

Evaluation of hepatoprotective activity of Bergamot orange in rats

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Abstract. Essential oil extract of Bergamot orange (BO) was investigated for its hepatoprotective effect on carbon tetrachloride-induced hepatotoxicity in rats. Six different groups were established. Silibinin was used as the reference agent. BO significantly reduced the serum ALT level when compared to CCl₄ group while it did not affect the serum AST level. The histopathological findings did not show any significant difference between the BO and CCl₄ groups. The results suggest that BO has a weak hepatoprotective effect in carbon tetrachloride induced acute liver toxicity.

Keywords: Bergamot orange, hepatoprotective activity, carbon tetrachloride, rats.

1. Introduction

The Bergamot orange (*Citrus aurantium* subsp. bergamia) is a member of the Rutaceae family. In Turkish folk medicine, Rutaceae family members have several uses such as for their appetizing, diaphoretic, antiseptic, analgesic and anti-inflammatory effects (1). The Bergamot orange (BO) is a pear-shaped citrus fruit. The fruit is produced in Italy, East Africa, Ivory Coast, Argentina, Brazil, Turkey and some other Mediterranean countries. The fruit is sour, and its aromatic peel is used to produce an essential oil that is used in Earl Grey tea, in perfumery, in candy-making (2). It is known empirically that inhalation of its essential oils causes physiological and psychological changes in humans (3). Graham et al reported that aromatherapy during radiotherapy with bergamot oil and some other kinds of plant oil was not beneficial (4). The BO oil has been linked to

several negative side-effects like photosensitivity (5-7). The natural essence is extracted from the peel of the fruit by a cold-pressing procedure or steam distillation. It consists of a volatile fraction, whose main components are, in approximate percentages, limonene (40%), linalool (8%), linalyl acetate (28%), bergapten, citropten, bergamottin, gamma-terpinen, alpha-pinene, beta-pinene, and a non-volatile fraction (4-7%) formed essentially by coumarins and psoralens (2,8).

It has been reported that Bergamot orange exhibited antifungal activity against some dermatophytes and antibacterial activity against *Campylobacter jejuni*, *Escherichia coli* O157, *Listeria monocytogenes*, *Bacillus cereus* and *Staphylococcus aureus* (9,10). Some of the components of BO, limonene and alpha-pinene, were shown to have hepatoprotective effects (11-13). Therefore it is likely that BO has hepatoprotective effect.

In this study, essential oil extract of Bergamot orange was investigated for its hepatoprotective effect on carbon tetrachloride-induced hepatotoxicity in rats (14,15).

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Table 1

Effects of BO on serum levels of AST and ALT of rats treated with CCl₄ (n=6)

Groups	Serum AST (U/L)	Serum ALT (U/L)
Control I (ISS*)	137.3±6.1	35.3±3.5
Control II (Olive oil)	127.8±16.9	46.8±3.3
CCl ₄	^{ab} 959.4±152.1	^{ab} 2172.2±362.2
Silibinin	^c 494.3±240.9	^{abc} 597.5±84.5
BO (0.1 mL/kg)	^{abd} 918.6±148.8	^{abc} 769.6±163.3

The values are given as the mean ± S.E.M. *ISS: Isotonic saline solution. Post-hoc LSD test: a: p<0.05 with respect to control I. b p<0.05 with respect to control II. c p<0.05 with respect to CCl₄ group. d p<0.05 with respect to silibinin group.

2. Material and method

2.1. Preparation of BO essential oil extract

Bergamot orange was collected in February, 2006 near Antalya (Turkey) and voucher specimens are kept in Yuzuncu Yil University, Faculty of Medicine (Specimen Nr: B-14).

The plant samples were kept at room temperature until they were processed. The fruits were peeled and the skin was sliced into small pieces and boiled in Clevenger device (Ildam, TURKEY). Collected BO essential oil was kept in test tubes and the yield was determined as 0.7%.

2.2. Drugs and chemicals

The following drugs were used; Carbon tetrachloride (Merck, Germany), olive oil (Fluka, Germany), Silibinin (Sigma, Germany).

2.3. Animals

Male and female Sprague-Dawley rats (180-220 g) were used in these experiments. The animals were housed in standard cages with food and water ad libitum, at room temperature (20 ± 2 °C) with artificial light from 7.⁰⁰ am to 7.⁰⁰ pm. The animals were 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. Prior to administration of the drugs, the rats were fasted for 12 h with free access to tap water.

Table 2

Histopathological changes in the liver of rats*

Microscopic observation	ISS**	Olive oil	CCl ₄	CCl ₄ +B. orange
Ballooning degeneration	0	0	++++	+
Centrilobular and bridging necrosis	0	0	++	++

*: 0, absent; +, mild; ++, moderate; +++ severe; ++++, extremely severe. **ISS: Isotonic saline solution.

2.4. Carbontetrachloride induced hepatotoxicity

The carbon tetrachloride (CCl₄) model described by Lershin (14) and Handa&Sharma (15) was used for scheduling the dose regimen. Intraperitoneal (i.p.) injection of 0.8 mL/kg of carbon tetrachloride diluted in olive oil (1:1 dilution) was employed for inducing liver toxicity.

Thirty rats of either sex were distributed into five groups of six animals each. Group I, which served as control, received isotonic saline solution (ISS). Group II (olive oil control) received olive oil (0.8 mL/kg). Group III received silibinin (50 mg/kg) (16). Group IV received CCl₄:olive oil (1:1) (0.8 mL/kg) and group V received essential oil extract of BO (0.1 mL/kg) + CCl₄:olive oil (1:1) (0.8 mL/kg). All chemicals were injected intraperitoneally once daily for 7 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 the marker enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT).

Body weights of the rats were measured daily for eight days. Daily changes in body weights were recorded as percentages. The serum AST and ALT concentrations were determined with commercial slides using a Vitros D60 II autoanalyzer.

2.5. 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. Histological damage was quantified using the following score system; 0:absent; +:mild; ++:moderate; +++:severe; ++++:extremely severe.

2.6. Statistical analysis

All data were presented as mean \pm standard error of the mean (SEM) or as percentages. Analysis of variance (ANOVA) used for the statistical analysis of data. LSD test (least significance difference test) was used for determining significance. Results with $p < 0.05$ were considered as statistically significant.

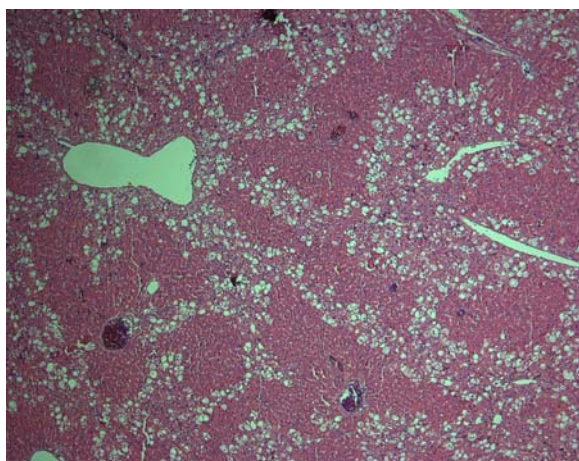


Fig. 1. Numerous ballooned hepatocytes are seen in the liver of the CCl₄ group (Hematoxylin-eosin stain, original magnification, X50)

3. Results

3.1. Effects of BO on AST, ALT and ALP levels

The results of hepatoprotective effect of BO on CCl₄-intoxicated rats are shown in Table 1. In the CCl₄:olive oil-treated group serum AST and ALT levels were quite high. In contrast, the BO treated group had significantly lower level of ALT towards normalization when compared with the CCl₄:olive oil group. But the level of AST in the BO group did not decrease significantly when compared with the CCl₄:olive oil group.

The percentage changes in weight were 8.87% in the ISS group, -1.72% in the olive oil group, -12.40% in the CCl₄ group, -4.60% in the silibinin group and -9.12% in BO group. The animals in the CCl₄ and BO groups showed a greater weight loss compared to those in the control group.

3.2. Histopathological examination

In carbon tetrachloride treated liver, drastic alterations were observed in histopathological examination. In the CCl₄:olive oil group (compared to the ISS control group and the olive oil control group) ballooning degeneration, centrilobular necrosis and bridging necrosis in

hepatocytes were induced (Table 2). Histopathological examination showed diffuse numerous ballooning degeneration. Ballooned hepatocytes were of different sizes and much larger than normal hepatocytes and occasionally appeared as confluent areas (Fig. 1). Centrilobular necrosis and bridging necrosis were occasionally present. BO + CCl₄ treated livers did not show significant recovery. Although ballooning degeneration was scarce, centrilobular and bridging necrosis were occasionally present (Fig. 2).

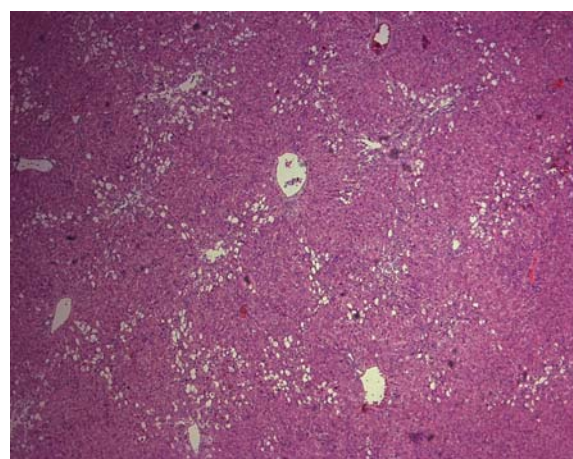


Fig. 2. Numerous ballooned hepatocytes are seen in the liver of the BO group (Hematoxylin-eosin stain, original magnification, X50).

4. Discussion

It was found that BO decreased the ALT level significantly compared with the CCl₄:olive oil group while it did not affect the serum AST level significantly (Table 1). ALT level is known to be more specific than AST level in showing liver damage (17). Weight loss in the BO group was less compared with that in the CCl₄:olive oil group. Although these findings suggest that BO has a weak hepatoprotective effect histopathological findings do not support this. BO only reduced the ballooning degeneration induced by hepatotoxicity and did not affect centrilobular necrosis and bridging necrosis in hepatocytes. The weak hepatoprotective effect of BO could be due to alpha-pinene and limonene found in its essential oil extract (13).

As a result, this study suggests that Bergamot orange essential oil extract has a weak hepatoprotective effect in carbon tetrachloride induced acute liver toxicity.

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