Food Chemistry 126 (2011) 429–434

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Protective effects of the crude extracts from yam (*Dioscorea alata*) peel on *tert*-butylhydroperoxide-induced oxidative stress in mouse liver cells

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ARTICLE INFO

Article history:
Received 26 February 2010
Received in revised form 5 September 2010
Accepted 1 November 2010

Keywords:
Yam
tert-Butylhydroperoxide
Lipid peroxidation
Antioxidative enzymes

ABSTRACT

Researchers have shown that yam extracts contain antioxidative activity; however, there are few reports regarding the antioxidant activities of yam peel. The effects of water and 50% ethanolic extracts from Darsan yam (*Dioscorea alata*) peel on the oxidative status of *tert*-butylhydroperoxide (t-BHP)-treated mouse Hepa 1–6 and FL83B liver cell lines were investigated. The cytosols were analysed for H_2O_2 and malondialdehyde (MDA) levels and antioxidative enzymes activities, including superoxide dismutase, glutathione peroxidase (GPx) and catalase activities. Both water and 50% ethanolic extracts from yam peel did not affect cellular MDA level in t-BHP-treated cells, but they altered the level of H_2O_2. Water extract from yam peel amplified the t-BHP-induced cytotoxicity in Hepa 1–6 whilst the ethanolic extract showed protection in FL83B cells. GPx activity might play an important role in the protective effect associated with t-BHP-induced oxidative stress.

1. Introduction

Yam is one of the important staples in the diets of many tropical countries because of its rich carbohydrate content (Waitt, 1963). Some yams are used as medication to prevent diarrhoea and diabetes in oriental countries (Hsu, Chen, Hsu, Chen, & Chang, 1984). Yam consumption would reduce blood sugar (Hikino et al., 1986) and lipid contents (Araghiniknam, Chung, Nelson-White, Eskelson, & Watson, 1996), as well as inhibit microbial activity (Kelmanson, Jager, & Van Staden, 2000). In addition, *Dioscrea japonica* yam was found to enhance serum IgG concentrations and promote lymphocyte proliferation (Fang & Kong, 2002).

Starch (75.6–84.3%) is the predominant fraction of the dry matter of yam tuber (Wanasundera & Ravindran, 1994), with small amounts of proteins, lipids and vitamins; it is also rich in minerals (Lasztity, Hidvegi, & Bata, 1998). The vitamin C content of yam tubers ranges from 13.0 to 24.7 mg/100 g wet weight and yam appears to be a moderately good source of minerals (Wanasundera & Ravindran, 1994). The active components of yam include steroidal sapogenin, glycan and storage proteins. Researchers have found that the steroid extract from yam could reduce serum lipid peroxidation, decrease serum triglyceride and phospholipid levels, and increase high density lipid level in the elderly (Araghiniknam et al., 1996). Dioscoran, a glycan isolated from *Dioscorea japonica*, was shown to markedly inhibit the hypoglycaemic effects in normal and alloxan-induced hyperglycaemic mice (Hikino et al., 1986). Dioscorin, the storage protein of yam tuber, exhibits scavenging activity toward the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical (Hou et al., 2001).

Researchers have shown that yam extracts contain antioxidative activity (Chan, Hsu, Wang, & Su, 2004; Chiang, Chou, & Hsu, 2005; Chou, Chiang, Chung, Chen, & Hsu, 2006). Although the antioxidative activities of yam extracts have been well documented, there are few reports regarding the antioxidant activities of yam peel. The total phenolic contents and antioxidant activities in the peel extracts of Darsan yam (*Dioscorea alata*) were determined and compared by Chung et al. (2008). The results indicated that the 50% ethanolic, hot water and water extracts from the peel all had high antioxidant activities. Amongst all of these extracts, the 50% ethanolic extract was found to be more effective in scavenging the DPPH radical than were the other extracts. Chen, Tu, Wu, Jong, and Chang (2004) also reported that all peel portions had better scavenging effects on the DPPH free radical than had the flesh portions of the Darsan yam.

Increasing evidence indicates that oxidative stress is related to the progression of carcinogenesis (Han, Mytilineou, & Cohen, 1996; Patel, Shah, Shukla, Shah, & Patel, 2009; Valko et al., 2007). Several enzymes are involved in the endogenous antioxidative system, such as superoxide dismutase (SOD), catalase and glutathione peroxidases (GPx) (Husain, Cillard, & Cillard, 1987; Oberley & Buettner, 1979; Wendel, 1980). The SOD is a ubiquitous

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doi:10.1016/j.foodchem.2010.11.004

metalloprotein that play a major protective role in living organisms by catalysing the dismutation of superoxide to H_2O_2 and an oxygen molecule (Oberley & Buettner, 1979). GPx and catalase prevent the formation of reactive oxygen species by catalysing the decomposition of H_2O_2 (Husain et al., 1987). The GPx, a GSH-related enzyme, plays a crucial role in the detoxification of H_2O_2 and lipid peroxidation, as well as providing the main enzymatic defence system against oxidative stress in mammalian cells (Wendel, 1980). Catalase is the enzyme that nonspecifically catalyses the decomposition of H_2O_2.

The oxidant, *tert*-butylhydroperoxide (t-BHP), is the chemical often used to induce oxidative stress in animals. The detoxification of t-BHP can be achieved by the GSH-mediated GPx catalysed reaction and/or by direct reaction with GSH to increase reactive oxygen species (ROS) formation, which further leads to lipid peroxidation (Davies, 1989). In addition, t-BHP can be metabolized to free radical intermediates by cytochrome P450 in hepatocytes, which affects cell integrity by initiating lipid peroxidation, and thus can result in cell damage due to oxidative stress (Rush et al., 1985).

Therefore, the main objective of this study was to investigate the effects of water or ethanolic yam peel extract on t-BHP-induced oxidative stress in mouse liver cells (Hepa 1–6 and FL83B).

2. Materials and methods

2.1. Materials

MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide), xylenol orange (3,3′-bis[N,N-di(carboxymethyl)-aminomethyl]-o-cresolsulfone-phthalein, sodium salt), *tert*-butylhydroperoxide (t-BHP), bovine serum albumin (BSA), glutathione reductase, reduced glutathione (GSH), and reduced beta-nicotinamide adenine dinucleotide phosphate (β-NADPH) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The media and reagents for cell culture were obtained from Hyclone (Logan, UT, USA) and GIBCO Life Technologies (Grand Island, NY, USA).

2.2. Preparation of yam peel extracts

The peel of Darsan yam (*Dioscorea alata*) was washed by immersing in a water tank for 30 min, and then dried using a wash dryer. Blanching was carried out by immersing the peel in an 85 °C water bath for 30 s. The peel was freeze-dried in a local food processing plant. The final moisture content was about 6% and the thickness was about 0.5 mm. The freeze-dried yam peel was stored in plastic bags at −20 °C until used. Before extraction, the peel was ground into powder and passed through a screener with mesh number of 80. Peel powder was extracted with de-ionized water or 50% ethanol by a blender at room temperature for 1 min, and then stirred at 4 °C for 1 h. Total solid yields of water and 50% ethanol extracts from yam peel powder were 11.6% and 13.4%, respectively. The water extract was concentrated and the ethanol in the ethanolic extract was removed in an evaporator at a temperature lower than 40 °C. The extracts were resolubilised in Dulbecco's modified Eagle medium (DMEM) with an ultrasonic processor (model UP-50H, Dr. Hielscher GmbH), and then passed through a 0.22 μm filter. After filtration, the extracts were diluted to a concentration of 0.05 mg dried peel/ml with DMEM for testing antioxidant activities.

2.3. Cell culture

The mouse hepatoma (Hepa 1–6) and mouse hepatocyte (FL83B) cells were purchased from Food Industry Research and Development Institute, Hsinchu, Taiwan. These liver cells were maintained routinely and passed in DMEM supplemented with 10% foetal bovine serum (FBS) under a humidified atmosphere of 5% CO_2 and 95% air at 37 °C. Microscopic examination was used to observe cell morphology and monitor cell growth. The culture medium was changed every 2–3 days until the cells reached confluence and the experiments were performed when the cells reached about 80% confluence.

2.4. Cytotoxicity assay

The mouse liver cells were plated onto 96-well plates at a density of 4×10^4 cells/well. Various experiments were performed after the cells reached about 80% confluence. The cells were incubated at 37 °C for about 72 h to allow them to adhere to the plate and reach about 80% confluence. Prior to the treatment of the water or ethanolic extract from yam peel, the cells were washed three times with ice-cold phosphate buffered saline (PBS). To investigate the cytotoxicity of t-BHP on mouse liver cells, graded concentrations of t-BHP (0–50 mM) were added to the culture media and the plates were incubated at 37 °C for 30 min. For testing the cytotoxicity of the water and ethanolic extracts from yam peel on mouse liver cells, graded concentration of the extracts (0–1 mg freeze-dried yam peel powder/ml) were added to the culture media and the plates were incubated at 37 °C for 48 h.

To investigate the effect of yam peel extracts on t-BHP-induced cytotoxicity, the cells were pretreated with the water or 50% ethanolic extract from yam peel at a concentration of 0.05 mg freeze-dried peel powder/ml at 37 °C for 48 h. The medium from one set of the plates was then changed to the culture medium without t-BHP, whilst the medium from the other set of the plates was changed to the culture medium containing 10, 20, 30, 40 or 50 mM t-BHP. The plates were incubated at 37 °C for 30 min in order to allow the action of t-BHP. The MTT assay was used to measure cytotoxicity (Mosmann, 1983). MTT (0.5 mg/ml of medium) was added to each well and the plates were then incubated at 37 °C for 4 h. After adding an equal volume of solubilisation solution (10% SDS in 0.01 M HCl) to each well, the plates were read on a microplate reader at the wavelength of 590 nm.

2.5. Cell treatments

For measurement of intracellular antioxidative enzyme activities, the mouse liver cells were cultured on 10 cm tissue culture plates. The experiments were performed after the cells reached 80% confluence. To investigate the effect of yam peel extracts or t-BHP alone on mouse liver cells, 0.05 mg/ml (mg freeze-dried peel powder/ml) of different yam peel extracts (water or 50% ethanolic extract) or 10 or 50 mM t-BHP were added to the culture media and the plates were incubated at 37 °C for 48 h or 30 min, respectively. To investigate the effect of different yam peel extract on t-BHP-induced intracellular antioxidative enzyme activities, the cells were pretreated with 0.05 mg/ml (mg freeze-dried peel powder/ml) of either water or 50% ethanolic yam peel extract at 37 °C for 48 h. The medium from one set of the plates was then changed to the culture medium without t-BHP, whilst the medium from the other set of the plates was changed to the culture medium containing 10 or 50 mM t-BHP. The plates were incubated at 37 °C for 30 min in order to allow the action of t-BHP. Following three washes with ice-cold phosphate-buffered saline (PBS), an appropriate amount of buffer was added to each plate and the cells were scraped into centrifuge tubes by a rubber policeman. After sonication for 10 s on ice, the mixtures were centrifuged at 17,000*g* for 10 min at 4 °C. Protein concentration of cell cytosols was determined by the Bradford assay (Bradford, 1976) using bovine serum albumin (BSA) as standard. The cell cytosols were then used for further analyses.