Saudi Journal of Biological Sciences (2014) 21, 342–354

King Saud University

Saudi Journal of Biological Sciences

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ORIGINAL ARTICLE

Biochemical and molecular studies on the possible influence of the *Brassica oleracea* and *Beta vulgaris* extracts to mitigate the effect of food preservatives and food chemical colorants on albino rats

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Received 25 July 2013; revised 10 November 2013; accepted 13 November 2013
Available online 28 November 2013

KEYWORDS
Gene expression;
Antioxidant enzymes;
Broccoli;
Beet;
Sunset yellow;
Sodium nitrite

Abstract This study aimed to investigate the biochemical influence of broccoli and beet extracts on selected individual additives $NaNO_2$ or sunset yellow treated rats, in addition to the gene expression of some antioxidant enzymes. Forty-two male rats were assigned to seven groups of six rats in each group. The control group was fed a diet without an additive for four weeks. Group (2) received $NaNO_2$, groups (3) received $NaNO_2$ co-administered with broccoli extract (4) $NaNO_2$ co-administered with beet extracts, Group (5) received sunset yellow, Group (6) received sunset yellow co-administered with broccoli extract, and Group (7) received sunset yellow co-administered with beet extract, for four weeks. At the end of the experiment, blood, liver, kidney, and brain samples were taken for biochemical and/or molecular analysis. The mRNA expression of antioxidant enzymes was determined by reversing transcriptase-polymerase chain reaction (RT-PCR). The obtained results revealed that rats co-administered with beet or broccoli extracts had a significant decrease in serum levels of AST, ALT, ALP, urea, total lipids, and triglycerides, as well as a significant increase in reduced glutathione (GSH), glutathione peroxidase (GSH-px), and superoxide

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.sjbs.2013.11.004

dismutase (SOD) enzyme activities, compared to the normal control group. Oral administration of $NaNO_2$ or sunset yellow caused a significant increase in serum levels of AST, ALT, ALP, urea, total lipids, and triglycerides, as well as a significant decrease in GSH, GSH-px, and SOD compared to the positive group. In conclusion, this study showed that broccoli and beet extracts have a protective effect against $NaNO_2$ or sunset yellow in rat treated groups.

1. Introduction

Traditionally, foods were grown, gathered, and eaten directly from a fairly unpolluted land. As the human population grew exponentially, farming progressed, trade developed, and local markets shared a variety of products among a variety of people. Techniques for food preparation and preservation were developed to deal with the new problems of storage, waste, and food-borne diseases (Pegg and Shahidi, 2000; Sindelar and Milkowski, 2012). Therefore, the modern food-industry dependence on processing and additives increases and has continually produced new chemicals to control, preserve, and transform our food. Food Additives are any substance added to food, not normally consumed as a food by itself and not normally used as a typical ingredient of the food, they may be natural or synthetic (El-Samrages, 2012; Yilmaz et al., 2009). Therefore, they are classified into various categories such as antimicrobial agents, antioxidants, artificial colors, artificial flavors and flavor enhancers, chelating agents and thickening and stabilizing agents (El-Samrages, 2012; Sasaki et al., 2002). The safety of repeated exposure to permitted synthetic food additives (colorants or preservatives) has been questioned. In relation to the toxicological limit, the FAO/WHO, Joint Expert Committee on Food Additive (JECFA) established an acceptable daily intake (ADIs) of many food additives that can be consumed everyday throughout the lifetime of an individual without any appreciable health effects (WHO, 1987). Certain food colors such as carmoisine (E122), amaranth (E123), sunset yellow (E110), Tartrazine (E102) and Allura red (E129) have been examined in bacterial and animal studies and it has been found that their mutagenicity varies widely, depending on the dose consumed, implying that they may also act as mutagenic and/or carcinogenic agents in humans (Macioszek and Kononowicz, 2004; Tsuda et al., 2001; Zeiger, 1993). Regardless of the significance of food colorants, there is great worry about the effects of synthetic food colorants on human health. Sunset yellow FCF (SY) (Disodium 6-hydroxy-5-[(4-sulfophenyl)azo]-2-napthalenesulfononate) is an azo dye permitted for food usage in several countries including Saudi Arabia (FAIRS Country Report, 2012). Many researchers (Tanaka, 2005; Zraly et al., 2006) studied the metabolic and toxicological disorders induced by the administration of specific food colorant additives to rats and other mammals. Many azo compounds are genotoxic in short-term tests and carcinogenic in laboratory animals (Sasaki et al., 2002). Permissible daily intake of the coloring matter per body weight is 0–2.5 mg/kg. Currently, sunset yellow is forbidden in many countries such as Norway and Finland. In 2008, European Union stated that food and drinks containing any of six artificial colorings that may be linked to hyperactive behavior in children will have to carry health warnings, including sunset yellow. Moreover, the European Food Safety Authority (EFSA) decided in 2009 to lower the acceptable daily intake for sunset yellow from 2.5 to 1.0 mg/kg bodyweight per day (McCann et al., 2007). Nitrates are naturally occurring chemicals that are a metabolic product of microbial digestion of wastes containing nitrogen, for example, animal feces or nitrogen-based fertilizers present in soil, air, surface water, and ground water. Nitrogen and oxygen combine to form the nitrate compound (NO_3^-), using three oxygen atoms and one nitrogen atom (WHO, 2011). A nitrite (NO_2^-) is formed from two oxygen atoms and one nitrogen atom. In nature, nitrates can easily be converted to nitrites and vice versa. Human exposure to nitrates and nitrites comes from water, food and air. However, the nitrate and nitrite exposure we receive from air is negligible. Our primary source of exposure to nitrates and nitrites is through the food we consume, however exposure to these compounds can also occur through drinking water (WHO, 2011). The World Health Organization estimates the mean daily dietary intake of nitrate is from 43 to 141 mg, humans generally consume 1.2–3.0 mg of nitrite per day (WHO, 2007, 2011). Nitrites are added to fix color, to the preserved meat flavor, and to inhibit the growth of bacterial spores, specifically *Clostridium botulinum* (Pearson and Gillett, 1996). Some scientific studies suggest that nitrites promote and induce cancers in animals. When nitrites combine with certain amino acids, N-nitroso compounds or nitrosamines are formed and these have been shown to be carcinogenic (Grosse et al., 2006). In response to these findings, and the concern that excess nitrate/nitrite may react with the protein in the meat when it is cooked, to form these compounds, the amount of nitrate or nitrite that can be added to meat products is limited. The toxic effects of nitrates and nitrites are well recognized in mammalians, including testicular toxicity (Aly et al., 2010), hepatotoxicity and methemogobenemia (Knobeloch et al., 2000; Nituc et al., 2010; Rehman, 2001), and endocrine disturbance (Chaoui et al., 2004; Radikova et al., 2008). The wide use of nitrates as preservatives in food technology promotes the significance of studying their effects.

Oxidative stress is the result of an increased amount of reactive oxygen species (ROS) which can cause extensive injury to cell structures through attacking DNA, proteins and lipids, and is considered a major cause chronic diseases (Roya et al., 2009). Under normal conditions, in humans and animals, ROS can be neutralized by antioxidant defense systems including antioxidant enzymes that are considered as the first line defense antioxidants such as superoxide dismutase (SOD), glutathione peroxidase (GSH-px), and catalase (Fang et al., 2002). The second line of defense against ROS is glutathione (GSH), vitamin C, uric acid, albumin, bilirubin, vitamin E (α-tocopherol), carotenoids and flavonoids (Catapano et al., 2000). Due to health concerns, natural antioxidants have been