Original Article

Antioxidant Machinery Related to Decreased MDA Generation by *Thymus Algeriensis* Essential Oil-induced Liver and Kidney Regeneration

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Abstract

Objective This study was conducted to determine the histopathological and biochemical effects of *Thymus algeriensis* essential oil (TEO) on hydrogen peroxide (H_2O_2)-induced oxidative stress in liver and kidney tissues of rats.

Methods Rats were treated in six groups and were exposed for 2 weeks to low (LD; 100 μmol/L) and high doses (HD; 1 mmol/L) of H_2O_2 in the presence or absence of TEO (180 mg/kg). Liver and kidney atrophy was measured by using biochemical and histopathological assays.

Results Our study demonstrated that H_2O_2 induced liver and kidney atrophy, as evidenced by the significant elevation of serum aminotransferase, urea, and creatinine levels compared with those in the control rats. Urea levels were estimated by evaluating the activity of serum urease that hydrolyzes urea into CO_2 and ammonia. However, TEO treatment significantly alleviated oxidative stress in the H_2O_2-induced liver and kidney toxicity model by reducing the levels of malondialdehyde concomitantly with marked elevations in superoxide dismutase, catalase, glutathione peroxidase, and glutathione S-transferase, as well as decrease in glutathione activity.

Conclusion Our data demonstrated that TEO protected against H_2O_2 toxicity by decreasing oxidant levels and DNA damage, as well as increasing antioxidant levels, indicating that TEO has a spectrum of antioxidant and DNA-protective properties.

Key words: Liver and kidney atrophy; Hydrogen peroxide; *Thymus algeriensis*; Antioxidants; Toxicity

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INTRODUCTION

Hydrogen peroxide (H_2O_2) is an important cause of oxidative injury because it can easily transform into a hydroxyl radical, which is one of the most destructive free radicals. Moreover, the half-life of H_2O_2 is comparatively longer than that of other reactive oxygen species (ROS)[1]. H_2O_2 uses aquaporins to cross cell membranes rapidly[2] and therefore has the ability to diffuse in and out through the cell membrane. In a previous study, it was found that H_2O_2, rather than the superoxide anion, is the most toxic species that induces alterations in cellular functioning[3].

The liver is very susceptible to toxicity produced by reactive metabolites because it is a major site of xenobiotic metabolism. Other than the liver, the kidney, which is susceptible to toxicity, is

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also a highly specialized organ that maintains the internal environment of the body through selective excretion of unwanted substances or retention of various substances according to the needs of the body[4]. ROS have been implicated in the pathogenesis of several harmful diseases, including multiple sclerosis and liver cirrhosis. It is also a universal risk factor for the development of liver cancer (human hepatocellular carcinoma)[5]. Because hepatocyte inflammation is the major cause of liver disorders, one important therapeutic strategy may include the use of anti-inflammatory and hepatoprotective interventions, either alone or in combination[6]. Furthermore, the use of safe natural compounds as anti-inflammatory agents could be a better alternative for the detoxification of the liver and kidney.

Numerous studies revealed that plant remedies are useful in treating liver diseases[7]. As many plants and herbs have effective natural antioxidants, such as carbolic acid, isoprene derivatives, carotene, terpene, and polyphenol,[8] extracts or essential oils from these plants could serve as effective hepatoprotective and nephroprotective agents. Collective evidence supports that H_2O_2-induced cell injury can be prevented by antioxidants such as the essential oil of *Thymus algeriensis* (TEO)[9]. As described in our previous reports, TEO has the potential to prevent acute gastric injury and testis toxicity in rats by correcting the cellular imbalance between oxidants and antioxidants[10-11].

In this study, we determined whether TEO has the potential to protect the liver and kidney from H_2O_2-induced injuries. We present extensive data showing the biochemical and pathological alterations induced by H_2O_2, and the preventive effects of TEO against H_2O_2-induced tissue damage in rats.

MATERIALS AND METHODS

Chemicals

All reagents, including Ellman's reagent, reduced glutathione (GSH), 5,5'-dithiobis-(2-nitrobenzoic acid), bovine serum albumin, H_2O_2, thiobarbituric acid (TBA), 2,4-dinitrochlorobenzene (CDNB), and Tris-HCl buffer were purchased from Sigma (St. Louis, MO, USA), Fluka Chemie (Buchs, Switzerland), and Merck (Nottingham, UK).

Plant Material

The aerial portions of wild *Thymus algeriensis* plants were collected during the flowering stage in March 2013 from Jebel Orbata in Gafsa, which is located on the middle west of Tunisia at a latitude and longitude of 34.39641 and 9.12914, respectively. Plant specimens (Voucher #1188) were identified and deposited in the herbarium of medicinal plants at the Agronomic National Institute of Tunis, Tunisia. The essential oil of TEO was extracted with a Clevenger apparatus[12] and dried over Na_2SO_4. Purified TEO was stored in sealed dark vials at 4 °C.

Experimental Animals

Thirty-six male Sprague Dawley rats (6-8 weeks old) weighing between 180 and 200 g were obtained from the animal laboratory of the Pasteur Institute of Tunis. The Ethical Committee for animal experiments of the Faculty of Sciences of Bizerte, University of Carthage, Tunisia, approved all animal protocols (Ethic# LNSP/Pro 152012) governing the experiments. Before the experiment, rats were acclimatized for 7 days to human contact to minimize their physiological responses to handling for subsequent protocols[12]. Rats were randomized into six groups comprising six animals each for treatments as follows: (1) control (C), (2) low dose (100 μmol/L) H_2O_2 (LD H_2O_2), (3) high dose (1 mmol/L) H_2O_2 (HD H_2O_2), (4) TEO (180 mg/kg per day dissolved in normal saline), (5) TEO and LD H_2O_2 (180 mg/kg per day and 100 μmol/L, respectively), and (6) TEO and HD H_2O_2 (180 mg/kg per day and 1 mmol/L, respectively). TEO was administered 1 h prior to H_2O_2 treatment in animals receiving both agents. Rats were then housed in a pathogen-free environment (clean polypropylene cages) with a 12 h light/dark cycle, relative humidity of 55%±10%, and a temperature of 20-25 °C. The animals were permitted access to standard pellets and water *ad libitum*. The dosage of TEO used had been optimized in our previous study[10]. All treatments were administered orally for 15 d.

Body weights were measured daily for 15 d. At the end of this experiment, rats were anesthetized and blood was collected from tail vein to measure the enzymatic activities. Subsequently, rats were euthanized by using cervical dislocation. The livers and kidneys of treated and untreated animals were carefully dissected, and portions were fixed in 10% buffered formaldehyde for routine histological evaluations after staining with hematoxylin and eosin (H&E). Unfixed portions of the livers and kidneys were weighed and subjected to extraction by using a mechanical rotary homogenizer in