Hepatoprotective Effects of *Pimpinella anisum* Seed Extract in Rats

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Summary

We investigated the role of *Pimpinella anisum* seed extract (PFO) in the prevention of carbon tetrachloride (CCl_4) induced liver injury.
Thirty-six Sprague-Dawley rats were allocated into five groups. Animals in group I, II, and III received isotonic saline solution, olive oil and CCl_4, respectively. Silibinin and CCl_4 were administered to the animals in the group IV while group V animals received PFO and CCl_4. The rats were sacrificed at the end of the eighth day and histopathological and biochemical examinations were performed. Body weights of the animals were measured daily.
CCl_4 induced acute liver damage observed by increases in serum ALT and AST levels and histopathological findings. In the PFO group serum AST and ALT levels were lower compared to those in the CCl_4 group, although this was not as low as those in the silibinin group. However there was no difference in the histopathologic findings between the CCl_4 and PFO groups. In conclusion, PFO could show hepatoprotective effects although less effective than silibinin.

Key words: *Pimpinella anisum* L., carbon tetrachloride; hepatoprotective effect, rats.

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Introduction

Pimpinella anisum is a member of the Umbelliferae and is found in the North-Eastern Anatolia (1). In Turkish folk medicine, *Pimpinella* species have been used as analgesic, antiinflammatory, appetizing, hypnotic, expectorant, antibacterial and hepatoprotective agents and to increase milk secretion (2-4). The seeds of *P. anisum* contain 1.5-6% essential oil, 10-20% fixed oil and 18% protein. The main constituents of the essential oil are 90% anethole, 2-4% gamma-himachalene, <1% p-anisaldehyde, 0.9-1.5% methylchavicol, 3% cis-pseudoisoeugenyl 2-methylbutyrate and 1.3% trans-pseudoisoeugenyl 2-methylbutyrate (5). The essential oil of *P. anisum* was not found effective against carbon tetrachloride-induced acute hepatotoxicity in rats (6). Hypoglycemic effect of the essential oil of P. anisum has been reported by Ceylan et al. (7). It was found acaricidal against *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* (8). Its anti-diuretic effect was reported by Kreydiyyeh et al (9). Essential oil of *P. anisum* was found effective some bacteriae (10). Sahraei et al. reported that the essential oil of the *P. anisum* may reduce the morphine effects via a GABAergic mechanism (11).

Bronchodilatory effects of the essential oil, aqueous, and ethanol extracts of *P. anisum* through its inhibitory effects on muscarinic receptors have been showed (12). It has been reported that the essential oil of the *P. anisum* suppressed tonic convulsions induced by pentylenetetrazole (PTZ) or maximal electroshock (MES) (13) and it has been considered to be an active estrogenic agent (14). Özbek et al. reported that median lethal dose of PFO was 3.152 mL/kg in mice (15).

No reports are available on the evaluation of *P. anisum* fixed oil for its hepatoprotective activity. In this study, the fixed oil of *P. anisum* was investigated for its hepatoprotective activity on carbon tetrachloride-induced liver injury in rats.

Materials and Methods

Plant material

The seeds of *P. anisum* were purchased from a local herbal store in Van, Turkey. The plant samples were kept at room temperature until grinding process. *P. anisum* was identified by the botanists in the herbarium of Yuzuncu Yil University, Van and the specimen number of the plant is B-16.

The seeds of *P. anisum* were ground in a mixer. Ground plant material was macerated in diethyl ether for two hours. The solvent was evaporated (Büchi RE 111 rotavapor and Büchi 461 water bath, Switzerland). The fixed oil content of the seeds was 14 %.

Chemicals

Carbon tetrachloride (CCl_4) was obtained from Merck KgaA (64271 Darmstadt, Germany), diethyl ether was obtained from Kimetsan (Ankara-Turkey), silibinin was obtained from Sigma-Aldrich (Steinheim-Germany) and olive oil was obtained from Fluka (Steinheim-Germany).

Animals

Sprague-Dawley rats of both sexes (150-200g) were used in this study. The animals were housed in standard cages with food and water ad libitum, at room temparature (20 ±2 ^{0}C) with 12h light-dark cycle. The animals were kept under controlled environment following the standard operating procedures of the animal house facility of the Faculty of Medicine (University of Yuzuncu Yil), and provided with pelleted food (Van Animal Feed Factory, Van-TURKEY). The approval of Animal Ethics Committee was obtained.

Carbon tetrachloride model for evaluation of acute hepatotoxicity

The CCl_4 model of hepatotoxicity described by Handa&Sharma and Lershin was used for scheduling the dose regimen (16, 17). Intraperitoneal (i.p.) injection of carbon tetrachloride (0.8 mL/kg) diluted in olive oil (1:1 dilution) was employed for inducing acute liver toxicity.

Experimental procedure

Thirty-six Sprague-dawley rats were divided into six groups of six animals each. Animals in group I (ISS group) received i.p. injection of 0.2 mL isotonic saline solution (ISS); Animals in group II and group III received olive oil (0.8 mL/kg, i.p.) and CCl_4 (0.8 mL/kg, i.p.), respectively. Group IV (Silibinin group) animals were injected with silibinin (50 mg/kg, i.p.) and CCl_4 (0.8 mL/kg, i.p.) (18), while Group V (PFO group) animals received PFO (0.5 mL/kg, i.p.) and CCl_4 (0.8 mL/kg i.p.). The first two groups served as control groups. Injections were given once a day for seven days. All animals were observed daily and 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) and alanine aminotransferase (ALT).

Body weights of the rats were measured everyday for eight days. Daily percentage changes in body weights were recorded.

Assessment of liver function

The serum AST and ALT concentrations were determined with a commercial slide (Vitros) by Vitros DT60II 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. Four µm thick sections were cut and stained using Haematoxylin-eosin (HE) stain.

Statistical analysis

Results of the biochemical analyses were reported as mean±Standard Error of Mean (SEM). The total variation was analysed by performing one-way analysis of variance (ANOVA). Tukey's Honestly Significant Difference test (Tukey's HSD test) was used for determining significance. Probability levels of less than 0.05 were considered significant.

Results

Effects of PFO on AST and ALT levels

Results of the biochemical analyses in all groups are presented in Table 1. There were no significant differences in AST and ALT levels between the ISS and olive oil groups. Serum AST and ALT levels were significantly higher in the CCl_4 group compared to the control groups. In the silibinin group serum AST and ALT levels were significantly lower compared to those in the CCl_4 group while serum AST level was higher compared to the control groups. Serum AST and ALT levels in the PFO group were significantly lower compared to those in the CCl_4 group while these were significantly higher compared to those in the control groups.

Percentage changes in weight were 8.87% in the ISS group, -1.72% in the olive oil group, -12.40% in the CCl_4 group, -4.60% in the silibinin group and -12.11% in PFO group. The animals in the CCl_4 and PFO groups showed a larger weight loss compared to those in the control group.

Table 1. The Effects of *P. anisum* extract (PFO) on serum levels of AST and ALT in rats.

Treatment	AST	ALT
	Serum (U/L)	Serum (U/L)
ISS*	177.0±015.6	43.5±2.1
Olive oil	127.8±16.9	46.8±3.3
CCl_4	1727.0±225.8 ^{ab}	969.0±166.4 ^{ab}
Silibinin	767.8±179.4 ^{abc}	248.2±93.1 ^{c}
PFO	1032.8±155.6 ^{abc}	417.4±69.8 ^{abc}
F-value	*22.246*	*19.059*
p-value	*0.000*	*0.000*

*ISS: Isotonic saline solution.
The values represent the mean ± S.E.M. (Standard Error of Mean).

Post-hoc Tukey's HSD test:
a : $p<0.05$ with respect to control (ISS) group,
b : $p<0.05$ with respect to olive oil group,
c : $p<0.05$ with respect to CCl_4 group,

Histopathological findings

There were no pathological changes in the livers of the rats in the ISS and olive oil groups. In the carbon tetrachloride group and the PFO group, drastic alterations were observed in the livers. Histopathological examinations showed diffuse ballooning degeneration (Fig. 1). Ballooned hepatocytes were of different sizes and much larger than normal hepatocytes and occasionally appeared as confluent areas. Histopathologic changes in the liver of the PFO group were similar to those of the CCl_4 group (Fig. 2).

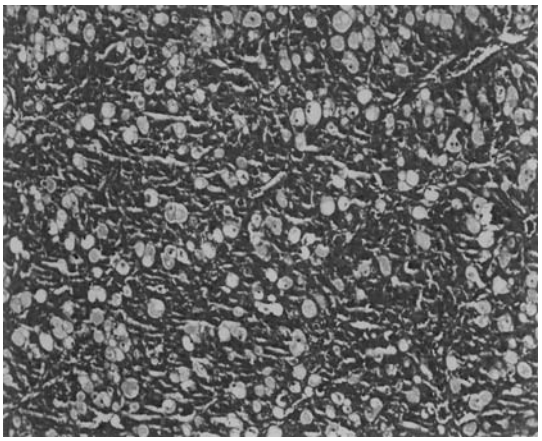

Fig. 1. Ballooning degeneration in liver in CCl_4 group (HE, x10).

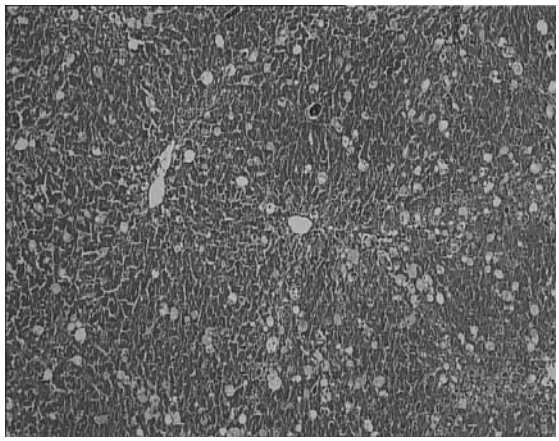

Fig. 2. Ballooning degeneration in liver in PFO group (HE, x5).

Discussion

CCl_4 induces centro lobular necrosis in rat liver and it is transformed to tricholoromethyle free radical (CCl_3) by sitocrom-P450. CCl_3 reacts with oxygen to form peroksil radical (CCl_3O_2), which powerfully induces lipid peroxidation. Thus, CCl_4 causes oxidative destruction of liver cell membranes and serious tissue damage in rats (19).

Biochemical findings showed that serum ALT and AST levels were significantly lower in the PFO group compared to CCl_4 group. Decreased serum AST and ALT levels suggest that PFO could prevent liver cell damage (20).

Silibinin, which is known to have hepatoprotective effects on liver significantly decreased serum AST and ALT levels compared to CCl_4 (18). Although serum ALT level was lower in the silibinin group than those in the PFO group the difference was not statistically significant. Considering the body weight changes and histopathological findings in the PFO group, it is suggested that silibinin has a better hepatoprotective effect than PFO.

Histopathological findings were similar in all groups although balloon degeneration in the groups other than the CCl_4 group was less widespread.

In the light of these findings it is concluded that PFO can have hepatoprotective effect in CCl_4 induced hepatotoxicity and it may be less effective than silibinin. To find out which chemical in the PFO is responsible for its effects a chromatographic analysis of PFO should be performed and it ingredients should be studied in in the CCl_4 induced hepatotoxicity model.

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