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Research Article
Impact of Pineapple Juice on Expression of CYP3A4, NAT2, SULT1A1 and OATP1B1 mRNA in HepG2 Cells

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Abstract

Background and Objective: Pineapple (*Ananas comosus*) is a popular fruit worldwide with natural antioxidant properties. This study examined how pineapple modified the expression of drug-metabolizing enzymes (CYP1A2, CYP2C9, CYP3A4, UGT1A6, NAT2 and SULT1A1) and a drug transporter (OATP1B1) in human hepatocarcinoma (HepG2) cells. **Materials and Methods:** HepG2 cells (2.5×10^5 cells/well in a 24-well plate) were incubated with pineapple juice extract (125-1,000 μg mL^{-1}) for 48 hrs in phenol red-free medium. Resazurin reduction, ROS, AST and ALT assays were performed. The mRNA expression of target genes was determined by RT/qPCR. **Results:** Pineapple juice slightly reduced HepG2 cell viability to 80% of the control, while ROS, AST and ALT levels were not changed. Pineapple juice did not alter the expression of CYP1A2, CYP2C9 and UGT1A6 mRNA. All tested concentrations of pineapple juice suppressed CYP3A4, NAT2 and OATP1B1 expression, while SULT1A1 expression was induced. **Conclusion:** Though pineapple juice slightly decreased the viability of HepG2 cells, cell morphology and cell function remained normal. Pineapple juice disturbed the expression of phase I (CYP3A4) and phase II (NAT2 and SULT1A1) metabolizing genes and the drug transporter OATP1B1. Therefore, the consumption of excessive amounts of pineapple juice poses a risk for drug interactions.

Key words: *Ananas comosus*, cytochrome P450, OATP1B1, HepG2, metabolism, drug interaction, cell morphology

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Pineapple (*Ananas comosus* L.) has been reported to possess numerous pharmacological activities such as antioxidant, antidiabetic, antibacterial, anticoagulant, analgesic and anticancer activities as well as promoting immune function and reducing gastric complications[1,2]. Pineapple juice contains high amounts of vitamin C with antioxidant properties, so it is well known as a healthy drink[3]. In addition, a mixture of cysteine proteases from pineapple was shown to have anti-allergic and anti-inflammatory effects and *A. comosus* leaf extract exhibited anti-inflammatory activity in carrageenan-induced inflammation in a rat model[4,5]. Cytochrome P450 (CYP) is a super-family of heme monooxygenase enzymes principally responsible for phase I metabolism in the human liver. Human CYP1A2, CYP2C9 and CYP3A4 are responsible for the biotransformation of more than 50% of clinical drugs[6]. Food-drug interactions are a particular concern for the safe and effective use of healthy foods or supplements. Common interactions arise due to inhibition or induction of drug-metabolizing enzymes, especially CYPs[7]. For example, green tea and grape seed have been reported to inhibit the activities of several CYPs, including CYP2C9, CYP2D6 and CYP3A4, in human hepatic microsomes[8].

For phase II metabolism, Uridine Diphosphate (UDP)-glucuronosyltransferase 1A6 (UGT1A6) metabolizes several drugs including naproxen, carvedilol, zidovudine and valproic acid[9]. *N*-acetyltransferase 2 (NAT2) catalyzes the conjugation of numerous carcinogens and arylamines and hydrazine drugs[10]. Human cytosolic sulfotransferase 1A1 (SULT1A1) is responsible for the sulfation of analgesics such as acetaminophen, desmethylnaproxen and tapentadol[11]. CYPs and phase II enzymes are involved in the metabolism of more than 90% of market available drugs. Moreover, phytochemicals and/or herbal products can also be substrates for CYPs and phase II enzymes[9,12,13]. In addition to phase I and II enzymes, the organic anion transporting polypeptide 1B1 (OATP1B1) is a key determinant for transporter-mediated drug interactions. Some herbal plants can also modulate the expression of OATP1B1[14,15].

The human hepatocarcinoma (HepG2) cell line is commonly used as an alternative to primary human hepatocytes for *in vitro* studies of drug metabolism and hepatotoxicity. HepG2 cells are characterized by their unlimited lifespan, high availability, stable phenotype and economical handling costs[16].

This study investigated the impact of pineapple juice extract on levels of Reactive Oxygen Species (ROS) and the Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) liver function biomarkers as well as expression profiles of CYPs, phase II metabolizing enzymes and a drug transporter in HepG2 cells.

MATERIALS AND METHODS

Study area: The experiment was performed at the Research Group for Pharmaceutical Activities of Natural Products using Pharmaceutical Biotechnology (PANPB), Faculty of Pharmaceutical Sciences, Khon Kaen University, Muang, Khon Kaen, Thailand from March-August, 2021.

Materials: Gibco® Dulbecco's Modified Eagle Medium (DMEM) with or without phenol red and DMEM/F-12 phenol red-free medium (Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12), Glutamax®, Fetal Bovine Serum (FBS), Dulbecco's Phosphate-Buffered Saline (DPBS) and Gibco® Penicillin-Streptomycin-Neomycin (PSN) were supplied by Gibco® (New York, USA). ReverTraAce®, Thunderbird™ Probe qPCR Mix and other reagents for RT/qPCR were products of Toyobo Co., Ltd. (Osaka, Japan). TaqMan™ gene expression assays were products of Applied Biosystem™ (Waltham, Massachusetts, USA). All other chemicals and reagents were of the standard purity for analytical grade from local commercial suppliers.

Preparation of pineapple juice extract: Pineapple (*Ananas comosus* L., cultivar Sriracha) plants were planted in Nakhon Phanom Province, Thailand in July, 2019 and the fruits were harvested in March, 2021. Fruits were washed with distilled water several times and waited to dry before peeling and weighing. The flesh was minced and squeezed before measuring the total volume of juice collected. The juice was frozen and lyophilized and then kept at -20°C[17]. The dry powder of juice extract was weighed and resuspended in de-ionized water and total phenolic, total flavonoid and anthocyanin contents, including percentage tannin contribution, were determined according to standard protocols[18]. Total phenolic, flavonoid and anthocyanin contents were 1.12±0.39, 0.89±0.16 and 333.33±0.01 mg g^{-1} dry weight, respectively. Tannin contribution was 74.42±1.52%. The dry pineapple juice powder was rehydrated in sterile distilled water, diluted in culture medium and filtered with a 0.22 µm-sterile filter before adding to the cells.

Culturing and treatment of HepG2: HepG2 cells (ATCC® HB-8065, Manassas, USA) were maintained in standard DMEM supplemented with 10% FBS, Glutamax® and PSN in a 5% CO_2

incubator (Forma™ Series 3, Thermo Scientific™, Waltham, Massachusetts, USA) at 37°C with 95% relative humidity. Cells were seeded at a density of 2.5×10^5 cells in 0.5 mL into 24 well-cell culture plates and incubated for 48 hrs before renewing the treated medium, namely 0.1% dimethyl sulfoxide (DMSO, control), 10 µM ketoconazole (Keto), 10 µM rifampicin (Rif) or pineapple juice extract (125, 250, 500 and 1,000 µg mL^{-1}) in DMEM/F12 supplemented with 10% FBS, Glutamax® and PSN for 48 hrs. After that the medium was collected for determination of cell viability, ROS, AST and ALT levels and the cells (n = 4-5 wells) were harvested for RT-qPCR.

Determination of cell viability: HepG2 cells were incubated with 1 mM resazurin (10:1) in 5% CO_2 at 37°C for 30 min. The living cells metabolized resazurin to the fluorescent resorufin and the fluorescence intensity was measured at an excitation of 530 nm and emission of 580 nm using a spectrofluorometer (EnSight™ multimode plate reader, PerkinElmer®, Santa Clara, CA, USA). The percentage of cell viability was calculated compared to the control as described by Chatuphonprasert *et al.*[19].

Determination of ROS level: The amount of ROS was determined by the DCFH-DA method based on the reaction of ROS with DCFH-DA to generate highly fluorescent dichlorofluorescein (DCF)[20]. The medium was incubated with 62.5 nM DCFH-DA in the dark for 40 min at room temperature. The fluorescence intensity of the DCF product was measured at an excitation wavelength of 484 nm and an emission wavelength of 530 nm using a spectrofluorometer (EnSight™ multimode plate reader). ROS level was calculated by comparison with a standard curve of hydrogen peroxide (2.5-20 µM).

Determination of AST and ALT: Cell medium was collected 48 hrs after treatment. Reaction mixtures of cell medium, α-ketoglutarate and either L-aspartate (for AST) or L-alanine (for ALT) substrates were incubated at 37°C for 30 or 20 min, respectively. And 2,4-dinitrophenylhydrazine was added to the mixture and left to react for 20 min. The reaction was stopped by the addition of sodium hydroxide. The absorbance of the coloured AST or ALT product complex was measured at 505 nm using a UV-Vis spectrophotometer (Sunrise™, Tecan Trading AG, Switzerland). Results are expressed as international units per litre (IU L^{-1}) and were calculated by comparison with a standard curve of sodium pyruvate (100-500 µM)[21].

Quantitative determination of mRNA expression by reverse transcription/real-time PCR (RT/qPCR): At 48 hrs after treatment, total RNA was isolated using the guanidinium thiocyanate-phenol-chloroform method[19]. Total RNA was reverse transcribed using ReverTraAce® kit (Toyobo Co., Ltd., Osaka, Japan). Expression of CYP1A2 (Hs00167927_m1), CYP2C9 (Hs02383631_s1), CYP3A4 (Hs00604506_m1), UGT1A6 (Hs01592477_m1), NAT2 (Hs01854954_s1), SULT1A1 (Hs00738644_m1) and OATP1B1 (Hs00272374_m1) mRNAs was determined using RT/qPCR and normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH, Hs02786624_g1) using probe-primers of TaqMan™ gene expression assays (Applied Biosystem™, Waltham, Massachusetts, USA) with Thunderbird™ reagents (Toyobo Co., Ltd.). The relative fold expression was calculated using ΔΔCt method[19].

Statistical analysis: The results are expressed as Mean±Standard deviation (n = 4-5 per group) and analyzed using one way analysis of variance (ANOVA) coupled with Tukey's honest significance test using IBM SPSS software (version 23, Chicago, IL, USA). A p-value less than 0.05 was considered statistically significant.

RESULTS

Effects of pineapple juice extract on cell viability, ROS, AST and ALT: All treatments significantly decreased the viability of HepG2 cells (Fig. 1a). However, as cell morphology remained normal, the study was continued using these concentrations. Keto strongly induced ROS level, while the Rif and the highest concentration of pineapple juice extract (1,000 ug mL^{-1}) significantly increased ROS level (Fig. 1b). AST and ALT levels are biomarkers indicating hepatic cell injury. Keto and Rif significantly increased both AST and ALT levels in HepG2 cells, while all tested concentrations of pineapple juice extract did not disturb either AST (Fig. 1c) or ALT (Fig. 1d) in the cells.

Effects of pineapple juice extract on the expression of CYP1A2, CYP2C9 and CYP3A4 mRNAs: The expression of CYP1A2 in HepG2 cells was significantly induced by Keto and suppressed by Rif (Fig 2a). In contrast, CYP2C9 expression was significantly suppressed by Keto and induced by Rif (Fig. 2b). All tested concentrations of pineapple juice extract did not alter the expression of CYP1A2 or CYP2C9 in HepG2 cells. Keto and Rif excessively induced expression of CYP3A4, while pineapple juice extract significantly down-regulated CYP3A4 expression (Fig. 2c).

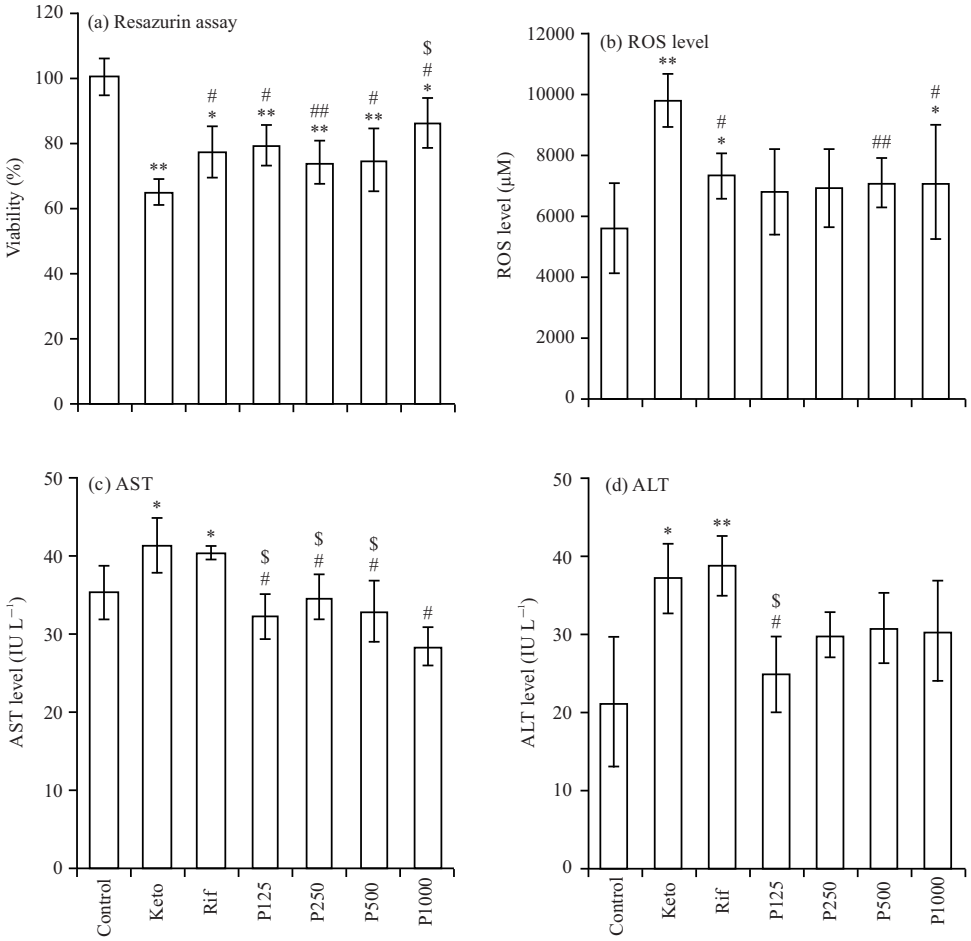

Fig. 1(a-d): Effects of pineapple juice extract on (a) cell viability, (b) ROS, (c) AST and (d) ALT levels

Keto, 10 μM ketoconazole, Rif, 10 μM rifampicin, P125-1000, pineapple juice extract 125-1,000 μg mL^{-1}. *p<0.05, **p<0.001 VS control, $^{\#}$p<0.05, $^{\#\#}$p<0.001 VS Keto and $^{\$}$p<0.05 VS Rif. (n = 4-5)

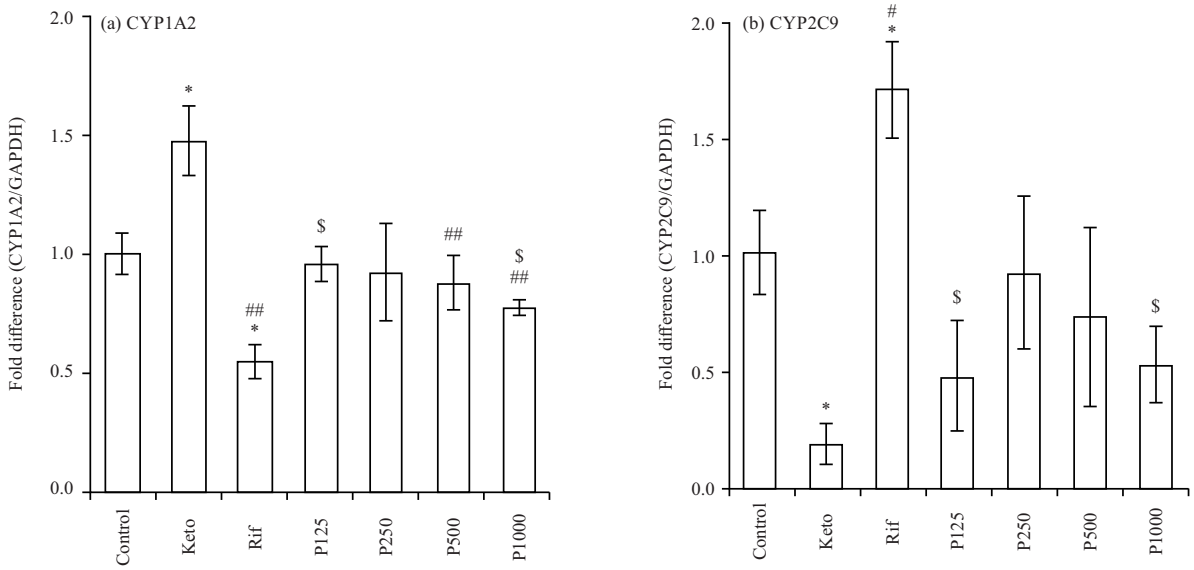

Fig. 2(a-c): Continue

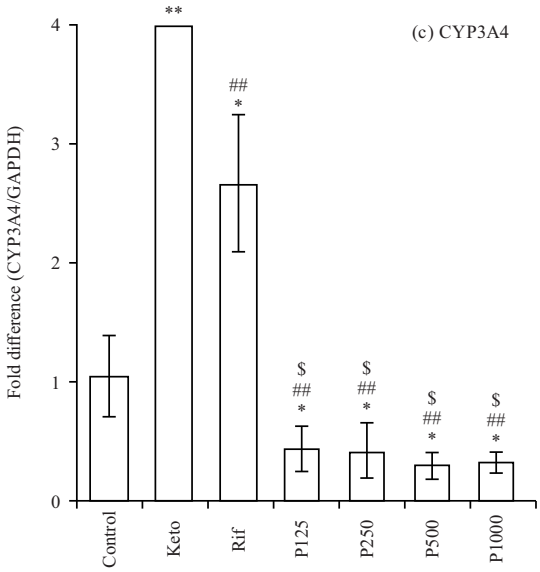

Fig. 2(a-c): Effects of pineapple juice extract on the expression of (a) CYP1A2, (b) CYP2C9 and (c) CYP3A4 mRNAs

Keto, 10 μM ketoconazole, Rif, 10 μM rifampicin, P125-1000, pineapple juice extract 125-1,000 μg mL^{-1}, *p<0.05, **p<0.001 VS control, $^{\#}$p<0.05, $^{\#\#}$p<0.001 VS Keto and $^{\$}$p<0.05 VS Rif. (n = 4-5)

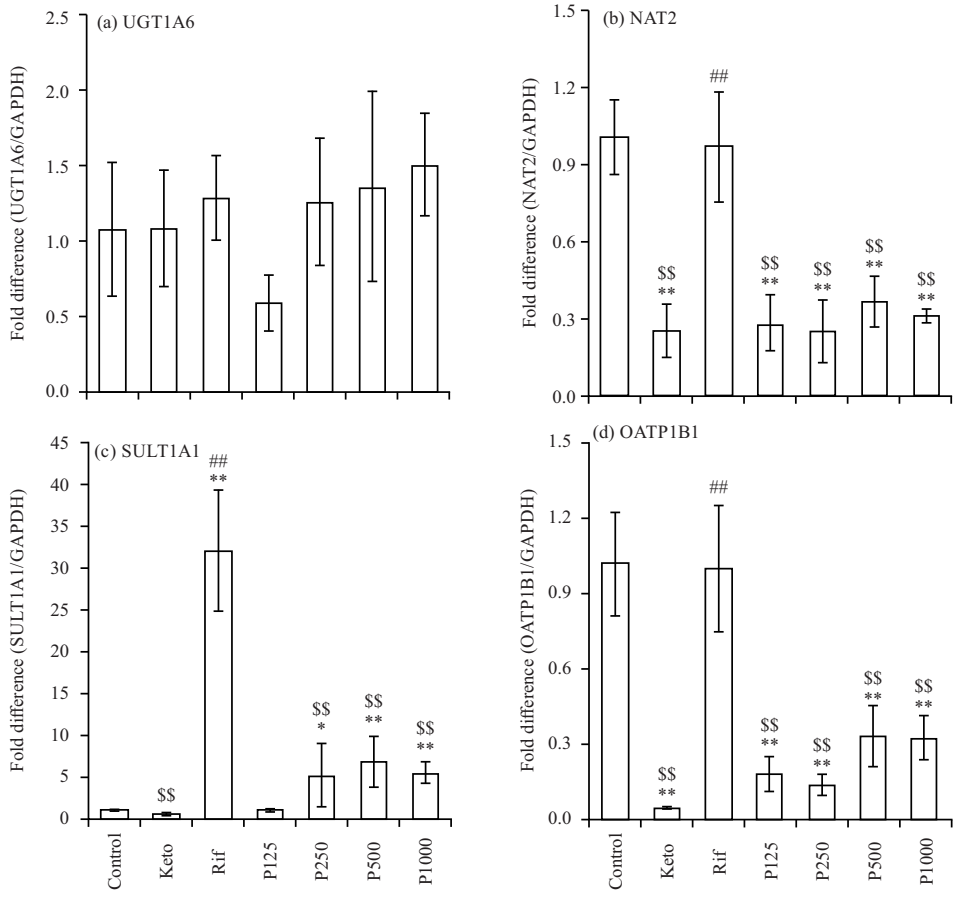

Fig. 3(a-d): Effects of pineapple juice extract on the expression of (a) UGT1A6, (b) NAT2, (c) SULT1A1 and (d) OATP1B1 mRNAs

Keto, 10 μM ketoconazole, Rif, 10 μM rifampicin, P125-1000, pineapple juice extract 125-1,000 μg mL^{-1}, *p<0.05, **p<0.001 VS control, $^{\#}$p<0.05, $^{\#\#}$p<0.001 VS Keto and $^{\$}$p<0.05 VS Rif. (n = 4-5)

Effects of pineapple juice extract on the expression of UGT1A6, NAT2, SULT1A1 and OATP1B1 mRNAs: The expression of UGT1A6 in HepG2 cells was not modified by any treatments (Fig. 3a). Keto and pineapple juice extract significantly suppressed expression of NAT2, while Rif did not alter NAT2 expression in HepG2 cells (Fig 3b). The expression of SULT1A1 was extensively induced by the Rif and by pineapple juice extract at concentrations of 250-1,000 μg mL^{-1} in HepG2 cells (Fig 3c). The drug transporter OATP1B1 was markedly down-regulated by Keto and pineapple juice extract in HepG2 cells, while Rif did not affect OATP1B1 expression (Fig. 3d).

DISCUSSION

The concentration range of pineapple juice extract employed in the present study was based on a previous study, in which consumption of 10 to 40 mg kg^{-1} day^{-1} of pineapple altered expression of CYPs in mice[17], for a comparable human blood volume equivalent of 120-480 μg mL^{-1}. Keto and Rif were employed as positive modifiers in the present study because they have been marked as high incidence of drug interaction[22,23]. Keto has been shown to up-regulate the expression of CYP1A2 and CYP3A4 mRNA via activation of the aryl hydrocarbon receptor and the pregnane X receptor, respectively[22]. Moreover, Keto is a CYP2C9 and drug transporter inhibitor[24] and Rif is a typical CYP3A4 inducer[25].

The resazurin fluorescent dye assay is an investigation for mammalian cell cytotoxicity that is normally measured as percent cell viability compared to control and can indicate cell proliferation or inhibition[26,27]. All treatments, especially Keto, showed reduced resorufin intensity representing reductions in several enzymes in HepG2 cells and reduced cell viability. Leakage of AST and ALT usually indicates hepatotoxicity in both cells and *in vivo*[20,21]. The present observations show that Keto and Rif both significantly increased levels of ROS, AST and ALT in the cell culture medium. These results suggested that Keto and Rif instigated HepG2 cell injury. The highest tested concentration of pineapple juice extract (1,000 ug mL^{-1}) slightly increased ROS level in the HepG2 cell culture medium. This phenomenon might be explained by the vitamin C contained in pineapple[28] acting as a pro-drug to deliver hydrogen peroxide and induce oxidative stress as a mechanism to bring death to cancer cells[29].

CYP3A4 metabolizes a large and diverse range of molecules that includes over 50% of clinical drugs including bronchodilators, antiviral, antibacterial, antifungal, lipid-lowering and anti-hypertensive drugs[6]. Our previous study reported that high amounts of pineapple juice suppressed expression of CYP1A2 but induced expression of CYP3A11 in mouse livers[17], which corresponds to the observations in the present study that indicate pineapple juice extract down-regulates CYP3A4 expression in HepG2 cells.

It is not an only phase I CYPs that are important for drug metabolism, the conjugation reactions during phase II are also crucial. NAT2 catalyzes the conjugation of hydrazine drugs (anti-depressant and anti-diabetic agents) and procarcinogens including arylamines and aromatic amines[10]. Expression of NAT2 mRNA is abundant in the liver, small intestine and colon and is readily detected in most other tissues[30]. A previous report found that polyphenols suppressed NAT2 expression in human livers and in cholangiocarcinoma cells[31], which is confirmed by the down-regulation of NAT2 expression in HepG2 cells by pineapple juice extract. SULT1A1 is responsible for the sulfation of analgesics including acetaminophen, O-desmethylnaproxen, tapentadol and natural phenolic compounds such as resveratrol[11,32]. This is the first report of the inductive effect of pineapple juice extract on the expression of SULT1A1 mRNA.

OATP1B1 is an important determinant of drug transporter-mediated drug interactions and some herbal plants can modulate the expression of OATP1B1[14,15]. OATP1B1 is majorly expressed in hepatocytes[33]. Current information regarding food/herbal drug interactions and OATP1B1 is limited. While dietary phenolic acids might interfere with the function of human organic anion transporters in general[33], there is a lack of detail about specific OATP1B1 interactions.

In summary, the present findings reveal that pineapple juice extract can potentially cause food-drug interactions via alteration of UGT1A6, NAT2 and OATP1B1 expression. On the other hand, pineapple juice extract reduced ROS generation by reducing mitochondrial depolarization and may help in lowering cancer risk via NAT2 down-regulation.

CONCLUSION

Pineapple juice extract did not interfere with the expression of CYP1A2, CYP2C9 and UGT1A6 in HepG2 cells. Nevertheless, it down-regulated the expression of CYP3A4, NAT2 and OATP1B1 in HepG2 cells and at high concentrations, it significantly elevated expression of SULT1A1. Therefore, excessive consumption of pineapple poses a risk for drug interactions via modulatory regulation of CYP3A4, NAT2 and SULT1A1-associated hepatic metabolism and OATP1B1-mediated drug transportation.

SIGNIFICANCE STATEMENT

The study revealed the effects of pineapple juice extract on phase I (CYP3A4) and phase II (NAT2 and SULT1A1) metabolism genes and a drug transporter OATP1B1 in a HepG2 cell model. Therefore, the consumption of a large amount of pineapple together with substrates of CYP3A4, NAT2, SULT1A1 and OATP1B1 could cause a drug interaction.

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