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ORIGINAL ARTICLE

Influence of olive and rosemary leaves extracts on chemically induced liver cirrhosis in male rats

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Abstract The current study was undertaken to evaluate the protective activity of olive and rosemary leaves extracts on experimental liver cirrhosis induced by thioacetamide (TAA) in Wistar male rats. Highly significant decline in the values of body weight gain and highly statistically increase of liver/body weight ratio were noted in rats treated with TAA. Furthermore, the levels of serum alanine aminotransferase, aspartate aminotransferase, gamma glutamyl transferase, alkaline phosphatase and total bilirubin were statistically increased. Additionally, light microscopic examination of liver sections from rats treated with TAA showed a marked increase in the extracellular matrix collagen content and bridging fibrosis was prominent. There were bundles of collagen surrounding the lobules that resulted in large fibrous septa and distorted tissue architecture. Interestingly, the findings of this experimental study indicated that the extracts of olive and rosemary leaves and their combination possess hepatoprotective properties against TAA-induced hepatic cirrhosis by inhibiting the physiological and histopathological alterations. Moreover, these results suggest that the hepatoprotective effects of these extracts may be attributed to their antioxidant activities.

1. Introduction

Liver or hepatic cirrhosis affects hundreds of millions of patients worldwide. Liver cirrhosis is the terminal stage of various chronic liver diseases (Gressner, 1996; Schuppan and Afdhal, 2008). Moreover, the majority of patients worldwide with hepatocellular carcinoma (HCC) have underlying liver cirrhosis, supported by the fact that in 80% of autopsies of patients with HCC, cirrhosis is found (Simonetti et al., 1991).

Thioacetamide (TAA), also known as thioacetimidic acid, or acetothioamide (CH_3CSNH_2), was originally used as a fungicide (Vadi and Neal, 1981). Moreover, many experimental studies showed that TAA induced liver fibrosis and cirrhosis in experimental animals (Al-Attar, 2011, 2012; Wang et al., 2012; Fatima and Mahboob, 2013; Shao et al., 2014).

Recently, World Health Organization (WHO) defined traditional medicine (including herbal drugs) as therapeutic practices that have been in existence, often for hundreds of years, before the development and spread of modern medicine and are still in use today (Kashaw et al., 2011). The olive tree (*Olea europaea* L.), family: *Oleaceae*, and in particular, its leaves have been used for the treatment of wounds, fever, diabetes, gout, atherosclerosis and hypertension since ancient

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times (Jänicke et al., 2003). Rosemary (*Rosmarinus officinalis* Linn.), mint (*Labiatae*) family, is a common household plant grown in many parts of the world. It is commonly used as a spice and flavoring agent in food processing (Saito et al., 2004). However, rosemary and its constituents have a therapeutic potential in treatment or prevention of many physiological, biochemical and histopathological alterations (al-Sereiti et al., 1999; Osakabe et al., 2004; Sancheti and Goyal 2006; Gaya et al., 2013). The purpose of the present study is to compare the effects of olive and rosemary leaves extracts on experimental liver cirrhosis induced by TAA in rats.

2. Materials and methods

2.1. Animals

Male albino rats of the Wistar strain (*Rattus norvegicus*), weighing 72.6–103.4 g were used in the present study. The experimental animals were obtained from the Experimental Animal Unit of King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia. Rats were acclimatized to the laboratory conditions for 10 days prior to the initiation of experimental treatments. The experimental animals were housed in standard plastic cages and maintained under controlled laboratory conditions of humidity (65%), temperature (20 ± 1 °C) and 12:12 h light:dark cycle. Rats were fed *ad libitum* on normal commercial chow and had free access to water. The experimental treatments were conducted in accordance with ethical guidelines of the Animal Care and Use Committee of King Abdulaziz University.

2.2. Olive and rosemary leaf extraction

Olive and rosemary leaves of fine quality were obtained from a commercial market, Jeddah, Saudi Arabia. The leaves were thoroughly washed and dried at room temperature. The methods of Sakr and Lamfon (2012), and Al-Attar and Abu Zeid (2013) were used to prepare the extracts with some modifications. The dried olive leaves (50 g) were powdered and added to 2 liters of hot water in a flask. After 6 h, the mixture was slowly boiled for 1 h. After boiling period, the mixture was cooled at room temperature and it was gently subjected to an electric mixer for 10 min. Also, the dried rosemary leaves (50 g) were powdered and added to 2 liters of hot water in a flask. After 6 h, the mixture was slowly boiled for 1 h. After boiling period, the mixture was cooled at room temperature and it was gently subjected to an electric mixer for 10 min. Thereafter the solutions of olive and rosemary leaves were filtered. Finally, the filtrates were evaporated in an oven at 40 °C to produce dried residues (active principles). With references to the powdered samples, the yields means of the olive and rosemary extracts were 18.7% and 20.6% respectively. Furthermore, these extracts were prepared every 2 weeks and stored in a refrigerator for subsequent experiments.

2.3. Experimental design

A total of forty-eight rats were randomly divided into eight experimental groups, six of rats each. The experimental groups were treated as follows:

1. Rats of group 1 were served as controls and intraperitoneally injected with saline solution (0.9% NaCl), twice weekly for twelve weeks.
2. Rats of group 2 were given 300 mg/kg body weight of TAA (Sigma–Aldrich Corp., St. Louis, MO, USA) by intraperitoneal injection, twice weekly for twelve weeks.
3. Rats of group 3 were intraperitoneally injected with TAA at the same dose given to group 2 and were orally supplemented with olive leaves extract at a dose of 200 mg/kg body weight/day for twelve weeks.
4. Rats of group 4 were intraperitoneally injected with TAA at the same dose given to group 2 and were orally supplemented with rosemary leaves extract at a dose of 200 mg/kg body weight/day for twelve weeks.
5. Animals of group 5 were intraperitoneally injected with TAA at the same dose given to group 2 and were orally supplemented with olive leaves extract (100 mg/kg body weight/day) and rosemary leaves extract (100 mg/kg body weight/day) for twelve weeks.
6. Rats of group 6 were intraperitoneally received saline solution at the same dose given to group 1 and were orally supplemented with olive leaves extract at the same dose given to group 3 for twelve weeks.
7. Animals of group 7 were intraperitoneally received saline solution at the same dose given to group 1 and were orally supplemented with rosemary leaves extract at the same dose given to group 4 for twelve weeks.
8. Rats of group 8 were intraperitoneally received saline solution at the same dose given to group 1 and were supplemented with olive and rosemary leaves extracts at the same dose given to group 5 for twelve weeks.

2.4. Body weight determinations

The body weights of rats were determined at the start of the experimental period and after twelve weeks using a digital balance. These weights were measured at the same time during the morning (Al-Attar and Zari, 2010). Moreover, the experimental animals were observed for signs of abnormalities throughout the period of study.

2.5. Blood serum analyses

After twelve weeks, the experimental animals were fasted for 12 h, water was not restricted, and then anaesthetized with diethyl ether. Blood samples were collected from orbital venous plexus in non-heparinized tubes, centrifuged at 2500 rpm for 15 min and blood sera were then collected and stored at 4 °C prior immediate determination of alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP) and total bilirubin. The method of Reitman and Frankel (1957) was used to determine the levels of serum ALT and AST. Serum GGT level was measured according to the method of Szasz (1969). The method of MacComb and Bowers (1972) was carried out to determine the level of serum ALP. Total bilirubin concentration was determined using the method of Doumas et al. (1973).