon tetrachloride (CCl4) induced liver injury in male albino rats. To achieve this purpose and to explain, the histological, hematological and biochemical parameters were included. Thirty male albino rats were used and randomly divided to in to three groups. The first group represented control group which received normal diet and intraperitoneal injection with oil (0.5 ml/kg). The second group injected with CCl4(1 ml/kg), represented the CCl4(1 ml/kg)model, the third group were treated like the second group with 15%/ day apricot kernel for a month. The apricot kernel extracts treated group showed significant differences in AST, ALT, ALP, direct bilirubin, GSH, liver SOD, WBC, LYM and PLT when compared to CCl4 treated rats. In the current study histological section through the liver of control rats showed normal architecture of hepatocytes. Although Paraffin section of the rat liver treated with CCl4 most of the hepatocytes were degenerated, congestion of blood and inflammatory leukocyte infiltration were observed, the results showed that CCl4 injection caused significant alterations in histological and biochemical parameters but our study showed that the apricot kernel administration showed improvement of histological analysis for four weeks (March to April 2015).

Keywords : Apricot kernel, CCL4 – Induced liver injury.
تأثيربذره المشمش على بعض المعايير النسيجية،الدم وبعض الفحوصات الكيميائية الحياتية في كبد الجرذان المتضرره والمستحدث بـ CCl4

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المختصر:

هدفت الدراسة الحالية معرفه تأثير مستخلص بذورالمشمش على كبد الجرذان المتضرره والمستحدث بـ CCl4 من خلال دراسه ومتابعه المعايير النسيجية، الديموبايوكيميائية. استخدمت في هذه الدراسة ثلاثين من ذكور الجرذان البيضاء فوضعت عشوائيا في ثلاث مجموع. المجموعة الأولى كانت معامله السيطره والتي أعطيت لها الغذاء الطبيعي وحقنت 0.5 كلغ/ كجم زيت الشفاف البيروتي. أما المجموعه الثانية فقد عمتل بنفس طريقه معامله المجموعه الأولى مع اضافه حقن ٠.١ كجم/ كجم CCl4 واخيرا المجموعه الثالثه تم حقنها ب٥٥ ٪ من مستخلص بذورالمشمش يوميا بعد معتمدها في طريقة المجموعه الثانية وبمكثها لمدة شهر. حيث اظهرت هذه المجموعه فروقات معنويه في AST , ALT , ALP , liver SOD , WBC , LYMPHOCYTES , PLT , GSH عند مقارن مع مجموعه الجرذان المعامله بـ CCl4. كما اظهرت الدراسة الحالية ان المقاطع النسيجية لخلايا الكبد في مجموعه السيطره دانترب طبيعية في حين ان المقاطع المعتمدة لـ CCl4 اظهرت تفشي شبعاتي في إضافة الى ان كريات الدم البيضاء الهامشية كزادة ولكن لم تظهر الدراسة الحالية تحسنات واضحة على بعض المقاطع النسيجية المتضرره بـ CCl4 عند المجموعه المعامله بمستخلص بذورالمشمش ولمدة شهر.

Introduction

Medicinal plants are important part of health care. Large varieties of plants (more than 1200) are available with known therapeutic effects [1]. Approximately 70–80% people worldwide depend on medicinal plants to cure various human ailments including viral diseases [2]. Moreover, herbal drugs have gained much importance due to their easily adaptability, low cost and fewer side reactions on patients [3]. Natural antioxidants can protect the body against the adverse effects of CCl4 and some other toxins [4, 5]. Apricot (Prunus armeniaca L.) is classified under the Prunus genus, Prunaidea sub-family and the Rosaceae family of the Rosales group. Apricot has
an important place in human nutrition and apricot fruits can be used as fresh, dried or processed fruit [6]. The kernel is a valuable byproduct. Sweet kernels taste like almond and are used as its substitutes in dried form. Apricot kernels (A.kernel), particularly rich in lipid and protein, they are potentially useful in human nutrition [7] along with significant amounts of oil and fiber [8]. Liver diseases are one of the major causes of mortality and morbidity worldwide, drug-induced liver toxicity is a major cause of hepatic dysfunction [9]. Oxidative stress, involving enhanced generation of reactive oxygen species (ROS), has been implicated in the etiology of many human diseases. Antioxidants capable of neutralizing ROS and their actions are considered beneficial. In this context, natural dietary components with antioxidant activities could be important [10]. Among environmental toxins, carbon tetrachloride (CCl4) dedicated most of conducted studies to itself [11]. Ground apricot kernel administration can effectively improve liver fibrosis caused by dimethylnitrosamine (DMN), and may be used as a therapeutic option and preventive measure against hepatic fibrosis due to its antioxidant nutrient (β-carotene and vitamin) contents and high radical-scavenging capacity[12].

Aim of this study was to evaluate the antioxidant activity of Apricot kernel plant, its hepatic protective activity, to determine the oxidative stress and antioxidant markers and some hematological parameters in CCL4 treated rat groups. Also the aim of this study was to study the effects of this plants with respect to the histological and ultrastructural alterations in toxic liver male rats.

Materials and Methods

Materials

Plant preparation
Apricots (PrunusarmeniacaL.) were purchased from local fruit market of Erbil. Apricot flesh was removed from fruits; the apricot outer shell was washed with tap water and air-dried the outer shell of apricot was cracked manually and the edible part (kernel) was stored at refrigerator in sealed plastic bags until used. The apricot kernels were soaked in warm distilled water, kernel thin layer coat was removed manually. The apricot kernels were placed on a sheet of filter paper dried and ground in a blender. In order to prevent the ground apricot kernel from possible rancidity and oxidation that may occur during storage, it was prepared freshly within 1 h before adding to basal diet [12].

Web Site:  http://www.isnra.com/ojs/index.php/KJPS   E-mail: kjps@uoalkitab.edu.iq
**Experimental animals**

Thirty male albino rats (*Rattusnorvegicus*), weighing about 250 – 350gm were used. The animals were given standard rat diet chow and housed in plastic cages bedded with wooden chips in a room with controlled temperature of 24±3°C, 12/12 hours light/dark schedule in an animal house belong to Biology department, College of Science, Salahaddin University-Erbil. Standard chow ingredients included (wheat 66.6% ,soya 25.6%, oil sun flower 4.4%, lime stone 1.5%, salt 0.63%, methionine 0.158%, Lysine 0.24%, choline chloride 0.062% and trace elements 0.05%).

**Experimental Design**

The experimental rats were divided randomly to three groups. This experiment was carried out for four weeks as explained below:

**Group 1: Control rats (n=10)** The rats of this group were given olive oil intraperitoneally (0.5 ml/kg body weight) for four weeks.

**Group 2: CCl₄ treated rats (n=10)**. The rats of this group were given CCl₄ intraperitoneally 1ml/kg b.w. (1:1 in olive oil) for four weeks.

**Group 3: Apricot kernel (n=10)**. The rats of this group were given CCl₄ intraperitoneally 1ml/kg b.w. (1:1 in olive oil) and 15% of apricot kernel combined with standard diet for four weeks.

**Methods :Tissue preparation**

**Anesthesia, dissecting of liver:** All animals were anesthetized with Ketamine hydrochloride 80mg/Kg (Trittau, Germany) and Xylazin 12mg/Kg (Interchem, Holland). The liver was removed then divided into two equal parts, one part cut into small pieces (less than 0.5cm³ thicknesses) then kept in formalin, while the other part stored at refrigerators until homogenized for estimation of SOD and GSH.

**Tissue homogenate**

Liver washed with cold saline. Pieces of each tissue used for homogenization by 20 mM cold phosphate buffer saline (pH 7.4). The liver tissues homogenized (10%w/v) using handheld glass
homogenizer [13]. Homogenates were centrifuged at 6000 rpm for 10 minutes. The supernatants were collected and stored at -80°C until assayed.

**Estimation of glutathione in liver tissue:** The procedure of [14] was followed with some modification. Weighting 1 gm of liver tissue and homogenate by using handled homogenizer with 10 ml of cold tris buffer solution. One ml of tissue homogenate was added to 0.25 ml of 25% trichloroacetic acid. After centrifugation for 5 minutes at 3000 rpm 0.2 ml of supernatant was taken in a test tube, adding one ml 0.15mole imidazole solution then adding 1.7 ml distilled water and 0.1 ml 5.5(DTNB) solution finally absorbance was read at 412nm after 3 minutes of adding DTNB. The concentration of GSH was calculated according to the absorbance of blank (B), test (T) and standard (S) solutions by the following equation:

\[ \text{GSH conc. (μmol/mg of tissue)} = \frac{\text{conc. Standard} \times 100}{\text{D}} \]

**Determination of liver tissue superoxide dismutase**

Liver samples were washed with 0.9% NaCl to remove red blood cells. The tissue was then blotted dry and weighed followed by homogenization in 200 μl buffer (0.05 M potassium phosphate and 0.1 mM EDTA, pH 7.8) and centrifuged at 15,000xg for 30 min at 4°C. The supernatant was used for determination of SOD. Superoxide dismutase was measured using the Superoxide Dismutase assay kit provided by Elabscience (Elabscience, WuHan P.R.C). The concentration of SOD was determined by competitive-ELISA method. The concentration of SOD in the samples is then determined by comparing the OD of the samples to the standard curve (Figure 1).

**Blood collection**

At the end of the treatment period, blood samples were collected from anesthetized rats through cardiac puncture. The collected blood samples were immediately placed into test tube and centrifuged and the sera were stored at -80°C (Sanyo – Ultra – Low Temperature, Japan) until assayed. While, for hematological analysis blood were collected in EDTA tube.

**Hematological analysis**

White blood cell (WBC) count, LYM and PLT count were determined automatically by using automated hematology analyzer (Sysmex model: K-1000, Japan).

**Determination of Liver Function Parameters**
Alkaline Phosphatase, Aspartate Aminotransferase, Alanine Aminotransferase and bilirubin were achieved automatically by using full automated (COBAS Integra 400plus-roche, Germany).

**Statistical analysis**

One way analysis of variance followed by Newman-Keuls post hoc test comparison procedures were used to compare between means of different groups. Data are represented as the mean±standard error (M±SE). Graphpad prism program, version 6.01, computer program was used for statistical analysis. P<0.05 was considered statistically significant. Citations and references were managed by Endnote X 7 (Endnote software, Thomson Reutter, Canada)

**Results and Discussion**

**Effect of Apricot kernel on liver function tests in carbon tetrachloride treated rats.**

Table (1) shows the effects of Apricot kernel on the liver function tests in CCl4 treated rats. The ALP level was significantly decreased in control (P<0.05) but there were no statistical difference of ALP level in A. kernel, also, serum AST,ALT levels were significantly decreased (P<0.001) in control and A. kernel groups when compared to the CCl4 treated rats. With respect to direct bilirubin level, control, Apricot kernel treated groups were significantly decreased (P≤ 0.001) compared to CCl4 treated rats. Results of the current data showed the increase in ALP, AST, ALT and bilirubin levels in CCl4 treated groups are in agreement with [15]. The mechanism of hepatic damage by CCl4 is well documented and reported that CCl4 is metabolized by Cytochrome P450 enzyme to (CCl3). This in turn reacts with molecular oxygen and gets converted to trichloromethylperoxy radical. This radical forms covalent bonds with sulfhydryl groups of several membrane molecules like GSH leading to their depletion and causes lipid peroxidation. The lipid peroxidation initiates a cascade of reactions leading to liver necrosis. Liver damage is detected by measuring the activities of liver function marker enzymes like AST, ALT and ALP, which are released into the blood from damaged cells. They are also indicators of liver damage [17].

Our results showed that extract of A. kernel can prevent the CCl4 induced toxicity in the liver by significantly reduction of AST, ALT, ALP and direct bilirubin levels, these results are in agreement with [17] they achieved that the normalization of the above enzyme levels in rat liver with the plant drugs establishes the hepato protective effect of T. foenum-graecum which may be
able to induce accelerated regeneration of liver cells by reducing the leakage of these enzymes into the blood. The results indicated that A. kernel significantly prevented the increased liver function marker enzyme activity induced by CCl4, indicating an improvement of the functional status of the liver by the A. kernel.

**Effect of Apricot kernel extracts on the some hematological parameters in carbon tetrachloride treated rats**

Table (2) shows the effect of A. kernel on the WBC, LYM and PLT counts in CCl4 treated rats. The results showed that WBC count significantly decreased in A. kernel (P≤ 0.01) but there were no statistical significant differences in control. Moreover, number of LYM significantly decreased in A. kernel (P≤ 0.01) and there were no significant differences in control. Furthermore, Table (2) shows the PLT count significantly decreased in control, A. kernel, (P≤ 0.05) when compared with CCl4 treated rats.

The present study demonstrated that the rats treated with apricot kernel significantly decreased WBC, LYM and PLT when compared with CCl4 treated rats.

**Effect of Apricot kernel extracts on the liver super oxide dismutase and liver glutathione levels in carbon tetrachloride treated rats.**

As shown in table (3), the level of liver GSH in Apricot kernel groups significantly increased (P≤ 0.001), but there was no statistical difference of liver GSH level in control when compared to CCl4 treated group. Also, liver SOD significantly increased in control (P≤ 0.001), but there were no significant changes in the A. kernel groups. Glutathion (GSH) is the most important of the sulfur-containing non-enzymatic antioxidant molecules. GSH can also conjugate with free radicals directly, marking them for renal excretion. The sulphhydryl (–SH) portion of the GSH can be used to reduce a variety of free radicals in a reaction catalyzed by the antioxidant enzyme, glutathione peroxidase [18]. In accordance with our present findings the treatment with apricot kernel significantly increased GSH level and improved the biochemical values may be due to increase levels of oleic acid and other polyphenols in apricot kernels. Treatment with apricot kernel in our study the SOD level does not reach statistical difference, however Vardi et al.
indicated that apricot diet provided a significant increase in SOD activity in the kidney[19]. This action may be due to an improvement in the antioxidant status and the scavenging of excessive free radicals such as O2− and the peroxyl radical. Therefore, these factors can protect cell or tissue from oxidative stress.

**Effect of Apricot kernel on the liver in carbon tetrachloride treated rats**

In the present study after 28 days of intraperitoneal injection of CCl4, several histological and ultrastructure alteration were observed in the structure of liver rats. Liver sections through the control group have shown healthy and normal histological liver structure with normal hepatocytes, blood sinusoids and central vein (Figure 2). Paraffin sections through the liver of CCl4 treated group showed hepatic damage revealed as dilation and congestion of the central vein, degeneration of hepatocytes and inflammatory leukocyte infiltration (Figure 3). Abdel-Moneim et al showed that treatment of CCl4 caused notable lesions including deformed cord arrangement, ballooning degeneration of hepatocytes, condensed nuclei, widespread hepatocellular necrosis[20]. Liver cross section in apricot kernel treated rats showed little degeneration of hepatocytes and vacuoles (Figure 4). As well as Vardi et al. (2013) indicated that apricot group was similar to the control.

<table>
<thead>
<tr>
<th>Groups</th>
<th>S. ALP (U/L)</th>
<th>S. AST(U/L)</th>
<th>S. ALT(U/L)</th>
<th>S.D. Bilirubin (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCl4</td>
<td>326±25.59</td>
<td>812.3±91.03</td>
<td>763.8±98.49</td>
<td>0.09625±0.006</td>
</tr>
<tr>
<td>Control</td>
<td>243.4±27</td>
<td>196.4±35.68</td>
<td>53.4±6.47</td>
<td>0.026±0.002</td>
</tr>
<tr>
<td>A. Kernel</td>
<td>333.8±15.81</td>
<td>169.9±25.42</td>
<td>58.5±7.8</td>
<td>0.025±0.005</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Groups</th>
<th>WBC *103/μL</th>
<th>LYM *103/μL</th>
<th>PLT*103/μL</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCl4</td>
<td>9.623±0.34</td>
<td>6.033±0.12</td>
<td>915.4±16.91</td>
</tr>
<tr>
<td>Control</td>
<td>8.3±0.7</td>
<td>4.65±0.15</td>
<td>522±117.5</td>
</tr>
<tr>
<td>A. Kernel</td>
<td>4.625±0.7</td>
<td>3.66±0.38</td>
<td>646.8±42.19</td>
</tr>
</tbody>
</table>
Table 3: Effect of Apricot kernel extracts on GSH and SOD in CCl4– liver injury rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>GSH (μmol)</th>
<th>SOD</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCl4</td>
<td>13.33±0.7</td>
<td>0.03576±0.0112</td>
</tr>
<tr>
<td>Control</td>
<td>25.19±1.33</td>
<td>0.2804±0.03531</td>
</tr>
<tr>
<td>A. Kernel</td>
<td>93.85±5.3</td>
<td>0.08597±0.02454</td>
</tr>
</tbody>
</table>

Figure (1): Standard curve of superoxide dismutase (SOD)

Figure (2): Histological section through the liver of control rat showing normal architecture of hepatocytes (black arrow) which polyhedral in shape with obvious nucleus, blood sinusoids (s) and central vein CV. 400X, H and E.
Figure (3): Paraffin section of the rat liver treated with CCl₄ in which most of the hepatocytes were degenerated (D), congestion of blood (black arrow) and inflammatory leukocyte infiltration (white arrow) were observed 400X, H and E.

Figure (4): Paraffin section of the rat liver treated with CCl₄ and apricot kernel in which few degenerated hepatocytes were observed 400X, H and E.
Conclusions: From the biochemical and physiological points of view, the model of CCl₄ caused several changes in the level of the oxidative parameters (decreasing of GSH) but the current plant was succeeded in attenuating these changes when added to the CCl₄ treated group. The model produced oxidative stress and rising in the levels of AST, ALT, ALP, direct bilirubin, while Apricot kernel edible plant lowered these levels and have shown hepatic protective effect and ameliorated inflammation caused by CCl₄ treatment via decreasing of WBC and LYM count. Moreover, it decreased thrombogenic activity of CCl₄ through decreasing of PLT count.

From the histological point of view, CCl₄ caused a hepatotoxic in male rats (degeneration of hepatocytes and inflammation in the liver sections). Administration of this plant extracts give good results in ameliorating these changes and normalized the liver structure.

References


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