Star Anise (Illicium verum) oil administration alleviates carbon tetrachloride-induced hepatotoxicity in rats

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Abstract

The purpose of this work was to evaluate the protective effect of star anise oil against carbon tetrachloride (CCL4)-induced hepatic injury and oxidative stress in rats. Twenty-four male Wistar rats were divided into four equal groups. Group I (control): rats administered corn oil 1 ml/kg orally every day for 5 weeks. Group II (star anise oil exposed group): rats received (1 ml/kg orally) every day via gastric tube over a period of 5 weeks. Group III (carbon tetrachloride): rats were injected IP with CCl4 (1:3 in corn oil, 2.5 ml/Kg, 3 doses at 72 hr interval) for 5 weeks. Group IV (CCl4 injected rats plus star anise oil) rats daily received star anise oil (1 ml/kg orally plus CCl4 (as in group III). Obtained results showed significant increase in serum ALT, AST and ALP activities, decrease in total protein, albumin and globulin concentrations and significant increase liver tissue MDA level in CCL4 exposed rats. In addition, a significant decrease in GSH and CAT concentrations were recorded when compared with control group. Administration of star anise oil with CCL4 induced significant improvement of all previous parameters towards their normal ranges. Various histopathological alterations were detected in the liver treated with CCL4. Interestingly, rats treated with star anise oil plus CCL4 showed marked reduction in these pathological alterations in comparison to CCL4 intoxicated rats. These results suggested that star anise oil has the potential to ameliorate CCL4 induced hepatic damage.

1. INTRODUCTION

Liver is a vital organ in the human body, playing an important role in digestion, metabolism, detoxification and biosynthesis (Dong et al., 2015). Nevertheless, liver injury is considering almost common liver disease which caused by different pathogenic factors, such as viruses, bacteria, chemical toxins and alcohol, and may consequently lead to liver fibrosis, cirrhosis and hepatocellular carcinoma (Bernal et al., 2010). So many drugs developed for treatment hepatic injury; unfortunately, these drugs caused limited efficacy with side effect (Li et al., 2018). On the other hand, many plants have been used for their medicinal values in folk medicine for a long time to fight the effects of some toxic substances due to their actual benefits (Mirmiran et al., 2014).

Therefore, in the present work, the hepatoprotective effect of star anise oil (Illicium verum) against CCl4-induced hepatic oxidative injury was assessed through evaluating hepatic functions, antioxidant parameters, as well as histopathology changes of hepatic tissue.

2. MATERIAL AND METHODS

2.1. Experimental animals:

Twenty-four male Wistar rats weighting about (200-250g) were purchased from the United Company for Chemicals, Abu-Zabal, Egypt. The rats were subjected to a normal light/dark cycle and room temperature (23 ± 3 °C) and allowed free access to chow and water. This work was approved by the institutional review board for animal experiments of the animal ethics committee, Faculty of Veterinary Medicine, Benha University, Egypt (BUFVTM 05-07-20). Rats were allowed one week to acclimatize before the commencement of the experiment. Animals were weighted weekly to adjust the dose of chemicals.

2.2. Drugs, chemicals and agents used in experimental protocol

Carbon tetrachloride CCl4 was obtained from ALAMIA company for chemicals (Cairo, Egypt). It was diluted with corn oil as CCl4 (30 % CCl4, 2.5 ml/kg b. wt. in corn oil). The diagnostic kits of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) were obtained from biosystems (Spain). Kits for albumin (ALB) and total protein were obtained from HUMAN Diagnostics (Germany). The diagnostic kits of malondialdehyde (MDA) concentration, catalase (CAT) and Glutathione (GSH) activities were obtained from Biodiagnostic Company (Cairo, Egypt).

2.3. Experimental design:

In this work, twenty-four male Wistar rats were randomly divided into four equal groups of 6 rats each as follows: Control rats (group 1): rats received corn oil orally daily and fed on standard diet for 5 weeks. Star anise oil administered rats (group 2); rats received star anise oil (1ml/kg orally) every day via gastric tube for 5 weeks.

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Carbon tetrachloride (CC\textsubscript{4}) injected rats (group 3): rats underwent the induction of hepatic injury by injecting CC\textsubscript{4} (1:3 in corn oil, 2.5 ml/Kg, 3 doses at 72 hr interval IP) for 5 weeks (Kumar et al., 2010).

CC\textsubscript{4} injected rats treated with star anise oil (group 4): rats received star anise oil (1 ml/kg orally and daily) plus CC\textsubscript{4} (as in group 3) for 5 weeks.

2.4. Sampling:

Blood and tissue sampling:

Five weeks after CC\textsubscript{4} administration, after 12 hours of fasting, the blood samples were obtained from retro-orbital venous plexus using a fine-walled Pasteur pipette. Blood was collected in plain clean well-dried centrifuge tube for separation of serum to be used in estimation of biochemical parameters. Serum samples were used for quantitative determination of ALT, AST, ALP, total protein and albumin. Subsequently globulin was calculated.

Rats were sacrificed by cervical decapitation, then the liver tissues of rats of all groups were collected for histopathological examination and tissue paraffin sections were routinely prepared and stained with H&E stain according to Bancroft and Layton (2013).

Tissue homogenization for oxidative stress parameters:

The liver specimens were quickly removed, then washed with cold saline then blotted on filter paper. The liver (1 gm) was suspended in 4 ml physiological saline (0.9 % NaCl) for homogenization (Teflon Homogenizer, India). The tissue homogenates were centrifuged 1500 xg for 20 minutes at 4\degree C. The supernatants were kept at -20\degree C till determination of oxidative parameters (Yang et al., 2010).

2.5. Statistical analysis

Statistical analysis was performed using the statistical software package SPSS (Version 18.0; SPSS Inc., Chicago, IL, USA). Differences between groups were evaluated using a one-way ANOVA with a post hoc test (Duncan). For each test, all the data are expressed as the mean ± standard deviation (SEM), and P-value <0.05 was considered significant.

3. RESULTS

3.1. Biochemical changes:

Tables (1&2) revealed changes of hepatic functions tests including AST, ALT, ALP total protein, albumin and globulin. In comparison with normal control rats (group 1), CCl\textsubscript{4} injected rats (group 3) showed significant increases in AST, ALT and ALP activities, while total protein, albumin and globulin values were significantly decreased.

Rats administrated star anise oil (group 2) showed non-significant changes in AST, ALT and ALP when compared with control rats. Also, non-significant changes in total protein, albumin and globulin values were recorded.

CC\textsubscript{4} injected rats treated with Star anise oil (group 4) when compared to CCl\textsubscript{4} injected rats (group3) showed significant decreases in AST, ALT and ALP and significant increase of total protein and albumin and non-significant changes in globulin.

### Table 1 Change in serum liver enzymes activities in different experimental groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>ALP (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (group 1)</td>
<td>47.00±2.21</td>
<td>169.86±10.39</td>
<td>351.00±18.59</td>
</tr>
<tr>
<td>Star anise oil (group 2)</td>
<td>54.80±4.14</td>
<td>189.60±12.04</td>
<td>365.20±37.87</td>
</tr>
<tr>
<td>CCl\textsubscript{4} (group 3)</td>
<td>1827.00±479.77</td>
<td>2796.80±533.91</td>
<td>657.20±90.56</td>
</tr>
<tr>
<td>CCl\textsubscript{4}+Star anise oil (group 4)</td>
<td>1022.60±304.83</td>
<td>1244.00±213.32</td>
<td>543.80±94.44</td>
</tr>
</tbody>
</table>

**Note:** There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter.

### Table 2 Change in serum protein concentrations in different experimental groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Total protein (g/dl)</th>
<th>Albumin (g/dl)</th>
<th>Globulin (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (group 1)</td>
<td>6.55±0.06*</td>
<td>3.53±0.29*</td>
<td>3.02±0.05*</td>
</tr>
<tr>
<td>Star anise oil (group 2)</td>
<td>6.64±0.59*</td>
<td>3.53±0.29*</td>
<td>3.11±0.41*</td>
</tr>
<tr>
<td>CCl\textsubscript{4} (group 3)</td>
<td>3.5±0.04*</td>
<td>2.0±0.09*</td>
<td>3.30±1.1*</td>
</tr>
<tr>
<td>CCl\textsubscript{4}+Star anise oil (group 4)</td>
<td>6.54±0.16*</td>
<td>3.23±0.24*</td>
<td>3.11±0.16*</td>
</tr>
</tbody>
</table>

**Note:** There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter.

3.2. Changes in antioxidant parameters:

Table (3) illustrated the changes in antioxidant parameters including CAT, MDA and GSH. MDA was significantly increased and level of GSH and CAT activity were significantly decreases in in CCl\textsubscript{4} injected rats (group 3) compared with control rats.

In comparison with control rats, rats administrated star anise oil (group 2) revealed non-significant changes in MDA, GSH and CAT. CCl\textsubscript{4} injected rats and treated with Star anise oil (group 4) showed increase in GSH and CAT with significant decrease in MDA when compared with CCl\textsubscript{4} injected rats (group 3).

3.3. Histopathological finding in liver tissue:

The liver of control rats (group 1) and rats administrated star anise oil (group 2) showed normal hepatocytes arranged in cords around the central vein to form hepatic plates which separated with blood sinusoids.

Rats injected with CCl\textsubscript{4} (group 3) there was marked hepatic tissue alteration represented by hepatic degeneration and necrosis. Most of hepatocytes around the central vein showed coagulative necrosis with congestion of portal blood vessels.

The liver of CCl\textsubscript{4} injected rats treated with star anise oil demonstrated noticeable decrease of fatty change (to mild and moderate degree), hepatic necrosis and inflammation.

4. DISCUSSION

In this work regarding to liver enzymes, CCl\textsubscript{4} injected rats (group 3) showed dramatic alterations in liver functions manifested by significant elevated in ALT and AST activities when compared to control rats. These results were supported by those recorded by Huang et al., (2012).

CC\textsubscript{4} caused injury of the hepatocyte’s membrane and leakage of the cytosol enzymes. It may be attributed to the reactive intermediate free radicals (trichloromethyl (CCl\textsubscript{3}), trichloromethylperoxy (CCl\textsubscript{3}O\textsubscript{2}) these radicals are produced
by CCl₄ bioactivation by cytochrome P450, resulting in lipid peroxidation (Weber et al., 2003). Also, CCl₄ injected rats (group 3) showed an increase in ALP level. This result was agreed with Deb, (1998) and Li et al., (2015). These alterations may be attributed to the increase synthesis ALP due to the biliary tract involvement (Muriel et al., 1992).

On the other hand, CCl₄ injected rats (group 3), showed decreases in total protein, albumin and globulin. Saba et al. (2010) reported this reduction due to decrease number of intact hepatocytes which result in decrease hepatic capacity to synthesize protein. These results confirmed by histopathological examination of liver of CCl₄ treated rats (group 3) which revealed coagulative necrosis and degeneration of hepatocytes with infiltration of the inflammatory cells (macrophages and lymphocytes). In addition, Congestion of the portal blood vessels with periportal attempts of hepatic proliferation and fibroblastic cells proliferation were recorded (Figure 1). These findings came in agreement with Al-Asmari et al.(2015).

Table 3  Changes in oxidative stress makers (CAT, MDA and GSH) in hepatic tissues of in different experimental groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>CAT (ng/mg tissues)</th>
<th>MDA (n mol/mg tissues)</th>
<th>GSH (ng/mg tissues)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (group 1)</td>
<td>20.7±1.97</td>
<td>23.0±1.99</td>
<td>30.4±2.46</td>
</tr>
<tr>
<td>Star anise oil (group 2)</td>
<td>22.9±3.1</td>
<td>20.5±2.3</td>
<td>34.2±3.1</td>
</tr>
<tr>
<td>CCl₄ (group 3)</td>
<td>13.0±1.00</td>
<td>51.6±4.41</td>
<td>9.3±0.33</td>
</tr>
<tr>
<td>CCl₄+Star anise oil (group 4)</td>
<td>18.5±0.50</td>
<td>32.1±4.05</td>
<td>14.3±2.98</td>
</tr>
</tbody>
</table>

*: There is no significant difference (P>0.05) between any two means, within the same column have the same superscript letter.

Table 4 Semiquantitative scoring of hepatic lesions within different treated groups on fifth week of the experiment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Vascular lesions</th>
<th>Degeneration</th>
<th>Necrosis</th>
<th>Inflammatory cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (group 1)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Star anise oil (group 2)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CCl₄ (group 3)</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>CCl₄+OIL (group 4)</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

(-) means no detectable lesions; (+) indicates mild lesions; (++) indicates moderate lesions; (+++) indicates severe focal lesions. Saber et al, (2019)

Fig. 1 Liver of rat in control group I (A) showing normal hepatic parenchyma (arrow indicates normal hepatocytes and PA means portal area). Liver of rats received star anise oil; group II (B) showing hepatic cells (arrow) around the portal area (PA). Liver of CCl₄ intoxicated rats; group III (C) showing necrosis and fatty degeneration of hepatocytes with proliferation of biliary epithelium and fibroblasts in the portal area and oval cells proliferation (arrowheads indicates necrotic cells, PA indicates portal area and arrow indicates oval cells). Liver of rats injected CCl₄ and treated with star anise; group IV (D) showing hepatic vacuolation (arrow) mixed few mononuclear cells infiltration mostly macrophages and lymphocytes (arrowhead), H&E, X200.

CCl₄ injected rats and treated with star anise (group 4) showed significantly decreased in liver enzymes (ALT, AST and ALP) and significantly increased in total protein, albumin and globulin compared to CCl₄ injected rats (group 3). These findings were in line with study of Mayer et al., (2005). This might be due to star anise oil contains natural
products ascorbic acid, sterols and squalene, which could reduce the membrane peroxidation and the leakage of enzymes Yavuc et al. (2004). Also, CCl4 injected rats treated with star anise (group 4) showed improvement in serum ALT and AST, ALP activities. These results were confirmed with those report of (Gbadegesin and Odunola., 2010). These results indicated that star anise could protected the injured hepatocytes from injuries and so improved the functions of liver (Bhattacharya et al., 2013). Star anise oil is rich sources of flavonoids which have various biological properties related to antioxidant mechanisms that were responsible for their hepatoprotective effects (Meera et al., 2009). These results confirmed by histopathological examination of liver of CCl4 treated rats star anise (group 4) which revealed improvement in the microscopic picture of the examined liver where mild hepatic degeneration were recorded. This results were supported by those mentioned by Morrison et al. (2015). The protective role of star anise oil in CCl4 hepatotoxicity, acted by antioxidative, anti-fibrotic, anti-lipid peroxidative, anti-inflammatory, membrane stabilizing, and liver regenerating mechanisms (Diaz, 2006). Also, star anise oil contained methyl eugenol which has antioxidant and anti-inflammatory properties (Krenkel and Tacke, 2017).

Concerning to antioxidants parameters, the increase of MDA in CCl4 injected rats (group 3) compared to control rats (group 1) could be attributed to the trichloromethyl radicals that resulted from CCl4 metabolism stimulate the lipid peroxidation process with formation of by-products such as MDA, leading to membrane integrity loss and the death of cell (Boll et al., 2001). Also, the hepatic antioxidant enzymes GSH and CAT activities were significantly decreased in CCl4 injected rats (group 3) compared with control rats. Decrease in enzyme activity of GSH and CAT activities, may be attributed to the deactivation of these isoenzymes by oxidation of a cysteine residue near the active center in the CCl4 injected rats (Tama et al., 1990). CCl4 injected rats treated with Star anise oil (group 4) showed a significant decrease in MDA levels. Although, CAT and GSH activities were significantly increased This findings could attribute to Star anise oil inhibits metabolic processes and thus lipid peroxidation and MDA decrease by its powerful free-radical scavenging properties chain-breaking antioxidant (Li et al., 2010). Also, the presence of flavonoids with strong antioxidant activities are responsible for scavenging the produced superoxide anion and hydroxyl radicals (Deleve et al., 1991). Anethole which present in star anise oil inhibited the reactive oxygen species (ROS) production therefore, anethole prevents oxidative damage and has therapeutic potential (Bouthillier et al., 1996), while increased GSH level and decreased MDA level in groups pretreated with either anise oil or anethole was due to enzymic activity of the oil (Domiciano et al., 2013)

5. CONCULSION

The bottom line here is that star anise oil has protective effect against CCl4 hepatotoxicity in rats.

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DISCLOSURE OF CONFLICT OF INTEREST:

None.

CONSENT FOR PUBLICATION:

All authors agreed to publish this manuscript.

COMPETING INTERESTS:

The authors declare that they have no competing interests.

6. REFERENCES


