Potential therapeutic effects of pomegranate peel on carbon tetrachloride induced liver injury in rat

Heba I. Ghamry
Faculty of Home Economics,
King Khalid University, Saudi Arabia

Abstract:

Pomegranate (*Punica granatum* L.) peel, a by-product of juice processing industries was reported to contain a series of bioactive compounds. This study was undertaken to investigate the putative antioxidant activity of the dried pomegranate peel and their extract clinically in carbon tetrachloride (CCl_4) induced liver damage in male rats was used. Intraperitoneal administration of CCl_4 (2 ml/kg) to rats for 4 days resulted in significantly elevated ($p \leq 0.05$) serum levels of liver enzymes compared to controls. In consequence, significant elevation ($p \leq 0.05$) of malondialdehyde (MDA) and a lowered level of reduced glutathione (GSH) were observed. When rats with CCl_4-induced hepatotoxicity were treated with the dried grape leaves and their water extract, the serum GOT, GPT and ALP levels reverted to near normal. Concurrently, the hepatic concentration of GSH was significantly increased ($p \leq 0.05$) and that of MDA significantly ($p \leq 0.05$) lowered when compared to CCl_4 exposed untreated rats. Histopathological studies confirmed the hepatoprotective effect profounded by the extracts. These results suggest that pomegranate peel extract is able to significantly alleviate the hepatotoxicity induced by CCl_4 and can protect liver against some pathological diseases.

Keywords: pomegranate peel; liver fibrosis; hepatoprotective; antifibrotic effect; histopathology.

Introduction

In the recent years, food scientists and nutrition specialists agree that fruits and vegetables, consumed daily, contribute to reducing risks of certain diseases, including cancer and cardio and cerebrovascular diseases **(Martin *et al.*, 2002).** These beneficial effects have been attributed to the various antioxidants in fruits and vegetables **(Peschel *et al.*, 2006),** including polyphenol, ascorbic acid, carotenoids, and tocopherols.

Consumption of fruits and vegetables is beneficial in both forestalling and reversing the deleterious effects of oxidative stress damage, e.g. cancer, neurodegenerative disorders, aging, neuronal communication and behavior (**Ames *et al.*, 1993 and Lau *et al.*, 2006).** These effects are attributed mainly to the antioxidant properties of the phenolic compounds found in fruits and vegetables (**Lau et al., 2006**).

Pomegranate (*Punica granatum* L.) is an important fruit of tropical and subtropical regions, which originated in the Middle East and India and it is one of the oldest known fruit. It is mentioned in the Ebers papyrus of Egypt written in about 1550 BC (**Faria *et al.*, 2006**) and has been used in various regions and folk or traditional medical systems as a food supplement or a medicine because of its enormous compounds with lots of activities and without toxicity. Pomegranate fruit consists of several anatomical parts including seed, juice, peel, leaf, flower, bark, and roots (**Orak *et al.*, 2012**). Pomegranate's by-products such as seeds and peel consisted 12% and 50% of the whole fruit (**Fawole *et al.*, 2012 and Tehranifar *et al.*, 2010**). Pomegranate peel are characterized by substantial amounts of phenolic compounds, including flavonoids (anthocyanins, catechins and other complex flavonoids) and hydrolyzable tannins (punicalin, pedunculagin, punicalagin, gallic and ellagic acid). These compounds are concentrated in pomegranate peel and juice, which account for 92% of the antioxidant activity associated with the fruit (**Zahin *et al.*, 2010**). The therapeutic potential of has been widely recognized by different cultures. **Wang *et al.*, (2011)** reported that these components can be extracted from peel by

using water which has the economic and safety merits as an environmental friendly method for food and pharmaceutical industry because it is nontoxic and gives an acceptable yield of those components. In Egyptian culture, several common ailments such as inflammation, diarrhea, intestinal worms, cough and infertility have been treated by exploiting pomegranate peel extract (**Lansky and Newman, 2007**).

Experimental observations indicated that pomegranate peel extract reversed thioacetamide-induced liver fibrosis, and significantly decreased the activity of liver enzymes, bilirubin and serum hepatocyte growth factor levels. These effects could be attributed to its antioxidant properties, antifibrotic and antiapoptotic activity **Salwe, *et al*. 2015**. Pomegranate peel extract prevents liver fibrosis in biliary-obstructed rats, and forecasted pomegranate peel extract antioxidant and antifibrotic properties, may be of potential therapeutic value in protecting the liver from fibrosis and oxidative injury due to biliary obstruction (**Sadeghipour *et al.*, 2014**). Carbon tetrachloride (CCl_4), a potent hepatotoxic agent, is biotransformed to a trichloromethyl radical by the cytochrome system in liver microsomes causing lipid peroxidation of membranes that leads to liver injury **(Recknagel, 1983 and McCay *et al.*, 1984).**

In this study, we aimed to evaluate the total putative antioxidant action of dried pomegranate peel and its water extract as dietary therapy. The liver inflammation and hepatofibrosis-improvement based on AST, ALT levels as well as the SOD, GSH, and MDA (antioxidant indicators) have been measured. Finally, the histopathological examination of rat's liver will be in the scope of this investigation..

Materials and Methods

Preparation of the Pomegranate peel and water extract

The whole plant of Pomegranate fruit was collected from the local market. Pomegranate peel were cut into small pieces, cleaned, washed by distilled water and dried in the hot air oven at 40 °C for 48 h. The dried peels were grinded to fine powder form. The dried pomegranate peel (200 g) was mixed with water (2000

mL) for 30 min, and subjected to continuous hot extraction (100 ^{0}C, 40 min). The resulting water extract was filtered and subsequently concentrated with a water bath (90 ^{0}C) until it became creamy, and was then dried in an oven (70^{0}C).

Animal experiment

Male albino rats (150g ±5), Sprague Dawely strain was obtained from Research Institute of Ophthalmology, Medical Analysis Department, Giza, Egypt. The animals were acclimated for 5 days prior to dosing, during which time they had free access to food and water ad *libitum*. Twenty-four acclimated rats were randomly divided into four groups of six each: Group I (normal) received only vehicle (olive oil; 1 ml/ kg b.w) for 4 days. Secondary group positive controls or toxin group received vehicle on the first and fourth days CCl_4 (20% solution of CCl_4 in olive oil, 2 ml/kg b.w) on the second and third days. Third group (test rats) received dried pomegranate peel 12% for diet with CCl_4 on the second and third days. Fourth group (test rats) received pomegranate peel extract (200 mg/kg b.w.) on the second and third days and pomegranate peel extract four times a week. All administrations were made intraperitoneally. At the end of the treatment blood samples were collected from the inferior vena cava, and the liver was removed. The serum was separated from the blood.

Proximate Analysis

A.O.A.C. (2005) methods were used to determine moisture, protein, fat, dietary fiber and ash contents, while carbohydrate was calculated by difference.

Determination of total phenolics

The content of total phenolic was estimated using the Folin–Ciocalteu's reagent (**Obanda and Owuor, 1997 and Singletion and Rossi, 1965)**. A calibration curve of gallic acid (range from 5 mg/ml to 30 mg/ml) was prepared and the results, determined gallic acid standard calibration curve, were expressed as milligram of gallic acid equivalents per gram of the extract.

Antiradical activity against DPPH

The antioxidant activity of the plant extract and standard was assessed based on the radical scavenging effect of the stable DPPH (1, 1-diphenyl-2 picrylhydrazyl) radical (**Cuendet *et al.*, 1997 and Burits & Bucar, 2000).** The pomegranate peel extract range from 50, 100, 150, and 200 µg/ ml in 80% of methanol was added 1 ml of DPPH radical in methanol solution (0.25 mM). After a 20 min incubation period at room temperature in dark, the absorbance was read at 517 nm. The extract concentration providing 50% inhibition (IC_{50}) was calculated from the plot of inhibition (%) against extract concentration **(Yen and Duh, 1994),** tests were carried out in triplicate.

Analytical Methods

The levels of serum cholesterol, total lipids and triglyceride were determined according to the methods outlined **by Roechlau *et al.*, (1974), Assmann (1979) and Frings and Dunn (1979).** Alanine amino transferase (ALT) activities, Aspartate amino transferase (AST) activities and serum alkaline phosphatase (ALP) activity (IU/L) were measured according to the methods described by **Bergmeyer and Harder (1986), Kachmar and Moss (1976)** and **Varley *et al.*, (1980),** respectively.

Determination of biochemical parameters in liver

Preparation of liver supernatants

Prior to biochemical analysis, each liver sample (100 mg/ml buffer) was homogenized in 50 mM phosphate buffer (pH 7.0); the homogenate was then centrifuged at 4,000 rpm for 15 min and the supernatant obtained was used for biochemical analysis. All liver parameters were expressed as activity per mg protein. The protein concentration in each fraction was determined by the method of **Lowry *et al.*, (1951)** using crystalline bovine serum albumin as a standard.

Determination of lipid peroxidation

The mean malondialdehyde (MDA) content (µmol/mg protein), a measure of lipid peroxidation, was assayed in the form of thiobarbituric acid-reacting substances (TABRS) by the method of **Ohkawa *et al.*, (1979).**

Determination of reduced glutathione (GSH)

The mean reduced glutathione concentration (µmol glutathione/mg protein) in the liver homogenate was determined by the method of **Moron *et al.*, (1979).**

Histopathological methods

At the end of the biological experiments, the rats were scarified, and the liver was embedded in paraffin wax. The organs were then sectiond at the thickness of 5-6 µ and stained in hoematoxylin and eosin mixture according to **Drury and Wallington (1980).**

Statistical analysis

The results obtained for each group of rats tested was expressed as the mean ± SD of six values. Statistical analysis of the data was performed by one-way ANOVA.

Results and Discussion

The proximate chemical composition of fresh and dried pomegranate peel

The proximate components of fresh and dried pomegranate peel are presented in Table 1. The fresh pomegranate peel contained 2.23±0.12, 1.16±0.27 and 1.08±0.09 g/100g of protein, fat and ash. Meanwhile, the dried pomegranate peel contained 3.76±0.49, 1.78 ± 0.22 and 3.85 ± 0.31 g/100g of protein, fat and ash, respectively. The dried pomegranate peel amounted 23.59±1.19 and 61.37±3.42 crud fiber and carbohydrates, respectively that can serve as good source of dietary fiber and carbohydrate at the same respect. These quantities are much higher than those found in the peels of lemons, oranges and grapefruit; 14, 13.9 and 13.9%, respectively (**Gorinstein *et al.*, 2001).**

Table (1). Proximate chemical composition of fresh and dried pomegranate peel

Component	Fresh pomegranate peel	Dried pomegranate peel
Moisture	70.59 ± 2.07	5.65±2.43
Protein	2.23±0.12	3.76 ± 0.49
Fat	0.66±0.27	1.78 ± 0.22
Crud fiber	9.19±0.86	23.59 ± 1.19
Ash	1.08±0.09	3.85 ± 0.31
Carbohydrates*	16.25±2.45	61.37 ± 3.42

* The carbohydrate content was calculated by difference.

Total phenol and antioxidant of pomegranate peel and its water extract

The antioxidant activity for both pomegranate peel and their extract is mainly phenolic compounds are reported in Table 2. The results showed that pomegranate peel contained 31.26±1.72 mg/100g phenolic compounds, its extracts contained 54.69±2.34 mg/100g. The antioxidative capacities of pomegranate peel and their extract were also determined by the in vitro assays, DPPH that uses for evaluation of antioxidant activity of compounds **(Maksimovic *et al.*, 2005).** The 50% inhibition (IC50) of DPPH radicals for pomegranate peel and pomegranate peel water extract was shown at level of 31.4±2.57 and 23.9±1.68 mg/ml, respectively.

Polyphenolic compounds are widely distributed in plants and known to be excellent antioxidants *in vitro*. They have the capacity to reduce free-radical formation by scavenging free radicals and protecting antioxidant defenses. In our study, the phenolic level estimation of pomegranate peel and their water extracts reveals that contains considerable amount of polyphenolic compounds. For evaluation of antioxidant capacity of various extracts of plant, DPPH assay was performed. The highest scavenging activity was found for the water extract of pomegranate peel with an IC50 value of 23.9 µg/ml. It can be concluded that the content of polyphenolic compounds of water

extract of Pomegranate could be responsible for the radical scavenging activity in liver toxicity. Pomegranate fruit and its peel exhibit a high antioxidant potential. They have gained a wide acceptance for their pharmacological activities against serious maladies such as prostrate, colon and liver cancers, stomach ulcers, cardiovascular diseases and digestive disorders (**Ismail *et al*., 2012**).

Table (2): Total phenols and antioxidant activity determined by scavenging of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) of pomegranate peel and their water extract

Material	Total phenol (mg/g)	DPPH (EC_{50} values; mg/ml)
pomegranate peel	31.26 ± 1.72^{b}	31.4 ± 2.57^{a}
pomegranate peel water extract	54.69 ± 2.34^{a}	23.9 ± 1.68^{b}

* Means $\pm$ standard deviation of means of three determinations. Means in the same column with different letters are significantly difference ($P \leq 0.05$).

Effect of pomegranate peel and water extract on total cholesterol, total lipids triglyceride and Liver index on liver rats

The effects of dried pomegranate peel and water extract on Total cholesterol, Total lipids Triglyceride and Liver index are presents in **Table (3)**. The results have showed that the rats receiving CCl_4 caused a significant elevation of liver index, while after treatment with pomegranate peel and there extract these indexes were markedly reduced. Moreover, liver lipids (TC, TG and TL) were found to be significantly higher ($P\leq0.05$) in rats receiving CCl_4 than that normal control group. The simultaneous ingestion of CCl_4 and dried pomegranate peel in diets and oral pomegranate peel extract significantly decreased ($P\leq0.05$) the liver lipids levels with no significant differences between group 3 and 4.

Administration of CCl_4 to rats produced hepatotoxicity showed by significant increase in the serum levels of serum triglycerides and cholesterol in comparison to control group **(Gopal and Sengottuvelu 2008).** The result came compatible with **Esmaillzadeh *et al.*, (2006)** who reported that consumption of concentrated pomegranate juice for type ll diabetic patient with hyperlipidemia caused significant reduction were seen in serum total cholesterol and low density lipoprotein LDL and had slight changes in serum HDL. On the other hand, dietary supplementation with nutrients rich in polyphenols and antioxidants is associated with inhibition of at herogenic modifications to LDL and atherosclerosis (**Aviram *et al.*, 2000).**

Table (3): Effect of pomegranate peel and water extract on Total cholesterol, Total lipids Triglyceride and Liver index on liver rats

Groups	Total cholesterol (mg/dl)	Total lipids (mg/dl)	Triglyceride (mg/dl)	Liver index
Normal	$85.64^{c}\pm1.06$	$341.83^{c}\pm4.28$	$69.45^{c}\pm2.90$	$2.25^{d}\pm0.17$
Positive control (CCL_4)	$130.52^{a}\pm2.02$	$429.75^{a}\pm6.12$	$127.67^{a}\pm3.29$	$3.39^{a}\pm0.19$
pomegranate peel +CCL_4	$105.35^{b}\pm1.55$	$356.44^{b}\pm5.39$	$85.19^{b}\pm3.17$	$2.67^{c}\pm0.16$
pomegranate peel extract+ CCL_4	$102.98^{b}\pm1.89$	$364.67^{b}\pm5.72$	$86.57^{b}\pm2.98$	$2.59^{c}\pm0.08$

* Means $\pm$ standard deviation of means of three determinations. Means in the same column with different letters are significantly difference ($P \leq 0.05$).

Effect of pomegranate peel and their water extract on liver enzyme in serum of rats

Several hepatic enzymes in serum were used for the biochemical markers to understand the early hepatic injury, such as GOT, GPT and ALP. **Table (3)** shows the effect of pomegranate peel and their water extract on the activities of serum GOT, GPT and ALP in rats for 28 days. Treatment with CCl_4 significantly ($p \leq 0.05$) elevated the levels of serum GOT, GPT and ALP compared with the control group (Table 3). Dried grape leaves and there water extract administration at during CCl4

treatment significantly ($p \leq 0.05$) lowered the serum GPT and ALP activities as compared with CCl_4 treatment group. Specially, water extract at the dose of 200 mg/kg b.w. with CCl_4 treatment showed a significant ($p \leq 0.05$) reduction in the serum GOT activities while compared with CCl4 treatment group and dried grape leaves. These results are in agreement with **Toklu *et al.* (2007)**, who studied the effect of chronic administration of PPE on liver fibrosis induced by bile duct legation in rats and found that serum ALT and AST were significantly decreased by PPE treatment.

Liver damages were assayed by biochemical studies. Four separate liver enzymes including aspartate aminotransferase (AST) and alanine aminotransferase (ALT), which are known together as transaminases and alkaline phosphatase (ALP), which are known together as cholestatic liver enzymes are the reliable indices of liver function. The increased levels of serum enzyme such as AST and ALT indicate the increased permeability and damage and/or necrosis of hepatocytes **(Goldberg and Watts, 1965)**. The membrane bound enzymes like ALP are released unequally in to bloodstream depending on the pathological phenomenon **(Sillanaukee, 1996).** Based on our results, the chronic CCL4 consumption caused a significant increased in the activities of AST, ALT and ALP, which could be to severe damage to tissue membrane. The result indicated that the pomegranate peel and their water extract had protective effects against liver injuries in rats injected with CCl4. Our data agree well with by **Abdel-Rahman and Abd El-Megeid (2006), Ibrahium (2010) and Hamad *et al.*, (2011).**

Table (4): Effect of pomegranate peel and their water extract on liver enzyme in serum of rats

Groups	GOT (IU/L)	GPT (IU/L)	ALP (IU/L)
Normal rats	$42.42^{d}\pm 2.84$	$29.48^{c}\pm 2.35$	$98.86^{c}\pm 5.08$
Positive control (CCL_4)	$96.35^{a}\pm 3.95$	$74.29^{a}\pm 4.46$	$145.03^{a}\pm 6.13$
pomegranate peel +CCL_4	$73.96^{b}\pm 3.27$	$41.26^{b}\pm 4.05$	$106.14^{b}\pm 4.75$
pomegranate peel extract +CCL_4	$58.47^{c}\pm 3.15$	$39.87^{b}\pm 3.19$	$101.89^{bc}\pm 4.82$

* Means $\pm$ standard deviation of means of three determinations. Means in the same column with different letters are significantly difference ($P \leq 0.05$).

Malondialdehyde (MDA) and reduced glutathione (GSH) concentration in liver

A marked increase in the mean MDA level was found in the liver of positive group (CCl_4 exposed) rats relative to normal Group rats (Table 5); this increase was statistically significant ($p \leq 0.05$). Treatment with pomegranate peel and their water extract in Group 3 and 4 rats was found to result in a significant ($p \leq 0.05$) lowering of the mean MDA concentration, presumably by limiting lipid peroxidation in the hepatic tissue. CCl_4 administration in Group 2 rats resulted in a marked decrease (relative to normal) in the level of reduced glutathione in liver (Table 5); this decrease was statistically significant ($p \leq 0.05$). Treatment with pomegranate peel and their water extract in Group 3 and 4 rats resulted in a significantly higher concentration of GSH ($p \leq 0.05$) than that in positive control Group. This result is in agreement with that of **Abdou *et al.*, (2012)**, who revealed that pomegranate possesses protective effects against CCl_4 genotoxicity and hepatotoxicity in animal models.

GSH is a major, non-protein thiol in living organisms which performs a key role in coordinating innate antioxidant defense mechanisms **(Gueeri, 1995).** Reduced glutathione (GSH) plays a key role in the detoxification of the reactive toxic metabolites of CCl^4; liver necrosis is initiated when reserves of GSH are markedly depleted **(Recknagel *et al.*, 1991).** Thus, the reduced levels of GSH (relative to normals) observed in the present investigation in positive Group rats (administered CCl_4) are

consistent with the results of other workers. MDA, a secondary product of lipid peroxidation, is used as an indicator of tissue damage involving a series of chain reactions (**Ohkawa *et al.*, 1979**). Lipid peroxidation has been implicated in the pathogenesis of increased membrane rigidity, osmotic fragility, reduced erythrocyte survival and perturbations in lipid fluidity. It has been hypothesized that one of the principal causes of CCl_4 induced hepatotoxicity is lipid peroxidation of hepatocyte membranes by free radical derivatives of CCl_4 **(Recknagel *et al.*, 1989 and 1991).**

Table (5): Effect of dried grape leaves and their water extract on malondialdehyde and reduced glutathione in liver of rats

Groups	MDA (µmol/ gm protein)	GSH (µg/gm protein)
Normal rats	$0.687^{c}\pm 0.058$	$7.382^{a}\pm 0.521$
Positive control (CCL_4)	$1.439^{a}\pm 0.112$	$5.265^{c}\pm 0.423$
pomegranate peel +CCL_4	$1.046^{b}\pm 0.098$	$6.143^{b}\pm 0.469$
pomegranate peel extract +CCL_4	$0.853^{bc}\pm 0.079$	$7.055^{a}\pm 0.517$

* Means $\pm$ standard deviation of means of three determinations. Means in the same column with different letters are significantly difference ($P \leq 0.05$).

Histopathological examinations

When compared to the histoarchitecture of the liver of Group I (normal) animals (Fig. 1a), liver cells of Group II rats (exposed to CCl_4) revealed extensive damage, characterized by the disruption of the lattice nature of the hepatocyte, damaged cell membranes, degenerated nuclei, disintegrated central vein and damaged hepatic sinusoids (Fig. 1b). In Group III rats (exposed to CCl_4 and pomegranate peel and their extract), only minimal disruption of the hepatic cellular structure was observed (Fig. 1c and 1_D). This observation was supplemented by histopathological examination in liver. Our data suggest that pomegranate peel its protective effect by decreased the lipid peroxidation and improving antioxidants status, thus proving itself as an effective antioxidant in alcohol induced oxidative damage in rats. This

result agreement with that of **Wei et al., 2015** treatment with extracts of pomegranate peels (EPP) and seeds (EPS) obviously alleviated the collagen deposition. showed that treatment with EPP and EPS significantly lower the graded of fibrosis as compared to CCl_4 group.

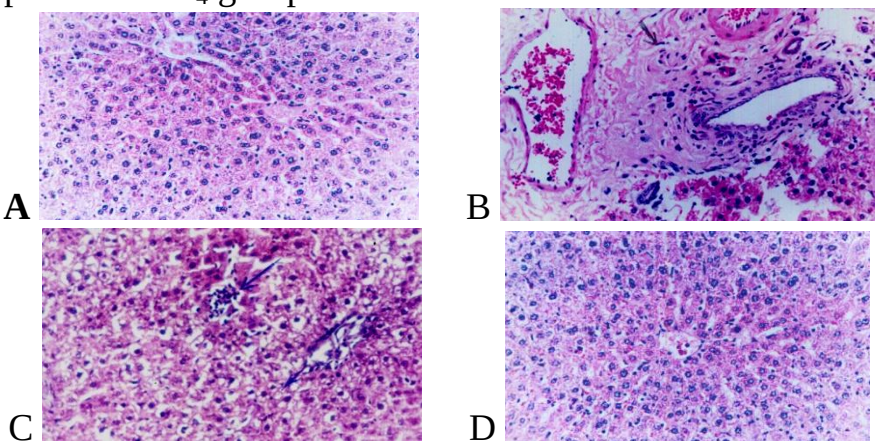

Fig (1): Liver of normal rats (A), Positive control (CCL_4) rat (B), pomegranate peel with CCL_4 (C) and pomegranate peel water extract with CCL_4 (D). (H and E × 200).

Histopathological studies were performed to provide direct evidence of the hepatotoxicity of CCl_4, and of the hepatoprotective effect of the extract of pomegranate peel and their extract. Marked disruption of the structure of hepatocytes was noted in liver tissue of positive Group rats (exposed to CCl_4 alone). Only minimal disruption of the structure of hepatocytes was noted in liver tissue of experiential Group rats (exposed to CCl_4 and grape leave dried and water extract); this minimal disruption of the hepatocyte structure complemented the results of the liver enzyme studies (GOT, GPT and ALP activities and MDA levels approximated to the levels in normal rats).

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التأثيرات العلاجية المحتملة لقشر الرمان على رباعي كلوريد كربون الكبد المستحث في الفئران

د. هبة ابراهيم غمري
قسم الاقتصاد المنزلى - كلية الاقتصاد المنزلى
جامعة الملك خالد ، المملكة العربية السعودية

تعتبر قشور الرمان منتج ثانوي من صناعات معالجة العصير والتى تحتوي على عدد كبير من المركبات النشطة حيويا. وقد أجريت هذه الدراسة للبحث في النشاط المضاد للأكسدة المتوقع لقشر الرمان المجفف وتأثيراته العلاجية على أضرار الكبد المستحثة برابع كلوريد الكربون (CCl_4) في ذكور الفئران. وقد أدت المعالجة بـ CCl_4 (٢مل / كغ) للفئران لمدة ٤ أيام الى مستويات الدم المرتفعة ($p\leq0.05$) من أنزيمات الكبد مقارنة مع الكنترول. ونتيجة لذلك ، لوحظ ارتفاع كبير ($p\leq0.05$) بمركب المالونالدهيد MDA (مؤشر أكسدة الدهون) ومستوى منخفض من الجلوتاثيون (GSH). وعندما عولجت الفئران المصابة بالسمية الكبدية الناتجة عن CCl_4 بقشور الرمان المجففة ومستخلصاتها المائية ، عادت مستويات إنزيمات الكبد (GOT وGPT و ALP) إلى ما يقرب من المعدل الطبيعي. في الوقت نفسه وازدادت GSH بشكل ملحوظ تركيز الكبد $p\leq0.05$ وانخفضت مستويات MDA بشكل كبير ($p\leq0.05$) بالمقارنة مع بالمجموعة المعاملة بـ CCl_4. كما أكدت الدراسات التشريحية النسيجية أن كبد الأمعاء تأثرت إيجابيا عند المعاملة بالمستخلصات. وتشير هذه النتائج إلى أن مستخلص قشور الرمان قادر على التخفيف بشكل كبير من التسمم الكبدي الناجم عن CCl_4 ، كما أن له تأثير وقائى كبير على الكبد من الإصابة ببعض الأضرار المرضية.

الكلمات المفتاحية: قشر الرمان - تليف الكبد – الكبد - تأثير المضاد الحيوي - التشريح المرضي.