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Antioxidant and hepatoprotective properties of dried fig against oxidative stress and hepatotoxicity in rats

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ABSTRACT

The aim of this study was to investigate the hepatoprotective effect and antioxidant role of dried fig (DF) (*Ficus carica* L.) against ethanol-induced oxidative stress. Experiment was designed as normal Control, 20% ethanol, 10% DF and 10% DF + 20% ethanol groups. The hepatoprotective and antioxidant role of the dried DF supplementation feed against ethanol induced oxidatif stress were evaluated by liver histopathological changes, measuring liver damage serum enzymes (LDSE), antioxidant defense system (ADS) and malondialdehyde (MDA) content in various tissues of rats following the exposure of experimental for 50 days. The biochemical analysis showed a considerable increase the LDSE in the ethanol group as compared to that of control group whereas, decreased in 10% DF + 20% ethanol group as compared to that of ethanol group. In addition, the DF supplementation diet restored the ethanol-induced MDA and ADS towards to control. The hepatoprotection of DF is further substantiated by the almost normal histologic findings of liver in 10% DF + 20% ethanol group against degenerative changes in ethanol group. The results indicated that the DF could be as important as diet-derived antioxidants and antihepatotoxicity in preventing oxidative damage in the tissues by inhibiting the production of ethanol-induced free radicals and hepatotoxicity in rats.

1. Introduction

Epidemiological studies consistently show that increased consumption of plant-based, antioxidant-rich foods, *i.e.*, fruits, vegetables, whole grains, and nuts, is associated with the reduced risk for several chronic diseases [1]. Biomolecules from plants have attracted a great deal of attention, mainly concentrated on their role in preventing diseases. In addition, epidemiological studies have consistently shown that there is a clear significant positive association between intake of these natural products and reduced rate of heart disease mortalities, common cancers and other degenerative diseases [2]. Many naturally occurring compounds with antioxidative action are now known to protect cellular components from oxidative damage and prevent diseases [3,4]. Numerous studies demonstrate that a great number of medicinal and aromatic herbs, as well as fruits and leaves of some berry plants biosynthesize phytochemicals possessing antioxidant activity and may be used as a natural source of free radical scavenging compounds [5,6]. In addition, a great number of spices and aromatic herbs contain chemical compounds exhibiting antioxidant properties. These properties are attributed to a variety of active phytochemicals including vitamins, carotenoids, terpenoids, alkaloids, flavonoids, lignans, simple phenols and phenolic acids, and so on [7]. Free radicals present in human organism cause oxidative damage to various molecules, such as lipids, proteins and nucleic acids, being involved in the initiation of those diseases [8]. Antioxidant compounds, such as phenolics, organic acids, vitamin E and carotenoids, scavenge free radicals, thus inhibiting the oxidative mechanisms that lead to degenerative illnesses [9,10]. Further, combinations of polyphenols naturally found in fruits and vegetables had been suggested to be optimal for cancer prevention [11–13] and their anticarcinogenic effects were well established [11]. Some antioxidants, such as resveratrol, are assumed to possess cancer-preventive and cancer-therapeutic properties and can induce apoptosis of cancer cells [14]. Study has suggested that many chemotherapeutic agents induce apoptosis of cancer cells through ROS-mediated cell damage [15].

Ficus carica L., a deciduous tree belonging to the Moraceae family, is one of the earliest cultivated fruit trees and an important crop world wide for both dry and fresh consumption. Mediterranean diets are characterized by abundant intake of this fruit [16], which can be eaten fresh, dried or used as jam. Figs are an excellent source of minerals, vitamins and dietary fibre; they are fat and cholesterol-free and contain a high number of amino acids [16,17]. Figs have been traditionally used for its medicinal ben-

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efits as laxative, cardiovascular, respiratory, antispasmodic and anti-inflammatory remedies [18]. Seventy percent of the worlds FC (*Ficus carica* L.) production of is grown in the countries of the Mediterranean coast. In these countries, figs are an important constituent of the Mediterranean diet, which is considered to be one of the healthiest and is associated with longevity [19]. Solomon et al. [16] showed that the higher the polyphenol content, particularly anthocyanins, in FC fruit, the higher their antioxidant activity. Antioxidants from FC can protect plasma lipoproteins from oxidation and significantly elevate plasma antioxidant capacity for 4 h after consumption [20].

The reactive oxygen species (ROS) are known to play a major role in either the initiation or progression of carcinogenesis by inducing oxidative stress [21]. Peroxides and superoxide anion ($^{\bullet}O_2^-$) produce cytotoxicity/genotoxicity in cellular system [4,22]. ROS and nitrogen species are formed in the human body and endogenous antioxidant defenses are not always sufficient to counteract them completely. A large number of studies support the hypothesis that oxidative damage to DNA, lipids and proteins may contribute to the development of cardiovascular disease, cancer and neurodegenerative diseases [21,23]. Diet-derived antioxidants may therefore be particularly important in protecting against chronic diseases [23,24].

There is a growing interest of natural products in human diet, both due to the possible negative effects of synthetic food additives on human health and the increased consumer perception of this problem in recent years. As far as our literature survey could ascertain, no studies have so far been reported on hepatoprotective role and antioxidant capacity of the DF supplementation used in this study. The objective of this study was to determine healthful potentials of DF against ethanol-induced oxidative stress and hepatotoxicity by evaluating their *in vivo* hepatoprotective and antioxidant role. Thus, in the present study, we have extensively studied the antioxidant activity of DF using *in vivo* models. For this aim, the treatments of DF was done orally as food containing 10% powdered DF because the effect of the functional plant represents a well characterized in nutrition and widely used as consumption by human in our country and worldwide. The preventive potential and antioxidant capacity of the DF was evaluated by liver histopathological changes, measuring LDSE as aspartate aminotransferase (AST), alanin aminotransferase (ALT), gamma glutamyltranspeptidase (GGT), lactate dehydrogenase (LDH), antioxidant defense systems (ADS) such as reduced glutathione (GSH), glutathione reductase (GR), superoxide dismutase (SOD), glutathione *S*-transferase (GST), catalase (CAT), glutathione peroxidase (GPx) and malondialdehyde (MDA) content in various tissues of rats. The LDSE, ADS and histopathologic changes were chosen due to their importance as index of hepatoprotective and antioxidant activity.

2. Materials and methods

2.1. Chemicals

Thiobarbituric acid (TBA), butylated hydroxytoluene (BHT), trichloroacetic acid (TCA), ethylenediaminetetraacetic acid (EDTA), reduced glutathione (GSH), metphosphoric acid, 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), trihydroxymethyl aminomethane (Tris), 1-chloro-2,4-dinitrobenzene (CDNB), oxidized glutathione (GSSG), β-Nicotinamide adenine dinucleotide phosphate (NADPH), potassium dihydrogen phosphate (KH_2PO_4) and sodium chloride (NaCl) of technical grade used in this study were supplied by Sigma Chemical Co. (St. Louis, MO, USA). Kits for antioxidant enzymes analysis were supplied by Randox Laboratories ltd.

2.2. Animals

Rats (*Wistar albino*) 4 months of age with an average weighing 200–300 g were provided from the Experimental Animal Research Center, Yuzuncu Yil University, and were housed in 4 groups, each group containing 6 rats. The animals were housed at 20 ± 2 °C in a daily light/dark (~16/8 h) cycle. All animals were fed a group wheat-soybean-meal-based diet and water *ad libitum* in stainless cages, and received humane care according to the criteria outlined in the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Science and published by the National Institutes of Health. The ethic regulations were followed in accordance with national and institutional guidelines for the protection of animal welfare during experiments. This study was approved by The Ethic Committee of the Yuzuncu Yıl University.

2.3. Preparation of foods

Briefly, DF was provided from a local winegrower in Batman, Binati (local name) variety-producing province of Turkey. The natural and organic DF of Binati variety used in the study was obtained from the Hasankeyf-Batman region. This variety was chosen because of the remarkable total production of DF in the region comes from Binati. DF was ground into powder and then the amount of powdered DF was adjusted 10% of rat food.

2.4. Experimental design

The rats were randomly divided into four groups each containing six rats.

Group I (*Control*): the rats received tap water and fed with standard pellet diet as *ad libitum*.

Group II (*Ethanol*): the rats received 20% ethanol water and fed with standard pellet diet as *ad libitum*. Dose of ethanol was selected on the basis of a 20% concentration at which caused oxidative stress administered orally [25–29].

Group III (*10% DF*): the rats received tap water and fed with 10% DF powder containing diet supplementation.

Group IV (10% *DF* + 20% *ethanol*): the rats received 20% ethanol water and fed with 10% DF powder containing diet supplementation.

2.5. Preparation of tissues supernatant and erythrocyte pellets

At the end of the 50 days experiments, the rats were anesthetized by injection of ketamine (5 mg/100 g body weight) intra-peritoneally. The blood samples were obtained from a cardiac puncture using syringe for the determination of LDSE levels and biochemical analysis. The serum samples were obtained by centrifuging blood samples at 4000xg for 15 min at 4 °C, and enzyme levels were measured in these serum samples. For biochemical analysis, blood samples were put immediately into silicon disposable glass tubes with EDTA as an anticoagulant and were centrifuged at 4000xg for 15 min at 4 °C and erythrocyte pellets were obtained. Then, the pellets were washed tree times with physiological saline (0.9% NaCl).

The tissues as brain, kidney, spleen, heart and liver were dissected and put in petri dishes. After washing the tissues with physiological saline (0.9% NaCl), samples were taken and kept at −78 °C during the analysis. The tissues were homogenized for 5 min in 50 mM ice-cold KH_2PO_4 solution (1:5 w/v) using stainless steel probe homogenizer (20 KHz frequency ultrasonic, Jencons Scientific Co.) and then centrifuged at 7000*g* for 15 min. All processes were carried out at 4 °C. Supernatants and erythrocyte pellets were used to determine ADS constituents and MDA contents [27–29].