Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 100 (2007) 81–90
Food Chemistry
www.elsevier.com/locate/foodchem

Modulation of oxidative damage by natural products

Rakshamani Tripathi [a], H. Mohan [b], J.P. Kamat [c,*]

[a] Department of Biotechnology, V.B.S. Purvanchal University, Jaunpur, UP 222001, India
[b] Radiation Chemistry and Chemical Dynamics Division, Bhabha Atomic Research Centre, Mumbai 400 085, India
[c] Radiation Biology and Health Sciences Division, Bhabha Atomic Research Centre, Mumbai 400 085, India

Received 2 May 2005; received in revised form 5 September 2005; accepted 5 September 2005

Abstract

Using rat liver membrane as a model system, modulation of oxidative damage induced by pathophysiological agents such as photosensitization and radiation was examined with two medicinal plant extracts, namely *Andrographis paniculata* (Ap) and *Swertia chirata* (Sc). Results showed that simultaneous addition of both the extracts (50 μg/ml) in independent experiments during generation of reactive oxygen species (ROS) could significantly prevent increased levels of products of lipid peroxidation, such as conjugated dienes, lipid hydroperoxide, TBARS and 4-hydroxylnonenals. The oxidative damage observed with depletion of major endogenous antioxidants, such as GSH, as well as enhanced formation of protein oxidation, was effectively reduced by these extracts. Similarly, degradation of mitochondrial proteins by ROS, induced during photosensitization, was effectively prevented by both the extracts (SDS–PAGE experiments). The antioxidative property of these extracts could be attributed to their scavenging ability with superoxide, hydroxyl radicals and singlet oxygen species, the major species generated during photosensitization and γ-radiation. The high scavenging ability of the extracts may be due to high phenolic contents, flavonoid constituents and considerable reducing equivalents. The pulse radiolysis studies showed high reactivity with $ABTS^{-}$. The reaction of the extracts of Ap and Sc with dimethyl *p*-phenylene diamine dihydrochloride (DMPD), one of the important stable synthetic radicals gave >30% inhibition at 50 μg/ml. In view of these observations, termination of the free radical reaction, and quenching of reactive oxygen are suggested to be, in part, responsible for the antioxidant activity of *Andrographis paniculata* and *Swertia chirata* extracts. Therefore, *Andrographis paniculata* and *Swertia chirata* extracts may emerge as effective antioxidative agents, protecting cells from pathophysiological oxidants, generated during UV–vis photosensitization/radiation-induced injury, and may be useful in the food industry as effective synthetic antioxidants.

Keywords: Antioxidant activity; Lipid peroxidation; Free radical scavenging ability; Photosensitization; Radiation; *Andrographis paniculata* and *Swertia chirata* extracts

1. Introduction

In recent years, the studies on "oxidative stress" and its adverse effects on human health have become a subject of considerable interest. It is a well-documented fact that exposure of organisms to exogenous and endogenous factors generates a wide range of reactive oxygen species (ROS), resulting in homeostatic imbalance (Bonnefont, Bastard, Jandon, & Delattre, 2000; Halliwell & Gutteridge, 1999; Sies, 1997; Thomas & Kalyanaraman, 1998). Polyunsaturated fatty acids of cell membranes are the critical components, susceptible to such insult (Esterbauer, 1996; Halliwell & Gutteridge, 1999). ROS induce alterations and loss of structural/functional architecture in the cell, leading directly to cytotoxicity and/or indirectly to genotoxicity, with numerous serious anomalies favouring disharmony and diseases (Esterbauer, 1996; Girotti, 1994; Halliwell & Gutteridge, 1999; Sies, 1997). Therefore, the factors that shift the physiological process,

* Corresponding author. Fax: +91 2225505151.
E-mail address: jkamat@magnum.barc.ernet.in (J.P. Kamat).

0308-8146/$ - see front matter © 2005 Published by Elsevier Ltd.
doi:10.1016/j.foodchem.2005.09.012

in the homeostatic balance are of considerable interest (Sies, 1997).

Antioxidant principles from herbal resources are multifaceted in their effects and provide enormous scope in correcting the imbalance through regular intake of a proper diet. It has been assumed that nutritional intervention to increase intake of phyto-antioxidants may reduce threat of free radicals (Arora, Kaur, & Kaur, 2003; Ng, Liu, & Wang, 2000). Plants play a significant role in maintaining human health and improving the quality of human life. They serve humans well as valuable components of food, such as seasonings and beverages as well as in cosmetics, dyes, and medicines. The World Health Organization estimated that <80% of the earth's inhabitants rely on traditional medicine for their primary health care needs, and most of this therapy involves the use of plant extracts and their active components (Winston, 1999).

Lipid peroxidation is of great concern to the food industry and consumers because it leads to the development of undesirable off-flavours and potentially toxic reaction products (Maillard, Soum, Meydani, & Berset, 1996). Many synthetic antioxidants, such as butylated hydroxyanisole, butylated hydroxytoluene, *t*-butylhydroquinone and propyl gallate, are used to retard lipid peroxidation (Wanita & Lorenz, 1996). However, the use of synthetic antioxidants is under strict regulation due to the potential health hazards caused by such compound (Hettiarachchy, Glenn, Gnanasambandam, & Johnson, 1996; Park, Jung, Nam, Shahidi, & Kim, 2001). In view of the beneficial role of herbs in the food industry and the present understanding about the role of oxidative stress in pathogenesis of multiple diseases, attempts have been made to examine the antioxidant status of some herbal products, *Andrographis paniculata* (Ap) and *Swertia chirata* (Sc), (Banerjee, Sur, Mandal, Das, & Sikdar, 2000; Ghosal, Sharma, & Jaswal, 1978; Saha & Das, 2003; Saha, Manadal, Das, Das, & Das, 2004), commonly known as Kalmegh (Sanskrit), and chirayata (Hindi) of the families, *Acanthaceae* and *Gentianaceae*, respectively. These herbs are found in many Asian countries and make significant contributions in ayurvedic preparations against a variety of diseases (Poolsup, Suthisisang, Prathanturarug, Asawamekin, & Chanchareon, 2004; Zhang & Tan, 2000). Hence, present investigations were carried out on antioxidant properties of these two important herbs against photosensitization (endogenous pigments, riboflavin act as sensitizers in presence of light and induced ROS) (Paillous & Fery-Forgues, 1994), and ionizing radiation (present in environmental flares) (Von Sonntag, 1987), the ROS-generating agents to which humans are frequently exposed. Oxidative damage was studied in rat liver mitochondria, one of the crucial sub-cellular organelles and the major site of oxidative reactions. No studies have so far been conducted in these plant extracts against such agents. The results demonstrated that the extracts of Ap and Sc have significant antioxidant activity against various reactive oxidants.

2. Materials and methods

2.1. Plant extracts

Aqueous extracts of *Andrographis paniculata* (Ap) and *Swertia chirata* (Sc) were obtained as a gift from Zandoo Research Laboratory, Mumbai.

2.2. Animals

The rats were bred in the BARC Laboratory Animal House Facility and obtained after getting clearance from the BARC Animal Ethics Committee. All the experiments were conducted with the ethical guidelines laid down by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) constituted by the Animal Welfare Division of Government of India on the use of animals in scientific research.

Female Wistar rats (weighing 250 ± 20 g, 10–12 weeks old), maintained, under controlled laboratory conditions (25 ± 2 °C; RH 60 ± 5%; 12 h photoperiod), fed standard animal food and tap water ad libitum were used for oxidative studies.

2.3. Chemicals

2-Thiobarbituric acid, vitamin C, GSH, xylem orange, 2-deoxyribose, mannitol, SOD, FAD, methionine, ABTS, dinitrophenyl hydrazine, *N*,*N*-dimethyl *p*-phenylene diamine dihydrochloride, methylene blue and hydrogen peroxide were purchased from Sigma Chemical Co. Tetraethoxypropane was used as the standard for estimating malonaldehyde equivalents. All other chemicals used in the study were of the highest purity commercially available.

2.4. Preparation of rat liver mitochondria

Rats were fasted overnight, sacrificed by cervical dislocation. Livers were removed, and homogenized in 0.25 M sucrose containing 1 mM EDTA. The homogenate was centrifuged at 3000*g* for 10 min to remove cell debris and the nuclear fraction. The resulting supernatant was centrifuged at 10,000*g* and for 10 min to sediment mitochondria in a Sorvall RC5C centrifuge. The mitochondrial pellets thus obtained were washed thrice with 5 mM potassium phosphate buffer pH 7.4 to remove sucrose and were suspended in the same buffer at a concentration of 10 mg protein/ml (Kamat, Sarma, Devasagayam, Nesaretnam, & Basiron, 1997).

2.5. Photosensitization as a source of ROS generation

The system for exposing mitochondria to photosensitization was very simple. The mitochondria (final concentration 0.5 mg protein/ml) were suspended in 50 mM phosphate buffer (pH 7.4) and kept in a 'trap' maintained at 37 °C, with or without the sensitizer and constant