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The protective effect of red beet powder against carbon tetrachloride induced hepatotoxicity in albino rats

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Abstract: Red beet consider as functional food and a good source of several bioactive compounds have antioxidant and inflammatory properties that may have health benefits in reducing the risk of injury diseases. Therefore, in the present work, the effectiveness of red beet in protecting against carbon tetrachloride (CCl_4)-induced liver toxicity in rats was studied. The rats were randomly divided into three groups, eight rats per each. First group serves as normal control, second group was treated with CCl_4 (1 ml/kg 1:1 mixture with paraffin oil, i.p. twice weekly for 6 weeks) and third group were induced with CCl_4 and 10% red beet powder (RBP) adding to diet**.** Results show that treatment with 10% (RBP) reduced blood biomarkers for liver functions (AST, ALT and ALP) and kidney functions (urea) that were increased by chronic CCl_4 intoxication. Also, treatment with RBP significantly increased ($P \leq 0.05$) in the final body weight, daily food intake which were diminish by CCl_4. In addition, RBP inhibited the elevated WBC_S, RBCS and PCV values which were high in intoxicated rats. Histological examination support the tested biochemical markers of organs injury. Therefore, the study concluded that RBP as a partial inhibitor and a significant reducer can be a preventive or therapeutic agent due to its antioxidant components.

Key words*:* Beta vulgaris, liver toxicity ,CCl_4, histopathological examination Biochemical parameters.

Introduction

Red beet root, a member of *Chenopodiaceae* family scientifically known as *Beta vulgaris* is cultivated throughout the world for its roots, which are used as a food and as a source of natural dye (Gokhale and Lele 2014). It is considered a popular vegetable and one of the most potent vegetable with respect to antioxidant property. It possess an excellent dietary supplement being not only rich in minerals, nutrients and vitamins but also has unique phytoconstituents, which possess several medicinal properties anti-depressant, anti-microbial, anti-fungal, anti-inflammatory, diuretic, expectorant and carminative, stimulation of hematopoietic, immune systems, kidney and liver protection and as a special diet during cancer treatment (Winkler *et al.,* 2005 and Georgiev *et al.,* 2010)

Red beet is one of the few vegetables that contain a group of highly bioactive pigments known as betalains and rich in phenolic compounds as phenolic acids (Clifford *et al.,* 2015). 4-hydroxy benzoic acid was the major constituent the phenolic acids from red beet extracts followed by cinnamic acid, vanillic, chlorogenic, trans ferulic acid and caffeic acid (Ravichandran *et al.,* 2012). Red beet has high concentration of betalains that used as food additives including that have antioxidant, anticarcinogenic and anti-inflammatory properties (Georgiev *et al.,* 2010). Besides betalains, red beet root also contains small amounts of other antioxidants, derivatives of ferrulic acid, hydroxycinnamic acid and cyclo-dopa-glucoside, so consumption of a natural product can provide an additive or synergistic effect (Frank *et al.,* 2005).

Red beet has become a staple food ingredient in some salads and food products. It contains fiber and very few calories, no fat, which has also been shown to have cholesterol lowering (Krajka-Kuźniak *et al.,* 2012 and Kumar, 2015). Moreno *et al.,* (2006) found consumed red beet in our daily diet improve health and may reduce the risk of degenerative diseases as contain a high percentage of flavonoids. The possibility beetroot's antioxidant potential is includes the ability to reinforce the endogenous antioxidant network and not limited to just scavenging. Red beet might be help in diseased states resulting from oxidative stress such as liver injury and cancer because is an excellent source of antioxidants that protects from oxidation (Clifford *et al.,* 2015).

Carbon tetrachloride (CCl_4) is used for experimental induction of liver injury on animals to induce liver damage. Also, it is nephron toxin which causes chronic toxicity injury in several organs (Kumar *et al.,* 2009 and Lien *et al.,* 2016). Intoxication with CCl_4 caused oxidative stress, cellular necrosis and inflammation, which lead to hepatic damages, such as fibrosis, cirrhosis, and atrophy (Hsu *et al.,* 2012). A large dose of CCl_4 causes severe necrosis, while a lower dose is inducing hepatic fibrosis (Jaeschke *et al.,* 2013). The principle causes of used carbon tetrachloride in inducing the hepatic damage are lipid peroxidation, decreased activities of antioxidant enzymes and generation of free radicals (Eshak *et al.,* 2015). CCl_4 is transformed to trichloromethy free radicals that are capable to attack cellular macromolecules such as lipids, proteins, and DNA (Campo *et al.,* 2004 and Hsu *et al.,* 2010). These reactive free radicals initiate cell damage through two different mechanisms including of covalent binding to the membrane proteins and cause lipid peroxidation (Kanter *et al.,* 2003). Therefore, lipid peroxidation is an important factor which connects inflammatory disease with fibrosis. Oxidative stress is involved in liver fibrosis, frequently associated with the lipid peroxidation amplification and the decline of the antioxidant status (Karadeniz *et al.,* 2009). Body producing antioxidants to counteract oxidative stress by endogenous antioxidants or exogenous antioxidants externally supplied through foods. Food antioxidants such as vitamins and polyphenols are useful in prevention and treatment diseases associated with free radical and oxidative stress that may cause damage in the cells and weakening the immune system (Thompson *et al*., 2010). Beet root might serve as a useful to strengthen endogenous antioxidant defines, helping to protect from oxidative damage (Pham-Huy *et al.,* 2008 and Clifford *et al.,* 2015). Therefore, the present work was designed to investigate the protective role of RBP against CCL_4-induced liver toxicity.

2. Materials and methods:-

2.1 Chemicals and red beet samples preparation:-

Assay kits for AST, ALT, ALP, total protein, albumin and blood urea nitrogen (BUN) were purchased from Biodiagnostic, Cairo, Egypt. Carbon tetrachloride was purchased from El-Nasr Chemical Co. Cairo, Egypt. All other chemicals and solvents were of highest grade commercially available.

The fresh red beet plants were purchased from the local vegetable market in Minia city, Egypt. The red beet roots were prepared according to (Emelike et al., 2015) cleaned, washed and sliced into thin pieces of about 1-2 mm thickness then was dried in sun for seven days.

2.2. Animals and experimental design:-

Twenty four adult male albino rats weighting (222±2g) were obtained from Agricultural Faculty, Minia University. Egypt and allowed to acclimatize for 7 days at room temperature (25± 2C°) in plastic cages at room temperature and photo period 12 cycles. Animals were fed a commercial balanced diet and tap water ad libitum till the end of the experiment (6 weeks). The experiments were conducted according to ethical guidelines of Minia University, Egypt.

The rats were randomly assigned into equal three groups with 8 animals per each as follows: The first group was fed standard chow diet and served as normal control group. The second group injected with CCl_4 (1 ml/kg b.wt, i.p according to Madkour et al., (2012), twice weekly for 6 weeks to induce liver toxicity. Third group were fed the same diet containing 10% (RBP) according to Rabeh, (2015) and injected with CCl_4 (1 ml/kg b.wt, i.p), twice weekly.

The daily feed intake was recorded every day and the body weight was measured weekly, at the end of experimental body weight gain and feed intake were calculated. Feed efficiency ratio (FER) was determined using the following equation. FER= Daily body weight gain (g)/Daily food consuming (g) × 100(Youssef and Abd El Azez 1995).

2.3 Blood samples:-

The rats were fasted overnight after six weeks and anesthetized to collect the blood samples from the retro-orbital plexus (Schermer, 1967) from all animals. Each sample was divided into two portions as follows: the first portion: was immediately taken in heparinized tube for hematological and Immunological studies. The second portion: was allowed to coagulate at room temperature and centrifuged at 3000 r.p.m for 15 min at $4^{o}C$, the obtained supernatant (serum) were separated and kept at $-20^{o}C$ until required for assay of biochemical parameters.

2.4.1. Hematological analysis:-

Blood collected was analyzed for red blood cells (RBCs) and white blood cells (WBCs) as described by Dacie and Lewis (1984) while hemoglobin concentration (Hb) was measured according to VanKampen and Zijlstra (1961). Hematocrit value (HCT%) or packed cell volume (PCV) was determined by centrifuging blood in heparinized micro hematocrit tube (capillary tubes of 1mm internal diameter and 7.5 cm length) for 5 minutes at 15000 r.p.m (Dacie and Lewis 1991).The percentage of each type of the total leucocyte population in relation to the total count of counted WBCs was determined according to Schalm *et al.,* (1975**).**

2.4.2. Biochemical assays:-

Serum levels of total protein, albumin and blood urea nitrogen were determined according to the methods of Gornall *et al.,* (1949), Doumas *et al.,* (1971) and Fawcett and Soctt, (1960) respectively. Globulin was determined by difference between total protein and albumin. Liver function AST, ALT and ALP were measured calorimetrically according toVan and Soons 1992, Henry, 1964 and Belfield and Goldberg 1971) respectively.

2. 5. Histopathological studies:-

Small pieces of liver, kidney and heart of each animal of control and treated groups were fixed in 10% formal saline solution for twenty four hours. Washing was done using tap water then serial dilutions of absolute ethyl alcohol were used for dehydration. After routine processing, paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness by slidge microtome. The obtained tissue sections were collected on glass slides, deparaffinized and stained by hematoxylin and eosin stain for histopathological examination through the light microscope. (Banchroft *et al.,* 1996).

2.6. Statistical analysis:-

Statistical analysis was performed with SPSS computer program (SPSS version 16.00 software for Windows) data were analyzed using one way analysis of variance (ANOVA). Results are reported as mean values ± SE and difference were considered significant at p<0.05(Rabeh and Ibrahim, 2014).

3. Results and Discussion:-

3.1 Changes in body weight gain and Feed efficiency ratio (FER)

The results present in Table (1) showed that there were no differences in body weight at the beginning amongst groups. CCl_4 exposure caused a remarkably significant decrease (P<0.05) in final body weight, reaching 203.90± 4.1 compared with control group (250.40± 6.12). Meanwhile co-treatment with RBP improved the final body weight reaching 231.85± 3.83 compared with control group. Daily food intake was diminishing in rat exposed to CCL_4 about 27.2% whereas concomitant with red beet improved this value but not completely. Alsuhaibani, (2013) advice to consume red beet as nutritious functional to hepatic disease patients. These results may be due to nutritional values of RBP is a rich source of vitamins A, C, B complex and minerals such as Mg, Ca, Fe and K (Chawla *et al.*, 2016).

Table (1): Effect of RBP on body weight gain, daily food intake and FER in the experimental rat groups.

Parameters	Control	CCL_4	CCl_4+ RBP
Initial weight(g)	221.72±2.14	221.45± 2.63	220.67±3.02
Final weight(g)	250.40± 6.12	203.90 ± 4.1 [a]	231.85 ± 3.83 [ab]
Body weight gain (g)	28.68 ± 1.91	17.55 ± 2.99 [a]-	11.1± 1.08 [ab]
Daily food intake(g)	13.97± 0.37	10.17 ± 0.19 [a]	12.23± 0.69 [ab]
FER(%)	4.27±0.27	- 4.1 ± 0.68 [a]	2.17 ±0.14 [ab]

Data represent the mean ±S.E. (a) significantly different from control group at P < 0.05, (b) significantly different from CCl_4 group at P < 0.05.

Data in Table (2) appeared that liver function enzymes ALT, AST and ALP activities in rats intoxicated by CCl_4 were markedly significant elevated (P<0.05) about 7.7 and 2.6 folds for ALT and AST respectively, while ALP 7.8% compared with normal control group.

Table (2): Effect of RBP on liver function in the experimental rat groups.

Parameters	Control	CCL_4	CCl_4+ RBP
ALT(IU/L)	14.13±0.13	108.52 ±0.99 [a]	89.04 ±0.95 [ab]
AST(IU/L)	44.92±0.80	118.84 ±0.25 [a]	98.84 ±0.86 [ab]
ALP(IU/L)	114.85±0.79	123.67 ±3.18 [a]	118.59±0.71

Data represent the mean ±S.E. (a) significantly different from control group at P < 0.05, (b) significantly different from CCl_4 group at P < 0.05.

Meanwhile Concomitant with red beet improved this result but not completely. The result agree with (Alsuhaibani, 2013) reported that addition of red beet root to biscuits increase nutritional values and acceptability and improved liver function enzymes and antioxidant in injured liver rats.

From Table (3), CCl_4 exposure caused a remarkably significant (P≤0.05) decrease (P<0.05) in protein, albumin and globulin level compared to control group. Concomitant treatment with RBP had a significant (P≤0.05) improvement in the activities in this parameters compared with CCl_4 intoxicated group. This results agree with Sahreen *et al.*, (2015). In the same table the blood urea level were significantly increase in group exposed to CCl_4 while treated with RBP caused a significant decrease in this level and restored it to nearly normal. These results may be due to red beet rich in nutrients, minerals and amino acids (Chawla *et al.*, 2016).

Table (3): Effects of RBP on serum, protein, albumin globulin and urea levels in the experimental rat groups.

Parameters	Control	CCL_4	CCL_4+ RBP
Protein(g/dl)	10.67± 0.34	7.41 ± 0.13 ^{a}	9.01 ± 0.24 ^{ab}
Albumin(g/dl)	5.36 ± 0.22	3.91 ± 0.19 ^{a}	5.09 ± 0.17 ^{b}
Globulin(g/dl)	5.34 ± 0.19	3.60 ± 0.21 ^{a}	4.26 ± 0.23 ^{ab}
Urea(mg/dl)	47.44 ± 4.04	55.97± 3.27	43.61 ± 1.43 ^{b}

Data represent the mean ±S.E. (a) significantly different from control group at P < 0.05, (b) significantly different from CCl_4 group at P < 0.05.

In similar study Krajka *et al.*, (2012) explained that beet root reduced creatinin level which is the marker of kidney damage and the protective activity of beet root may be comparable to that of garlic extracts or silymarin, attributed to the maintaining integrity of the plasma membrane which suppresses the leakage of enzymes and proteins.

3.2 Hematological parameters

The results in table (4) show that the exposure to CCl_4 caused a significant decrease (P < 0.05) in the Hb concentration in rats compared to control group. While concomitant with RBP improved the Hb concentration but not completely, this result was similar to the obtained data by (Eshak *et al.*, 2015).

Table (4): Effect of RBP on some blood parameters in the experimental rat groups.

Parameters	Control	CCL_4	CCl_4+ RBP
Hb(g/dl)	14.76 ± 0.17	11.12 ± 0.31 ^{a}	12.89 ± 0.21 ^{ab}
PCV(ml/dl)	41.40 ± 0.75	43.86 ± 0.81	40.17 ± 0.90
RBC_S (10^6)	3.93 ± 0.13	5.36 ± 0.11 ^{a}	4.01 ± 0.07 ^{b}
WBC_S (10^3)	4.38 ± 0.05	5.56 ± 0.12 ^{a}	4.56 ± 0.04 ^{b}

Data represent the mean ±S.E. (a) significantly different from control group at P < 0.05, (b) significantly different from CCl_4 group at P < 0.05.

Treatment with CCl_4 showed significant increase (P≤0.05) in WBC_S and RBC_S values compared with control group. Meanwhile simultaneous treatment with RBP resulted in significant reduction of WBC_S and RBC_S

values comparable to intoxicated group. On other hand PCV was elevated in CCl_4 group but not significantly and RBP restored this elevation to nearly normal ,the results are in agreement with (Saba *et al.,* 2010).

From Table (5), CCl_4 exposure caused a remarkably decrease ($P<0.05$) in neutrophil percent compared to control group. Treatment with RBP offered significant protection against CCl_4 induced liver toxicity in the experimental rats. On the other hand lymphocyte and eosinophil percent were significantly high ($P<0.05$) in CCl_4 intoxicated group whereas concomitant with RBP restored eosinophil percent to normal while lymphocyte was decreased but still high than control.

Table (5):Effect of RBP on some blood parameters in the experimental rats group.

Parameters	Control	CCL_4	CCl_4+ RBP
Neutrophil (%)	47.66 ± 1.71	37.11 ±2.13 [a]	41.12 ± 0.25 [a]
Lymphocyte (%)	33.86 ±1.33	45.81 ±1.68 [a]	41.78 ± 1.08 [a]
Monocyte (%)	5.65 ±0.53	6.40 ± 0.39	5.08 ± 0.46
Basophil (%)	1.51 ±0.16	1.82 ± 0.12	1.56 ± 0. 17
Eosinophil (%)	5.48 ±0.26	8.67 ± 0.70 [a]	5.16 ± 0.29 [b]

Data represent the mean ±S.E. (a) significantly different from control group at P < 0.05, (b) significantly different from CCl_4 group at P < 0.05.

Additionally no significant differences were observed among all groups in monocyte and basophil percent. These results may be due to that red beet rich in betalains and phenolic compounds which have antioxidant, anti-inflammatory properties (Georgiev *et al.*, 2010 and Clifford *et al.*, 2015).

3.3. Histopathological Examination:

Histopathological changes results appeared significant correlation with the biochemical study results. The microscopic examination showed normal histological structure of hepatic lobule in the control group (Fig. 1). Meanwhile, exposure to CCl_4 revealed fatty change of hepatocytes multi lobular strands of fibroblasts proliferation and apoptosis of hepatocytes in intoxicated liver rats. These due to CCl_4 caused hepatocellular damage (Heeba and Mahmoud 2014) and massive damage to liver tissue in the form of excessive fibrosis, cellular infiltration and vacuolar degeneration of hepatocytes (Eshak *et al.*, 2015). Nevertheless, these finding were ameliorated by feeding rats with 10% red beet dried in diet.

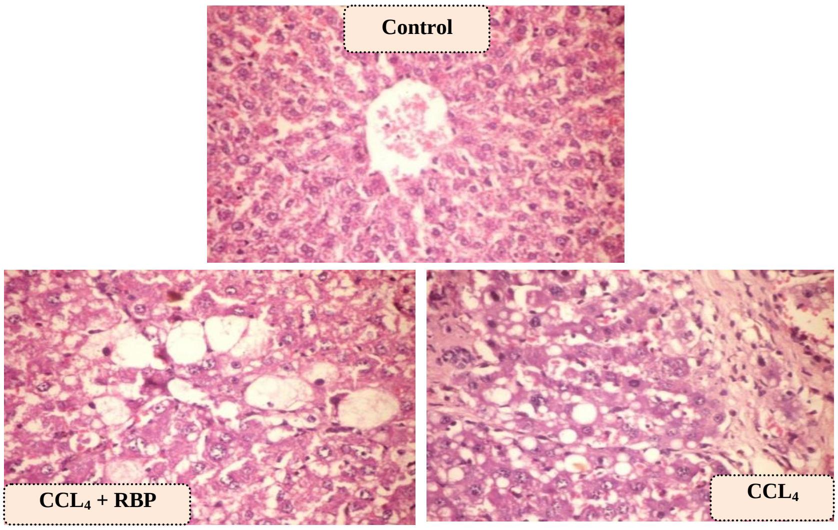

Fig .1. Histological examinations of liver tissues of rats in different groups

Microscopically, sections taken from kidneys normal control rat showed normal histological structure of renal parenchyma. In contrast the CCl_4 leading to vacuolation of epithelial lining glomerular tuft and epithelial lines renal tubules (Fig 2).

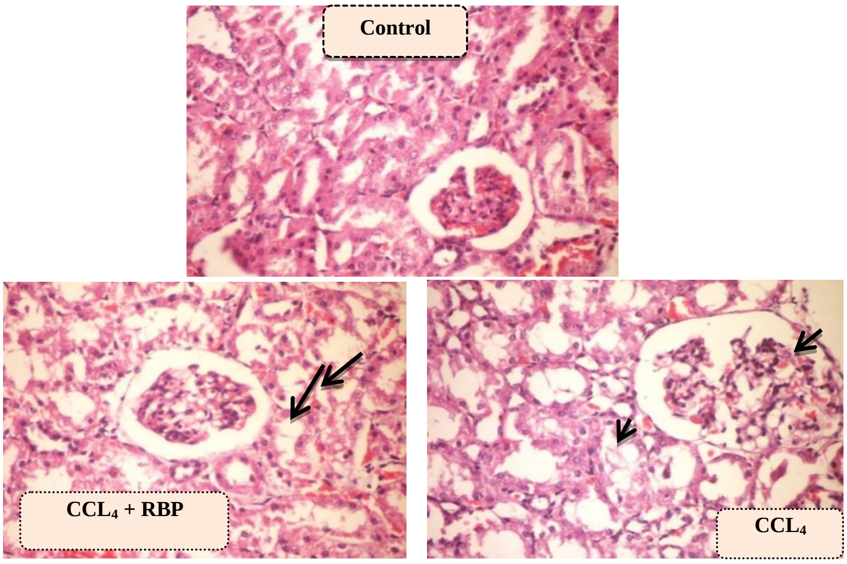

Fig. 2 Histological examinations of liver tissues of rats in different groups

Similar histopathological chenges were observed by Sahreen *et al.*, (2015) in renal tissue of rats in response to chronic administration of CCl_4 for 8 weeks. However, Simultaneous treatment with red beet ameliorated these change. Red beet as nutritional approach preserves or restores endothelial function (Clifford *et al.*, 2015).

Histopathological studied showed normal histological structure of cardiac myocytes in the control group. In contrast the CCl_4 showed perivascular oedema (Figure 3). CCl_4 causes oxidative damage in organs such as heart and kidney and liver (Çetin and Çetin 2011). However, concomitant intoxicated rat feeding with RBP 10% return heart histopathological changes to normal structure.

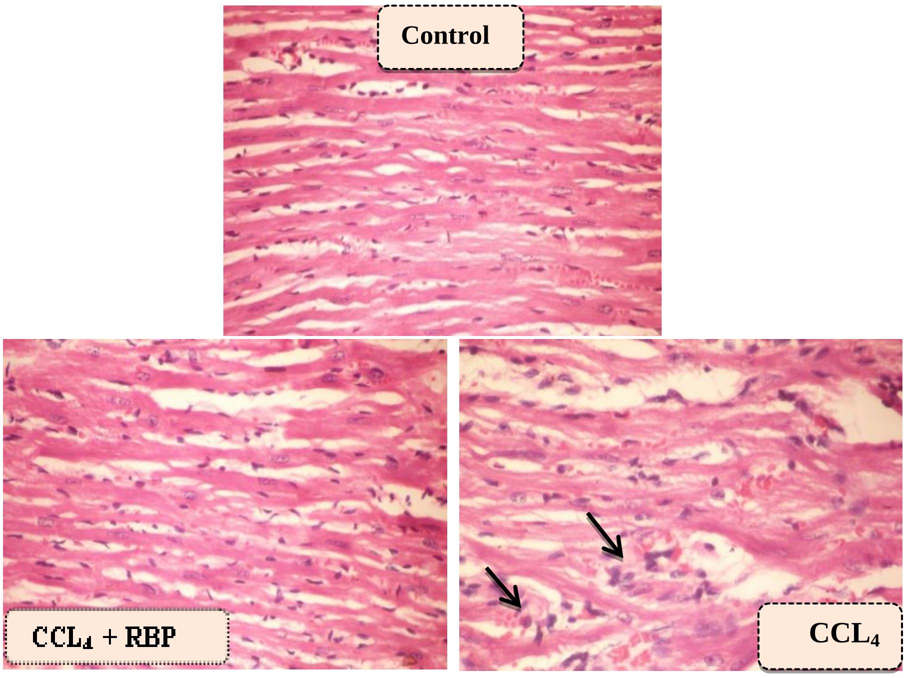

Fig. 3 Histological examinations of liver tissues of rats in different groups

In conclusion, the results of this study suggest that feeding with RBP improved hematological parameters and have protective effects for the prevention of CCl_4-induced damage organs in rats.

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التأثير الوقائي لمسحوق البنجر الأحمر ضد التسمم الكبدي المستحدث برابع كلوريد الكربون في فئران الألبينو

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الملخص العربى :

يعتبر البنجر الأحمر غذاء وظيفي ومصدر غني بالعديد من المركبات النشطة حيويا التي تمتلك خصائص مضادات الأكسدة والالتهابات وبذلك قد تكون لها فوائد صحية في تقليل خطر الإصابة بالأمراض، لذلك في هذا العمل تم دراسة فاعلية البنجر الأحمر في الحماية من تسمم الكبد الناجم عن رابع كلوريد الكربون في الفئران. تم تقسيم الفئران عشوائيا إلى ثلاث مجموعات كل مجموعة بها ثماني فئران, المجموعة الأولى هي الضابطة العادية، تم معاملة المجموعة الثانية بواسطة CCl_4 (١مل / كجم) مخلوط مع زيت البارا فين مرتين أسبوعيا, المجموعة الثالثة تم معاملتها بواسطة CCl_4 وإضافة ١٠٪ من مسحوق البنجر الأحمر إلى الوجبة الغذائية. أظهرت النتائج أن المعاملة بمسحوق البنجر الأحمر بنسبة ١٠٪ أدى إلى تخفيض المؤشرات الحيوية في الدم لوظائف الكبد (AST, ALT, ALP) ووظائف الكلى (اليوريا) و التي ارتفعت من خلال التسمم المزمن بـــ CCl_4. علاوة على ذلك، المعاملة بمسحوق البنجر الأحمر أظهرت زيادة معنوية($P \leq 0.05$) في وزن الجسم النهائي، تناول الطعام اليومي التي كانت انخفضت بواسطة CCl_4. بالإضافة إلى ذلك، منع مسحوق البنجر الأحمر ارتفاع قيم (WBC_S, RBCS , PCV) التي كانت مرتفعة في الفئران المصابة. كما أن نتائج الفحص النسيجي قد دعمت النتائج البيو كيميائية لاختبار إصابة الأعضاء, لذلك خلصت الدراسة إلى أن مسحوق البنجر الأحمر كمانع جزئي ومخفض يمكن أن يكون عامل وقائي أو علاجي بسبب مكوناته المضادة للأكسدة.

الكلمات المفتاحية: Beta vulgaris - سمية الكبد - رابع كلوريد الكربون– الفحص النسيجي- القياسات البيو كيميائية.