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Physiological investigations on the effect of olive and rosemary leaves extracts in male rats exposed to thioacetamide



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Abstract Physiologically, it is known that thioacetamide (TAA) toxicity is generally associated with hepatic fibrosis induction, complicated metabolic disorders and health problems. The capability of extracts of olive and rosemary leaves to attenuate the severe physiological disturbances induced by thioacetamide (TAA) intoxication in male rats has been evaluated. Healthy male Wistar rats were used in the present study and were divided randomly into eight groups. Rats of the first group were served as normal control. Rats of the second group were administrated with TAA. Rats of the third, fourth and fifth groups were exposed to TAA plus olive leaves extract, TAA plus rosemary leaves extract and TAA plus olive and rosemary leaves extracts respectively. The sixth, seventh and eighth groups were supplemented with olive leaves extract, rosemary leaves extract, and olive and rosemary leaves extracts respectively. After 12 weeks of experimental treatments, the levels of serum glucose, total protein, albumin and high density lipoprotein cholesterol were significantly decreased, while the levels of triglycerides, cholesterol, low density lipoprotein cholesterol, very low density lipoprotein cholesterol, creatine kinase and lactate dehydrogenase were statistically increased in rats exposed to TAA. Administration of the studied extracts inhibited the hematobiochemical parameters and improved the physiological disturbances induced by TAA intoxication. Additionally, most improvements were noted in rats administrated with rosemary leaves extract followed by olive and rosemary leaves extracts and olive leaves extract. These results suggested that the effect of these extracts might be due to their antioxidant activities against TAA toxicity.

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1. Introduction

The World Health Organization (WHO) estimates that more than 25% of the global burden of disease is linked to environmental factors, including exposures to toxic chemicals. Tens of thousands of chemicals are currently in use, and hundreds more are introduced every year. Because current chemical

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testing is expensive and time consuming, only a small fraction of chemicals have been fully evaluated for potential human health effects. Thioacetamide (TAA) was originally used to control the decay of oranges and then as a fungicide (Childs and Siegler, 1945). TAA is a potent hepatotoxicant which requires metabolic activation by the mixed-function oxidases. For its toxicity, thioacetamide requires oxidation to its S-oxide and then further to reactive S,S-dioxide form which ultimately attacks lipids and proteins (Hajovsky et al., 2012). Furthermore, the effects of TAA are not limited to the liver as profound structural and functional changes have been described in the thymus (Barker and Smuckler, 1974), the kidney (Barker and Smuckler, 1974; Caballero et al., 2001), the intestine (Ortega et al., 1997; Caballero et al., 2001), the spleen (Al-Bader et al., 2000) and the lung (Latha et al., 2003).

The pharmacological treatment of disease began long ago with the use of herbs (Schulz et al., 2001). Herbal medicine is the use of plants, plant parts, their water or solvent extracts, essential oils, gums, resins, exudates or other forms of advanced products made from plant parts used therapeutically to provide proactive support of various physiological systems; or, in a more conventional medical sense, to treat, cure, or prevent a disease in animals or humans (Weiss and Fintelmann, 2000). About 70–80% of the world populations, particularly in the developing countries, rely on non-conventional medicine in their primary healthcare as reported by the World Health Organization (Akerele, 1993). The use of herbal medicines and phytonutrients or nutraceuticals continues to expand rapidly across the world with many people now resorting to these products for treatment of various health challenges in different national healthcare settings (WHO, 2004). This interest in drugs of plant origin is due to several reasons, namely, conventional medicine can be inefficient (e.g. side effects and ineffective therapy), abusive and/or incorrect use of synthetic drugs results in side effects and other problems, a large percentage of the world's population does not have access to conventional pharmacological treatment, and folk medicine and ecological awareness suggests that natural products are harmless.

Olive tree (*Olea europaea*, Oleaceae) is a longevous plant, anciently known in the Mediterranean basin (Melillo, 1994). The olive tree has been widely accepted as one of the species with the highest antioxidant activity via its oil, fruits, and leaves. It is well known that the activity of the olive tree byproduct extracts in medicine and food industry is due to the presence of some important antioxidant and phenolic components to prevent oxidative degradations. Olive leaves are considered a cheap raw material and a useful source of high-added value products (Briante et al., 2002; Jemai et al., 2008). The main phenolic compound in olive leaves is the glycosylated form of oleuropein (Amro et al., 2002; Visioli et al., 2002). It is a natural phenolic antioxidant, which is present in high concentration in olives, olive oil and olive tree leaves (Andreaddou et al., 2007). Rosemary plant with the scientific name of *Rosmarinus officinalis* belongs to the Lamiaceae family. Four main categories of compounds found in rosemary include flavonoids, phenols, volatile oil, and terpenoids (Barnes et al., 2007). Leaves of rosemary possess a variety of bioactivities, including antioxidant, antitumor, antiinflammatory, treat headaches and anti-HIV (Aruoma et al., 1996; Altinier et al., 2007). The present study is aimed to investigate the role of olive and rosemary leaves extracts against TAA toxicity in Wistar male rats.

2. Materials and methods

2.1. Extraction of olive and rosemary leaves

The methods of Sakr and Lamfon (2012), and Al-Attar and Abu Zeid (2013) were used for the preparation of olive and rosemary leaves extracts with some modifications. Fine qualities of olive and rosemary leaves were obtained from a commercial market, Jeddah, Saudi Arabia. The leaves were scientifically defined by the herbarium of Biological Sciences Department, Faculty of Sciences, King Abdulaziz University, Jeddah, Saudi Arabia. The leaves were thoroughly washed and dried at room temperature. The dried olive leaves (50 g) were powdered and added to 2 L 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 L 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 yield 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 experimentation.

2.2. Animal models

Forty-eight Wistar male rats weighing 72.6–103.4 g were obtained from the Experimental Animal Unit of King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia. Rats were acclimatized to the experimental laboratory having temperature 20 ± 1 °C, controlled humidity conditions (65%) and 12:12 h light:dark cycle. Rats were housed in standard plastic cages, fed with standard diet, and water *ad libitum*. All experimental procedures were conducted in accordance with ethical guidelines of the Animal Care and Use Committee of King Abdulaziz University.

2.3. Experimental design

The animals were divided into eight groups of six animals each and then subjected to one of the following treatments:

Group 1: Rats were served as controls and intraperitoneally injected with saline solution (0.9% NaCl), twice weekly for 12 weeks.

Group 2: Rats were given 300 mg/kg body weight of TAA (Sigma–Aldrich Corp., St. Louis, MO, USA) by intraperitoneal injection, twice weekly for 12 weeks.

Group 3: Rats 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 12 weeks.

Group 4: Rats were intraperitoneally injected with TAA at the same dose given to group 2 and were orally supplemented

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