Hepatoprotective effect of *Foeniculum vulgare* essential oil: A carbon-tetrachloride induced liver fibrosis model in rats.

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Summary

Hepatoprotective activity of *Foeniculum vulgare* (fennel) essential oil was studied using a carbon tetrachloride-induced liver fibrosis model in rats. The hepatotoxicity produced by chronic carbon tetrachloride administration was found to be inhibited by *Foeniculum vulgare* essential oil with evidence of decreased levels of serum aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and bilirubin. Histopathological findings also suggest that *Foeniculum vulgare* essential oil prevents the development of chronic liver damage. The changes in body weights in the rats assigned to the study groups supported these biochemical and histopathological findings. The results of this study clearly indicate that *Foeniculum vulgare* essential oil has a potent hepatoprotective action against carbon tetrachloride-induced liver fibrosis in rats.

Keywords: Foeniculum Vulgare Miller; Fennel essential oil; FEO, Carbon tetrachloride; Liver fibrosis; Hepatoprotective effect.

Introduction

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. Each fruit weighs between 6 and 7 mg, has conspicuous vittae, is about 6 mm long and 2 mm wide in central portion (*Warrier et al*). The dried, aromatic fruits are widely employed in culinary preparations for flavoring bread and pastry, in candies, and in alcoholic liqueurs, as well as in cosmetic and medicinal preparations (*Farrell, 1985; Hänsel et al., 1993*). 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 (*Embong et al., 1977; Miura et al., 1986; Akgül et al., 1988; Katsiotis et al., 1988; Verghese 1988; Arslan et al., 1989; Gupta et al., 1995; Venskutonis et al., 1996*). Trans-anethole and fenchone are the most important volatile components of *Foeniculum vulgare* essential oil (FEO). In the essential oil of sweet fennel the fenchone content usually does not exceed 5%, whereas in the bitter types its content can be as high as 20%. In sweet fennel oil the anethole content reaches 84-90%, whereas its proportion in bitter fennel is about 61-70% (*Lawrence 1994; Bernath et al. 1996*). Volatile components of fennel seed extracts by chromatographic analysis include trans-anethole, fenchone, methylchavicol, limonene, α-pinene, camphene, ß-pinene, ß-myrcene, α-phellandrene, 3-carene, camphor, and cisanethole (*Simándi et al., 1999; Özcan et al., 2001*). Özbek (*Özbek; 2001*) suggested that the value of LD_{50} of FEO was 1.038 ml/kg in mice. Fennel and its herbal drug preparations are used for dyspeptic complaints such as mild, spasmodic

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gastric-intestinal complaints, bloating, and flatulence. It is also used for the catarrh of the upper respiratory tract (*Czygane 1989; Madaus 1976; Merkes 1980; Forster et al. 1980; Forster 1983; Weib 1991*). It has been reported that fennel essential oil is used in the pediatric colic and some respiratory disorders due to its anti-spasmodic effects (*Reynolds 1982*). The seeds of this plant have been known as a promoter of menstruation, to alleviate the symptoms of the menopause, and increase libido (*Albert-Puleo 1980*). Özbek et al have reported that FEO has a potent hepatoprotective action against CCl_4-induced acute liver injury in rats (*Özbek et al, 2003*). The liver is the key organ of metabolism, secretion and excretion and it is continously and variedly exposed to xenobiotics, environmental pollutants and chemotherapeutic agents because of its strategic location in the body. Liver disease is a worldwide problem. 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. In this publication, we report the hepatoprotective effects of FEO on carbon tetrachloride-induced liver fibrosis in rats.

Materials and Methods

Plant materials

The fennel seeds used were purchased from a local market from Van in Turkey. A voucher specimen (B-02) has been kept in our laboratory for future reference.

Chemicals

Carbon tetrachloride (CCl_4) was obtained from Merck KgaA, 64271 Darmstadt, Germany. All other chemicals were obtained from local sources and were analytical grade.

Animals

Male, outbred, Sprague-Dawley rats weighing 180-200 g were maintened in the Animal House of YuzuncuYil University, Faculty of Medicine. The rats were bred in our institutional animal house but the lineage originally came from Ankara Health Protection Institute (a governmental organisation). The animals were housed in standard cages with food and water *ad libitum*, at room temparature (22±2°C), with artificial light from 7.00 am to 7.00 pm, and provided with pelleted food (Van Animal Feed Factory, Van-TURKEY). Ambient temp was 22±2°C, ambient RH was % 55-60 and the rats housed in groups. The approval of the Animal Ethics Committee was obtained.

Isolation of FEO

The seeds were powdered in a mixer, placed in a distillation flask and the oil was collected by steam distillation, to obtain the essential oil with 1% yield.

Analysis of essential oil

Analysis of the the oil was carried out by *"Anadolu Üniversitesi Tibbi ve Aromatik Bitki ve Ilaç Arastirma Merkezi, Eskisehir/TURKEY"*. Gas chromatography analysis was çarried out on a Shimadzu GC-9A gas chromatograph with FID detector and a Thermon-600 T capillary column (50 mL., 0.25 mm I.D.). The operating condition was as follows. Carrier gas was nitrogen with a split rate of 60:1, the oven temperature for first 10 min was kept at 70°C and then increased at a rate of 2°C/min until 180°C, injector and detector temperature were set at 250°C.

Carbon tetrachloride model for evaluation of anti-hepatotoxic activity

The CCl_4 model described by Rojkind (*Rojkind 1973*) was used for scheduling the dose regimen. 1.5 ml/kg i.p. of carbon tetrachloride diluted in olive oil (1:7 dilution) was employed for inducing liver toxicity.

Experimental procedure

Twenty-four albino rats were divided into three groups of eight animals each. Group I, which served as normal control, received isotonic saline solution 0.2 ml intraperitoneally (i.p.), group II received CCl_4:

olive oil (1:7) 1.5 ml/kg i.p. and group III received CCl_4 1.5 ml/kg + FEO 0.3 ml/kg i.p. (all three times a week for seven weeks). The dose of FEO was determined according to Özbek et al (*Özbek et al; 2003*). CCl_4 and FEO were not put in the same injector. They were injected into different region of the rat abdomen. All the animals were observed daily and any dead animals were subjected to post-mortem examination to find the cause of death. At the end of the treatment, blood samples were collected by direct cardiac puncture and the serum was used for the assay of marker enzymes, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and bilirubin. Body weights of the rats were measured three times a week during seven weeks. Daily changes in body weights as percentages were recorded. The percentage of daily changes in body weights was calculated according to the following formula:

Change in body weights (%) = 100 X (Weight$_n$ - Weight$_{initial}$) / Weight$_{initial}$

Assessment of liver function

The serum AST, ALT, ALP and bilirubin concentrations were determined with a Roche Modular Autoanalyzer.

Histopathological examination of the liver

The livers of the experimental animals were fixed in 10 % neutral buffered-formalin prior to routine processing in paraffin-embedded blocks. Sections (4 µm thick) were cut and stained using hematoxylin-eosin (HE), reticulin and Masson's Trichrome stain.

Statistical analysis

Results of the biochemical estimations and the body weights of the rats are reported as mean ± S.E.M. (Standard Error of Mean). The total variation was analysed by performing one-way analysis of variance. "LSD (Least Significant Difference) test" was used for determining significance (*Sümbüloğlu 1998*). Probability levels of less than 0.05 were considered significant.

Results

Analysis of essential oil

The composition of the essential oil we used is summarised in Table 1. (E)- anethole and limonene are the two major constituents.

Table 1. Gas chromatography analysis of *F. vulgare* essential oil.

Compound	%
(*E*)-anethole	74.8
limonene	11.1
methyl chavicol	4.7
fenchone	2.5
α-pinene	1.3
(Z)-ß-ocimene	1.2

Effects of FEO on AST, ALT, ALP and bilirubin levels

The results of the hepatoprotective effect of FEO on CCl_4-intoxicated rats are shown in Table 2. In the CCl_4-treated group, serum AST, ALT and ALP levels were quite high. In contrast, the CCl_4 group treated with FEO had significantly lower levels of AST and ALT when compared with the CCl_4 control group.

Histopathological examination

Histopathological studies demonstrated that CCl_4 (compared to normal) induces ballooning degeneration and apoptotic bodies in hepatocytes, cirrhotic nodules and lymphocytic infiltration in portal areas. In carbon tetrachloride-treated livers, drastic alterations were observed. Histopathological examination showed extensive diffuse ballooning degeneration. Ballooned hepatocytes were of different sizes and much larger than normal hepatocytes and occasionally appeared as confluent areas (Fig. 1). Apoptotic bodies, cirrhotic nodules (Fig 2) and lymphocytic infiltration in portal areas were frequently present. FEO + CCl_4 treated livers showed significant recovery. In histopathological examination, ballooning degeneration, apoptotic bodies in hepatocytes and lymphocytic infiltration in portal areas

Table 2. Effect of FEO on serum levels of AST, ALT, ALP and bilirubin of rats treated with CCl_4 (*n*=8)

Treatment	AST	ALT	ALP	Bilirubin
	Serum (U/L)	Serum (U/L)	Serum (U/L)	mg/dl
Control	103.33±026.41	77.33±019.79	361,50±15.20	0.125±0.015
CCl_4	[a] 1392.50±300.50	[a] 1269.66±231.39	[a] 757.66±70.94	0.153±0.035
FEO	[c] 512.60±108.32	[c] 552.00±119.18	[b] 640.40±25.50	0.128±0.016
F-value	*12.234*	*15.887*	*20.226*	*0.416*
p-value	*(p<0.01)*	*(p<0.001)*	*(P<0.001)*	*(p>0.05)*

The values represent the mean ± S.E.M. (standard error mean).
Post-hoc LSD (least significant difference) test:
a : p<0.001 with respect to control group.
b : p<0.01 with respect to control group.
c : p<0.01 with respect to CCl_4 group.

Table 3. Weekly changes as percentage in body weights of the rats.

	Measurements					
Groups	**1**	**2**	**3**	**4**	**5**	**6**
Control	0.57±0.45	5.10±0.66	4.62±0.82	4.08±0.83	5.68±0.84	6.67±1.10
CCl4	[a] 4.92±0.90	[a] 11.56±1.61	[a] 14.76±3.00	[a] 16.62±2.66	[a] 22.05±3.68	[a] 24.46±3.60
FEO	[d] -1.31±1.36	[d] 1.29±1.84	[d] -0.11±2.13	[d] 0.16±2.64	[c] 5.17±3.31	[b] 12.85±5.44
F-value	*11.561*	*13.068*	*11.942*	*15.605*	*11.459*	*6.507*
p-value	*P<0.01*	*P<0.001*	*P<0.01*	*P<0.001*	*P<0.01*	*P<0.01*

The values represent the mean ± S.E.M. (standard error mean).
Post-hoc LSD (least significant difference) test:
a : p<0.01 with respect to control group.
b : p<0.05 with respect to CCl4 group.
c : p<0.01 with respect to CCl4 group.
d : p<0.001 with respect to CCl4 group.

were scarce (Fig. 3). Apoptotic bodies and cirrhotic nodules were not observed.

Effects of FEO on body weights of the rats

The effect of FEO on the body weights of CCl_4-intoxicated rats are shown in Table 3 and chart 1. The weekly body weight changes as percentages indicated that the CCl_4 group had a significant increase compared to the control and the FEO groups ($p<0,001$).

Discussion

Histopathological studies demonstrated that CCl_4 (compared to normal) induces degeneration in hepatocytes. According to microscopic examinations, severe hepatic lesions induced by CCl_4 were remarkably reduced by the administration of the extract obtained from fennel, in good agreement with the results of the biochemical tests. The observation of a significant corrective effect of FEO on biochemical parameters was supported by histopathological examination and the changes in the body weights of the rats. In this study, we have shown that FEO extract has a protective effect against the chronic toxicity induced by CCl_4 in rats. Moreover Özbek et al have reported that FEO has a potent hepatoprotective action against CCl_4-induced acute liver injury in rats (*Özbek et al 2003*). What component(s) of the extract is responsible for this effect, however, was not investigated. Volatile components of fennel seed extracts contain, by chromatographic analysis, trans-anethole, fenchone, methylchavicol, limonene, α-pinene, camphene, ß-pinene, ß-myrcene, α-phellandrene, 3-carene, camphor, and cisanethole (*Simándi et al. 1999*). Among these, d-limonene and ß-myrcene have been shown to affect liver function. D-limonene increases the concentration of reduced glutathione (GSH) in the liver (*Reicks & Crankshaw 1993*). Glutathione in

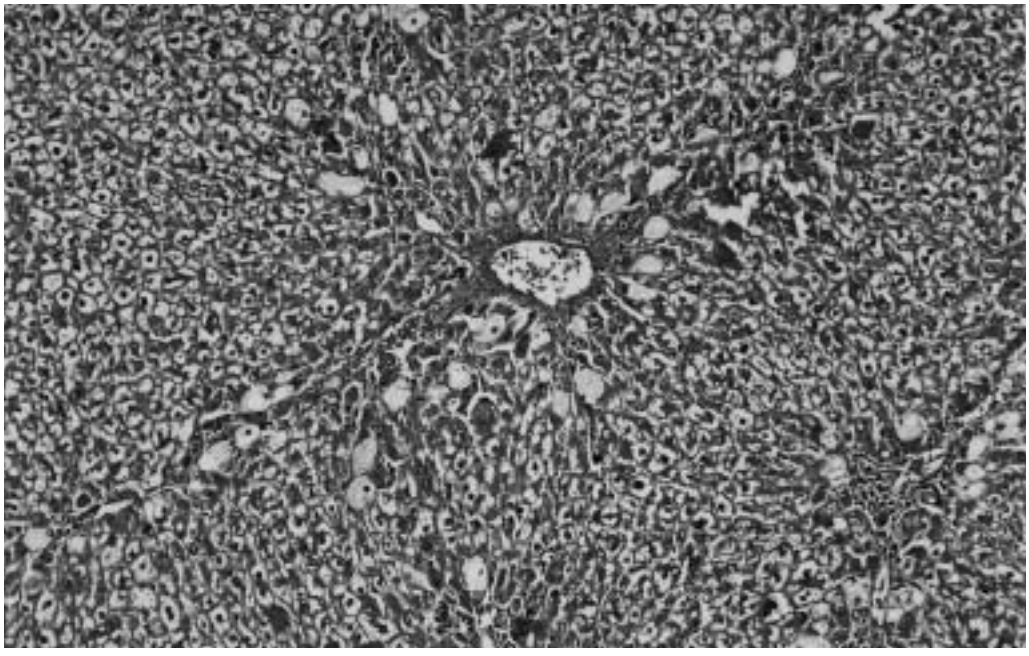

Figure 1: Numerous ballooned hepatocytes are seen in the liver (Hematoxylin-eosin stain, original magnification, X25)

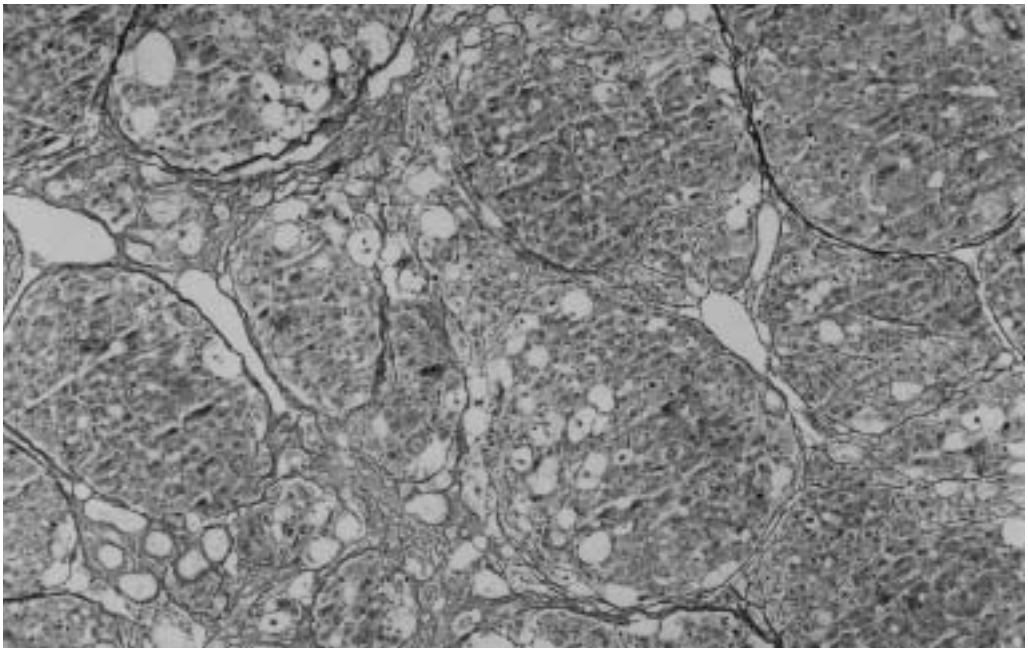

Figure 2: Numerous cirrhotic nodules are seen in the liver (Reticulin stain, original magnification, X10)

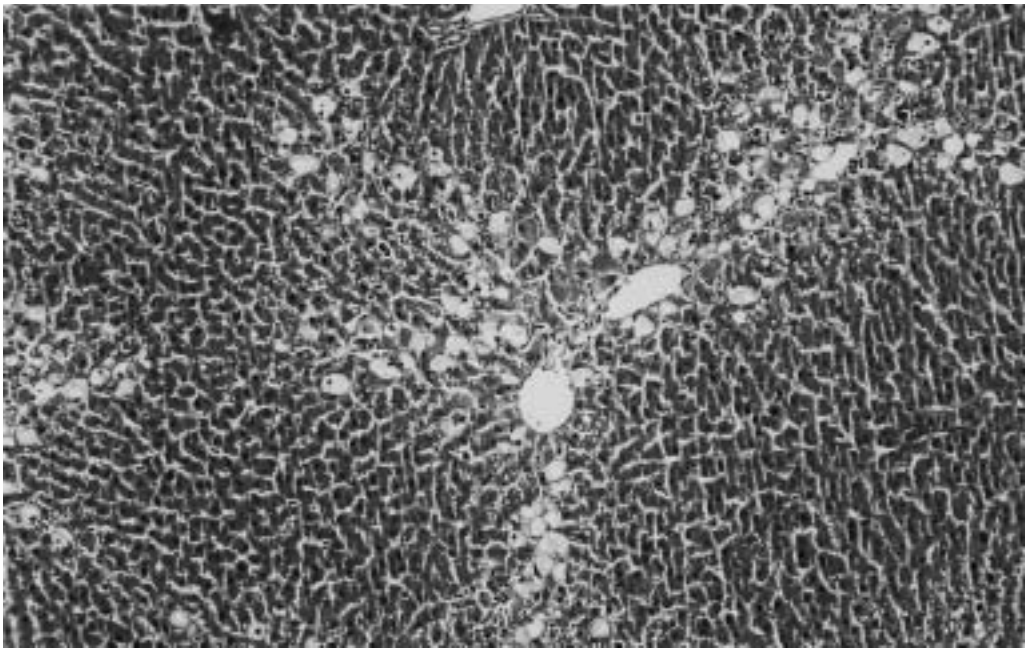

Figure 3: A few ballooned hepatocytes are seen in the liver (Hematoxylin-eosin stain, original magnification, X25)

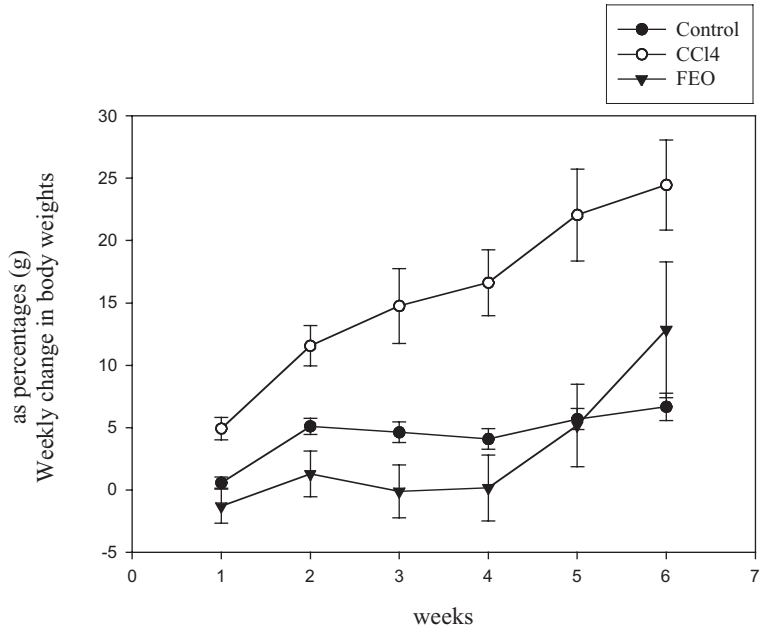

Chart 1. Weekly changes in body weights as percentages in the rats during the study.

which the N-terminal glutamate is linked to cystine via a non-α-peptidyl bond, is required by several enzymes. Glutathione and the enzyme glutathione reductase participate in the formation of the correct disulfide bonds of many proteins and polypeptide hormones and participate in the metbolism of xenobiotics (Rodwell 1993). ß-myrcene on the other hand elevates the levels of apoproteins CYP2B1 and CYP2B2, which are subtypes of the P450 enzyme system (*De-Oliveira 1997*). The cytochrome P450(CYP) enzyme system consists of a superfamily of hemoproteins that catalyse the oxidative metabolism of a wide variety of exogenous chemicals including drugs, carcinogens, toxins and endogenous compounds such as steroids, fatty acids and prostaglandins (*Shimada et al. 1994*). In order to elucidate the mechanism(s) by which FEO extract components exhibit the hepatoprotective effect which we demonstrated in this study, further studies with the isolated components will follow.

Acknowledgements

The authors would like to thank to Prof. Dr. M. Koyuncu for identification of the plant material. This study was supported by a grant (2002-TF-074) from Yüzüncü Yil University, The Directorate of Research Fund.

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