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Effects of carvacrol on defects of ischemia-reperfusion in the rat liver

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Abstract

Many plants found in nature have been used to treat various illnesses. One such plant is oregano (Kekik in Turkish). Health beneficial effects of carvacrol obtained from oregano oil have been shown scientifically. We have investigated the comparative effects of carvacrol in the liver of rats subjected to ischemia-reperfusion defect, with silymarin. To test the effects we formed four groups using male Wistar albino rats. Group I was control. The other three groups of animals were administered 60 min prior to surgical operation single doses of physiological serum, carvacrol and silymarin, respectively. Group II, III and IV animal were subjected to 45 min long liver ischemia and 60 min reperfusion. Blood and tissue samples were collected for biochemical and histological analysis following the test.

AST and ALT values obtained after biochemical analysis of the serums showed statistically significant difference in group II than the other three groups. A statistical evaluation of the serum AST levels among the groups II, III and IV showed that both groups III and IV which had no difference in between were significantly different in a positive way from group II ($p<0.001$). As to the serum ALT levels, difference between group II and group III ($p<0.001$) and group II and group IV ($p<0.01$) was found significant. No statistical difference was observed in groups I, III and IV for GSH, MDA and CAT levels of the liver. A statistical evaluation of the GSH level in group III and group IV was found to be significantly different from group II ($p<0.001$) without any difference between them. A similar evaluation for MDA and CAT levels among the revealed no difference between group III and group IV, however, group II showed difference with group II and group IV ($p<0.05$).

Histological findings were in harmony with the biochemical results. We conclude that carvacrol protects the liver against defects caused by ischemia and reperfusion, and carvacrol is not hepatotoxic at the applied dosage.

Keywords: Liver; Ischemia/reperfusion; Carvacrol; Silymarin; Rat

Abbreviations: I/R, ischemia/reperfusion; H&E, haematoxylin and eosin; DNA, deoxyribonucleic acid; MDA, malondialdehyde; GSH, reduced glutation; CAT, catalase; PMNL, polymorphonuclear leukocytes; AST, aspartate transaminase; ALT, alanine transaminase; ALP, alkaline phosphatase; GCMS, gas chromatography and mass spectrometer analyses.

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Introduction

Any functional defect occurring in the liver which is one of the most important organs of the human body affects all the systems. Chemical substances, medicines, alcohol, liver tumors, viral liver diseases and ischemia occurring after surgical operations are likely causes of liver injury. Hepatic ischemia which is a frequent problem encountered in clinical conditions such as liver transplantations, liver deficiency and liver surgery results in functional and structural defects in hepatocytes (Debonera et al., 2001; Okatani et al., 2003). Blockage of the veins to control bleeding during partial hepatectomy results in the undesired ischemia (Watanabe et al., 2001; Seo and Lee, 2002). Prolongation of the period of ischemia is an important risk factor for complications in liver transplantations (Kaibori et al., 2000). As in many surgical operations, ischemia occurring during hepatectomy (min. 60 min in Pringle's maneuver) causes serious tissue injury (Nakano et al., 1998; Zapletal et al., 2001). Reperfusion following ischemia results in the passage of toxic products to the systemic circulation. This worsens the liver damage (Ozturk et al., 2002). Furthermore, oxidants formed and circulated as a result of I/R in another organ bring about negative effects especially in the lungs and the liver (Tang et al., 1997; Iijima et al., 1997; Serteser et al., 2002).

Medicinal treatment is necessary for a rapid recovery of liver injury due to various reasons. However, medicines used in the treatment of liver have too many side effects. Therefore, natural products offer hope for the development of new liver medicines. Most of such medicines are plant based. For instance, silymarin has been used for liver health. Positive effects of silymarin in liver regeneration is well documented. Silymarin is a plant derived flavonoid which is extracted from the fruits and seeds of the milk thistle (*Silybum marianum* L. Gaertn.) belongs to the family of Asteraceae. Silymarin is a flavonolignan and consists of a mixture of mainly three flavonoids, silybin (silibinin), silydianin and silychristin. Silibinin is the major component (70–80%) found in silymarin and is thought to be the most biological active compound. Pharmacological studies revealed that silymarin is non-toxic even at higher physiological doses, which suggests its safe use for the treatment of various diseases (Katiyar, 2005). Oregano water (kekik suyu), a hydrosol of oregano, is used as a folk medicine in Turkey for liver health. Oregano oil contains carvacrol as the main constituent. Several useful attributes of carvacrol have been scientifically proven in *in vivo* studies, however, there is no research into liver protective and liver healing effects of carvacrol. We, therefore, have decided to investigate *in vivo* effects of carvacrol in the livers of rats subjected to I/R, in comparison with silymarin.

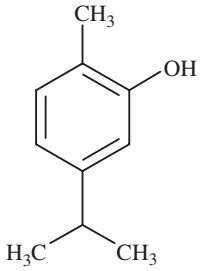

Fig. 1. The structural formula of carvacrol.

Materials and methods

Plant extract

The plant substance tested in this study, carvacrol (2-methyl-5-(1-methyl ethyl) phenol), was isolated from steam distillated essential oil of *Origanum onites* L. collected from West Anatolia (Fig. 1). For the isolation, fractional distillation was performed using a lab-size glass fractional distillation unit containing column packed with S/S Knit Mesh packing material (2.8 cm × 1.35 m). Reflux ratio was adjusted at 10/1 to 20/1 and the medium pressure was 8–10 mm Hg. Carvacrol-rich fractions were bulked to obtain carvacrol with 99% purity (GCMS).

Silymarin used in our experiment containing 70–80% silibinin was purchased from Sigma (Cat no S0292).

Animals

Male Wistar albino rats, weighing between 230 ± 30 g, were used after 2 weeks of adaptation. They were housed in polycarbonate cages in an air-conditioned room (12L/12D, 22 ± 2°C, 50 ± 5% humidity). Water and food supplied *ad libitum*.

The experimental design and procedures were approved by the Institutional Ethical Committee for Animal Care and Use at the Eskisehir Osmangazi University, Eskisehir, Turkey.

Experimental protocols

The rats were randomly divided into four groups, each containing 8 animals.

Group I normal (sham operation). Each animal received 0.5 ml physiological serum 60 min prior to surgical operation and was subjected only to laparotomy in order to make hepatic veins and bile duct clearly visible. The animals were killed after 105 min.

Group II (I/R-untreated). The animals were subjected to I/R and received 0.5 ml saline solution intraperitonally.