Hypoglycemic and Hepatoprotective Effects of *Foeniculum vulgare* Miller Seed Fixed Oil Extract in Mice and Rats

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Objective: **We aimed to investigate median lethal dose (LD_{50}) and hypoglycemic effect of fixed oil of *Foeniculum vulgare* Miller seed fixed oil (FFO) in mice and its hepatoprotective effect on carbon tetrachloride (CCl_4) induced liver injury model in rats.**

***Method*: Extract of FFO, glibenclamide (as a reference group) and physiologic saline (control group) were administrated to the healthy and diabet occured mice with alloxan. Before treatment in the first, second, third, fourth and 24th hours, blood was taken from the vena coccygea of mice. Blood glucose levels were measured.**

Twenty-four Sprague-Dawley rats were divided into four groups (*n=6*), and the groups treated daily for seven days, by i.p. injections, of isotonic saline solution (ISS), olive oil, carbon tetrachloride (CCl_4), CCl_4 + FFO respectively.

Results: **FFO did not significantly reduced blood glucose in alloxane-induced diabetic mice compared to ISS control group. In contrast, glibenclamide effectively reduced blood glucose of alloxane-induced mice in first, second, fourth and 24th hours as expected. In the CCl_4-treated group and FFO-treated group serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) levels were quite high. In contrast, the control groups (group I and group II) had significantly lower levels of AST and ALT when compared with the CCl_4 and FFO groups.**

Conclusion: **The results of this study indicate that FFO has neither a potent hepatoprotective effect against CCl_4-induced hepatic damage in rats nor a hypoglycemic action in mice. The LD_{50} of FFO was determined as 5.52 mL/kg.**

Key words: *Foeniculum vulgare Miller, fennel fixed oil, median lethal dose, hepatoprotective effect, hypoglycemic effect.*

Fennel (*Foeniculum vulgare* Miller, 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 cylinderical, straight or slightly curved, 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 (1). The dried, aromatic fruits are widely employed in culinary preparations for flavoring bread and pastry, in candies, and in alcoholic liqueurs of French type, as well as in cosmetic and medicinal preparations (2,3). 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 (4-11). 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 % (12,13). Volatile components of subsequent fennel seed extracts by chromatographic analysis contain *trans*-anethole, fenchone, methylchavicol, limonene, α-pinene, camphene, β-pinene, β-myrcene, α-phellandrene, 3-carene, camphor, and cisanethole (14,15). Major components of oil samples (essential oil, hexane extract and alcoholic extract) are α-pinene, β-pinene, limonene, fenchone, methychavicol and *trans*-anethole. The essential oil contains more α-pinene, limonene, and methylchavichol and less anethole than the extracted oils (14).

Özbek previously demonstrated hypoglycemic activiy of FEO in mice with a LD_{50} of 1.038 ml/kg (16). The authors also suggested that FEO has a potent hepatoprotective action in rats and has analgesic activity in mice (17-19).

Fennel and its herbal drug preparations are used for dyspeptic complaints such as mild, spasmodic gastric-intestinal complaints, bloating, and flatulence (20-24). It is also used for its mucolytic effect of the upper respiratory tract (25). Fennel seed oil emulsion is superior to placebo in alleviating infantile colic (26). It has been reported that fennel essential oil is used in infantile colic and some respiratory disorders due to its anti-spasmodic effects (27). The seeds of the plant have been known to promote menstruation, alleviate climacteric symptoms, and increase libido (28).

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Table I. The Effects of *F. vulgare* fixed oil (FFO) on serum levels of AST, ALT, ALP and bilirubin in rats treated with CCl_4 *(n=6)*

Treatment	AST	ALT	ALP	Indirect bilirubin
	Serum (U/L)	Serum (U/L)	Serum (U/L)	(mg/dl)
Control (ISS*)	137.33±0006.09	35.33±0003.48	274.00±40.99	0.016±0.003
Control (olive oil)	127.83±0016.90	46.83±0003.38	[a] 539.66±45.63	0.035±0.008
CCl_4	1169.00±0306.56	988.33±0266.73	405.50±26.29	0.171±0.018
FFO	[bce] 8916.40±1992.16	[bce] 6381.80±1499.11	[d] 525.80±44.72	[bcd] 0.498±0.103
F-values	*21.794*	*19.590*	*9.759*	*21.944*
p-values	*0.000*	*0.000*	*0.000*	*0.000*

**ISS: Isotonic saline solution.*
The values represent the mean ± S.E.M.
a: p<*0.01 with respect to control (ISS) (Tukey's test).*
b: p<*0.001 with respect to control (ISS) (Tukey's test).*
c: p<*0.001 with respect to control (olive oil) (Tukey's test).*
d: p<*0.01 with respect to CCl_4 group (Tukey's test).*
e: p<*0.001 with respect to CCl_4 group (Tukey's test)*

Table II. Blood glucose levels in glibenclamide, FFO and control groups of mice with alloxane-induced diabetes. Groups Fasting blood glucose (mg/dL)(Percent reduction in blood glucose compared to the beginning)

Groups	Fasting blood glucose (mg/dL) (Percent reduction in blood glucose compared to the beginning)				
	Before treatment	1st hour	2nd hour	4th hour	24th hour
Control (ISS*)	337.2±23.4	318.4±25.3 (-4.79±4.4)	308.0±34.2 (-5.81±8.8)	225.0±34.4 (-29.40±9.6)	205.4±19.3 (-38.08±4.2)
Glibenclamide	267.3±37.7	197.8±47.3 [a] (-30.16±4.9)	150.5±39.7 [c] (-47.10±4.9)	101.8±10.6 [b] (-59.56±3.3)	90.1±15.4 [a] (-57.21±2.9)
FFO	282.8±27.1	277.0±26.9 [e] (0.53±7.9)	232.4±23.5 [e] (-16.76±4.9)	216.6±28.8 [e] (-25.08±4.5)	166.6±26.7 [d] (-40.58±6.5)
F values		*8.109*	*11.760*	*9.764*	*5.292*
P values		*0.002*	*0.000*	*0.001*	*0.011*

Data were represented as mean ± Standart error mean.
**ISS: Isotonic saline solution.*
Post-hoc Tukey's HSD test:
a: p<0.05 comparision of the related group to controls.
b: p<0.01 comparision of the related group to controls.
c: p<0.001 comparision of the related group to controls.
d: p<0.05 comparision of the related group to glibenclamide group.
e: p<0.01 comparision of the related group to glibenclamide group.

The liver is the key organ of metabolism, secretion and excretion. It is continously and variably exposed to xenobiotics, environmental pollutants and chemotherapeutic agents because of its strategic placement in the body. Liver disease is a worldwide problem. Conventional drugs used in the treatment of liver diseases are generally inadequate and have some serious adverse effects. It is, therefore, necessary to search for alternative drugs for the treatment of liver diseases to replace currently used drugs of doubtful efficacy and safety.

The use of medicinal plants for the treatment of diabetes mellitus dates back from the Ebers papyrus of about 1550 BC. Many traditional herbal treatments for diabetes are still used throughout the world (29). After the introduction of insulin and oral antidiabetic agents, the use of the traditional treatments for diabetes mellitus greatly declined in the occidental societies, although some traditional practices are continued for prophylactic purposes and as

Table III. Blood glucose levels in glibenclamide, FFO and control groups of healthy mice.

Groups	Blood glucose levels (mg/dL) (Percent reduction in blood glucose compared to the beginning)				
	Before treatment	1st hour	2nd hour	4th hour	24th hour
Control (ISS*)	91.50±12.8	72.75±7.2	60.50±04.1	61.25±04.1	54.50±3.0
		(-19.04±3.6)	(-31.34±7.0)	(-30.0±9.0)	(-38.13±5.9)
Glibenclamide	68.75±01.3	59.25±4.8	59.00±3.6	53.25±2.9	49.75±2.0
		(-14.08±5.5)	(-14.34±4.0)	(-22.60±3.6)	(-27.66±2.3)
FFO	116.80±06.4	122.20±3.0	89.60±7.9	76.00±6.6	73.20±6.8
		[a] (5.96±6.6)	(-21.38±9.6)	(-34.29±6.5)	(-36.96±6.0)
F values		*5.668*	*1.101*	*0.761*	*1.101*
P values		*0.023*	*0.370*	*0.492*	*0.370*

Data were represented as mean ± Standart error mean.
**ISS: Isotonic saline solution.*
Post-hoc Tukey's HSD test:
a: p<0.05 comparision of the related group to controls.

adjuncts to conventional therapy (30). In the areas of developing world in which conventional medicines are not readily available, traditional treatments for diabetes remain the major mode of therapy. Few of the traditional herbal treatments for diabetes have received scientific or medical scrutiny, and the World Health Organization has recommended that this area warrants attention (31).

Extracts of the fennel seeds are used in Turkish traditional medicine as a remedy for liver diseases (32). In this study, we studied the hepatoprotective and hypoglycemic effects and median lethal dose (LD_{50}) of *Foeniculum vulgare* Miller seed fixed oil extract (FFO).

Material and Methods

Plant

The fennel seeds used were purchased from a local market from Van in Turkey. It was identified by Dr. M. Koyuncu, a plant taxonomist, Deparment of Medicinal Biology, Faculty of Medicine, Yüzüncü Y1l University. A voucher specimen (B-02) has been kept in our laboratory for future reference.

Isolation of tested material

The seeds of *F. vulgare* were ground in a mixer. Ground plant material was macerated with diethyl ether for 2 hours. The solvent was evaporated (Büchi RE 111 rotavapor and Büchi 461 water bath, Switzerland). The content of the fixed oil of the seeds is 10 %.

Chemicals

Carbon tetrachloride (CCl_4) was obtained from Merck KgaA, 64271 Darmstadt, Germany. Diethyl ether was obtained from KOMETSAN, Ankara-Turkey. Alloxan was obtained from SIGMA, Steinheim, Germany. Glibenclamide was obtained from Nobel, İstanbul, Turkey. All other chemicals were obtained from local sources and were analytical grade.

Animals

Sprague-Dawley rats (250-300 g) and Swiss albino mice (22-28 g) were used in these experiments. The animals were housed in standard cages with food and water ad libitum, at room temparature (20 ±2 ^{0}C) with artificial light from 7.^{00} am to 7.^{00} pm. The animals kept under controlled environment following the standard operating procedures of the animal house facility of the Faculty of Medicine (University of Yüzüncü Yıl), and provided with pelleted food (Van Animal Feed Factory, Van-TURKEY). The approval of Animal Ethics Committee was obtained.

Acute toxicity

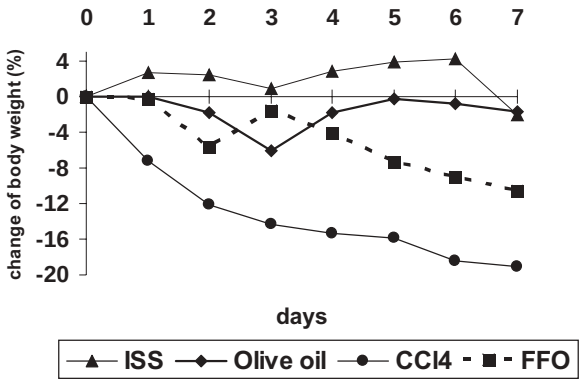
Male and female Swiss albino mice were randomly assigned to 7 groups with 8 animals in each group. First group was treated with normal saline and considered as control and the other six groups were treated with FV extract given intraperitoneally (i.p.) in increasing dosages of 0.2, 0.4, 0.8, 1.6, 3.2 and 6.4 ml/kg body weight. The mortality in each cage was assessed 72 h after administration of FFO. The percentage mortalities were converted to probits. Regression lines were fitted by the method of least squares and confidence limits for the LD_1, LD_{10}, LD_{50}, LD_{90} and LD_{99} values were calculated by the method of Litchfield and Wilcoxon (33) and Kouadio et al. (34).

Carbon tetrachloride model for evaluation of acute hepatotoxicity

The CCl_4 model described by Handa&Sharma (35) and Lershin (36) was used for scheduling the dose regimen. 0.8 mL/kg, i.p. of carbon tetrachloride diluted in olive oil (1:1 dilution) was employed for inducing acute liver toxicity.

Experimental procedure

Twenty-four Sprague-dawley rats were divided into four groups of six animals each. Group I, which served as control I, received only isotonic saline solution (ISS) 0.2



ISS: Isotonic saline solution.

Figure 1. Daily loss of body weight in percentage in the rats during the study.

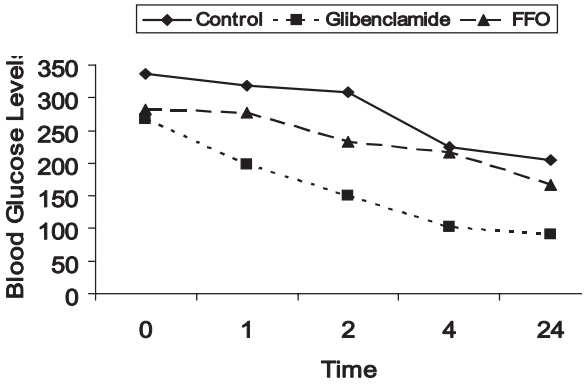


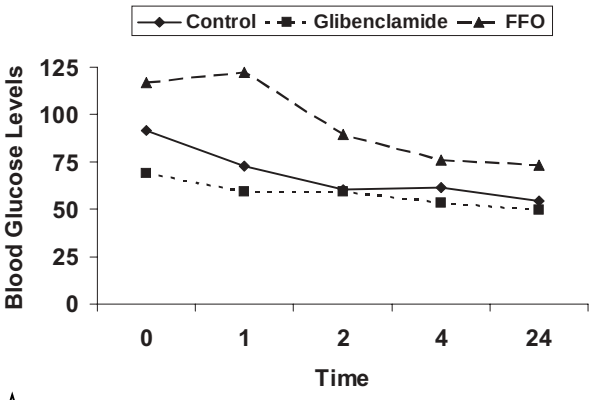
Figure 2. Blood glucose levels in glibenclamide, FFO and control groups of mice with alloxane-induced diabetes.

mL intraperitoneally (i.p.), group II which served as control II, received only olive oil 0.8 mL/kg, i.p., group III received CCl_4:olive oil (1:1) 0.8 mL/kg, i.p., and group IV received CCl_4 0.8 mL/kg + FFO 0.5 mL/kg, i.p. once a day for seven days. 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 once a day during seven days. 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 as percentage = 100 X ($Weight_n$ - $Weight_{initial}$) / $Weight_{initial}$

$Weight_{initial}$: measurement of first day.



☆ *: $p<0.05$*

Figure 3. Blood glucose levels in glibenclamide, FFO and control groups of healthy mice.

$Weight_n$: measurement of 2., 3., … 8. days.

Assessment of liver function

The serum AST, ALT, ALP and bilirubin concentrations were determined with a commercial kit (Roche) by 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) stain.

Preparation of alloxan diabetic mice

Mice were fasted for 18 h. diabetes was induced by an i.p. injection of 150 mg/kg of alloxan monohydrate in normal saline. This procedure were repeated three times (37). After 7 days of the last treatment, mice with blood glucose levels of 200 mL/dL and over were taken into the study (38).

Hypoglycemic activity in normal mice

Animals were randomly divided into three groups of eight animals each. Group I mice received 0.1 ml normal saline i.p. The animals of group II were treated i.p. with glibenclamide, used as a standard at a dose of 3.0 mg/kg. Group III received i.p. with 1 ml/kg body weight of FV extract. Blood glucose levels were determined before treatment, 1, 2, 4 and 24 h after treatment by applying glucose oxidase peroxidase method (Abbott, United Kingdom).

Hypoglycemic activity in diabetic mice

The same protocol described above for normal mice was applied in mice made diabetic by administering 150 mg/kg i.p. of alloxan monohydrate. Also in this case, three groups of eight animals each were used.

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 (ANOVA).

Tukey's HSD test (Tukey's honestly significant difference test) was used for determining significance (39). Probability levels of less than 0.05 were considered significant.

Results

Acute toxicity

The results of acut toxicity are given as below:

LD_1 : 1.100 ml/kg,

LD_{10} : 2.270 ml/kg,

LD_{50} : 5.519 ml/kg,

LD_{90} : 13.417 ml/kg,

LD_{99} : 27.680 ml/kg.

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

The results of FFO on CCl_4-intoxicated rats are shown in Table 1. In the CCl_4-treated group and FFO-treated group serum AST, ALT and ALP levels were quite high. In contrast, the control groups (group I and group II) had significantly lower levels of AST and ALT when compared with the CCl_4 and FFO groups.

Daily changes in body weights as percentages are shown in Figure 1. The daily body weight changes as percentages indicated that CCl_4 group and FFO group had a significant decrease compared to the controls.

Histopathological findings

In the carbon tetrachloride group (group III) and the FFO group (group IV), drastic alterations were observed in the livers. Histopathological examinations showed diffuse ballooning degeneration. Ballooned hepatocytes were of different sizes and much larger than normal hepatocytes and occasionally appeared as confluent areas. In FFO group, Concilman bodies were observed occasionally. One rat was died in FFO group at fifth day of the study. Histopathologic changes in the liver of that animal were similar to those of CCl_4 group.

Hypoglycemic activity in healthy and diabetic mice

The blood glucose levels of the alloxan diabetic mice are given in Table 2 and Figure 2. Table 3 and Figure 3 demonstrate the levels of blood glucose in normal mice. *Foeniculum vulgare* Miller fixed oil extract did not have any hypoglicemic effect in healthy or alloxane-induced diabetic mice.

Discussion

In the present study hepatoprotective effect of FFO was investigated on CCl_4-intoxicated rats and the results were presented in Table 1. In the CCl_4-treated group and FFO-treated group serum AST, ALT and ALP levels were quite high. In contrast, the control groups had significantly lower levels of AST and ALT when compared with the CCl_4 and FFO groups. Daily changes in body weights are shown in Figure 1. The daily changes of body weight indicated that CCl_4 group and FFO group had a significant decrease compared to the controls.

In this study hypoglycemic effect of FFO was investigated in healthy and diabetic mice and the results were presented in Table II and III. As that can be seen in Table I FFO did not significantly reduced blood glucose in alloxane-induced diabetic mice compared to ISS control group. In contrast, glibenclamide effectively reduced blood glucose of alloxane-induced mice in first ($p<0.05$), second ($p<0.001$), fourth ($p<0.01$) and 24^{th} hours ($p<0.005$) as expected. The only effect of FFO extract on blood glucose levels in healthy mice was a significant increment in first hour compared to controls (see Table III).

The essential oil contains more ±-pinene, limonene, and methylchavichol and less anethole than the fixed oil (14). In contrast to FEO which had hepatoprotective, hypoglycemic and analgesic activities, FFO did not have any beneficial effects. Instead FFO significantly increased the hepatic injury. The difference may be due to different chemical compositions of FEO and FFO (14). Further studies are needed to define the constituents which make the difference.

Our results revealed that histopathological and biochemical findings and daily changes in body weights did not suggest a hepatoprotective activity for FFO on CCl_4-induced liver toxicity in rats. In addition FFO did not have any hypoglycemic effect in healthy or alloxane-induced diabetic mice. The median lethal dose (LD_{50}) of FFO was determined as 5.519 mL/kg.

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