HEPATOPROTECTIVE EFFECT OF PIMPINELLA ANISUM AND FOeniculum vulgare AGAINST CARBON TETRACHLORIDE INDUCED FIBROSIS IN RATS

El-Sayed, M. G. A.*, Elkomy, A.*, Sahar Samer** and ElBanna, A. H.***

*Department of Pharmacology, Faculty of Veterinary Medicine Banha University.
**Department of Pathology, Faculty of Veterinary Medicine, Cairo University.
***Student Hospital, Cairo University.

ABSTRACT

Medicinal plants have been used traditionally worldwide for the prevention and treatment of liver disease. Pimpinella anisum (Anise) or Foeniculum vulgare (Fennel) are used frequently as spices. The present work aimed to investigate the possible potential protective effect of Anise and Fennel essential oils, against carbon tetrachloride (CCL4) induced fibrosis in rats. Administration of ccl4 (1.5ml/kg /kg.b.wt ) intra-peritoneally (IP) in olive oil (1:7 dilution) for 7 successive weeks resulted in liver damage manifested by significant increase in serum AST,ALT, ALP, decreased total protein and increased triglycerides, total cholesterol, LDL while decreased the HDL level. Rats treated orally with essential oil of Pimpinella anisum (Anise, 125 &250mg/kg) or Foeniculum vulgare (Fennel, 200 &400kg/b.wt.) for 7 successive weeks and intoxicated with CCL4 showed a significant protection against-induced increase in serum liver enzyme (AST,ALT, ALP), restored total protein level and ameliorate the increased triglycerides, total, cholesterol, LDL and decreased the HDL. A significant corrective effect of either Anise or fennel oils on biochemical parameters were supported by histopathological examination of the rats. In conclusion, these data indicated that essential oils of Pimpinella anisum (Anise) or Foeniculum vulgare (Fennel) possessed a hepatoprotective activity against hepatotoxicity induced by CCL4 induced fibrosis model in rats.

KEYWORDS: Foeniculum vulgare, Pimpinella anisum.
2- INTRODUCTION
Liver is a vital organ play a an important vital function in metabolism, maintenance, performance and regulating homeostasis of the body and excretion of xenobiotics from the body. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction. And its functions as a centre of metabolism of nutrients such as carbohydrates, proteins and lipids and excretion of waste metabolites. Therefore, maintenance of a healthy liver is essential for the overall well being of an individual.[1]

Liver diseases are a major worldwide health problem, with high endemicity in developing countries. Liver cell injury caused by various toxicants such as certain chemotherapeutic agents, carbon tetrachloride, thioacetamide, chronic alcohol consumption and microbes are common. Enhanced lipid per oxidation during metabolism of ethanol may result in development of hepatitis leading to cirrhosis.[2] Despite advances in modern medicine, there is no effective drug available that stimulates liver function, offer protection to the liver from damage or help to regenerate hepatic cells. Herbal drugs have gained importance and popularity in recent years because of their safety, efficacy and cost effectiveness. One of the important and well documented uses of plant products is their use as hepatoprotective agents. Hence, there is an ever increasing need for safe hepatoprotective agent.[3] Several medicinal plants have been extensively used in traditional system of medicine for the management of liver disorder. Some of these plants have already been reported to posse’s strong antioxidant activity.[4] But management of liver disorders by a simple and precise herbal drug is still an intriguing problem. Essential oils (EOs), also known as volatile oils, are concentrated natural plant products which contain volatile aroma compounds.

These mixtures of volatile compounds (mainly mono- and sesquiterpenoids, benzoids, phenylpropanoids, etc.) exert different biological actions on humans, animals, and other plants.[5]

Fennel (Foeniculum vulgare Mill., family Umbelliferae) is an annual, biennial or perennial aromatic herb, depending on the variety, which has been known since antiquity in Europe and Asia Minor. The leaves, stalks and seeds (fruits) of the plant are edible. Foeniculum vulgare is an aromatic herb whose fruits are oblong, ellipsoid or cylindrical, straight or slightly curved and greenish or yellowish brown in colour.[6] Much work has recently been done on
the yield and composition of both extracts and essential oils of fennel of several varieties from several locations.[7]

*Pimpinella anisum* is an annual herb in digeneous to Iran, India, Turkey and Egypt and many other warm regions in the world. As a medicinal plant, anise has been used as stimulating effect of digestion and antiparasitic[8], antibacterial[9] and antipyretic[10] and could have some direct antiviral effects. In folk medicine, *Pimpinella* species have been used as analgesic, antiinflammatory, appetizing, hypnotic, expectorant, antibacterial and hepatoprotective agents and to increase milk secretion.[11]

The aim of the present study is to extract the essential oils of the tested plants, determine the acute toxicity study of the extracted oils and to investigate the hepatoprotective effect of the extracted oils.

3. MATERIAL AND METHODS

3.1. Plants

*Foeniculum vulgare* (Fennel) or *Pimpinella anisum* (anise) seed were obtained from local market, and taxonomic identifications were established by the staff members of the Department of botany, Faculty of science, Cairo university.

Essential oils of *Pimpinella anisum* (anise) and *Foeniculum vulgare* (Fennel) were prepared by steam distillation as described by.[12]

3.2. Animals

Adult albino rats weighing around 120-140 grams, and mice of 20-25 gram were purchased from Faculty of Veterinary Medicine, Cairo University. They were acclimatized to animal house conditions. Animals were provided with standard diet and water *ad libitum*. Rats and mice were kept under constant environmental condition and observed daily throughout the experiments.

3.3. Determination of LD$_{50}$

LD50 of the studied essential oil of Anise or Fennel were determined as described by Karber.[13]
3.4. Experimental Design
Forty two adult male albino rats were allocated into 7 groups of 6 animals each. Group I (control healthy) received distilled water orally (1 ml per day) for 49 days. The other six groups were given carbon tetrachloride CCL4 (1.5 ml/kg) I.P. in olive oil (1:7 dilution) 3 times per week for 7 successive weeks. Group II was used as a control positive (intoxicated non treated). Group III was used as a standard group and received silymarin orally at a dose of 50 mg/kg b.wt. for same period. Group IV and V given essential oils of Anise (125 and 250 mg/kg b.wt.) while group VI and VII given essential oil of Fennel (200 nad 400 mg/kg.b.wt) orally for 7 consecutive weeks.

Assessment of hepatotoxicity
Liver marker enzymes (alanine aminotransferase (ALT), aspartate aminotransferase (AST) as described by Reitman and Frankel[15], alkaline phosphatase (ALP) [16], Total protein [17], lipid profile [total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL) and triglyceride] were estimated by using standard A diagnostic kits.[18]

3.5. Histopathological examination
At the end of the experiment, the rats were decapitated and livers were removed for histopathological examination. Liver were then fixed in 10 % buffered formalin solution. The fixed specimens were then trimmed, washed and dehydrated in ascending grades of alcohol, cleared in xylene, embedded in paraffin, and sectioned at 4-6 μ thickness and stained with Haematoxylen and Eosin as a routine stain according to the method described by Carleton.[19]

3.6. Statistical Analysis
Statistical analysis of all result, were carried out using analytical software SPSS (version 8.0.) according to.[20]

4. RESULTS
The obtained results showed that LD50 of Pimpinella anisum (Anise) was found to be 2520mg/kg body weight while that of Foeniculum vulgare (Fennel) essential oils was found to be 4000 mg/kg body weight

Administration of CCl4 increased triglycerides, total, cholesterol, LDL while decreased the HDL as shown in, Table 1. Oral administration of Anise in both tested doses 125 mg/kg bw and 250 mg/kg bw significantly restored the increased level of triglycerides, total,
cholesterol, LDL and increased level of HDL. In addition, concentration of HDL was significantly (P<0.05) increased by Fennel administration in both tested dose (200&400 mg/kg. body weight) whereas concentration of triglycerides, total cholesterol and LDL was appreciably (P<0.05) augmented to compensate the CCl4-induced toxicity.

The results of the hepatoprotective effect of Anise in both tested dose 125 and 250 mg/kg bw.t against CCl4-intoxicated rats are shown in Table 2. In the CCl4-treated group, serum AST, ALT and ALP levels were quite high while total protein was low. In contrast, the CCl4 group treated with Anise in both tested doses significantly lower levels of AST and ALT and increased total protein level when compared with the CCl4 control group. In addition, administration of Fennel in both tested doses (200&400 mg/kg. body weight) significantly decreased (P<0.05) serum AST, ALT and ALP levels and increased total protein level as compared to the CCl4 group.

The examined livers of rats intoxicated with CCL4 induced fibrosis model, revealed that septa of bridging fibrosis consisted of inflammatory cells, fine collagen fibers and dilated vessels. Notice apoptotic cells among the degenerated hepatocytes (Fig. 1b). Oral administration of Anise –treated rats showing restoration of the hepatic architecture(fig1,c) and Fennel -treated rats showing mild degree of macro- and microvesicular-steatosis among the hepatocytes ( Fig1,d).

Table (1): Effect of Pimpinella anisum (Anise) or Foeniculum vulgare (Fennel) on Lipids profile (Mean ±SE., n=5).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose mg/kg body weight</th>
<th>Triglycerides (mg/dl)</th>
<th>Total cholesterol (mg/dl)</th>
<th>High density lipoprotein (mg/dl)</th>
<th>Low density lipoprotein (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Healthy</td>
<td>0.0</td>
<td>7.4±0.37b</td>
<td>6.10±0.29b</td>
<td>3.51±0.26a</td>
<td>2.40±0.38b</td>
</tr>
<tr>
<td>Control Positive</td>
<td>3ml/kg CCl4</td>
<td>12.4±0.79a</td>
<td>11.95±0.57a</td>
<td>2.54±0.15b</td>
<td>8.66±0.28a</td>
</tr>
<tr>
<td>Anise</td>
<td>CCl4 +125</td>
<td>8.51±0.44b</td>
<td>6.92±0.55b</td>
<td>3.12±0.32a</td>
<td>3.0±0.3b</td>
</tr>
<tr>
<td></td>
<td>CCl4 +250</td>
<td>7.88±0.98b</td>
<td>6.55±0.95b</td>
<td>3.47±0.024a</td>
<td>2.68±0.29b</td>
</tr>
<tr>
<td>Fennel</td>
<td>CCl4 +200</td>
<td>7.85±0.67b</td>
<td>6.44±0.63b</td>
<td>3.44±0.28a</td>
<td>2.81±0.21b</td>
</tr>
<tr>
<td></td>
<td>CCl4 +400</td>
<td>7.52±0.58b</td>
<td>6.23±0.17b</td>
<td>3.6±0.31a</td>
<td>2.54±0.17b</td>
</tr>
<tr>
<td>Silymarin</td>
<td>CCl4 +50</td>
<td>7.92±0.22b</td>
<td>6.45±0.23b</td>
<td>3.32±0.15a</td>
<td>2.94±0.28b</td>
</tr>
</tbody>
</table>
Table (2): Effect of *Pimpinella anisum* (Anise) or *Foeniculum vulgare* (Fennel) on AST, ALT, ALT and Total protein levels, (Mean ±SE., n=5).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose mg/kg body weight</th>
<th>AST (U/L)</th>
<th>ALT (U/L)</th>
<th>ALP (U/L)</th>
<th>Total protein (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Healthy</td>
<td>0.0</td>
<td>30.96 ± 1.92c</td>
<td>21.28 ± 1.43 c</td>
<td>32.4±0.26c</td>
<td>3.94 ± 0.16 a</td>
</tr>
<tr>
<td>Control Positive</td>
<td>3 ml/kg CCl4</td>
<td>94.87 ± 3.74 a</td>
<td>71.54 ± 3.19 a</td>
<td>115.7 ± 6.54a</td>
<td>3.1±0.17b</td>
</tr>
<tr>
<td>Anise</td>
<td>CCl4 +125</td>
<td>49.5±1.455b</td>
<td>32.58±1.68b</td>
<td>45.8±1.78b</td>
<td>3.5±0.3a</td>
</tr>
<tr>
<td></td>
<td>CCl4 +250</td>
<td>35.87±2.34c</td>
<td>23.7±1.24c</td>
<td>35.2±2.31c</td>
<td>3.74±0.26a</td>
</tr>
<tr>
<td>Fennel</td>
<td>CCl4 +200</td>
<td>36.8±3.57c</td>
<td>24.8±2.57c</td>
<td>33.5±2.58c</td>
<td>3.77±0.23a</td>
</tr>
<tr>
<td></td>
<td>CCl4 +400</td>
<td>31.2±1.24c</td>
<td>20.4±1.36c</td>
<td>31.8±1.38c</td>
<td>3.88±0.15a</td>
</tr>
<tr>
<td>Silymarin</td>
<td>CCl4 +50</td>
<td>33.8±2.58c</td>
<td>24.9±2.78c</td>
<td>33.4±2.45c</td>
<td>3.8±0.21a</td>
</tr>
</tbody>
</table>

Values within a column with different letters are significantly different (≥0.05)

Figure 1: a- Liver of control negative rats, showing normal hepatic architecture. b- Liver of CCl4-treated intoxicated rats showing septa of bridging fibrosis consisted of inflammatory cells, fine collagen fibers and dilated vessels. Notice apoptotic cells (arrow) among the degenerated hepatocytes. c- Liver of Anise -treated rat (250mg/kg.) Showing restoration of the hepatic architecture. d- Liver of Fennel -treated rats (200mg/kg.b.et) showing mild degree of macro- and microvesicular-steatosis among the hepatocytes.
5. DISCUSSION

The obtained results showed that LD50 of *Pimpinella anisum* essential oils was found to be 2525mg/kg body weight while LD50 of *Foeniculum vulgare* essential oils was found to be 4000 mg/kg body weight. These values for LD50 was consistent with that previously reported\[^{21}\] for Anise essential oil 2.7 g. In addition, values of the oral LD50 corresponding to 3.8 g/kg b.w. had been reported previously for fennel oil in rats.\[^{22}\] These results may indicate safety of essential oils isolated from either Anise or Fennel when administrated orally as the acute administration of 2 g/kg dose was reported to be the ceiling point for acute oral toxicity of medicinal plants.\[^{23}\]

The liver is the key organ of metabolism, secretion and excretion and it is continuously and variedly exposed to xenobiotics, environmental pollutants and chemotherapeutic agents because of its strategic location in the body. Conventional drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effects. It is, therefore, necessary to search for alternative drugs for the treatment of liver disease to replace currently used drugs of doubtful efficacy and safety. Hepatoprotective activity of either Pimpinella Anisum (Anise) or *Foeniculum vulgare* (fennel) essential oil was studied using a carbon tetrachloride-induced liver fibrosis model in rats.

CCl4 induces centro-lobular necrosis in rat liver and it is transformed to tricholoromethyle free radical (CCl3) by sitocrom-P450. CCl3 reacts with oxygen to form peroksil radical (CCl3O2), which powerfully induces lipid peroxidation. Thus, CCl4 causes oxidative destruction of liver cell membranes and serious tissue damage in rats.\[^{24}\]

The obtained results showed that, administration of CCl4 increased triglycerides, total, cholesterol, LDL while decreased the HDL, which is restored after Anise or fennel treatment. In addition, concentration of HDL was significantly increased by Anise (125 and 250 mg/kg.b.wt) or Fennel (200&400 mg/kg. body weight), administration whereas concentration of triglycerides, total cholesterol and LDL was appreciably (P<0.05) augmented to compensate the CCl4-induced toxicity. Importantly, the increased serum concentrations of triglycerides, total cholesterol and LDL, and the decreased level of HDL, were restored to normal values with Anise or Fennel co-treatment. This may be explained on the basis that Anise or Fennel has a strong ability to chelate multivalent metal ions, especially zinc, calcium and iron. Indeed, its ability to chelate minerals has been reported to have some protective effects, such as decreasing iron mediated free radical formation and lowering
serum cholesterol, triglycerides and lipid peroxides in experimental animals. Similar findings were reported in another study that investigated the hepatoprotective effects of *pimpinella anisum oil* (PFO) for the prevention of carbon tetrachloride (CCl4) induced liver injury in rats.

The present study revealed that CCl4-induction in rats remarkably increased the level of ALT, AST, ALP and decreased protein levels. CCl4 causes hepatocyte injuries, altered membrane integrity and as a result enzymes in hepatocytes leak out. However, after treatment with either Anise or Fennel essential oils, the pathological increases in ALT, AST, and ALP were significantly restored.

These results indicate that Anise or Fennel essential oils has the ability to protect against CCl4-induced hepatocyte injury, which is in agreement with a previous study that reported the protective consequence of polyphenolic compounds against CCl4-induced liver cirrhosis. The abnormal high level of serum ALT, AST, ALP observed in our study are the consequence of CCl4 induced liver and denotes the damage to the hepatic cells dysfunction. Treatment with essential oils of Anise or fennel reduced the enhanced level of serum ALT, AST and ALP which seem to offer the protection and maintain the functional integrity of hepatic cells. A reduction in total serum protein observed in the CCl4-treated rats may be associated with the decrease in the number of hepatocytes which in turn may result into decreased hepatic capacity to synthesize protein and consequently decrease in the liver weight. But, when the essential oils of Anise or fennel were given along with CCl4, the significant increase in total protein was observed indicating the hepatoprotection activity of the essential oil. In addition, Diaaz-Maroto et. al., reported that, *F. vulgare* has been reported to contain 6.3% of moisture, 9.5% protein, 10% fat, 13.4% minerals, 18.5% fiber and 42.3% carbohydrates. The minerals and vitamins present in *F. vulgare* are calcium, potassium, sodium, iron, phosphorus, thiamine, riboflavin, niacin and vitamin C. *F. vulgare* is well known for its essential oil. CCl4 induced liver damage observed by increases in serum ALT and AST levels and histopathological findings. Biochemical findings showed that serum ALT and AST levels were significantly lower in the Anise essential oil group compared to CCl4 group. Decreased serum AST and ALT levels suggest that PFO could prevent liver cell damage.

In view of the present findings it was observed that Anise and Fennel provided good degree of protection against CCl4-induced hepatic toxicity as was evident by histopathological
evaluation. The restorative effect of Fennel on the CCl4-induced hepatic histopathological changes was more obvious than that achieved by Anise. Both of the two essential oil significantly improved the state of steatosis with a significant reduction in the number of macro- and microvesicular steatosis. The normal hepatic architecture was restored and both compounds apparently suppressed hepatic fibrogenesis as there was no obvious fibrous proliferation and bridging fibrosis nor pseudolobulation of the hepatic parenchyma. Only variable degrees of vacuolar degeneration with mild fatty change, necrosis of the hepatocytes. While, proliferation in the portal triad was still exist.

The observation of a significant corrective effect of either fennel or Anise oils on biochemical parameters were supported by histopathological examination and the changes in the body weights of the rats. In the present study, we have shown that essential oils obtained from Fennel or Anise have a protective effect against the chronic toxicity induced by CCl4 in rats. In this respect, Özbek et al[14] reported that Fennel essential oil and Cengiz, et. al.,[26] found that Anise essential oil has a potent hepatoprotective action against CCl4-induced acute liver injury in rats.

In conclusion, these data indicate that essential oils of Pimpinella anisum (Anise) or Foeniculum vulgare (Fennel) possessed a hepatoprotective activity against hepatotoxicity induced by CCL4 induced fibrosis model in rats.

6. REFERENCES


