Role of Psyllium Husk (*Plantago ovata*) on Liver Function Alterations Induced by Carbon Tetrachloride (CCl₄) in Adult Male Albino Rats

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INTRODUCTION

Carbon tetrachloride was once used widely as a cleaner, solvent, and degreaser both for home and industrial use. The hepatotoxicity and carcinogenicity of this drug were discovered in humans who were exposed to it. So, it is eventually used as an experimental model (weber *et al.*, 2003). In the liver, the mechanism of CCl₄-induced liver damage is due to the metabolism of CCl₄ into the trichloromethyl free radical, CCl₃* through the cytochrome P450 oxygenase system of the liver endoplasmic reticulum.
Various biologically important substances such as amino acids, nucleotides, and fatty acids, as well as proteins, nucleic acids and lipids, react with CCl$_3^*$ radical initiating the process of lipid peroxidation leading to a complex series of reactions that end with the complete disintegration of the polyunsaturated fatty acids molecule with the formation of aldehydes, other carbonyls, and alkanes (Brattin et al., 1995; Weber et al., 2003; Al Amin and Menezes, 2021).

Liver disease is considered the major reason of death every year. Approximately 29 million people suffer from a chronic liver condition and more than 30 million Americans have liver diseases. Liver diseases are the fifth biggest killer in England. Liver disease is the tenth most common death cause in India, as per the World Health Organization. Liver diseases do not usually cause any clear signs or symptoms until it’s fairly progressed, and the liver is damaged (Sivakrishnan and Pharm, 2019). The increase in the number of patients who suffer from liver dysfunction due to massive usage of drugs leads to an increase in the use of herbal medicines because of their perceived effectiveness in the treatment and prevention of disease and it is believed that these treatments are safe because they are ‘natural’ (Rajaratnam et al., 2014).

A medicinal plant is any plant that, in one or more of its parts, contains substances that can be used for therapeutic purposes, because of the wide biological and medicinal activities of medicinal medicines, higher safety margins and low costs, they have a great demand in the developed as well as in the developing countries for primary health care (Yudharaj et al., 2016). In liver diseases, inflammation is considered the main cause of the development of the disease chronicity in which oxidants affect all inflammation stages where oxidative stress plays a crucial role in its development. Therefore, keeping a balanced state of oxidants and antioxidants to prevent oxidative stress is a crucial part of reasonable health keeping (Widodo et al., 2019).

Plantago ovata is an important medicinal plant. in Pak-Indo subcontinent, the seed and husk of Plantago ovata are used in a popular household folk medicine, the alcoholic extract of the husk has an antioxidant activity more than the seed of the plant (Jabbar et al., 2020); therefore, Plantago ovata husk can be used for liver diseases. Plantago ovata is rich in bioactive compounds and different primary and secondary metabolites such as polyphenols, fatty acids, amino acids, flavonoids (polyphenols and flavonoids have a prominent antioxidant activity), alkaloids, terpenoids, and vitamin C. These compounds have good antioxidant and anti-inflammatory effects (Rafiee et al., 2022). Species of Plantago are characterized by the presence of phenolic compounds that play important roles in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. Phenolics, flavonoids and condensed tannins have shown ideal structural chemistry for free radical scavenging activity (Khedher et al., 2022).

The present investigation evaluates the ameliorative effects of crude powder of Plantago ovata husk on CCl$_4$-induced liver injuries.

MATERIALS AND METHODS

Materials:

1. **Plantago Ovata** Forssk husk (gluten-free, pesticide-free, GMO-free and raw) utilized in this experiment were bought from a hypermarket in Cairo, Egypt.
2. **Carbon Tetrachloride** (CCl$_4$) of 99 % concentration was obtained from PIOCHEM company.
3. **Olive Oil** was obtained from a local market in Qena.
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4. ALT, AST and ALP determination kits were purchased from bio-diagnostic co. Giza, Egypt.

5. Total Protein, Albumin, Bilirubin (BIL) and Nitric Oxide determination kits were purchased from bio-diagnostic co. Giza, Egypt.

Methods:

Preparation of Plantago Ovata Husk Dose:

PSH was ground to make powder. Each rat received orally 0.5g/kg b. wt. (Ahmed *et al.*, 2010) in 2ml of water, rats were weighed weekly.

Preparation of CCl₄ and Olive Oil Solution:

2 mg of CCl₄ were dissolved in 2 mL of olive oil. So each rat was given (0.5mg/ kg b. wt.) of the prepared solution intraperitoneally twice a week (EL Sayed *et al.*, 2019).

Animal Experimental Design:

Thirty white male Albino rats (*Rattus norvegicus*) from the order Rodentia and family Muridae were used in the present study, at a weight of about (144-176 g). Rats were divided into five groups (6 rats/group):

- Group 1 (Control group): included rats that received nothing.
- Group 2 (Oil-treated group): They were given an equivalent volume of olive oil by intraperitoneal injection at a dose of (0.5mg/ kg b. wt.) in the third week for four consecutive weeks.
- Group 3 (CCl₄-treated group): They were given by intraperitoneal injection CCl₄ at a dose of (0.5mg/ kg b. wt.) in the third week for four consecutive weeks.
- Group 4 (PS+ CCl₄-treated group): was given a daily oral dose of PSH (0.5g/ kg b. wt.) in addition to the PSH dose for another four consecutive weeks.
- Group 5 (CCl₄+PS-treated group): was given an intraperitoneal injection of CCl₄ (0.5mg/ kg b. wt.) in the third week for four consecutive weeks, then they were given a daily oral dose of PSH (0.5g/ kg b. wt.) for six weeks.

Hematological Examination:

The examination of complete blood picture red blood cell counts (RBCs), the white blood cells count (WBCs), total hemoglobin and hematocrit assays were done by Automated Hematology Analyzer (Diff3) Mek-6410/Mek-6420.

Serum Biochemical Analysis:

At the end of the experiments, blood samples were collected from the eye medial canthus. Blood samples were divided into 2 portions. The first portion was collected on Disodium salt of ethylene diamine tetra-acetic acid (EDTA) for hematological analyses. The other portion of blood was left in clean tubes (collected without anticoagulant) at room temperature to clot then after 20 minutes; serum was separated by centrifugation for 30 minutes at 3000 rpm (Dacie and Lewis, 1975). Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT), total protein and bilirubin were assayed according to the method of Reitman and Frankel,1957 using a reagent kit purchased from Biodiognostic (Egypt).

Histochemical Study:

The liver was dissected and fixed in 10% neutral buffered formalin, dehydrated in ascending series of alcohols, cleared in xylene substitutes and embedded in paraffin wax. Paraffin sections of 6 micrometers in thickness were prepared for Periodic Acid-Schiff (PAS) reaction.

Morphometric Study and Statistical Analysis:

Images were obtained using a (Research Microscope & Image analysis system in the Central Laboratory of South Valley University – Faculty of...
Science, and quantitative analysis were done using the ImageJ software, version 1.53a (for Windows). The results were expressed as mean ± SEM. Statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by the student Newman-Keuls T-test, prism, and image analyzer software. Values of P<0.05 were statistically significant.

RESULTS

Hematological Parameters:

WBCs count was increased (63.02%) in the CCl₄ group than those in the normal group. PS+CCl₄ and CCl₄+PS groups had a decreasing effect (7.7%) & (29.4%), respectively versus those of CCl₄ group (Table 1).

CCl₄ group showed, decreased (18.8%) in RBCs count versus those of the control group, but PS+ CCl₄ and CCl₄+PS groups had significant effects (20.5%) & (11.9%), respectively in increasing RBCs count versus those of CCl₄ group (Table 1).

HB count was decreased (18.75%) in the CCl₄ group than those in the control group. PS+ CCl₄ and CCl₄+PS groups had a significant effect (23.1%) & (19.43%), respectively in increasing HB versus those of CCl₄ group. (Table 1).

HCT count was decreased (18.9%) in the CCl₄ group than those in the control group. PS+CCl₄ and CCl₄+PS groups had shown a significant increase in HCT count (23.4%) & (16.9%), respectively versus those of CCl₄ group (Table 1).

Table 1: Effects of PSH on some hematological parameters indices in CCl₄-induced hepatotoxicity in rats. Each value represents the mean ± standard error of means. Statistically significant in the Newman-Keuls Multiple Comparison Test at < 0.05.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control group Mean ± S.E.</th>
<th>CCl₄-treated group Mean ± S.E.</th>
<th>PS+CCl₄-treated group Mean ± S.E.</th>
<th>CCl₄+PS-treated group Mean ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBCs count (mill/cmm)</td>
<td>9.300 ± 0.5</td>
<td>7.550 b ± 0.05</td>
<td>9.100 a ± 0.10</td>
<td>8.450 a ± 0.05</td>
</tr>
<tr>
<td>WBCs count (×10³/cmm)</td>
<td>5.950 a ± 0.05</td>
<td>9.700 c ± 0.05</td>
<td>8.950 c ± 0.05</td>
<td>6.850 d ± 0.05</td>
</tr>
<tr>
<td>Hb content (g/dL)</td>
<td>15.20 a ± 0.70</td>
<td>12.35 b ± 0.05</td>
<td>15.20 a ± 0.8</td>
<td>14.75 a ± 0.05</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>44.25 a ± 0.75</td>
<td>35.85 c ± 0.15</td>
<td>48.50 d ± 0.50</td>
<td>47.50 d ± 0.50</td>
</tr>
</tbody>
</table>

Liver Functions Parameters:

ALT activity was increased (64.5%) in the CCl₄ group than those in the control group. PS+ CCl₄ and CCl₄+PS groups had significant effects in decreasing ALT activity (58.8%) & (19.6%) respectively versus those of CCl₄ group (Table 2).

AST activity was increased (20.6%) in the CCl₄ group than those in the control group. PS+ CCl₄ and CCl₄+PS group had non-significant effects in decreasing AST activity (5.4%) & (9%) respectively versus those of CCl₄ group (Table 2).

T. Protein was increased (17.3%) in the CCl₄ group than those in the control group. PS+ CCl₄ and CCl₄+PS groups had significant effects in decreasing T. Protein (29.7%) & (21.98%) respectively versus those of CCl₄ group (Table 2).

T. Bilirubin was increased (12.8%) in the CCl₄ group than those in the control group. PS+ CCl₄ and CCl₄+PS groups had significant effects in decreasing T. Protein (11.9%) (Table 2).
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Table 2 Effects of PSH on changes in liver function tests associated with CCl4 toxicity in rats. Each value represents the mean ± standard error of means. Statistically significant in the Newman-Keuls Multiple Comparison Test at < 0.0.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control group Mean ± S.E.</th>
<th>CCl4-treated group Mean ± S.E.</th>
<th>PS+CCl4-treated group Mean ± S.E.</th>
<th>CCl4+PS-treated group Mean ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (units/ml)</td>
<td>15.50 ± 0.50</td>
<td>25.50 c ± 0.50</td>
<td>10.50 d ± 0.50</td>
<td>20.50 e ± 0.50</td>
</tr>
<tr>
<td>AST (units/ml)</td>
<td>46.00 ± 10.00</td>
<td>55.50 a ± 0.50</td>
<td>52.50 a ± 2.50</td>
<td>50.50 a ± 0.50</td>
</tr>
<tr>
<td>Total protein (g/dl)</td>
<td>6.217 ± 0.2685</td>
<td>7.290b ± 0.0058</td>
<td>5.128 a ± 0.4938</td>
<td>5.687 a ± 0.0033</td>
</tr>
<tr>
<td>Total Bilirubin mg/dl</td>
<td>0.1675 ± 0.0005</td>
<td>0.1890b ± 0.0007</td>
<td>0.1665 ± 0.0005</td>
<td>0.1665 ± 0.0005</td>
</tr>
</tbody>
</table>

**Glycogen Estimation:**

PAS-reaction sections of the liver of control groups showed deeply pink granules of a strong PAS reaction in the pole of the cytoplasm of hepatocytes (glycogen flight) (Fig. 1).

PAS-reaction stain of liver sections of rats of CCl4 group showed weak PAS reaction in some hepatocytes, and an absence of the reaction in other cells, reflecting a reduction in the glycogen content of the hepatocytes (Fig.2).

PAS-reaction stain of liver sections of rats of PS+ CCl4 group showed that most of the liver cells retained their content of carbohydrates compared to those of CCl4- treated rats (Fig.3).

PAS-reaction stain of liver sections of rats of CCl4 + PS group showed no restoration to the number of carbohydrates (Fig.4).

**Estimation of Liver Glycogen Content:**

The optical density of liver glycogen statistically demonstrated a significant decrease in CCl4 group (47.2%) liver sections when compared with control rats (Fig. 39). While PS+ CCl4 group had a significant increase in liver glycogen content by percentage (73.6%) as compared to CCl4 group, while in CCl4+PS group had a decreased percentage of (15.1 %) as compared to CCl4 group (Fig.5).
Fig. 1: A photomicrograph in the liver section from the control group showing: the normal distribution of glycogen in the cytoplasm of hepatocytes. (PAS reaction, Bar = 50 µm)

Fig. 2: A photomicrograph in the liver section from CCl4 group showing: a weak PAS reaction in a large number of hepatocytes. (PAS reaction, Bar = 50 µm)

Fig. 3: A photomicrograph in the liver section from PS+CCl4 group showing nearly normal distribution of glycogen in hepatocytes (arrows). (PAS reaction, Bar = 50 µm)

Fig. 4: A photomicrograph in the liver section from CCl4+PS group showing weak PAS reaction in a large number of hepatocytes. (PAS reaction, Bar = 50 µm)

Fig. 5. Liver histochemical changes in the mean values ±S.E.M of glycogen content in control and different treatments in male rats were observed. The data are expressed as mean ± SE, and values of different Values in the same column with unlike superscript letters are significantly different at P < 0.05.
DISCUSSION
Carbon tetrachloride (CCl₄) is a xenobiotic industrial solvent that is used for inducing chemical hepatitis and liver injuries in experimental animals (Zamzami et al., 2019). It is used as an experimental model for monitoring the hepatoprotective and therapeutic activity of PSH. In the present study, intraperitoneal injection of CCl₄ resulted in a significant decrease in RBC, Hb and HCT of rats and a significant increase in total WBCs Neutrophils, Lymphocytes, Monocytes and Eosinophils count compared to the control group.

The increase in the WBCs count might be due to the defensive mechanism of the immune system as introduced by Asmaa et al., 2018, Ubhunin et al., 2019, Udobang et al., 2019 and Abu et al., 2022, where white blood cells function primarily in body defense against foreign bodies, and this is often achieved through leukocytosis and antibody production. The decrease in RBC may be attributed to the metabolism of CCl₄ that releases free radicals causing liver injury and some of CCl₄ free radicals liberated from the liver into the blood causing destruction of RBC. The low values of HCT may be attributed to anemic conditions. Also, the decreasing of hemoglobin may be attributed to the formation of methemoglobin; a compound resulting from the autoxidation of oxyhemoglobin to methemoglobin as a result of oxidative stress induced by CCl₄ in RBCs these findings agree with Abdalla et al., 2013, Asmaa et al., 2018, Emam et al., 2020, Bikheet et al., 2022 and Gazwi and Mahmoud, 2018.

It was found that the treatment of rats with PSH powder in PS+CCl₄ decreased WBCs number and increased RBCs, Hb and HCT these results agreed with (Amer et al., 2019, Abbas and Sheikh, 2016). This may be due to the antioxidant properties of PSH where it was found that antioxidants can counteract oxidative stress and cause a remarkable improvement in hematological parameters (Kelkar et al., 2008). Also in this study, PSH powder administration in CCl₄+PS group, after CCl₄ toxicity induction reversed the effect on the hematological parameters, suggesting that the PSH may have the ability to reverse the harmful effect caused by CCl₄. A similar result has been reported by other researchers after treatment of CCl₄-induced toxicity with other antioxidants (Zahran et al., 2020 and elekwa et al., 2021).

AST and ALT are intracellular enzymes that are excreted outside the cells because they are the most sensitive markers, so they are used in the diagnosis of liver damage (Cosgun et al., 2019, Kango et al., 2008). AST is localized in the mitochondria whereas ALT is distributed throughout the cytoplasm, ALT is more specific to the liver and is thus a better parameter for detecting liver injury (Lee et al., 2017, Palanivel et al., 2008). Lipid peroxidation caused by CCl₄ causes cell membrane disruption leading to increased cell membrane permeability and AST and ALT leakage out from the liver into the bloodstream which explains the elevation of these enzymes level in blood after administration of CCl₄ (Hus et al., 2020, Kanawati et al., 2021). The treatment of intoxicated rats with PSH in PS+CCl₄ reduced ALT and AST serum activities to the normal level. This referred to the antioxidant power and free radical scavenging ability or the restoration might be attributed to the stabilization of cellular membrane due to regression of leakage of the liver enzymes into the cytosol (Giannini et al., 2005). These results agree with Sadeghian et al., 2018, Wahid et al., 2020 and Elhassaneen et al., 2021.

The treatment of the intoxicated group with husk powder as a therapeutic agent in CCl₄+PS restored
ALT, AST, and ALP serum activities in the control group. Therefore, these results confirmed that PSH has a significant preventive and therapeutic potential for hepatic injuries. These results were in agreement with Naz et al., 2020 who described the therapeutic effect of a plant with antioxidant properties against CCl₄-induced liver injury.

It was found that the intraperitoneal injection of CCl₄ resulted in a significant increase in total protein The level of serum proteins is a vital indicator of impaired or normal functions of the hepatocyte (Ibrahim et al., 2020). This result is in agreement with Yacout et al., 2012 and Bikheet et al., 2022. Treatment of rats with PSH in PS+CCl₄ and CCl₄+PS preserves the normal range and treats the toxic effect of CCl₄ on serum total protein to the normal range that reflects the hepatoprotective and hypotherapeutic effect of PSH probably through stabilizing endoplasmic reticulum or through neutralizing reactive free radicals by scavenger compounds.

A significant increase in serum total bilirubin level was detected in groups that received CCl₄ alone compared to the control group. Serum bilirubin provides useful information on how well the liver is functioning (Mahesh et al., 2010, Usunobun et al., 2020). The accumulation of bilirubin in the circulation was attributed to the liver's inability to conjugate bilirubin with glucuronide resulting in unconjugated bilirubin accumulation in the blood which reflects liver damage (Usunobun et al., 2020). This result agrees with (Biomy et al., 2003, Mahesh et al., 2010, Shahwan and Zain Al Abdin, 2018, Hadayat Ullah et al., 2020 and Usunobun et al., 2020).

A significant decrease in serum total bilirubin was found in PS+CCl₄ group that was treated with psyllium powder, this may be attributed to the ability of PSH as an anti-oxidant to prevent the free radicals from the damage of liver cells as it increases the stability of cells membrane, preventing liver damage and improvement in the liver secretory function, these results agree with Wahid et al., 2020 and Rafiee et al., 2022. Also, the post-treatment of rats with PSH powder in CCl₄+PS significantly decreased the t. bilirubin strongly indicates that the plant can ameliorate liver damage. A number of studies referred to the use of antioxidants as therapeutics and showed the restoration of bilirubin by an antioxidant effect such as Atawodi et al., 2011 and Meharie et al., 2020.

Histochemical results obtained in our study showed that glycogen content was depleted markedly in the hepatocytes after administration of CCl₄; were agree with Sakr et al, 2007, Abdel-Salam et al., 2012 and Sakr et al., 2017 who attributed the reduction to the elevated hepatocyte stress or to the liver cells' decreased capability to store glycogen as a result of the toxicity of CCl₄. Also, they explained the central role of the liver in metabolism. As the primary source of endogenous glucose through the breakdown of glycogen (glycogen glycogenolysis) and the de novo synthesis of glucose (gluconeogenesis) from proteins, it is crucial for maintaining glucose homeostasis. As a result of decreased hepatic gluconeogenesis and significantly diminished glycogen storage, patients with chronic liver illness frequently have problems with glucose homeostasis. After receiving CCl₄, studies have shown that the liver produces less gluconeogenesis, which results in a drop in hepatic glycogen content. Hepatocyte viability is anticipated to continue to decline in a damaged liver due to depleted glycogen stores and the liver's inability to provide...
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Glucose in the post-absorptive condition.

Group treated with PSH powder (PS+CCl4) showed replenishing glycogen stores; these results agree with Abdel-Salam *et al.*, 2012 who found that the restoration of glycogen to the normal is considered an indicator of improved liver function and preservation of hepatic architecture integrity against toxic insult. Badawi, 2018 explained that oxidative stress causes glycogen depletion which occurred as a secondary event to the lipid peroxidative damage to the liver mitochondria and showed that the antioxidants cause inhibition of the lipid peroxidation damage to the mitochondria, thus inhibiting glycogenolysis. And restoration of glycogen as a result of that.

The post-treatment of rats with PSH in PS+CCl4 group showed a significant enhancement in quantitative analysis of glycogen content in the liver while PSH in CCl4+PS group didn’t show a significant restoration of glycogen in the liver, this may be attributed to the short time in which the intoxicated rats were received PSH and the amount of psyllium that received in CCl4+PS group was less than that of PS+CCl4 group was received. Sharma and Shukla, 2010 and Sancheti *et al.*, 2013 found that the antioxidant wasn’t effective at low doses in the treatment of the liver and showed mild generation in histological results.

**Conclusion**

The presented study showed that CCl4 caused, hematological, and biochemical alterations and depletion of glycogen content in the liver. Psyllium husk showed a protective and therapeutic role against this CCl4-induced hepatotoxicity.

**Ethical number:**

The animals were cared for in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The study was approved by the Research Ethics Committee of the Faculty of Veterinary Medicine, South Valley University, Egypt (N.43/05.07.20224).

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*Pharmaceutical Sciences and Research, 10*(11), 2800-2804.


**ARABIC SUMMARY**

دور قشور السيليوم (لسان الحمل البيضوي) في تغيرات وظائف الكبد التي يسببها رابع كلوريد الكربون (CCL4) في ذكور الفئران البالغة

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2-قسم علم الحيوان-كلية العلوم-جامعة أسيوط

تناولت هذه الدراسة التأثير الوقائي والعلاجي المحتمل لقشور السيليوم على السمية التي يسببها رابع كلوريد الكربون في ذكور الفئران البيضاء. تم استخدام ثلاثين فارا من الفئران البيضاء، بوزن حوالي (144-176 جم). تم تقسيم الفئران إلى خمس مجموعات (6 فارا/ مجموعة): المجموعة 1 (المجموعة الضابطة) لم تحصل على أي شيء. المجموعة 2 (المجموعة المعالجة بالزيت) أعطيت زيت الزيتون. المجموعة 3 (مجموعة رابع كلوريد الكربون) وهي المجموعة المصابة، المجموعة 4 (المجموعة المعالجة بقشور السيليوم قبل واثناء رابع كلوريد الكربون) والمجموعة 5 (المجموعة المعالجة بقشور السيليوم بعد الإصابة برابع كلوريد الكربون).

تم تخدير جميع المجموعات وذبحها. تم تحديد عدد كريات الدم الحمراء، خلايا الدم البيضاء، تركيز الهيماتوكريت والهيموجلوبين. تم تقدير مستوى بعض النيازات الكبد، البروتينات الكلي بالإضافة إلى محتوى الجليكوجين في الكبد. تسبب رابع كلوريد الكربون في انخفاض كبير في عدد خلايا الدم الحمراء وتركز النيازات الكبد، البروتينات الكلي بالإضافة إلى محتوى الجليكوجين في الكبد. تسبب رابع كلوريد الكربون تأثيرات سلبية على خلايا الدم البيضاء. علاوة على ذلك، زادت إنزيمات الكبد والبروتينات الكلي. وضع في المجموعة المعالجة برابع كلوريد الكربون مقارنة بالضابطة، وقد أدى علاج الفئران بمسحوق قشور السيليوم إلى درجة ملحوظة من التحسن في القياسات الخاصة بالدم وزائدة ملحوظة في محتوى الجليكوجين. علاوة على ذلك، انخفضت مستويات إنزيمات الكبد والبروتينات الكلي في الدم.