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Transcriptional response of genes involved in cell defense system in human cells stressed by H_2O_2 and pre-treated with (Tunisian) *Rhamnus alaternus* extracts: Combination with polyphenolic compounds and classic *in vitro* assays

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Abstract

The ability of three *Rhamnus alaternus* leaves extracts on antigenotoxic and gene expression level effects was respectively investigated in a bacterial assay system, i.e. the SOS chromotest with *Escherichia coli* PQ37 and in human K562 lymphoblast cell line. Total oligomers flavonoids (TOF) enriched, methanol and ethyl acetate extracts were prepared from powdered *R. alaternus* leaves and characterized quantitatively for the presence of polyphenolic compounds. We explored the response to oxidative stress using the transcriptional profile of genes in K562 cells stressed with H_2O_2 after incubation with plant extracts. For this purpose, we used a cDNA microarrays containing 82 genes related to cell defense, essentially represented by antioxidant and DNA repair genes. Analysis revealed that SOD1, AOE 372, TXN genes involved in the antioxidant defense system and XPC, LIG4, POLD2, PCNA genes implied in the DNA repair system were among the most expressed ones in the presence of the tested extracts. These results were in accordance with those obtained when we tested the antigenotoxic and antioxidant effects of the same extracts with, respectively the SOS chromotest and the xanthine/xanthine oxidase enzymatic assay system. The effect of the tested extracts on SOS response induced by both Aflatoxin B1 (AFB1: 10 μg/assay) and nifuroxazide (20 μg/assay) showed that the TOF extract exhibited the highest antimutagenic level towards the indirect mutagen AFB1. Whereas ethyl acetate extract showed the highest antimutagenic effect towards the direct mutagen, nifuroxazide.

None of the tested extracts induced mutagenic activity. However all the tested extracts exhibited xanthine oxidase inhibiting and superoxide anions scavenging effects.

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R. alaternus extracts contain compounds with significant antioxidant and antigenotoxic activities. These compounds modulate gene expression as detected by using cDNA arrays.

Keywords: *Rhamnus alaternus* leaf extracts; Polyphenolic content; Gene expression modulation; Antigenotoxic effect; Antioxidant activity; cDNA microarrays

1. Introduction

Environmental mutagens may interact with the organism cells and produce Reactive Oxygen Species (ROS) which cause molecular damage to proteins [1], lipids [2] and nucleic acids [3]. Fortunately, numerous defense systems protect cellular macromolecules against oxidation. Among them, antioxidant enzymes, DNA repair and redox control systems.

DNA repair systems take charge of oxidized bases, abasic sites and single strand breaks generated by oxidative process. However, cell defenses against oxidative stress are also known to decrease through changes in gene expression in response to oxidative stress [4]. Oxidative stress is believed to play important roles in several human diseases [5,6].

There is an increasing interest in the natural antioxidants contained in medicinal and dietary plants, which are candidates for the prevention of oxidative damages. Throughout investigations on plant antioxidants, it became necessary to appreciate antioxidant activity of plant extracts [7]. The role of free-radical reactions in biology becomes an area of intense interest, and it is generally accepted that free radicals play an important role in the development of tissue damage and pathological events in living organisms [8].

The plant used in this study, *Rhamnus alaternus* (Rhamnaceae), was selected based on its traditional use in Tunisian medicine. This plant has been used as a laxative, purgative, diuretic, antihypertensive and depurative. Mediterranean Buckthorn, *R. alaternus* L. (Rhamnaceae) inhabits the Mediterranean area and grows in Tunisia. The genus *Rhamnus* is characterized from a phytochemical point of view by the abundance of phenolic substances, especially flavonoids, anthraquinones and tannins [9,10], which are described by numerous authors as antioxidant molecules [11–13].

2. Materials and methods

2.1. Plant material

Aerial part of *R. alaternus* was collected from Ain Sobh, situated in the North West of Tunisia in November 2004. Identification was carried out by Dr. Ben Tiba (Horticulture Institute of Chott-Mariam, Tunisia), according to the flora of Tunisia [14].

A voucher specimen (Ra-12-004) has been kept in the Laboratory of Pharmacognosy, Faculty of Pharmacy of Monastir, Tunisia for future reference. The leaves were shade, dried, powdered and stored in a tightly closed container for further use.

2.2. Extraction method

Ethyl acetate (EtOAc) and methanol (MeOH) extracts were obtained by Soxhlet extraction (6 h). The two types of extracts, with different polarities were concentrated to dryness and the residue was kept at 4 °C. In order to obtain an enriched total oligomers flavonoids (TOF) extract, the powdered leaves were macerated in a water/acetone mixture (1:2, v/v) during 24 h, with continuous stirring. The extract was filtered and the acetone was evaporated under low pressure to obtain an aqueous phase. The tannins were partially removed by precipitation with an excess of NaCl during 24 h at 5 °C, then we recovered the supernatant. This latter was extracted with ethyl acetate, concentrated and precipitated with an excess of chloroform. The precipitate was separated and yielded the TOF extract which was dissolved in water [15]. In the present study, three extracts were investigated. The doses of extracts we tested in both SOS chromotest (10, 50 and 250 μg/assay) and xanthine oxidase enzymatic system (15, 50, 150, 200, 250 and 300 μg/ml) are in accordance with previous investigations in our laboratory [16–21], where a number of preliminary dose-finding tests involving a number of plant extracts were conducted. This means that the doses were suitable for testing the majority of the extracts, however, not necessarily all. Therefore, some extracts may be toxic at one or more of the applied doses.

2.3. Preliminary phytochemical analysis

Plant extracts were screened for the presence of flavonoids and tannins, by using the methods previously described by Kilani et al. [19] and Tona et al. [22]. Two