ANTIOXIDANT PROPERTIES OF ARTICHOKE LEAVES AND ONION SKIN ETHANOLIC EXTRACTS ON CARBON TETRACHLORIDE-INDUCED RAT LIVER INJURY.

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Key Words: Artichoke leaves, Onion skin, Carbon tetrachloride, Thiobarbituric, Glutathione-S-transferase

ABSTRACT

Artichoke leaves, onion skin ethanolic extracts were evaluated for their ability to inhibit carbon tetrachloride (CCL₄)-induced liver function, kidney function and lipid peroxidation in rats. The antioxidant enzyme activity (glutathione-S-transferase) was significantly decreased while the level of liver enzymes activities and thiobarbituric acid reactive substances, was significantly increased following treated with hepatotoxic dose of CCL₄ as compared to control negative. In contrast, the antioxidant enzyme activities was significantly increased while, the level of liver enzymes activities and thiobarbituric acid was significantly decreased by orally administration of artichoke leave, onion skin extracts as compared to control positive. After and before of CCL₄-intoxicated rats with artichoke leaves and onion skin extracts for 4 week showed a significant increase in the activity of antioxidant enzyme with simultaneous significantly decreased in the level of liver enzymes activities and thiobarbituric acid as compared to control positive. We speculate that artichoke leaves and onion skin extracts its effect by decreasing lipid peroxidation and enhancing the activities of antioxidant enzymes.

INTRODUCTION

Recently an increasing impact of liver disease constitutes a major problem at worldwide, especially liver injury due to pharmacological treatment that plays a significant role, thus a new insights have been brought into the pathomechanisms of liver injury, and this provides the basis for novel therapeutic strategies (Gerbes et al., 2006).

Carbon tetrachloride (CCL₄) is a well known hepatotoxin that is widely used to induce acute-toxic liver injury, acute hepatotoxicity, inflammation, necrosis and oxidative stress of hepatocytes in laboratory
animals (Muriel, 2007). Moreover, overproduction of free radicals is
toxic to hepatocytes and initiate reactive oxygen species (ROS) mediated
cascade causing hepatocyte death, leading to acute hepatic damage
(Jaeschke, 2000). Therefore, anti-oxidative treatment was proposed to be
a potential means of preventing or attenuating liver injury (Higuchi and
Gores, 2003).

In the last few years, it has been suggested that, most cases of
hepatocellular injury is not due to damaging agent itself but to the
inflammatory cells which have been attacked by the stressed hepatocytes
(Ramadori and Armbrust, 2001).

Artichoke (Cynara scolymus L.) is an ancient herbaceous perennial
plant, originating from the southern Mediterranean parts of North Africa
and widely grown around the world. Ethanolic extracts from artichoke
have been used in folk medicine against liver complaints and the extracts
or their constituents have been claimed to exert a beneficial action
against hepato-biliary diseases (Gebhardt, 1997), Wang et al. (2003)
and Nuutila et al. (2003). Improve liver regeneration after partial
hepatectomy (Adzet et al., 1987). Artichoke leaves which have a high
content of polyphenolic compound and considered as a source for
antioxidant compounds Coinu et al. (2007). Identified and calibrated the
polyphenol content of leaves and outer bracts of Violettto di Toscania by
HPLC/DAD and HPLC/MS. The following compounds were identified:
1-O- caffeoylquinic acid, 3-O-caffeoylquinic acid, chlorogenic acid,
caffeic acid, luteolin 7-O-rutinoside, luteolin 7-O-glucoside, 1,5-O-
dicaffeoylquinic acid, luteolin 7-O-malonilglucoside and luteolin
(agycone). Artichoke leaves extracts have been used to reduce plasma
lipid levels, including total cholesterol (Bundy et al., 2008).

Onion (Allium cepa L.) is one of the major vegetable crops grown
in all over of the world which production and more than 450,000 tones of
onion wastes is produced annually in the European Union. Nowadays,
the food and agricultural products processing industries generate
substantial quantities of phenolic- rich by-products, which could be
valuable natural sources of antioxidant to be employed as ingredients.
Some of these by-products have been the subject of investigation and
have proven to be sources of phenolic antioxidants (Peschel et al., 2006).
Red onion skin possesses high content of phenolic compounds and
flavonoids (Singh et al., 2009). The identified phenolic compounds were
gallic acid, ferulic acid, protocatechuic acid, quercetin and kampferol.
The effects of onion skin on lipid peroxides and DNA damage in aged
rats was studied by Park et al., (2007) who found that onion skin powder
extract reduced liver thiobarbituric reactive substances relative to those
of the control diet in aged rats.
The present work aims at extracting natural potentially active substances with hepatoprotective activity from some agricultural wastes and determining the effect of artichoke leaves and onion skin extracts on CCl₄-induced lipid peroxidation.

**MATERIAL AND METHODS**

**A-MATERIALS**

1-Wastes material:
A- Artichoke (*Cynara scolymus* L.) leaves were obtained from Faculty of Agriculture, Benha University.
B- Onion (*Allium cepa* L.) skin were obtained from local market

2-Chemicals:-
Radical 2,2-diphenylpicrylhydrazyl (DPPH) was purchased from Aldrich Chemical Co. Folin-ciocalteu reagent was purchased from Sigma. Diagnostic kits from Bio Meriêuex Laboratory Reagents and Products, France. Different solvents purchased from El-Nasr Chemical Co.

3-Carbon tetrachloride (CCl₄) solution:
Carbon tetrachloride (5 ml) was dissolved in corn oil (45 ml) to obtain a solution of CCl₄ which was used for treating rats as cancer inducer (0.8 ml/Kg B.W) as recommended by Qin *et al.* (1998) and Aziz *et al.* (2005)

3- Experimental animal:
Sixty adult male albino rats (Wister Strain), weighing approximately 120-140 gram each were purchased from Organization of Biological Products and Vaccines (Helwan Farm), Egypt. They were housed under ambient temperature of 22±2°C and kept in wire-bottomed stainless steel cages. Animals were allowed free access of water and fed on standard diet according to Reeves *et al.* (1993).

**B-METHODS**

1- Ethanolic extraction of artichoke leaves and onion skin:
Artichoke leaves and onion peel were dried in an oven at 70°C and ground in a Wiley mill to path through a 0.5 mm screen and were extracted according to Onyeneho and Hettiarachchy, (1991).

2- Determination of phenolic compounds: The amounts of total phenolics in the ethanolic extracts of artichoke leaves and onion skin were determined according to the Folin-Ciocalteu procedure (Singleton and Rossi, 1965).

3- Free radical scavenging assay:
The free radical scavenging effect of the wastes extracts were assessed by the decolouration of a methanolic solution of DPPH
(Diphenylpicryl hydrazyl) according to Feresin et al. (2002) and Lee et al. (2002).

4- Experimental Design:-

In this study sixty rats were used and divided randomly into 6 equal groups (10 rats each).

Group 1: Rats were fed on the standard synthetic diet and served as a negative control group (-ve) for 8 weeks.

Group 2: Rats were fed on the standard synthetic diet and received CCl₄ orally (twice a week) by gastric gavage at a dose of 0.8 ml/Kg b.w for 8 weeks and served as positive control group (+ve).

Group 3: Rats were fed on the standard synthetic diet and received ethanolic extract of artichoke leaves orally (twice a week) by gastric gavage at a dose of 0.6 mg/Kg b.w after the first 4 weeks CCl₄ was gastric gavage (each third day) at a dose of 0.8 ml/Kg b.w for a period of 4 weeks.

Group 4: Rats were fed on the standard synthetic diet and received CCl₄ orally (twice a week) by gastric gavage at a dose of 0.8 mg/Kg b.w. Four weeks latter, ethanolic extract of artichoke leaves was given by gastric gavage (each third day) for a period of 4 weeks.

Group 5: Rats were fed on the standard synthetic diet and received ethanolic extract of onion skin orally (twice a week) by gastric gavage at a dose of 0.6 mg/Kg b.w after the first 4 weeks CCl₄ was gastric gavage (each third day) at a dose of 0.8 ml/Kg b.w for a period of 4 weeks.

Group 6: Rats were fed on the standard synthetic diet and received CCl₄ of orally (twice a week) by gastric gavage at a dose of 0.8 mg/Kg b.w. Four weeks latter, ethanolic extract of onion skin was given by gastric gavage (each third day) for a period of 4 weeks.

5- Blood samples:

Blood samples were collected before treatment (at zero time) and then after 4 and 8 weeks from the administration of the different treatments. Blood samples were obtained from the retro-orbital plexus veins from the individual rat according to the procedure described by Schermer (1967) by means of fine capillary heparinized tubes. Blood samples were divided into two parts; one was collected into a plain
centrifuge tube for serum preparation to assay the biochemical parameters of blood including liver function tests, kidney function tests and serum cholesterol. The other part was collected into heparinized tube for assay of the complete blood picture.

6- Tissue samples:

At end of the experimental, rats were sacrificed and the post mortem findings were recorded. Tissues specimens from some organs were fixed in 10% saline formalin for histopathological examination. Routine histopathological procedures were carried out.

7- Biochemical analysis:

Serum enzyme activities of alanine transaminase (ALT) and aspartate transaminase (AST), as well as the serum total bilirubin, direct bilirubin, total protein and serum albumin were determined according to the methods of Reitman and Frankel (1957), Tietz (1983), Doumas (1975), and Doumas et al. (1971), respectively. The globulin was calculated by subtracting the albumin from serum total protein. Also, kidney function parameters as urea, uric acid and creatinine were measured by using the method of Tabacco et al. (1979), Haisman and Muller (1977) and Henery et al. (1974), respectively. In addition, lipid profile including triglycerides (TG), total cholesterol (TC), high density lipoprotein (HDL-C) and low density lipoprotein (LDL-C), was determined according to the methods of Fossati and Precipe (1982); Finely (1978) and Lopes-Virella et al. (1977), respectively.

Antioxidant markers:

Glutathione-S-transferase activity was determined using aromatic substrate by monitoring the change in absorbance due to thioether formation as described by Habig et al. (1974). Lipid peroxide (malondialdehyde, (MDA) was measured by using the method of Uchiyama and Mihara (1978).

Hematological studies:

Total erythrocyte count (RBCs count), hemoglobin percentage (Hb%), hematocrit (packed cell volume, PCV) value and total leucocytes count. This determined according to Dacie and Lewis, (1991).

Histopathological examination:

Small tissue specimens were collected from livers and kidneys of the rats in all groups were rapidly fixed in 10% neutral buffered formalin. After proper fixation, thin paraffin sections were routinely prepared and stained with hematoxylin and eosin stain for the histopathological lesions
in hepatic and renal tissues according to Drury and Wallington (1986). These histopathological were determined at Faculty of Vet. Med., Benha University.

**Statistical analysis of data:**

Data were analyzed using SAS program (SAS, 1996). Differences between means were tested (\( P < 0.05 \)) based on Duncon’s method using PROC MEANS (SAS, 1996).

**RESULT & DISCUSSION**

Phenolic compounds and DPPH Scavenging activity of artichoke leaves and onion skin ethanolic extracts.

From the data presented in Table (1) it is clear that values of the ethanolic extract of artichoke leaves and onion skin were 17.53\% and 24.57\%, respectively. Total phenolic contents of artichoke leaves and onion skin were 8.46 and 8.78 \%, respectively. These results are in good agreement with those reported by Wang et al. (2003) and Nuutila et al. (2003). Free Radical Scavenging Capacity to extracts from artichoke leave and onion skin showed strong scavenging activity against DPPH* radicals (Table, 1). Samples content low phenolic compound also lower antioxidant activity. The antioxidant activities of phenolic compounds are reported to be largely determined by the number of hydroxyl groups on the aromatic ring. The higher number of hydroxyl groups, the greater expected antioxidant activity.

**Table (1): Extract yield, phenolic content and antiradical activities of artichoke leaves and onion skin extracts.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Material</th>
<th>Artichoke leaves</th>
<th>Onion skin</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Total ethanolic extracts</td>
<td></td>
<td>17.53</td>
<td>24.54</td>
</tr>
<tr>
<td>% Total phenolic content</td>
<td></td>
<td>8.46</td>
<td>8.78</td>
</tr>
<tr>
<td>%Antiradical activity</td>
<td></td>
<td>33.61</td>
<td>78.82</td>
</tr>
</tbody>
</table>

Effect of artichoke leaves and onion skin extracts on liver function in rats treated with CCl\(_4\):

Data presented in Table (2) show that in the rats fed on artichoke leaves and onion skin extracts during the first four weeks, (hepatoprotective effect) i.e., before CCl\(_4\) application there were significant decreases in serum ALT and AST, total bilirubin and direct
bilirubin activity concentration compared with normal rats (control negative). On the other hand, total protein, albumin and globulin significantly increased in the serum of these rats. Slight in significant increases differences occurred after 8 weeks in values of ALT, AST, total bilirubin and direct bilirubin compared with the normal rats. While total protein, albumin and globulin non significantly decreased compared with the normal rats.

Administration of CCl₄ by normal rats caused significant increases in serum ALT, AST, total bilirubin and direct bilirubin concentration allover the experimental period as compared with the other groups but on the other hand caused total protein, albumin and globulin to significantly decrease.

Data presented in Table (2) show also that in the rats fed on artichoke leaves and onion skin extracts during the last four weeks, (therapeutic effect) after CCl₄ application, significant increases in serum ALT, AST, total bilirubin and direct bilirubin activity occurred after four weeks compared with the normal rats (control negative). On the other hand, total protein, albumin and globulin a significant decreased. Orally administration of artichoke leaves and skin extracts the obtained results indicated that significant decreased serum contents of ALT, AST, total bilirubin and direct bilirubin activity compared with the positive control. How ever, total protein, albumin and globulin significantly increased.

From the obtained result, it could be attributed to the biotransformation of CCl₄ by the cytochrom p-450 in the liver endoplasmic reticulum to the highly reactive trichloromethyl free radical. This free radical reacts with oxygen to form a trichloromethylperoxy radical; which may attack lipids on the membrane of endoplasmic reticulum more readily than the trichloromethyl free radical. The trichloromethylperoxy radical leads to elicit lipid peroxidation, disruption of Ca²⁺ homeostasis, elevation of hepatic enzymes and finally results in death (Clawson, 1989). Also, Halliwell (1991) attributed such increase in serum ALT, AST concentration to enzymatic oxidation of CCl₄ to a CCl₃⁻ free radical within the membrane, triggers the progressive destruction of polyunsaturated fatty acids (PUFA) ultimately to membrane destruction.

Higher level of marker enzymes indicates that, CCl₄ induced liver dysfunction and denotes damage to the hepatic cell. The therapeutical activity of the crude extract is probably first of all due to the phenolic structure of these substances that is responsible for the free radical mediated processes inhibition (Hertog and Hollmann, 1998).
Table (2): Changes in serum liver enzymes activities and protein concentration of experimentally induced hepatotoxicity with CCl₄ in rats (hepatoprotective & therapeutic groups).

<table>
<thead>
<tr>
<th>Tests</th>
<th>Time</th>
<th>CN</th>
<th>CP</th>
<th>ALE + CCl₄</th>
<th>CCl₄ + ALE</th>
<th>OSE + CCl₄</th>
<th>CCl₄ + OSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (U/ml)</td>
<td>0 Time</td>
<td>25.00±1.19&lt;sup&gt;gb&lt;/sup&gt;</td>
<td>22.00±1.19&lt;sup&gt;gb&lt;/sup&gt;</td>
<td>21.00±1.19&lt;sup&gt;h&lt;/sup&gt;</td>
<td>24.00±1.19&lt;sup&gt;fgb&lt;/sup&gt;</td>
<td>23.00±1.19&lt;sup&gt;gb&lt;/sup&gt;</td>
<td>24.00±1.19&lt;sup&gt;fgb&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>4 Weeks</td>
<td>25.07±1.19&lt;sup&gt;lb&lt;/sup&gt;</td>
<td>44.00±1.19&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>22.00±1.19&lt;sup&gt;hb&lt;/sup&gt;</td>
<td>42.00±1.19&lt;sup&gt;d&lt;/sup&gt;</td>
<td>22.00±1.19&lt;sup&gt;hb&lt;/sup&gt;</td>
<td>46.00±1.19&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>8 weeks</td>
<td>26.00±1.19&lt;sup&gt;lb&lt;/sup&gt;</td>
<td>101.67±1.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23.33±1.19&lt;sup&gt;fgb&lt;/sup&gt;</td>
<td>24.00±1.19&lt;sup&gt;fgb&lt;/sup&gt;</td>
<td>25.00±1.19&lt;sup&gt;fgb&lt;/sup&gt;</td>
<td>25.00±1.19&lt;sup&gt;fgb&lt;/sup&gt;</td>
</tr>
<tr>
<td>AST (U/ml)</td>
<td>0 Time</td>
<td>26.67±0.89&lt;sup&gt;gb&lt;/sup&gt;</td>
<td>26.33±0.89&lt;sup&gt;gb&lt;/sup&gt;</td>
<td>27.67±0.89&lt;sup&gt;gb&lt;/sup&gt;</td>
<td>26.33±0.89&lt;sup&gt;gb&lt;/sup&gt;</td>
<td>28.00±0.89&lt;sup&gt;cgb&lt;/sup&gt;</td>
<td>28.33±0.89&lt;sup&gt;fgb&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>4 Weeks</td>
<td>26.77±0.89&lt;sup&gt;gb&lt;/sup&gt;</td>
<td>47.00±0.89&lt;sup&gt;c&lt;/sup&gt;</td>
<td>26.00±0.89&lt;sup&gt;gb&lt;/sup&gt;</td>
<td>49.67±0.89&lt;sup&gt;ch&lt;/sup&gt;</td>
<td>27.00±0.89&lt;sup&gt;fgb&lt;/sup&gt;</td>
<td>51.00±0.89&lt;sup&gt;b&lt;/sup&gt;</td>
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<td></td>
<td>8 weeks</td>
<td>28.67±0.89&lt;sup&gt;fg&lt;/sup&gt;</td>
<td>125.00±0.89&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29.00±0.89&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>29.67±0.89&lt;sup&gt;efg&lt;/sup&gt;</td>
<td>29.00±0.89&lt;sup&gt;c&lt;/sup&gt;</td>
<td>30.33±0.89&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total bilirubin (mg%)</td>
<td>0 Time</td>
<td>0.85±0.04&lt;sup&gt;efgh&lt;/sup&gt;</td>
<td>0.82±0.04&lt;sup&gt;efgh&lt;/sup&gt;</td>
<td>0.90±0.04&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>0.88±0.04&lt;sup&gt;efgh&lt;/sup&gt;</td>
<td>0.91±0.04&lt;sup&gt;defg&lt;/sup&gt;</td>
<td>0.81±0.04&lt;sup&gt;fgb&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>4 Weeks</td>
<td>0.80±0.04&lt;sup&gt;fgbhi&lt;/sup&gt;</td>
<td>1.04±0.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.74±0.04&lt;sup&gt;hj&lt;/sup&gt;</td>
<td>0.96±0.04&lt;sup&gt;ck&lt;/sup&gt;</td>
<td>0.74±0.04&lt;sup&gt;hj&lt;/sup&gt;</td>
<td>1.02±0.04&lt;sup&gt;ef&lt;/sup&gt;</td>
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<td></td>
<td>8 weeks</td>
<td>0.78±0.04&lt;sup&gt;fgbhi&lt;/sup&gt;</td>
<td>1.44±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.73±0.04&lt;sup&gt;hj&lt;/sup&gt;</td>
<td>0.76±0.04&lt;sup&gt;hj&lt;/sup&gt;</td>
<td>0.87±0.04&lt;sup&gt;efgh&lt;/sup&gt;</td>
<td>0.78±0.04&lt;sup&gt;fgbhi&lt;/sup&gt;</td>
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<tr>
<td>Direct bilirubin (mg%)</td>
<td>0 Time</td>
<td>0.14±0.01&lt;sup&gt;defghi&lt;/sup&gt;</td>
<td>0.12±0.01&lt;sup&gt;defghi&lt;/sup&gt;</td>
<td>0.15±0.01&lt;sup&gt;def&lt;/sup&gt;</td>
<td>0.15±0.01&lt;sup&gt;defghi&lt;/sup&gt;</td>
<td>0.19±0.01&lt;sup&gt;bcd&lt;/sup&gt;</td>
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<td>0.21±0.01&lt;sup&gt;bc&lt;/sup&gt;</td>
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<td>0.17±0.01&lt;sup&gt;defgh&lt;/sup&gt;</td>
<td>0.10±0.01&lt;sup&gt;j&lt;/sup&gt;</td>
<td>0.18±0.01&lt;sup&gt;bcd&lt;/sup&gt;</td>
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<td>8 weeks</td>
<td>0.11±0.01&lt;sup&gt;fgbhi&lt;/sup&gt;</td>
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<td>0.13±0.01&lt;sup&gt;efgh&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total protein (g/dl)</td>
<td>0 Time</td>
<td>7.57±0.15&lt;sup&gt;cdef&lt;/sup&gt;</td>
<td>7.87±0.15&lt;sup&gt;abcded&lt;/sup&gt;</td>
<td>7.83±0.15&lt;sup&gt;abced&lt;/sup&gt;</td>
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<td>8.00±0.15&lt;sup&gt;ab&lt;/sup&gt;</td>
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<td>7.57±0.15&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>Total albumin (g/dl)</td>
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<td>3.90±0.15&lt;sup&gt;cdef&lt;/sup&gt;</td>
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<td>3.36±0.15&lt;sup&gt;phi&lt;/sup&gt;</td>
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<td>3.77±0.15&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>Total globulin (g/dl)</td>
<td>0 Time</td>
<td>3.67±0.07&lt;sup&gt;def&lt;/sup&gt;</td>
<td>3.70±0.07&lt;sup&gt;cdef&lt;/sup&gt;</td>
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<td>3.33±0.07&lt;sup&gt;gb&lt;/sup&gt;</td>
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<td>4 Weeks</td>
<td>3.65±0.07&lt;sup&gt;def&lt;/sup&gt;</td>
<td>3.36±0.07&lt;sup&gt;h&lt;/sup&gt;</td>
<td>3.73±0.07&lt;sup&gt;def&lt;/sup&gt;</td>
<td>3.70±0.07&lt;sup&gt;def&lt;/sup&gt;</td>
<td>3.83±0.07&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>3.47±0.07&lt;sup&gt;fg&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>8 weeks</td>
<td>4.08±0.07&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.13±0.07&lt;sup&gt;h&lt;/sup&gt;</td>
<td>3.93±0.07&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>4.17±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.88±0.07&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>3.80±0.07&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

CN: Control negative (Normal Diet)  
ALE: Artichoke leaves extract  
CP: Control positive  
OSE: Onion skin extract  

<sup>a, b, c, .... etc. : There is no significant difference (P>0.05) between any two means with the same letter in each column.</sup>
Effect of artichoke leaves and onion skin extracts on kidney functions and lipid profile in rats treated with CCl₄:

Data presented in Table (3) showed that rats fed on artichoke leaves and onion skin extracts during the first four weeks, (hepatoprotective effect) i.e., before CCl₄ application, significant decreases occurred in serum urea, uric acid, creatinine, total cholesterol, triglyceride and LDL-cholesterol concentration compared with the normal rats (control negative). On other the hand HDL-cholesterol significant increased. Slight differences were achieved after 8 weeks, where urea, uric acid and creatinine non-significant increased in these rats as compared with the normal rat however HDL-cholesterol non-significantly decreased as compared with the normal rats.

Administration of CCl₄ by normal rats caused significant increases in serum urea, uric acid, creatinine, total cholesterol, and triglyceride and LDL-cholesterol concentration all over the experimental period as compared with other groups. On other hand, HDL-cholesterol a significantly decreased.

Data presented in Table (3) show that in the rats fed on artichoke leaves, onion skin and extracts during the last four weeks, (therapeutic effect) i.e., after CCl₄ application, significant increases occurred in serum urea, uric acid, creatinine, total cholesterol, triglyceride and LDL-cholesterol after four weeks compared with the normal rats (control negative). On other hand, HDL-cholesterol significantly decreased. Orally administration of artichoke leaves and skin extracts significant decreased contents of serum urea, uric acid and creatinine as compared with the positive control. On the other hand, HDL-cholesterol significantly increased.

Enhanced levels of urea, uric acid and creatinine in serum might occur due to CCl₄ induced physiological imbalance in liver and kidney as shown by Mehmetçik et al. (2008). Speroni et al. (2003) attributed the increase in serum urea level to increase in renal disorders.

Carbon tetrachloride induced liver injury is due to the conversion of CCl₄ to CCl₃⁺ and CCl₃OO⁺ by the cytochrome p450 enzyme. These highly reactive free radicals cause cell damage (Park et al., 2007). Since the kidney has an affinity for CCl₄ (Abraham et al., 1999) and contains cytochrome p450 predominantly in the cortex (Ronis et al., 1998), so the mechanism of CCl₄ nephrotoxicity is probably the same as that of the liver and also independent from the diminished functionality of the liver.
Table (3): Changes in serum kidney Function and lipid profile concentration of experimentally induced hepatotoxicity with CCl₄ in rats (hepatotrophic & therapeutic groups).

<table>
<thead>
<tr>
<th>Tests</th>
<th>Time</th>
<th>CN</th>
<th>CP</th>
<th>ALE+ CCl₄</th>
<th>CCl₄+ALE</th>
<th>OSE+ CCl₄</th>
<th>CCl₄+OSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea (mg/dl)</td>
<td>0 Time</td>
<td>27.12±1.03</td>
<td>26.92±1.03</td>
<td>26.85±1.03</td>
<td>27.09±1.03</td>
<td>26.96±1.03</td>
<td>27.14±1.03</td>
</tr>
<tr>
<td></td>
<td>4 Weeks</td>
<td>27.65±1.03</td>
<td>52.46±1.03</td>
<td>26.48±1.03</td>
<td>26.14±1.03</td>
<td>26.22±1.03</td>
<td>46.29±1.03</td>
</tr>
<tr>
<td></td>
<td>8 weeks</td>
<td>27.38±1.03</td>
<td>79.95±1.03</td>
<td>27.76±1.03</td>
<td>30.77±1.03</td>
<td>32.04±1.03</td>
<td>32.56±1.03</td>
</tr>
<tr>
<td>Uric acid (mg/100ml)</td>
<td>0 Time</td>
<td>3.60±0.20</td>
<td>3.47±0.20</td>
<td>3.50±0.20</td>
<td>3.50±0.20</td>
<td>3.73±0.20</td>
<td>3.40±0.20</td>
</tr>
<tr>
<td></td>
<td>4 Weeks</td>
<td>3.83±0.20</td>
<td>4.83±0.20</td>
<td>3.10±0.20</td>
<td>4.53±0.20</td>
<td>3.42±0.20</td>
<td>4.33±0.20</td>
</tr>
<tr>
<td></td>
<td>8 weeks</td>
<td>3.97±0.20</td>
<td>7.94±0.20</td>
<td>3.50±0.20</td>
<td>3.73±0.20</td>
<td>4.07±0.20</td>
<td>3.60±0.20</td>
</tr>
<tr>
<td>Creatinine (mg/100ml)</td>
<td>0 Time</td>
<td>0.44±0.02</td>
<td>0.43±0.02</td>
<td>0.44±0.02</td>
<td>0.44±0.02</td>
<td>0.45±0.02</td>
<td>0.49±0.02</td>
</tr>
<tr>
<td></td>
<td>4 Weeks</td>
<td>0.53±0.02</td>
<td>0.79±0.02</td>
<td>0.43±0.02</td>
<td>0.71±0.02</td>
<td>0.42±0.02</td>
<td>0.79±0.02</td>
</tr>
<tr>
<td></td>
<td>8 weeks</td>
<td>0.55±0.02</td>
<td>1.58±0.02</td>
<td>0.52±0.02</td>
<td>0.50±0.02</td>
<td>0.55±0.02</td>
<td>0.52±0.02</td>
</tr>
<tr>
<td>Total cholesterol (mg/100 ml)</td>
<td>0 Time</td>
<td>157.67±1.84</td>
<td>161.33±1.84</td>
<td>161.00±1.84</td>
<td>158.00±1.84</td>
<td>159.33±1.84</td>
<td>154.33±1.84</td>
</tr>
<tr>
<td></td>
<td>4 Weeks</td>
<td>160.33±1.84</td>
<td>185.00±1.84</td>
<td>154.33±1.84</td>
<td>181.00±1.84</td>
<td>152.67±1.84</td>
<td>182.33±1.84</td>
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<tr>
<td></td>
<td>8 weeks</td>
<td>163.33±1.84</td>
<td>255.00±1.84</td>
<td>162.33±1.84</td>
<td>170.00±1.84</td>
<td>167.67±1.84</td>
<td>167.67±1.84</td>
</tr>
<tr>
<td>Triglyceride (mg/100ml)</td>
<td>0 Time</td>
<td>175.00±3.22</td>
<td>179.33±3.22</td>
<td>181.33±3.22</td>
<td>181.00±3.22</td>
<td>177.67±3.22</td>
<td>174.33±3.22</td>
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<td></td>
<td>4 Weeks</td>
<td>180.00±3.22</td>
<td>247.00±3.22</td>
<td>173.67±3.22</td>
<td>241.67±3.22</td>
<td>168.33±3.22</td>
<td>241.00±3.22</td>
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<tr>
<td></td>
<td>8 weeks</td>
<td>182.67±3.22</td>
<td>297.67±3.22</td>
<td>171.67±3.22</td>
<td>191.00±3.22</td>
<td>195.67±3.22</td>
<td>192.33±3.22</td>
</tr>
<tr>
<td>HDL- cholesterol (mg/100ml)</td>
<td>0 Time</td>
<td>50.67±1.36</td>
<td>47.67±1.36</td>
<td>47.67±1.36</td>
<td>50.67±1.36</td>
<td>50.00±1.36</td>
<td>51.33±1.36</td>
</tr>
<tr>
<td></td>
<td>4 Weeks</td>
<td>47.67±1.36</td>
<td>34.67±1.36</td>
<td>34.67±1.36</td>
<td>37.67±1.36</td>
<td>56.33±1.36</td>
<td>35.67±1.36</td>
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<tr>
<td></td>
<td>8 weeks</td>
<td>44.33±1.36</td>
<td>29.00±1.36</td>
<td>37.67±1.36</td>
<td>43.67±1.36</td>
<td>46.33±1.36</td>
<td>43.00±1.36</td>
</tr>
<tr>
<td>LDL- cholesterol (mg/100ml)</td>
<td>0 Time</td>
<td>72.00±2.46</td>
<td>77.80±2.46</td>
<td>77.07±2.46</td>
<td>69.13±2.46</td>
<td>73.80±2.46</td>
<td>68.13±2.46</td>
</tr>
<tr>
<td></td>
<td>4 Weeks</td>
<td>76.67±2.46</td>
<td>100.93±2.46</td>
<td>68.93±2.46</td>
<td>95.00±2.46</td>
<td>62.67±2.46</td>
<td>98.47±2.46</td>
</tr>
<tr>
<td></td>
<td>8 weeks</td>
<td>82.46±2.46</td>
<td>166.47±2.46</td>
<td>81.13±2.46</td>
<td>88.13±2.46</td>
<td>81.87±2.46</td>
<td>86.20±2.46</td>
</tr>
</tbody>
</table>

CN: Control negative (Normal Diet)  
ALE: Artichoke leaves extract  
CP: Control positive  
OSE: Onion skin extract

a, b, c, .... etc.: There is no significant difference (P>0.05) between any two means with the same letter in each column.
as stated by Ogeturk et al. (2005). Rincon et al. (1999) showed that effects of CCl₄ on kidney structure and function depended on the functional state of the liver.

The recorded results may be attributed to the increase in the non essential fatty acids resulted from lipid peroxidation induced by CCl₃⁻ free radical. These free fatty acids enter in the synthetic pathway of triacylglycerol, total cholesterol and LDL-Cholesterol. Bundy et al. (2008) attributed this increase to oxidative stress which increases the supply of non-essential fatty acids, that in turn increases triacylglycerol and cholesterol levels in serum and tissues. This suggestion was supported by the findings of Park et al. (2007) who concluded that the significant increase in lipid profile indicates severe lipid peroxidation and physiological defects in the organs after CCl₄ administration. Bundy et al. (2008) reported that, changes in blood cholesterol and triglyceride levels are indicative of disorders of the lipid metabolism.

Effect of different treatments on antioxidant markers:--

Data presented in Table (4) show that in the rats fed on artichoke leaves and onion skin extracts during the first four weeks, (hepatoprotective effect) i.e., before CCl₄ application, significant decreases occurred in serum malondialdehyde concentration as compared with the normal rats (control negative). On the other hand, glutathione-S-transferase concentration significantly increased. A slight difference was achieved after 8 weeks, where malondialdehyde non-significantly increased compared with the normal rats. On the other hand glutathione-S-transferase non-significantly decreased compared with the normal rats.

Administration of CCl₄ to normal rats caused a significant increase in serum malondialdehyde concentration allover the experimental period as compared with the other groups but on the other hand caused glutathione-S-transferase significantly decrease.

Data presented in Table (4) show that in rats fed on artichoke leaves, onion skin and extracts during the last four weeks, (therapeutic effect) i.e., after CCl₄ application, there were significant increases in serum malondialdehyde after four weeks compared with the normal rats (control negative). On the other hand glutathione-S-transferase significantly decreased.

These results may be attributed to the exhaustion of antioxidant enzyme GST in detoxification of CCl₃⁻ free radical resulting from CCl₄ toxicity via its conjugation with other antioxidant substrates as GSH.
This suggestion is confirmed by the findings of Lee et al. (2008) who stated that, administration of CCl₄ significantly reduces the antioxidant enzyme GST and attributed these findings to the inactivation of antioxidant enzymes by lipid peroxides as lipid peroxidation is one of the principal causes of CCl₄–induced liver injury that mediated by the free radical derivatives of CCl₄. In addition, the antioxidant activity and / or the inhibition of free radical generation are important in terms of protecting the liver from CCl₄-induced damage (Manibusan et al., 2007).

Effect of different treatments on some hematological parameters in rats treated with CCl₄.

Data presented in Table (5) showed that rats fed on artichoke leaves and onion skin extracts during the first four weeks, (hepatoprotective effect) i.e., before CCl₄ application, there were significant increases in blood RBCs, Hb%, PCV and WBCs×10⁹/L compared with the normal rats (control negative). Slight differences were achieved after 8 weeks in blood RBCs non-significantly decreased compared with the normal rats.

Administration of CCl₄ by normal rats caused significant decreases in blood RBCs, Hb%, PCV and WBCs×10⁹/L concentration all over the experimental period as compared with the other groups.

Data presented in Table (5) show that in rats fed on artichoke leaves and onion skin extracts during the last four weeks, (therapeutic effect) i.e., after CCl₄ application, caused a significant decreases occurred in blood RBCs, Hb%, PCV and WBCs×10⁹/L concentration after four weeks compared with the normal rats (control negative). Orally administration of artichoke leaves and onion skin extracts significantly increased blood RBCs, Hb%, PCV and WBCs×10⁹/L concentration compared with positive control.

CONCLUSION

We highly recommend the use of natural antioxidant e.g. ethanolic extracts of artichoke leaves and onion skin separately or in combinations in order to avoid the different hazards which can happen due to (CCl₄) the presence of free radicals.

Histopathological Findings:
1- Control negative group

The microscopic examination of the liver of rats in control group revealed no abnormal changes in the hepatic parenchyma where the
### Table (4): Changes in serum glutathione-S-transferase (U/ml) & malondialdehyde (nmol/ml) of experimentally induced hepatotoxicity with CCl_4 in rats (hepatoprotective & therapeutic groups).

<table>
<thead>
<tr>
<th>Tests</th>
<th>Time</th>
<th>CN</th>
<th>CP</th>
<th>ALE+ CCl_4</th>
<th>CCl_4+ALE</th>
<th>OSE+ CCl_4</th>
<th>CCl_4+OSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutathione-S-transferase (U/ml)</td>
<td>0 Time</td>
<td>3.65±0.03</td>
<td>3.70±0.03</td>
<td>3.67±0.03</td>
<td>3.71±0.03</td>
<td>3.74±0.03</td>
<td>3.71±0.03</td>
</tr>
<tr>
<td></td>
<td>4 Weeks</td>
<td>4.04±0.03</td>
<td>2.87±0.03</td>
<td>5.88±0.03</td>
<td>2.75±0.03</td>
<td>6.05±0.03</td>
<td>2.78±0.03</td>
</tr>
<tr>
<td></td>
<td>8 weeks</td>
<td>4.12±0.03</td>
<td>1.81±0.03</td>
<td>4.74±0.03</td>
<td>4.93±0.03</td>
<td>4.88±0.03</td>
<td>5.07±0.03</td>
</tr>
<tr>
<td>Malondialdehyde (nmol/ml)</td>
<td>0 Time</td>
<td>0.62±0.09</td>
<td>0.61±0.09</td>
<td>0.61±0.09</td>
<td>0.62±0.09</td>
<td>0.62±0.09</td>
<td>0.61±0.09</td>
</tr>
<tr>
<td></td>
<td>4 Weeks</td>
<td>0.65±0.09</td>
<td>0.73±0.09</td>
<td>0.53±0.09</td>
<td>0.83±0.09</td>
<td>0.52±0.09</td>
<td>8.43±0.09</td>
</tr>
<tr>
<td></td>
<td>8 weeks</td>
<td>1.68±0.09</td>
<td>15.47±0.09</td>
<td>1.50±0.09</td>
<td>1.73±0.09</td>
<td>1.40±0.09</td>
<td>1.90±0.09</td>
</tr>
</tbody>
</table>

### Table (5): Changes in hematological parameters of experimentally induced hepatotoxicity with CCl_4 in rats (hepatoprotective & therapeutic groups).

<table>
<thead>
<tr>
<th>Tests</th>
<th>Time</th>
<th>CN</th>
<th>CP</th>
<th>ALE+ CCl_4</th>
<th>CCl_4+ALE</th>
<th>OSE+ CCl_4</th>
<th>CCl_4+OSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBCs×10^6</td>
<td>0 Time</td>
<td>5.28±0.27</td>
<td>5.67±0.27</td>
<td>5.00±0.27</td>
<td>5.25±0.27</td>
<td>5.03±0.27</td>
<td>5.29±0.27</td>
</tr>
<tr>
<td></td>
<td>4 Weeks</td>
<td>5.00±0.27</td>
<td>3.30±0.27</td>
<td>4.70±0.27</td>
<td>3.02±0.27</td>
<td>6.47±0.27</td>
<td>3.62±0.27</td>
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<tr>
<td></td>
<td>8 weeks</td>
<td>4.90±0.27</td>
<td>2.50±0.27</td>
<td>4.98±0.27</td>
<td>4.64±0.27</td>
<td>4.71±0.27</td>
<td>6.40±0.27</td>
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<tr>
<td>Hb%</td>
<td>0 Time</td>
<td>11.77±0.54</td>
<td>10.60±0.54</td>
<td>10.05±0.54</td>
<td>11.91±0.54</td>
<td>12.32±0.54</td>
<td>12.63±0.54</td>
</tr>
<tr>
<td></td>
<td>4 Weeks</td>
<td>11.30±0.54</td>
<td>8.77±0.54</td>
<td>14.53±0.54</td>
<td>9.23±0.54</td>
<td>14.97±0.54</td>
<td>9.32±0.54</td>
</tr>
<tr>
<td></td>
<td>8 weeks</td>
<td>11.93±0.54</td>
<td>7.77±0.54</td>
<td>16.07±0.54</td>
<td>14.27±0.54</td>
<td>10.74±0.54</td>
<td>14.47±0.54</td>
</tr>
<tr>
<td>PCV (ml/d)</td>
<td>0 Time</td>
<td>44.47±0.64</td>
<td>45.00±0.64</td>
<td>45.83±0.64</td>
<td>43.50±0.64</td>
<td>45.17±0.64</td>
<td>44.63±0.64</td>
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<tr>
<td></td>
<td>4 Weeks</td>
<td>45.20±0.64</td>
<td>38.53±0.64</td>
<td>48.30±0.64</td>
<td>39.87±0.64</td>
<td>46.70±0.64</td>
<td>40.27±0.64</td>
</tr>
<tr>
<td></td>
<td>8 weeks</td>
<td>46.35±0.64</td>
<td>37.93±0.64</td>
<td>44.73±0.64</td>
<td>39.93±0.64</td>
<td>43.27±0.64</td>
<td>44.97±0.64</td>
</tr>
<tr>
<td>WBCs×10^3/L</td>
<td>0 Time</td>
<td>10.56±0.46</td>
<td>10.57±0.46</td>
<td>9.43±0.46</td>
<td>7.65±0.46</td>
<td>11.20±0.46</td>
<td>7.49±0.46</td>
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<tr>
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<td>4 Weeks</td>
<td>10.56±0.46</td>
<td>9.72±0.46</td>
<td>9.93±0.46</td>
<td>7.65±0.46</td>
<td>9.51±0.46</td>
<td>9.07±0.46</td>
</tr>
<tr>
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<td>8 weeks</td>
<td>11.30±0.46</td>
<td>4.63±0.46</td>
<td>10.40±0.46</td>
<td>8.22±0.46</td>
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<td>8.49±0.46</td>
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</table>

CN: Control negative (Normal Diet)
ALE: Artichoke leaves extract
OSE: Onion skin extract

\* There is no significant difference (P>0.05) between any two means with the same letter in each column.
(A): Liver of rats in control negative group showing normal histologic structure of hepatic parenchyma. H&E stain x 200

(B): Kidney of rats control negative group showing normal histological structure of renal tubules (T) and glomeruli (G). H & E stain x 400

(C): Liver of rats in control positive showing massive degenerative change in hepatocytes, these changes represented by focal. H&E stain x 200

(D): Kidney of rats in control positive showing mononuclear inflammatory cellular infiltration of the renal parenchyma. H&E stain x 400

(E): Liver of rats protected with artichoke leave extract and sequently given CCl₄ showing dilated bile duct filled with eosinophilic debris. H & E stain x 400

(F): Kidney of rats protected with artichoke leave extract and sequently given CCl₄ showing cellular casts in lumen of renal tubules. H&E stain x 200

Fig. (1A, B, C, D, E & F): Photomicrographs showing histopathological effects of different treatment on liver & kidney of rats.
Fig. (2a, b, c, d, e & f): Photomicrographs showing histopathological effects of different treatment on liver & kidney of rats.
the liver revealed focal aggregation of inflammatory cells among normal hepatic cells. In few cases, some hepatic cells were suffered from hydropic degeneration (Fig. 2A). These results were agreed with Speroni et al. (2003).

The microscopic examination of the kidneys of rats given CCl₄ and subsequently curative with artichoke leave extract showed vacuolar degeneration of some renal tubules with aggregation of few lymphocytes around blood vessels (Fig. 2B). Congestion of some intertubular blood capillaries with accumulation of cellular casts in lumen of renal tubules was also noticed. These results were agreed with Speroni et al. (2003).

5- Onion skin extract + CCl₄

The pathological examination of the liver of rats protected with onion skin extract and subsequently given CCl₄ showed congestion of central veins and portal vessels. Focal areas of degeneration of hepatocytes were seen (Fig. 2C). Moreover, bile ductal hyperplasia was also recorded.

In this group, the microscopic examination of the kidneys of rats protected with onion skin extract and subsequently given CCl₄ revealed no microscopic pathological changes except congestion of some renal blood vessels with cystic dilation of some renal tubules (Fig. 2D).

6- CCl₄ + Onion skin extract

The histopathological examination of the liver of rats given CCl₄ and subsequently curative with onion skin extract showed congestion of the central veins and portal vessels with perivascular lymphocytic cellular aggregations (Fig. 2E). Multiple areas of degeneration of hepatocytes were also detected.

Microscopically the examined kidneys of rats given CCl₄ and subsequently curative with onion skin extract showed congestion of blood vessels with preivascular inflammatory cellular aggregation. Some of renal tubules showed hydropic and vacuolar degeneration of their lining epithelium while the other revealed dilation of their lumens (Fig. 2F).

REFERENCES


خواص المستخلصات الكحولية لأوراق الخرشوف وقشرة البصل كمضادات للأكسدة في الفئران المعاملة ببراع كلوئيد الكربون.

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الهدف من هذا البحث هو دراسة استخدام المستخلص الكحولي لأوراق الخرشوف وقشرة البصل كمضادات للأكسدة لحماية أو علاج الفئران المعاملة ببراع كلوئيد الكربون. وقد أظهرت النتائج المحتملة عليها حدوث انخفاض معنوي في نشاط الإنزيمات المضادة للأكسدة (جلوتايتون، ناد، ناد ترانسفيريز) بينما وجد زيادة معنوية في نشاط إنزيمات الكبد الكلى، الكولسترول الكلي، الجلسيديات الثلاثية والكولسترول منخفض الكثافة وأيضاً زيادة الشفوق الحرة في كبد الفئران المعاملة ببراع كلوئيد الكربون بمقارنة بينهما بالمجموعة الضبطة. وعلى العكس ذلك فقد وجد أن تجربة الفئران بواسطة المستخلص الكحولي لأوراق الخرشوف أو قشرة البصل لمدة أربعة أسابيع ساءت أو قبل بعد المعاملة ببراع كلوئيد الكربون بسبب ارتفاع ملحوظ في نشاط الإنزيمات المضادة للأكسدة ومستوي جلوتاتيون، ناد، ناد ترانسفيريز وانخفاض مستوى في نشاط إنزيمات الكبد الكلى والكولسترول الكلي والجلسيديات الثلاثية والكولسترول منخفض الكثافة وأيضاً انخفاض الشفوق الحرة الكربون بالمقارنة بالمجموعة الضبطة وذلك قبل أو بعد المعاملة ببراع كلوئيد الكربون. و أظهرت هذه المستخلصات حماية ضد الأكسدة أفضل من استخدامها للعلاج. ومن النتائج التي شملتها هذه الدراسة يمكننا استنتاج أن المستخلص الكحولي لأوراق الخرشوف أو قشرة البصل تعمل على تقليل نشاط إنزيمات الكبد، الكولسترول الكلي، الدهون الثلاثية والدهون منخفضة الكثافة وأيضاً أكسدة الدهون في كبد الفئران المعاملة ببراع كلوئيد الكربون وذلك بخفض مستوى الشفوق الحرة وزيادة الإنزيمات المضادة للأكسدة.