ABSTRACT

The present study aimed to examine the pre- and post-treatment effect of Nigella sativa water extract and crude oil against hepatotoxicity - induced by carbon tetrachloride in rats. The Nigella sativa extract or crude oil was screened for estimating their total phenolic contents and free radical scavenging activity. They have a high antioxidant activity due to phenolic compounds. Protective role of the Nigella sativa water extract and crude oil against carbon tetrachloride -induced alternation in blood biochemical and liver was evaluated. The examined extracts were administered 28 days before and 28 days after concomitantly with carbon tetrachloride for water extract and crude oil. Blood samples were determination of serum aminotransferases activity (ALT and AST), total protein (TP) albumin (Alb), urea, creatinine, and the activities of antioxidants such as glutathione-S-transferase (GST), superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) content. The results detected that the intraperitoneal injection of carbon tetrachloride motivate the increase in ALT and AST activity also urea, creatinine and liver MDA, meanwhile a high decrease in the levels of total protein, albumin and liver GST, SOD, CAT were obtained. The results revealed that Nigella sativa water extract or crude oil had a protective effect against the carbon tetrachloride -induced.

Keywords: Nigella sativa, crude oil extract, antioxidant markers, hepatic injury

INTRODUCTION

Liver is the most sensitive organ in the body for detoxification; trouble to this organ is the most serious health problems (Samuel et al., 2012). Liver diseases still on of the dangerous health problems and control of the liver disease still a challenge to the novel medicine. Liver plays a major role in organization of many physiological processes, implicated in many vital functions like secretion, storage and metabolism. Also detoxifies an assortment of drugs and xenobiotics and plays a main role in transforming, clearing the chemicals because of that liver susceptible to the toxicity from all of these agents (Pal and Manoj 2011). Forever Humans exposed to various kinds of Harmful substances such as pesticides, food additives, industrial chemicals, and other contaminants in the food, air, and soil (Hasegawa et al., 1995). Carbon tetrachloride (CCL4) is a famous hepatotoxicant and has been extremely used for produced liver damage in animals models (Minami et al., 2005).The liver is a axial organ in human body which detoxifies exogenous xenobiotics, viral infections, drugs, and chronic alcoholism. During the time that carry out the detoxifications, liver undergoes high stress, this risk leading to liver diseases finally to liver failure and dangerous health problems and death Hepatotoxicity is a common reason of sharp metabolic trouble and even death (Patel et al., 2008). Hepatic harm happens due to its multidimensional functions, several oxidative stress and xenobiotics leading to distortion of all of its functions (Wolf, 1999). Herbal medicines derived from plant extracts are being used widely to remedy a wide type of clinical diseases, with relatively a few information concerning their modes of action (Matthews et al., 1990).The selected seed has inclusive phytochemical investigations, and the presence of many type of chemical constituents such as phenolic acid, quercetin, epicatechin, and flavones from Nigella sativa shoots and roots has been studied (Bourgou et al., 2008). Nigella sativa seed oil contains high amounts of sterols; also the oil is rich in b-sitosterol that inhibits the absorption of dietary cholesterol (Atta, 2003). Similarly, phytopharmacological evaluation showed that it has antiasthmatic (Boskabad et al., 2010), neuropharmacological (AI-Naggar et al., 2010), and immune modulatory effects (Swamy and Tan 2000).

Badawi et al., (2015) found that the fatty acids composition of fixed oil extracted from Nigella sativa was W-6 fatty acid C18:2; (55.2%), followed by C18:1; (23.9%), C16:0; (14.8%), C18:0, (2.6%) and C20:0, (1.9%). They found that total polyunsaturated fatty acids (PUFAs); (56.7%) of all fatty acids, total monounsaturated fatty acids (MUFAs); (23.9%) and total saturated fatty acids (SFAs); (16.2%) also stated that total phenolic content (mg GAE/G) was 2.93 and antioxidant activity was 39 %.

The aim of the present study was to explore the effects of Nigella sativa extract and crude oil on the protective and remedy of liver injury induced by carbon tetrachloride in rats; in addition, the levels of liver marker enzymes, aspartate transaminase (AST), alanine transaminase (ALT) in serum, malondialdehyde (MDA) content, and the activities of antioxidants such as glutathione-S-transferase (GST), superoxide dismutase (SOD), catalase (CAT) were measured in order to investigate Nigella sativa possible mechanisms.

MATERIALS AND METHODS

Materials

Nigella sativa seeds were obtain from local market of Zagazig city, Sharkia governorate, Egypt and identified by botanical members of the Department of Botany, Faculty of Agriculture, Zagazig University. Carbon tetrachloride and standards used were purchased from Merck (Coimbatore, Tamilnadu, India). Kits and other chemicals were of the highest purity available.

Male albino rats weighing 140–160 g rats were purchased from Faculty of Veterinary Medicine, Zagazig University.

Methods

Preparation of seeds extract

The seeds were cleaned, dried, and powdered using blender (Philips, Japan) and then one kilogram were soaked in distilled water for three days finally the water extract was lyophilized 96 g. The powder of
lyopholized extract was kept in a vial in a refrigerator until use (Nwangwa and Ekhoye, 2013). The concentrated sample was dissolved in distilled water before oral administration by gavage to the experimental animals.

**Preparation of seeds oil**

*Nigella sativa* seeds crude oil was extracted using n-hexane and the solvent was then removed exclusively by rotary evaporator under reduced pressure and the obtained crude oil was kept in a clean brown vial and cooled in a refrigerator until use.

**Determination of Total phenolics content**

Total phenolic contents in *Nigella sativa* water extract or oil were calculated as mg gallic acid/g DW according to Ghasemzadeh et al., (2010).

**Determination of total flavonoid content**

The total flavonoid contents were determined in *Nigella sativa* water extract or oil according to Ahn et al. (2007).

**Determination of radical scavenging activity**

The free radical scavenging activity of the *Nigella sativa* water or crude oil extract was determined as described by Sarikurkcu et al., (2008). The inhibition activity was determined by using following equation:

\[
\%\text{ inhibition} = \frac{[\text{absorbance of control} - \text{absorbance of test sample}]}{\text{absorbance of control}} \times 100.
\]

**Biological assay:**

**Animals and treatment**

Rats were kept in wire-bottomed stainless steel cages, which were environmentally controlled (25 °C, 12-h light dark cycle); with free access to water and food.

Ten days before the beginning of the experiment all rats were fed basal diet American institute of nutrition standard reference diet (AIN-93M) (Reeves et al., 1993) as adaptation period after that the rats were divided into 6 groups every one included 6 rats. Treatments used in this study were as shown in Table1.

At the end of the experimental period, rats were anaesthetized with diethyl ether, and the blood samples were withdrawn from retro-orbital venous plexus through capillary tube and collected into heparinized tubes. Animals were deprived of food day before they were sacrificed. Livers were rapidly removed, washed in ice-cold saline, and kept in ice to use. Liver homogenate was prepared according to the method described by El-Demerdash et al., (2005).

**Table 1. Treatments used in the experiment**

<table>
<thead>
<tr>
<th>Group No</th>
<th>Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>Negative group (normal control) fed basal on diet and daily doses of 1ml/200g B.W, oral (0.9% saline)</td>
</tr>
<tr>
<td>G2</td>
<td>Positive control received carbon tetrachloride 1 ml/kg/ B.W (0.18 ml, intraperitoneal injection, at 72-hour intervals for 15 days).</td>
</tr>
<tr>
<td>G3</td>
<td>Received CCl₄ (0.18 ml, intraperitoneal injection, at 72-hour intervals for 15days)-induced liver damage were additionally given 20% water extract of <em>Nigella sativa</em> orally for 28 days.</td>
</tr>
<tr>
<td>G4</td>
<td>Received 20% water extract of <em>Nigella sativa</em> for 28 days and then injected with CCl₄ (0.18 ml, intraperitoneal injection, at 72-hour intervals for 15 days).</td>
</tr>
<tr>
<td>G5</td>
<td>Received CCl₄ (0.18 ml, intraperitoneal injection, at 72-hour intervals for 15days)-induced liver damage were additionally given 6.0 ml/kg) seeds crude oil of <em>Nigella sativa</em> orally for 28 days.</td>
</tr>
<tr>
<td>G6</td>
<td>Received (6.0 ml/kg) seeds crude oil of <em>Nigella sativa</em> for 28 days and then injected with CCl₄ (0.16 ml, intraperitoneal injection, at 72-hour intervals for 15 days).</td>
</tr>
</tbody>
</table>

**Biochemical analysis:**

The method of determined parameters which were used to evaluate the hepatotoxicity of *Nigella sativa* described below. Assays of aspartate transaminase (AST), alanine transaminase (ALT) as described by Reitman and Frankel (1957), Albumin (ALP) was determined as described by Doumas et al., (1979), Total protein (TP) was determined as described by Reitman and Frankel (1957), Albumin (ALP) was determined as described by Doumas et al., (1979) and creatinine according to Allston (1993).

Determination of antioxidant activity in liver homogenate included superoxide dismutase was described by Das et al., (2007) catalase, was determined as described by Sinha, (1972) glutathione S-transferases was determined as described by Wilce and Parker (1994). MDA was determined by their action with thiobarbituric acid and used as an index of lipid peroxidation (LPO) was determined as described by Buege and Aust (1978).

**Statistical Analysis**

All experimental values were expressed as mean SD. Results were assessed by analysis of variance and Dunnett’s multiple comparison tests. Differences were considered significant at p < 0.05.

**RESULTS AND DISCUSSION**

In this work the Total Phenol content was 44± 3.1 (mg GAE/g) for water extract and 42±5.1 ( mg GAE/g) for crude oil extract while the flavonoid content was 16 ± 2.2 mg QE/g for Nigella sativa water extract and 18 ± 1.4 (mg QE/g) for Nigella sativa crude oil extract.

Data presented in fig 1. Show that the changes in absorbance of DPPH scavenging activity were detected for *Nigella sativa* aqueous and crude oil extract recorded a higher scavenging activity for both *Nigella sativa* aqueous and crude oil extract.

As indicated from the results in Table (2), the effect of *Nigella sativa* aqueous or oil extract on pre-and post-treatment of hepatotoxicity - induced by carbon tetrachloride appearance in ALT and AST levels, CCl₄ caused hepatotoxicity in rats confirmed by an increase in levels of AST and ALT activities before treatment of carbon tetrachloride treatment and after carbon tetrachloride treatment, whereas rats pretreated...
with *Nigella sativa* aqueous or oil extract recorded a high decrease in the activities of these enzymes levels, *Nigella sativa* aqueous or oil extract also reversed the change of ALT and AST levels when compared with the hepatotoxic group. Reduced in the level of protein was recorded in the carbon tetrachloride - treated group when compared to the control group, while *nigella sativa* aqueous or oil extracts pre- and post-treated groups showed good level compared to the control group this result was in the same line with Reham Hamza and Al-Harbi (2015) who stated that treatment with *Nigella sativa* improved liver Biomarkers.

### Table 2. Effect of *Nigella sativa* water and crude oil extract on serum ALT and AST activities (µ/ml) as well as T.P (g/dl) and Alb contents (g/dl) activity of hepatotoxic rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ALT (µ/ml)</th>
<th>AST (µ/ml)</th>
<th>Total protein (g/dl)</th>
<th>Albumin (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>38.00±3.72</td>
<td>46.00±4.84</td>
<td>7.9±0.6</td>
<td>3.8±0.36</td>
</tr>
<tr>
<td>G2</td>
<td>99.00±9.34</td>
<td>110.00±6.25</td>
<td>4.8±0.2</td>
<td>2.7±0.26</td>
</tr>
<tr>
<td>G3</td>
<td>53.00±4.88</td>
<td>67.50±7.4</td>
<td>6.3±0.23</td>
<td>3.4±0.13</td>
</tr>
<tr>
<td>G4</td>
<td>63.00±1.96</td>
<td>75.00±4.72</td>
<td>5.6±0.35</td>
<td>3.1±0.31</td>
</tr>
<tr>
<td>G5</td>
<td>43.00±4.39</td>
<td>60.00±6.35</td>
<td>6.6±0.27</td>
<td>3.7±0.09</td>
</tr>
<tr>
<td>G6</td>
<td>54.00±3.67</td>
<td>70.00±8.48</td>
<td>6.1±0.5</td>
<td>3.2±0.42</td>
</tr>
</tbody>
</table>

Values with different letters in the same column are significantly different (*P* < 0.05)

G1: normal group   G2: Received CCl4   G3: Received CCl4 then 20% water extract of *N. sativa* orally G4: Received 20% water extract of *Nigella sativa* then injected with CCl4 G5: Received CCl4 -then given 6.0 ml/kg seeds crude oil of *Nigella sativa* orally G6: Received (6.0 ml/kg) seeds crude oil of *Nigella sativa* then injected with CCl4

Hepatic GST level was reduced after carbon tetrachloride treatment. On the other hand, treatment with 20% aqueous extract or oil of *Nigella sativa* reversed the lowered level of GST caused by carbon tetrachloride treatment near to the normal level, leading to the results *Nigella sativa* helped to keep the level of GST near normal level despite carbon tetrachloride injection. Data confirmed that lowered levels of hepatic SOD and CAT activities are the result of carbon tetrachloride injection on the other hand treatment with *Nigella sativa* and activated increase in SOD and CAT activities compared with hepatotoxic group.

Increased the level of hepatic MDA in the hepatotoxic group, confirms that oxidative damage was induced. After treated rats with *Nigella sativa* aqueous or oil extracts levels of MDA in liver were significantly reduced when compared with hepatotoxic group treated and pretreated groups show a resistance of oxidative damage in carbon tetrachloride -injured liver by *Nigella sativa* aqueous or oil treatment. This result suggests that *Nigella sativa* aqueous or oil is antioxidative and hepatoprotective the same trend indicated by Seval et al., (2014).

The Polyphenols and its derivatives are the widely substances in plants also these substances are the most effective compounds in biological system because of their properties as antioxidant and the ability of remedy many disease (Landete, 2013). The highest active compounds of the antioxidant group in plants are phenolic compounds (Bors et al., 2001). Phenolic is a great antioxidant because the ability to give electrons or hydrogen (Maillard et al., 1996). Phenolic acid and flavonoids are great participating agents to the hepatoprotective and antioxidant effects in the human being diets. However, most of the phenolic compounds absorption is extensively affected by biotransformation in the body being.

### Table 3. Effect of *Nigella sativa* water and crude oil extract on serum creatinine and urea (mg/dl)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Creatinine (mg/dl)</th>
<th>Relative (%)</th>
<th>Urea (mg/dl)</th>
<th>Relative</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>0.57±0.02</td>
<td>100</td>
<td>24.7±1.07</td>
<td>100</td>
</tr>
<tr>
<td>G2</td>
<td>1.32±0.02</td>
<td>231</td>
<td>56.2±1.16</td>
<td>233</td>
</tr>
<tr>
<td>G3</td>
<td>0.69±0.04</td>
<td>121</td>
<td>38.2±1.16</td>
<td>158</td>
</tr>
<tr>
<td>G4</td>
<td>0.85±0.09</td>
<td>149</td>
<td>43.3±1.54</td>
<td>179</td>
</tr>
<tr>
<td>G5</td>
<td>0.64±0.1</td>
<td>112</td>
<td>31.3±2.1</td>
<td>126</td>
</tr>
<tr>
<td>G6</td>
<td>0.73±0.2</td>
<td>128</td>
<td>37.6±1.45</td>
<td>154</td>
</tr>
</tbody>
</table>

Values with different letters in the same column are significantly different (*P* < 0.05)

G1: normal group   G2: Received CCl4   G3: Received CCl4 then 20% water extract of *Nigella sativa* orally G4: Received 20% water extract of *Nigella sativa* then injected with CCl4 G5: Received CCl4 -then given 6.0 ml/kg seeds crude oil of *Nigella sativa* orally G6: Received (6.0 ml/kg) seeds crude oil of *Nigella sativa* then injected with CCl4

All over the world the use of active component in the diet and herbs which contains many bioactive compounds has increased for the protection and remedy of many serious diseases such as liver diseases and also protect from adverse reactions (Yang et al., 2010 and Bhoopat et al., 2011). The chemical compounds that occur naturally in plants, diet which containing polyphenols such as flavonoids have a great issue of phenolic hydroxyl groups which explain the role of and the responsibility of the beneficial effect, which is at most because of their scavenging activity against free radical or reactive oxygen species (ROS) (Shimoda et al., 2008 and Halliwell et al., 1992). It is known that cell harm can cause by ROS under the reaction of subsequent tissue injury with lipid peroxidation (Halliwell et al., 1992). Because of that, today from important ways to protect or remedy liver from toxic is to use plant antioxidants especially polyphenol. Plant extracts play a great role in the remedy of hepatotoxicity. Relationship between the levels of blood antioxidant and many diseases was recorded by many new studies. In the absence of effective protection of liver in modern medicine, in many countries several of herbal plants are used to remedy hepatic troubles (Girish et al., 2009). Many studies were indicate that modern medicine have many side effects on the other hand natural extracts treatment are more safe and effective without any side effects for hepatotoxicity.

The investigation of plant extracts becomes more important when it improve disease condition to get better. In this investigation, the hepatoprotective effect of *Nigella sativa* was studied based on carbon tetrachloride which motivates liver damage in rats. Phytochemical analyses of *Nigella sativa* confirm the existence of flavonoids, phenols, and many other bioactive compounds. The hepatoprotective effect of *Nigella sativa* against carbon tetrachloride while damage is established by lowering the increased of serum liver marker enzymes, also related to the
inhibition of increase hepatic lipid peroxide content and a serious increase in total sulphydryl content in liver tissues. many results mentioned that the inhibition of lipid peroxidation is the premier step in the mechanism of *Nigella sativa* protective effect against toxicity of carbon tetrachloride also could be improve many other parameter which inhibited by carbon tetrachloride (Chenoweth and Hake, 1962 and Mansour, 2000) reported that increases of AST and ALT levels in blood have been refers to injury in the structural of the liver. Chenoweth and Hake (1962) they may be quickly released from the cytoplasm to be presence in blood circulation after harm of the plasma membrane and cellular damage. High dose of CCl₄ causes inclusive necrosis in the liver around the central veins (Sallie et al., 1991). The hepatotoxicity of CCl₄ can be explain by the respective specific isozyme of the cytochrome P450 system in the endoplasmic reticulum of hepatocytes to Produce the reactive metabolite, trichloromethyl radical (CCl₃), which covalently linked to protein, macromolecules, and lipid and also react with O₂ to Produce highly reactive trichloromethylperoxy radical (CCl₃O₂) (Walker et al., 1980)).

Extracts of *Nigella sativa* maybe play an important role in maintain the structural integrity of plasma cellular membrane of the hepatocytes to guard against breakage by the reactive metabolites created from carbon tetrachlorided treatment. Also prevented damage to more hepatocytes and this may be a reason of reduced AST and ALT due to cell damage. This may be explain the lower in the levels of transaminases observed in animals treated with the *Nigella sativa* extracts before and after exposure to the toxin. Lipid peroxidation is one of the main causes of carbon tetrachloride encourage liver damage and is intercede by the free radical derivatives which produced by carbon tetrachlorid. The liver cell damage by the treatment of CCl₄ was associated with a high increase in hepatic lipid peroxide content and a high decrease in hepatic total sulphydryl content. The results confirmed that *Nigella sativa* can reduced malondialdehyde generation. In other way, the mechanism of *Nigella sativa* in the inhibitory effects of lipid peroxidation, may back to radical scavenging ability. This result of *Nigella sativa* extracts may provide various biologically active compounds like polyphenol and flavonoid which can inhibit lipid peroxidation in the rat liver (Younes and Siegers, 1980). Also, the antioxidative activity and the resistance of free radical production are the main agent in terms of protecting the liver from carbon tetrachloride encourage damage (Manibusan et al., 2007). Also an important role played by the antioxidative enzymes in the detoxification of xenobiotics, stimulating their role in reduced GSH. The antioxidative and free radical scavenging activities of numerous substances have been evaluated, and many substances that have antihepatotoxic activity also show high antioxidative activity (Hwang et al., 2009). In this investigation treatment rats with carbon tetrachloride caused a decrease in activities of SOD and CAT as well as an increased in level of Lipid peroxidation in liver, implying the down regulation of numerous antioxidative reactions in the liver. The enzyme antioxidant system plays a major role in cellular protection versus reactive free radicals and other oxidant species. This system depend on GSH and other functionally related enzymes, of which GR is responsible for the renovation of GSH, whereas GPx works with GSH in the decay of hydrogen peroxide and other organic hydroperoxides.

![Graph](image.png)

**Fig. 1.** DPPH scavenging activity of *Nigella sativa* water and crude oil extract

![Graph](image.png)

**Fig. 2.** Effect of *Nigella sativa* water and crude oil extract on serum ALT, AST activities as well as T.P and Alb activity of hepatotoxic rats

G1: normal group  G2: Received CCl₄  G3: Received CCl₄ then 20% water extract of *Nigella sativa* orally  G4: Received 20% water extract of *Nigella sativa* then injected with CCl₄  G5: Received CCl₄ then given 6.0 ml/kg seeds crude oil of *Nigella sativa* orally  G6: Received (6.0 ml/kg) seeds crude oil of *Nigella sativa* then injected with CCl₄
Table 4. Effect of Nigella sativa water and crude oil extract on liver homogenate glutathione S-transferases GST (Mm/mg protein), SOD (µg/mg protein), CAT (µmol/mg protein), MDA (µmol/mg protein) activity of hepatotoxic rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>GST (Mm/mg protein)</th>
<th>SOD (µg/mg protein)</th>
<th>CAT (µmol/mg protein)</th>
<th>MDA (µmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>28.8±2.16</td>
<td>40.42±5.2</td>
<td>18.23±0.6</td>
<td>4.3±0.23</td>
</tr>
<tr>
<td>G2</td>
<td>13.3±3.2</td>
<td>15.65±6.6</td>
<td>10.2±0.43</td>
<td>10.2±0.46</td>
</tr>
<tr>
<td>G3</td>
<td>18.9±2.01</td>
<td>25.37±4.3</td>
<td>12.48±0.23</td>
<td>5.8±0.12</td>
</tr>
<tr>
<td>G4</td>
<td>16.6±3.69</td>
<td>20.14±3.3</td>
<td>10.41±0.35</td>
<td>6.3±0.09</td>
</tr>
<tr>
<td>G5</td>
<td>21.1±1.83</td>
<td>27.82±5.0</td>
<td>13.27±0.11</td>
<td>5.1±0.35</td>
</tr>
<tr>
<td>G6</td>
<td>31.3±1.67</td>
<td>31.3±1.83</td>
<td>12.25±0.44</td>
<td>5.9±0.13</td>
</tr>
</tbody>
</table>

Values with different letters in the same column are significantly different (P<0.05)

G1: normal group. G2: Received CCl4. G3: Received CCl4 then 20% water extract of Nigella sativa orally. G4: Received 20% water extract of Nigella sativa then injected with CCl4. G5: Received CCl4 then given 6.0 ml/kg seeds crude oil of Nigella sativa orally. G6: Received (6.0 ml/kg) seeds crude oil of Nigella sativa then injected with CCl4.

Fig.3. Effect of Nigella sativa water and crude oil extract on liver homogenate glutathione S-transferases GST, SOD, CAT, MDA activity of hepatotoxic rats

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Awad, A. E.


نتأثير الوقاية لذعر حية الردية ضد الإختلال الكبدية المستحدث بواسطة رابع كليوريدي الكربون

أحمد السيد عوض محمد

قسم الكيمياء الحيوية - كلية الزراعة - جامعة القاهرة

هذه دراسة للاختبار تأثير المعاملاة مستمعية حية الردية المازية المحمولة في البرك وفي بعد مضي الإختلال الكبدية - الناجم عن رابع كليوريدي الكربون (Radical)

الكرتون في الفئران. المستمعي المسمى لحية الردية أو الزيت المحمول تم تجريب الفيتامينات النباتية والتفاعليات النترورية . وقد كان النتائج الممعناء للدراسة Scavenging activity من خلال القياسات المحمولة في الفئران . وعند الكود خليطة الكرتون عند القيمة ملحوظة لriage الطريقة، لكي تكون مثل مكونات الكرتون: مثلاً مكونات الكرتون للاكسدة مثل مكونات ثاني الهيدروجين، مكونات أكسدة الدهون، مكونات أولاق الدهون - مكونات الأولاق الدهون - مكونات أولاق الدهون - مكونات أحد مكونات الكرتون مكونات ثاني الهيدروجين لـ جبلونو، آخر، تساعدي، و مكونات ثاني الهيدروجين في الفئران. وفي الوقت نفسه فادحة، كان هناك نتائج ملموس في بيئات الفئران، وكان هناك نتائج ملموس في البيئات الفئران - مكونات أولاق الدهون - و مكونات ثاني الهيدروجين - رمزهم: السوية، و أكسيد الكابراميد - كما كانت النتائج ملموس في البيئات الفئران، وكان هناك نتائج ملموس في البيئات الفئران - مكونات أولاق الدهون - و مكونات ثاني الهيدروجين.