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Comparison of protective effects of three varieties of sugarcane leaves on oxidative stress in Clone 9 cells

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ABSTRACT

The protective effects of water extracts of sugarcane (*Saccharum officinarum* L.) leaves among three varieties, including 28NG256, wild type, and ROC10, on *t*-BHP-induced oxidative stress in Clone 9 cells were systematically compared. Among these three varieties, 28NG256 showed the highest protective effect against 0.2 mM *t*-BHP-induced oxidative stress in Clone 9 cells. In addition, 28NG256 displayed higher inhibitory effects on ROS generation than wild type and ROC10. Moreover, 28NG256 showed higher positive regulated GSH levels and antioxidant enzymes as well as higher protective potential against cell death by inhibiting caspase-3 activity and mitochondrial membrane depolarization. Chlorogenic and caffeic acids present in 28NG256 decreased significantly the generation of ROS, which may partly be responsible for the effect of Clone 9 cell growth. Thus, 28NG256, among the three varieties studied, showed the most protective effects against *t*-BHP-induced oxidative stress in Clone 9 cells.

1. Introduction

Liver is an important organ involved in xenobiotic metabolism (Sahu et al., 2008). Hepatocytes make up the majority of liver structure and they are active in the metabolism of exogenous chemicals, thus causing a liver to be the target for toxic substances (Kim et al., 2007). Nowadays, liver-related diseases are still among the leading causes of morbidity and mortality in the world. Among causes of liver-related diseases, oxidative stress has been shown to be linked to hepatoxicity and other liver pathological conditions (Leal, Begoña Ruiz-Larrea, Martínez, & Lacort, 1998). Many synthetic compounds like thalidomide, corticosteroids, and natural compounds such as curcumin, glycyrrhizin, and silymarin from plants are regarded as drugs for the treatment of hepatic diseases, however, only a few of these compounds were successfully used in humans (Muriel & Rivera-Espinoza, 2008). Thus, more research efforts are needed to study drugs for liver-related diseases drugs.

It is well known that oxidative stress caused by the reactive oxygen species (ROS) may oxidize cellular DNA, proteins and lipids and contribute to disease progression through interfering with signal cascade systems. However, many studies have shown that intervention of bioactive compounds from foods or dietary supplements such as vegetables, fruits and other natural sources may eliminate or decrease ROS, leading to protection against liver injury, because they exhibit one or a combination of antioxidant, antifibrotic, immunomodulatory, or

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antiviral activities (Yang, Li, Wang, & Wu, 2010). In other words, compounds that are able to suppress or retard oxidative stress in hepatic tissues can be potential candidates for enhancing hepatoprotective activity against liver-related disease.

Sugarcane (*Saccharum officinarum* L.), wildly distributed in the subtropics, is one of the most important sources of sugar. During sugar production, the sugarcane leaf is first removed. Byproducts of agroindustries such as seed testa, hulls, peels and leaves, are either disposed of or used as feedstuff and fertilizer. Sugarcane leaves are often discarded or burned. However, the disposal has become a matter of great concern. Hence, more rational uses of sugarcane leaves are necessary.

In a previous study, the water extracts of sugarcane leaves at 1500 and 2500 μg/ml promoted effectively the accumulation of ROS generation, consequently inducing apoptosis, and thereby pushing HepG2 cells toward cell death. Clearly, the pro-oxidant activity of sugarcane leaves at 1500 and 2500 μg/ml can contribute to their antiproliferative effects on HepG2 cells. Bioactive constituents such as caffeic, chlorogenic and hydroxybenzoic acids present in sugarcane leaves could in part contribute to antiproliferative effects on HepG2 cells (Lee, Chen, Yu, Wang, & Duh, 2012).

The water extracts of sugarcane leaves in the range of 0–500 μg/ml have been described as displaying antioxidant activity, having the potential to decrease ROS generation (Lee et al., 2012). In other words, sugarcane leaves tested with proven antioxidant activity may have pro-oxidant activity as well, depending on the concentrations used. This finding is in agreement with the results of Tian and Hua (2005) who showed that aloin and aloe-emodin displayed antioxidant or pro-oxidant activity on plasmid DNA, depending on their nature (structure and concentrations). Given that sugarcane leaves can reduce ROS generation, they may also ameliorate oxidative insult in cellular systems. Thus, the aim of this study was to explore the hepatoprotective effect of sugarcane leaves of three varieties, namely 28NG256, wild type, and ROC10, which are widely processed for sugarcane in Taiwan, against *tert*-butyl hydroperoxide (t-BHP)-induced oxidative stress in Clone 9 cells. The mechanism of action was also elucidated.

2. Materials and methods

2.1. Materials

Peroxidase and 2-aminoethyl were purchased from Sigma (St. Louis, MO, USA). Sodium carbonate, Folin–Ciocalteu, rutin, methanol (100%), acetonitrile (>99%), *tert*-butyl hydroperoxide (t-BHP), and 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) were obtained from MERCK (Dermstadt, Germany). 2′,7′-Dichlorofluorescein diacetate (DCFH-DA) was obtained from Calbiochem (Calbiochem, San Diego, CA, USA). Caspase-glotm 3/7 assay was purchased from Promega Co. (Madison, WI, USA).

2.2. Preparation of samples

Sugarcane leaf donated by Taiwan Sugar Corporation, Tainan, Taiwan was cut into small pieces and stored at cooling room (4 °C) until used. Sugarcane leaf (20 g) was extracted with boiling water (200 ml) for 30 min, and the filtrate was freeze–dried. The yields of the extracts for 28NG256, wild, and ROC10 were 8.6%, 5.7%, and 13.9%, respectively.

2.3. Clone 9 cell viability

Clone 9 cells (ATCC number: CRL-1439) were purchased from Bioresources Collection and Research Center (Shin-chu, Taiwan) and cultured in Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F12) containing 10% fetal bovine serum and maintained in humidified 5% CO_2/95% air at 37 °C. The tetrazolium dye colorimetric test (MTT test) was used to determine the viability of Clone 9 cells. Cells were plated in 24-well flat-bottom plate at a density of 5×10^4 cells/ml and grown. After 24 h incubation, cells were treated with or without extracts. After a period time of incubation, 50 μl 0.1% MTT solution were added and cells were incubated for 1 h. Subsequently the reaction was terminated and the plates were incubated for 30 min to solubilize the formazan dye by addition of dimethyl sulfoxide, the optical density of each well was determined at 570 nm (Denizot, Wilson, Battye, Berke, & Shortman, 1986).

2.4. Intracellular ROS measurement

Intracellular ROS was estimated by using a fluorescent probe, DCFH-DA (Wang & Joseph, 1999). In brief, at the end of incubation, the cells (10^6 cells/ml) were collected and re-suspended with PBS. An aliquot of the suspension (195 μl) was loaded into a 96-well plate and then 5 ml DCFH-DA was added (final concentration 5 μM). The DCF fluorescence intensity was detected using a Bio-Tek FLx800 microplate fluorescence reader (Winooski, VT, USA) with an excitation wavelength of 485 nm and emission wavelength of 530 nm.

2.5. Determination of antioxidant enzyme activity

Antioxidant enzyme activities of liver homogenate supernatants were determined as previously described. Hepatic glutathione peroxidase (GPx) and glutathione reductase (GRd) activities were measured by following the decrease in the absorbance due to oxidation of nicotinamide adenine dinucleotide phosphate (NADPH) (Wang, Yu, Chang, Yen, & Duh, 2008). Briefly, a reaction mixture containing 1 mM glutathione (GSH), 1 unit/ml of GRd, 1 mM NaN_3, 1 mM ethylenediaminetetraacetic acid (EDTA), 0.2 mM NADPH and 0.1 ml of liver supernatants was mixed with 0.1 ml of 2.5 mM hydrogen peroxide (H_2O_2) for GPx activity determination. To another mixture containing 1 mM $MgCl_2$, 1 mM oxidized glutathione (GSSG), 0.2 mM NADPH, was added 0.1 ml of liver supernatants for GRd activity determination. The decreased absorbance at 340 nm was measured for 3 min. The superoxide dismutase (SOD) activity was determined using the xanthine–xanthine oxidase-iodophenyl nitrophenyl phenyltetrazolium chloride (INT) system, as previously described (Durackova & Labuda, 1995). The reaction mixture containing 0.05 mM xanthine, 0.025 mM INT, 1 mM EDTA, and 0.1 ml of liver supernatants was mixed with 0.1 ml xanthine oxidase (80 unit/ml). The change in absorbance at 510 nm in 3 min was monitored. The activity of catalase (CAT) was measured