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Research Article
Impact of Pineapple on Mitochondrial Permeability Transition and Drug Metabolizing Genes in Caco-2 Cells

[1,2]Waranya Chatuphonprasert, [2]Nadta Sukkasem, [3]Wipawee Tukum-mee, [3]Jintanaporn Wattanathorn and [2,4]Kanokwan Jarukamjorn

[1]Faculty of Medicine, Mahasarakham University, Muang, Maha Sarakham, 44000, Thailand
[2]Research Group for Pharmaceutical Activities of Natural Products using Pharmaceutical Biotechnology, Khon Kaen University, Khon Kaen, 40002, Thailand
[3]Research Institute for Human High Performance and Health Promotion, Khon Kaen University, Khon Kaen, 40002, Thailand
[4]Division of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen, 40002, Thailand

Abstract

Background and Objective: Pineapple (*Ananas comosus* L.) has antioxidant and other pharmacological properties. This study examined how pineapple modified mitochondrial permeability transition and expression of drug-metabolizing enzymes, i.e., CYP1A2, CYP2C9, CYP3A4, UGT1A6, NAT2 and the drug transporter OATP1B1 in human colorectal adenocarcinoma (Caco-2) cells. **Materials and Methods:** Caco-2 cells (2.5×10^5 cells well^{-1} in 24-well plates) were incubated with pineapple (125 to 1,000 μg mL^{-1}) for 48 hrs in a phenol red-free medium. Mitochondrial permeability transition, resazurin cell viability and AST and ALT levels were investigated. The mRNA expression of target genes was determined by RT/qPCR. **Results:** Pineapple significantly reduced depolarized mitochondria, slightly decreased cell viability and did not change AST and ALT levels. Pineapple did not modify the mRNA expressions of CYP1A2, CYP2C9 and CYP3A4 but markedly induced UGT1A6 expression. The highest tested concentration of pineapple (1,000 μg mL^{-1}) significantly suppressed NAT2 and OATP1B1 expression. **Conclusion:** Although pineapple slightly decreased cell viability to ~80% of control, the morphology and functions of the cells were unaffected. Pineapple showed a beneficial effect to reduce depolarized mitochondria, which consequently decreased reactive oxygen species production. Pineapple did not modify the expression of CYPs, whilst it altered the expression of phase 2 metabolizing genes UGT1A6 and NAT2 and the transporter OATP1B1. Therefore, the consumption of large amounts of pineapple is of concern for the risk of drug interaction via alteration of UGT1A6, NAT2 and OATP1B1 expression.

Key words: *Ananas comosus*, Caco-2, cytochrome P450, NAT2, OATP1B1, UGT1A6, reactive oxygen species

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Corresponding Author: Kanokwan Jarukamjorn, Research Group for Pharmaceutical Activities of Natural Products using Pharmaceutical Biotechnology, Khon Kaen University, Khon Kaen, 40002, Thailand
Division of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen, 40002, Thailand

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., family Bromeliaceae) is a tropical fruit well known worldwide and its juice contains a high amount of vitamin C and has antioxidant properties[1]. Several studies have reported on the health benefits of pineapple due to its pharmacological properties such as anticoagulant, analgesic, immune system stimulation, reducing gastric complications and tumour suppressor activities[2]. In addition, bromelain, a mixture of cysteine proteases from pineapple, showed anti-allergic and anti-inflammatory effects in a murine allergic airway disease model and *A. comosus* leaf extract showed anti-inflammatory activity in carrageenan-induced paw oedema in rats[3,4].

Mitochondrial Permeability Transition (MPT) pore opening is a cellular event that causes sudden and severe damage to cells. Recently, MPT has been implicated in several human diseases, such as neuromuscular diseases, ischaemia reperfusion injury and neurodegenerative diseases[5]. Moreover, the heavy metal lead (Pb^{2+}) was shown to induce MPT dysfunction by causing Reactive Oxygen Species (ROS) overproduction and oxidative stress[6]. Although there is abundant evidence of the antioxidant properties of pineapple, its effect on MPT remains uninvestigated.

Cytochrome P450 (CYP) is a superfamily of monooxygenase enzymes responsible for phase I metabolism in the liver. Human CYP1A2, CYP2C9 and CYP3A4 are responsible for the biotransformation of more than 50% of clinical drugs[7]. Food or supplements sometimes interact with the metabolism of drugs by interfering with the regulatory expression of CYPs. For example, concomitant consumption of grapefruit juice with the antihypertensive drugs felodipine and nifedipine causes an increase in the bioavailability of both drugs due to a rise in the drug concentration in plasma. Flavonoids in grapefruit juice suppress CYPs, which subsequently decreases the metabolism of felodipine and nifedipine. This food-drug interaction affects the efficacy or toxicity of these drugs and since citrus fruit or its juice is a common breakfast drink, this has clinical significance[8].

Uridine diphosphate (UDP)-glucuronosyltransferase 1A6 (UGT1A6) is a phase II enzyme responsible for metabolizing many drugs such as naproxen, carvedilol, zidovudine and valproic acid[9]. N-acetyltransferase 2 (NAT2) is another phase II metabolizing enzyme that catalyzes the biotransformation of arylamine and hydrazine drugs and many procarcinogens[10]. Together, CYP and UGT enzymes are involved in the metabolism of 90% of market drugs and many phytochemicals or herbal products[9,11,12]. In addition to the phase, I and II metabolizing enzymes, the organic anion transporting polypeptide 1B1 (OATP1B1) is an important determinant for transporter-mediated drug interactions and some herbs have been shown to modulate the expression of OATP1B1[13,14].

The human colorectal adenocarcinoma (Caco-2) cell line is the most suitable *in vitro* model representing the processes of absorption and metabolism of compounds in the human small intestine[15]. Moreover, the Caco-2 cell monolayer is an excellent model to study the bioactivity of food supplements[16]. Hence, this study investigated the effects of pineapple juice on the mRNA expression of several important drug-metabolizing enzymes, i.e., CYP1A2, CYP2C9, CYP3A4, UGT1A6, NAT2 and drug transporter OATP1B1 and on mitochondrial permeability using Caco-2 cells.

MATERIALS AND METHODS

Study area: The study was carried out at the Research Group for Pharmaceutical Activities of Natural Products using Pharmaceutical Biotechnology, Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen 40002 Thailand from March-May, 2021.

Materials: Dulbecco's modified Eagle medium (+) phenol red (DMEM with phenol red, Cat. No. 11885-084), DMEM/F12 (1:1) phenol red-free medium, Cat. No. 2104-025), fetal bovine serum (FBS), 1×Glutamax®, DPBS and 1×penicillin, streptomycin and neomycin antibiotics (PSN) were purchased from Gibco® (New York, USA). MitoPT JC-1 assay kit was from ImmunoChemistry Technologies, LLC. (Bloomington, MN, 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 Biosystems™ (Waltham, Massachusetts, USA). Other laboratory chemicals and materials were provided by commercial suppliers with analytical or molecular grade.

Pineapple juice preparation: Pineapple plants (*Ananas comosus* L., cultivar Sriracha) were planted in Nakhon Phanom province, Thailand in July, 2019 and the fruit was harvested in March, 2021. The fruit was rinsed with distilled water 5 times, weighed and peeled to expose the edible portion. The flesh was juiced and the volume of juice extract was measured using a graduated cylinder. The fruit juice was frozen and lyophilized using a freeze-dryer and the dry powder was kept at -20°C[17]. The dry powder was accurately weighed and suspended in distilled water before dilution in culture medium followed by filtration with 0.22 µm-sterile filter before addition to the cells.

The content of chemical markers, including total phenolic, total flavonoid and anthocyanin contents and the percent tannin contribution were determined according to the standard protocols[18]. Total phenolic, total 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%.

Caco-2 cell culture and treatments: Caco-2 cells (RBRC-RBC0988) were supplied by the RIKEN cell bank (Wako, Saitama, Japan). The cells were maintained in DMEM supplemented with 1 g L^{-1} D-glucose, L-glutamine, 110 mg mL^{-1} sodium pyruvate, 1×Glutamax®, 20% FBS and 1×PSN at 37°C with 5% CO_2 and 95% relative humidity. The cells were seeded into 24 well-plates (2.5×10^5 cells/well in 0.5 mL) for 48 hrs before incubation with 0.1% dimethyl sulfoxide (DMSO, control), 10 µM ketoconazole (Keto) or rifampicin (Rif) or 125, 250, 500, 1000 µg mL^{-1} of pineapple juice (P) for 48 hrs. The medium was collected for determination of cell viability and Aspartate Transaminase (AST) and Alanine Aminotransferase (ALT) assays. The cells were harvested for quantitative analysis by RT-qPCR (n = 4-5 replicates per group) and Mitochondrial Permeability Transition (MPT) with the MitoPT JC-1 assay kit (n = 3 replicates per group).

Determination of cell viability: Cell viability was determined by resazurin assay based on the ability of viable cells to reduce non-fluorescent blue resazurin to fluorescent pink resorufin. The medium was mixed with 1 mM resazurin (10:1) before incubation in 5% CO_2 at 37 for 30-45 min. The percentage of cell viability was calculated from an increasing rate of resorufin spectrofluorometric intensity at excitation of 530 nm and emission of 580 nm as previously described[19].

Determination of AST and ALT levels: At 48 hrs after treatment, the culture medium was incubated at 37°C with either AST substrate (10 mM L-aspartate and 1.7 mM α-ketoglutarate) for 30 min or ALT substrate (300 mM L-alanine and 0.7 mM α-ketoglutarate) for 20 min. Then 2,4-dinitrophenylhydrazine was added and the plates were left to stand at room temperature for 20 min, followed by the addition of sodium hydroxide. The mixture has measured the absorbance using a spectrophotometer at 505 nm. The levels of AST and ALT were determined as international units per liter (IU L^{-1}) by comparison with a standard curve of sodium pyruvate[20].

Mitochondrial Permeability Transition (MPT) assay: MPT assay was determined in Caco-2 cells after 48 hrs of treatment, following the instructions of the MitoPT JC-1 assay kit. The mitochondrial depolarization inducer carbonyl cyanide 3-chlorophenylhydrazone (CCCP, 50 µM) was added to the culture medium for 1 h before starting the measurement as a positive control. The culture medium was removed, then MitoPT buffer (0.5 mL) was added and the plates were incubated at 37°C in the dark for 15 min. MitoPT buffer was aspirated and replaced with 1x assay buffer (0.5 mL) before incubation at 37°C in the dark for 10 min. The 1x assay buffer was aspirated and replaced with PBS. The cells were immediately photographed with a ZOE™ Fluorescent cell imager (Bio-Rad, Hercules, California, USA) with red and green fluorescence filters. The fluorescence intensity was quantified by a spectrofluorometric plate reader at excitation of 490 nm and emission of 527 nm for the depolarized state and excitation of 490 nm and emission of 590 nm for the polarized state.

Quantitative analysis of mRNA expression by RT/qPCR: At 48 hrs after treatments, cells were harvested for total RNA extraction using the guanidinium thiocyanate-phenol-chloroform method[19]. Total RNA was reverse transcribed using the ReverTraAce® kit. Expression of CYP1A2 (Hs00167927_m1), CYP2C9 (Hs02383631_s1), CYP3A4 (Hs00604506_m1), UGT1A6 (Hs01592477_m1), NAT2 (Hs01854954_s1) and OATP1B1 (Hs00272374_m1) mRNAs was analyzed using RT/qPCR and normalized to a reference gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH, Hs02786624_g1), using the probe-primers of TaqMan™ gene expression assays with Thunderbird™ reagents. The relative fold expression was calculated using the delta-delta Ct method[19].

Statistical analysis: The results are expressed as Mean±SD. (n = 3-5 per group) and analyzed using one way analysis of variance (ANOVA) followed by Tukey's post hoc test (SPSS version 23, Chicago, IL, USA). $p\leq0.05$ was considered as statistically significant.

RESULTS

Effects of pineapple on cell viability, AST and ALT levels: All treatments (Keto, Rif and P125-1000) significantly reduced the viability of Caco-2 cells ($p<0.5$, Fig. 1a), with Keto showing the greatest reduction ($p<0.001$). Since the cell morphology was normal, these concentrations were employed in the study.

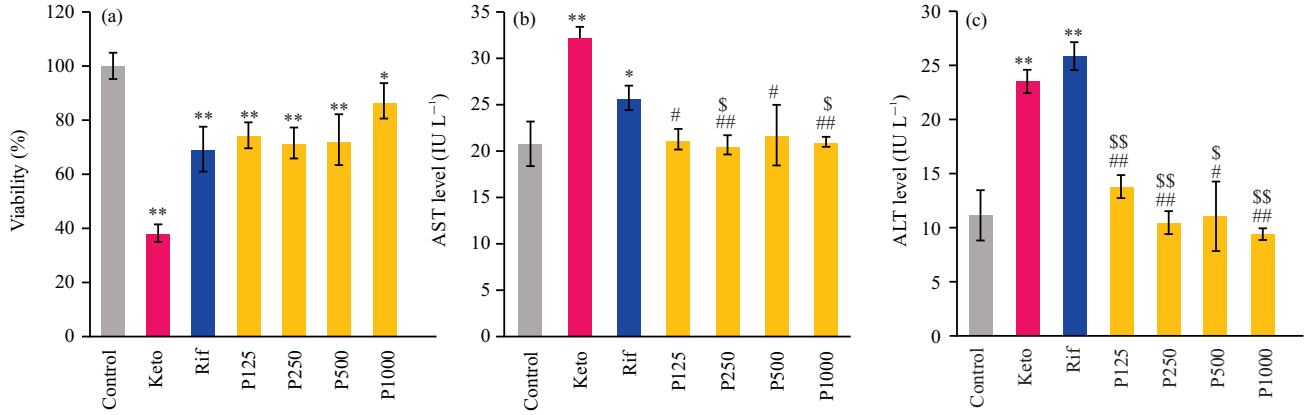

Fig. 1(a-c): Effects of pineapple on (a) Cell viability, (b) AST and (c) ALT levels

Keto: Ketoconazole (10 µM), Rif: Rifampicin (10 µM), P: Pineapple juice (125, 250, 500 and 1,000 µg mL^{-1}), n = 4-5, *p<0.05, **p<0.001 vs. control, $^{\#}$p<0.05, $^{\#\#}$p<0.001 vs. Keto; $^{\$}$p<0.05, $^{\$\$}$p<0.001 vs. Rif.

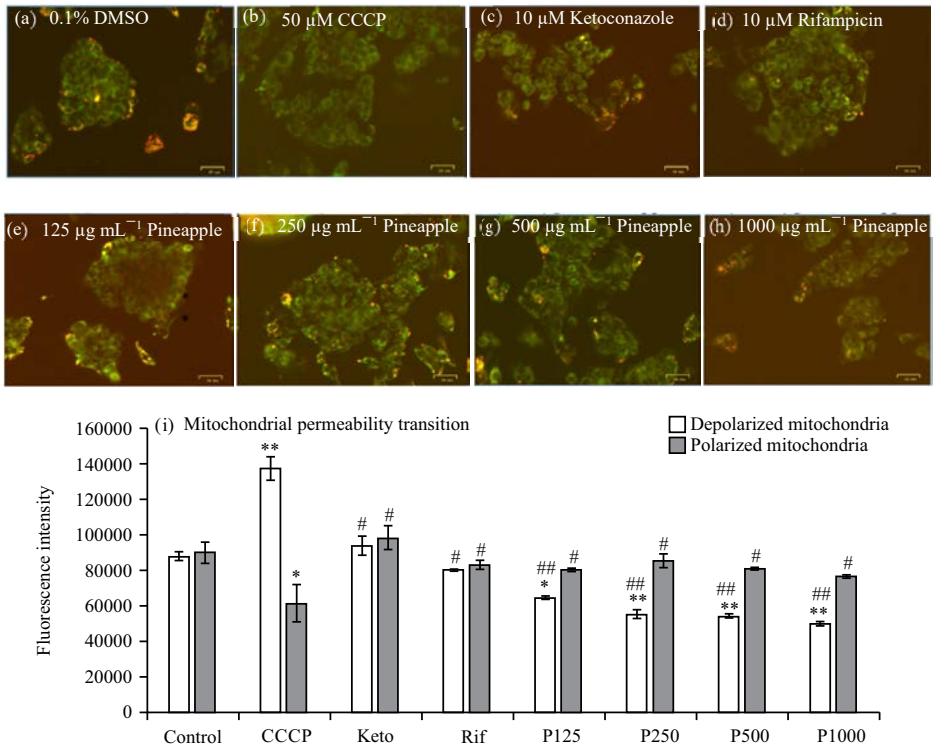

Fig. 2(a-i): Effects of pineapple on mitochondrial permeability transition

(a-h) The fluorescence photograph of Caco-2 cells in each treatment and (i) the quantitative mitochondrial permeability transition. Scale bar is 34 µm in length, n = 3, *p<0.05, **p<0.001, vs. control; $^{\#}$p<0.05, $^{\#\#}$p<0.001 vs. CCCP (50 µM carbonyl cyanide 3-chlorophenylhydrazone)

Keto and Rif significantly elevated AST and ALT, which are biochemical parameters of cell injury (Fig. 1b-c), while P did not affect either AST or ALT level.

Effects of pineapple on Mitochondrial Permeability Transition (MPT): From the fluorescence photographs (Fig. 2a-h), orange/red spots indicate cells with polarized mitochondria, while green spots indicate cells with depolarized mitochondria. CCCP (50 µM, a depolarization inducer) induced depolarized mitochondria (p<0.001, Fig. 2i) and reduced depolarized mitochondria (p<0.05). Keto and Rif did not alter MPT compared to control. Interestingly, all concentrations of pineapple decreased depolarized mitochondria but did not affect polarized mitochondria.

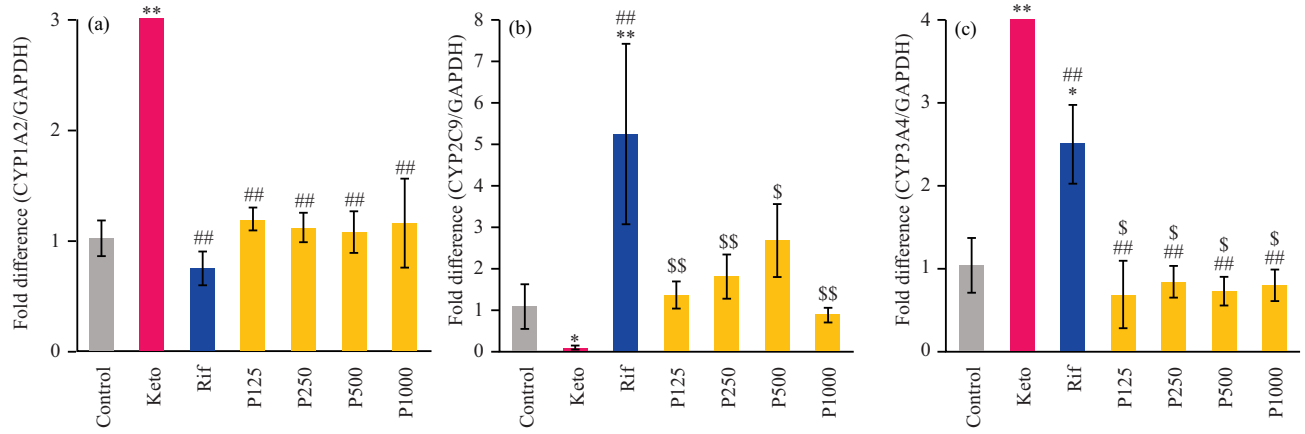

Fig. 3(a-c): Effects of pineapple on the expression of (a) CYP1A2, (b) CYP2C9 and (c) CYP3A4 mRNAs

Keto: Ketoconazole (10 μM), Rif: Rifampicin (10 μM), P: Pineapple juice (125, 250, 500 and 1,000 μg mL^{-1}), n = 4-5, *p<0.05, **p<0.001 vs. control; $^{\#}$p<0.05, $^{\#\#}$p<0.001 vs. Keto; $^{\$}$p<0.05, $^{\$\$}$p<0.001 vs. Rif.

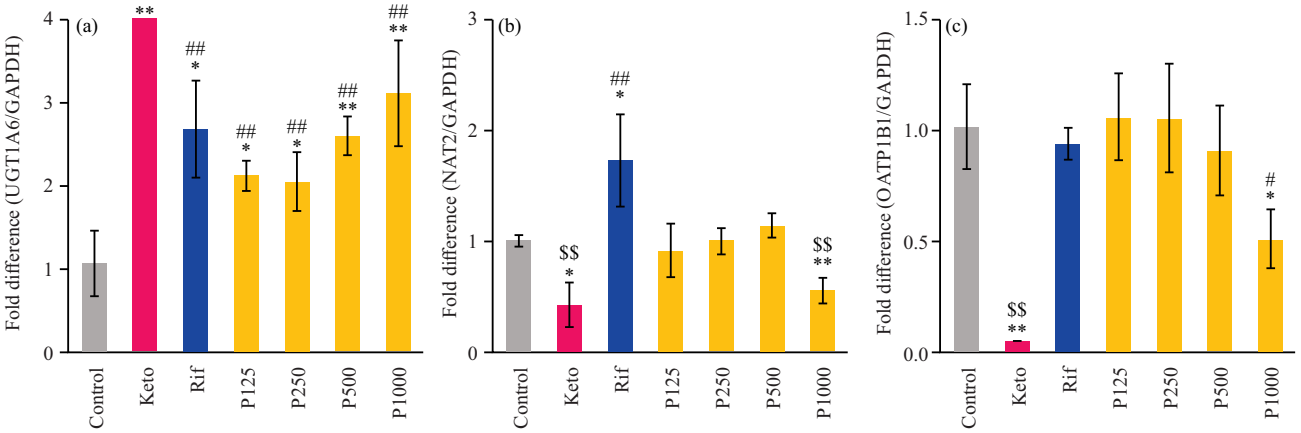

Fig. 4(a-c): Effects of pineapple on the expression of (a) UGT1A6, (b) NAT2 and (c) OATP1B1 mRNAs

Keto: Ketoconazole (10 μM), Rif: Rifampicin (10 μM); P: Pineapple juice (125, 250, 500 and 1,000 μg mL^{-1}), n = 4-5, *p<0.05, **p<0.001 vs. control; $^{\#}$p<0.05, $^{\#\#}$p<0.001 vs. Keto; $^{\$}$p<0.05, $^{\$\$}$p<0.001 vs. Rif.

Hence, pineapple appeared to protect the cells via a reduction in the amount of depolarized mitochondria.

Effects of pineapple on the expression of CYP1A2, CYP2C9 and CYP3A4 mRNAs: Keto extensively induced expression of CYP1A2 mRNA (p<0.001), while Rif and pineapple did not (Fig. 3a). On the other hand, Keto strongly suppressed the expression of CYP2C9 mRNA (p<0.05), while the Rif significantly induced CYP2C9 expression (p<0.001, Fig. 3b). Both the Keto and Rif CYP modifiers significantly up-regulated the expression of CYP3A4 mRNA (p<0.001) (Fig. 3c). All concentrations of pineapple did not modify the expression profile of the investigated CYPs.

Effects of pineapple on the expression of UGT1A6, NAT2 and OATP1B1 mRNAs: All treatments (Keto, Rif and P125-1000) significantly up-regulated the expression of UGT1A6 mRNA (p<0.05, Fig. 4a). Keto and P1000 significantly suppressed the expression of NAT2 (p<0.05), while Rif induced NAT2 expression (p<0.05, Fig. 4b). Expression of the OATP1B1 transporter was suppressed by Keto and P1000 but other treatments did not affect it (Fig. 4c).

DISCUSSION

The concentrations of pineapple used in the current study were estimated from a previous study that reported

consumption of 10-40 mg/kg/day of pineapple modified several CYP expression profiles[17], which is equivalent to 120-480 μg mL^{-1} in normal human blood volume. Keto and Rif were employed as positive modifiers as they have been marked as "high alert" for drug interactions[21,22]. Keto induces CYP1A2 and CYP3A4 expression via activation of the aryl hydrocarbon receptor and pregnane X receptor, respectively[21]. Moreover, Keto was reported as a CYP2C9 inhibitor and a transporter inhibitor[23], while Rif was noted as a typical CYP3A4 inducer[24].

The resazurin fluorescence dye assay is a test for mammalian cell viability that is normally used to determine cytotoxicity and proliferation in cell cultures. The principle is that resazurin (blue non-fluorescence) is reduced to resorufin (pink fluorescence) by living cells[25,26]. All treatments, particularly Keto, reduced resorufin intensity representing an intracellular change of several enzymes in Caco-2 cells. The extra-cellular levels of AST and ALT can be employed to determine toxicity in cells or *in vivo*[20]. Both positive modifiers, Keto and Rif, elevated ASL and ALT levels in the medium of Caco-2 culture, while pineapple did not. The evidence indicates that Keto and Rif induced Caco-2 cell injury, while pineapple did not.

The antioxidant properties of pineapple or its juice have been claimed to be from its phenolic and flavonoid constituents[27-29]. The phenolic and flavonoid contents of pineapple in the present study were different from those reported in a previous study carried out by our research group[17]. This is likely to be due to differences in strain, source and age of pineapple and including the harvesting period. MPT has been implicated in several human oxidative stress-related diseases and the cell protection capabilities of MPT inhibitors have been investigated[5,6]. For example, gallic acid is a phenolic antioxidant that was shown to reduce oxidative damage in isolated mouse brain mitochondria[30]. MPT is defined by the sudden increase in the permeability of the inner mitochondrial membrane to different solutes with a molecular size of less than 1.5 kDa. Depolarization of the inner mitochondrial membrane leads to an opening of the MPT pore, which results in the leakage of intermembrane proteins and cytochrome c, facilitating the induction of apoptosis. Moreover, a feedback mechanism that results in the generation of ROS further accelerates the rate of cell death[31,32]. CCCP is used as a positive depolarizer due to its potential to induce mitochondrial depolarization[33]. This is the first report of the cytoprotective potential of pineapple to allay depolarized mitochondria and lessen ROS production. It might bring beneficial effects for chronic oxidative stress-related diseases.

Humans constitutively express CYP1A2 in the liver[7]. CYP1A2 is responsible for the metabolism of anti-depressants and anti-psychotics as well as anti-inflammatory, anaesthetic and analgesic drugs[34], whilst CYP2C9 is responsible for the metabolism of fluoxetine, losartan, phenytoin, tolbutamide, torsemide, warfarin and non-steroidal anti-inflammatory drugs (NSAIDs)[35]. CYP2C9 is inducible by Rif[35], which concurs with the present findings. CYP3A4 metabolizes a large and diverse range of molecules that includes over 50% of clinical drugs including bronchodilators and antiviral, antibacterial, antifungal, lipid-lowering and anti-hypertensive drugs[7]. Pineapple at a high dose was reported to suppress Cyp1a2 expression but induce Cyp3a11 expression in mice[17]. Despite these previous findings, pineapple made no significant changes to CYP expression in Caco-2 cells. This might be due to the different regulatory pathways of mice and humans[36]. Moreover, differences in the organ-derived tissue expression of CYPs between the liver and intestine could explain this divergence[37,38].

Not only are the CYPs in phase I important for drug metabolism but conjugation reactions during phase II are also a key for drug metabolism. UGT1A6, a member of the UDP-glucuronosyltransferases, is responsible for the metabolism of many drugs such as aspirin, naproxen, carvedilol, zidovudine and valproic acid[9]. CYP and UGT enzymes are together involved in the metabolism of more than 90% of market drugs and phytochemicals or herbal products are also substrates of CYPs and UGTs[11]. For example, prenylflavonoids and the well-known flavonoid quercetin upregulated expression of UGT1A6 mRNA in Caco2 cells[11,39]. Therefore, it is possible that the pineapple contained flavonoids that might also induce UGT1A6 expression. N-acetyltransferase 2 (NAT2) catalyzes the biotransformation of numerous hydrazine drugs (anti-depressant and anti-diabetic agents) and arylamine and aromatic amine procarcinogens[10]. Expression of NAT2 mRNA is high in the liver, small intestine and colon and is readily detected in most other tissues[40]. For heterocyclic amine-related colon cancers, the NAT2 rapid acetylator phenotype is at higher risk[41]. In the present study, pineapple reduced the risk of cancer by reducing NAT2 expression, which corresponds to a previous report that polyphenols inhibited NAT2 expression in human livers and human cholangiocarcinoma cells[42]. Furthermore, a study in healthy volunteers showed that NAT2 activity was inhibited by quercetin[43]. Hence, suppression of NAT2 mRNA in Caco-2 by pineapple at the highest concentration might be due to its phenolic and flavonoid constituents[44]. OATP1B1 is an important determinant of transporter-mediated drug interactions and some herbal plants modulate the expression

of OATP1B1[13,14]. OATP1B1 is mainly expressed in hepatocytes but with nominal levels in colonic or intestinal cells[45]. Currently, the information regarding food/herbal drug interaction and OATP1B1 is limited. While dietary phenolic acids might interfere with the function of human organic anion transporters[45], details about the specific OATP1B1 are insufficient.

In summary, the present findings revealed that pineapple may cause food-drug interaction via alteration of UGT1A6, NAT2 and OATP1B1 expression. On the other hand, pineapple decreased ROS generation by suppression of mitochondrial depolarization and may help lower the risk of cancer via NAT2 suppression.

CONCLUSION

Pineapple revealed potential health benefits through inhibiting the depolarization of mitochondria and consequently lowering the ROS production in Caco-2 cells. Pineapple did not interfere with phase I CYP1A2, CYP2C9 or CYP3A4 expression, however, it modified the expression of phase II UGT1A6 and NAT2 and the transporter OATP1B1 in Caco-2 cells. Expression of UGT1A6 was up-regulated, while NAT2 and OATP1B1 were down-regulated. Therefore, excessive consumption of pineapple is of concern for drug interaction via alteration of UGT1A6, NAT2 and OATP1B1 profiles, which might subsequently interfere with the absorption, metabolism and transportation of concomitant drug(s).

SIGNIFICANCE STATEMENT

This study disclosed the differential potentials of pineapple on mitochondrial permeability transition, phase I metabolizing genes (CYP1A2, CYP2C9, CYP3A4), phase II enzymes (UGT1A6 and NAT2) and the transporter OATP1B1 in a Caco-2 cell model. Pineapple provided promising benefits to reduce depolarized mitochondria, which might be interesting for further examination of its impact on chronic oxidative stress-related diseases. While the profile of CYPs was not disturbed, the expression of UGT1A6, NAT2 and OATP1B1 was altered. Therefore, the consumption of a large amount of pineapple is a considerable risk for interaction with drug substrates of UGT1A6, NAT2, and/or OATP1B1.

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REFERENCES

1. Bamidele, O.P. and M.B. Fasogbon, 2017. Chemical and antioxidant properties of snake tomato (*Trichosanthes cucumerina*) juice and pineapple (*Ananas comosus*) juice blends and their changes during storage. Food Chem., 220: 184-189.
2. Wali, N., 2019. Pineapple (*Ananas comosus*). In: Nonvitamin and Nonmineral Nutritional Supplements. Nabavi, S.M. and A.S. Silva (Eds.)., Elsevier Inc., pp: 367-373.
3. Kargutkar, S. and S. Brijesh, 2018. Anti-inflammatory evaluation and characterization of leaf extract of *Ananas comosus*. Inflammopharmacol., 26: 469-477.
4. Secor, E.R., S.M. Szczepanek, C.A. Castater, A.J. Adami and A.P. Matson *et al*., 2013. Bromelain inhibits allergic sensitization and murine asthma via modulation of dendritic cells. Evidence-Based Compl. Alt. Med., 2013. 10.1155/2013/702196.
5. Briston, T., D.L. Selwood, G. Szabadkai and M.R. Duchen, 2019. Mitochondrial permeability transition: A molecular lesion with multiple drug targets. Trends Pharmacol. Sci., 40: 50-70.
6. Ma, L., J.Y. Liu, J.X. Dong, Q. Xiao, J. Zhao and F.L. Jiang, 2017. Toxicity of PB^{2+} on rat liver mitochondria induced by oxidative stress and mitochondrial permeability transition. Toxicol. Res., 6: 822-830.
7. Zanger, U.M. and M. Schwab, 2013. Cytochrome P450 enzymes in drug metabolism: Regulation of gene expression, enzyme activities and impact of genetic variation. Pharmacol. Therapeut., 138: 103-141.
8. Kafle, A., S.S. Mohapatra, J. Sarma and I. Reddy, 2018. Food-drug interaction: A review. Pharma. Innov., 7: 114-118.
9. Kim, S.B., K.S. Kim, D.D. Kim and I.S. Yoon, 2019. Metabolic interactions of rosmarinic acid with human cytochrome P450 monooxygenases and uridine diphosphate glucuronosyltransferases. Biomed. Pharmacother., 110: 111-117.
10. Salazar-González, R.A., E. Turiján-Espinoza, D.W. Hein, R.C. Milán-Segovia, E.E. Uresti-Rivera and D.P. Portales-Pérez, 2018. Expression and genotype-dependent catalytic activity of *N*-acetyltransferase 2 (NAT2) in human peripheral blood mononuclear cells and its modulation by Sirtuin 1. Biochem. Pharmacol., 156: 340-347.

11. Lněničková, K., M. Šadibolová, P. Matoušková, B. Szotáková, L. Skálová and I. Boušová, 2020. The modulation of phase II drug-metabolizing enzymes in proliferating and differentiated caco-2 cells by hop-derived prenylflavonoids. Nutrients, 12. 10.3390/nu12072138.
12. Kim, S.B., I.S. Yoon, K.S. Kim, S.J. Cho and Y.S. Kim *et al*., 2014. *In vitro* and *in vivo* evaluation of the effect of puerarin on hepatic cytochrome p450-mediated drug metabolism. Planta Med., 80: 561-567.
13. Kayesh, R., T. Farasyn, A. Crowe, Q. Liu and S. Pahwa *et al*., 2021. Assessing OATP1B1 and OATP1B3-mediated drug-drug interaction potential of vemurafenib using r-value and physiologically-based pharmacokinetic models. J. Pharm. Sci., 110: 314-324.
14. Chen, L., L. Liu, Y. Chen, M. Liu, Y. Xiong, H. Zhang, S. Huang and C. Xia, 2019. Modulation of transporter activity of OATP1B1 and OATP1B3 by the major active components of *Radix ophiopogonis*. Xenobiotica, 49: 1221-1228.
15. Vaessen, S.F.C., M.M.H. van Lipzig, R.H.H. Pieters, C.A.M. Krul, H.M. Wortelboer and E.V. de Steeg, 2017. Regional expression levels of drug transporters and metabolizing enzymes along the pig and human intestinal tract and comparison with Caco-2 cells. Drug Metab. Dispos., 45: 353-360.
16. Iftikhar, M., A. Iftikhar, H. Zhang, L. Gong and J. Wang, 2020. Transport, metabolism and remedial potential of functional food extracts (FFES) in Caco-2 cells monolayer: A review. Food Res. Int., 136. 10.1016/j.foodres.2020.109240.
17. Chatuphonprasert, W. and K. Jarukamjorn, 2012. Impact of six fruits-banana, guava, mangosteen, pineapple, ripe mango and ripe papaya-on murine hepatic cytochrome P450 activities. J. Applied Toxicol., 32: 994-1001.
18. Sriset, Y., W. Chatuphonprasert and K. Jarukamjorn, 2021. *In vitro* antioxidant potential of *Mallotus repandus* (Willd.) Muell. Arg stem extract and its active constituent bergenin. Songklanakarin. J. Sci. Technol., 43: 24-30.
19. Chatuphonprasert, W., T. Kitisripanya, W. Putalun, I. Ellinger and K. Jarukamjorn, 2020. *Pueraria candollei* var. *mirifica*-induced *CYP1A1* and *CYP1A2* expression in human choriocarcinoma beWo cells. Pharmacogn. Mag., 16: 506-512.
20. Sriset, Y., W. Chatuphonprasert and K. Jarukamjorn, 2021. Bergenin attenuates sodium selenite-induced hepatotoxicity via improvement of hepatic oxidant-antioxidant balance in Hepg2 cells and ICR mice. J. Biolo. Active Prod. Nat., 11: 97-115.
21. Novotna, A., M. Korhonova, I. Bartonkova, A.A. Soshilov and M.S. Denison *et al*., 2014. Enantiospecific effects of ketoconazole on aryl hydrocarbon receptor. PLOS ONE, 9. 10.1371/journal.pone.0101832.
22. den Bergh, A.V., J. Snoeys, L.D. Zwart, P. Ward and A. Lopez-Gitlitz *et al*., 2020. Pharmacokinetic drug-drug interaction of apalutamide, part 2: Investigating interaction potential using a physiologically based pharmacokinetic model. Clin. Pharmacokinetics, 59: 1149-1160.
23. Nikulin, S.V., E.A. Tonevitsky and A.A. Poloznikov, 2017. Effect of ketoconazole on the transport and metabolism of drugs in the human liver cell model. Russian Chem. Bull., 66: 150-155.
24. Kuncharoenwirat, N., W. Chatuphonprasert and K. Jarukamjorn 2020. Effects of phenol red on rifampicin-induced expression of cytochrome P450s enzymes. Pharmacophore, 11: 13-20.
25. Präbst, K., H. Engelhardt, S. Ringgeler and H. Hübner, 2017. Basic Colorimetric Proliferation Assays: MTT, WST and Resazurin. In: Cell Viability Assays. Gilbert, D.F. and O. Friedrich (Eds.)., Humana Press., New York, pp: 1-17.
26. O'Brien, J., I. Wilson, T. Orton and F. Pognan, 2000. Investigation of the alamar blue (resazurin) fluorescent dye for the assessment of mammalian cell cytotoxicity. Eur. J. Biochem., 267: 5421-5426.
27. Mhatre, M., J. Tilak-Jain, S. De and T.P.A. Devasagayam, 2009. Evaluation of the antioxidant activity of non-transformed and transformed pineapple: A comparative study. Food Chem. Toxicol., 47: 2696-2702.
28. Hossain, M.A. and S.M.M. Rahman, 2011. Total phenolics, flavonoids and antioxidant activity of tropical fruit pineapple. Food Res. Int., 44: 672-676.
29. Martínez, R., P. Torres, M.A. Meneses, J.G. Figueroa, J.A. Pérez-Álvarez and M. Viuda-Martos, 2012. Chemical, technological and *in vitro* antioxidant properties of mango, guava, pineapple and passion fruit dietary fibre concentrate. Food Chem., 135: 1520-1526.
30. Parihar, P., D. Jat, P. Ghafourifar and M.S. Parihar, 2014. Efficiency of mitochondrially targeted gallic acid in reducing brain mitochondrial oxidative damage. Cell. Mol. Biol., (Noisy-le-grand), 60: 35-41.
31. Zorov, D.B., C.R. Filburn, L.O. Klotz, J.L. Zweier and S.J. Sollott, 2000. Reactive oxygen species (Ros-induced) ros release. J. Exp. Med., 192: 1001-1014.
32. Rottenberg, H. and J.B. Hoek, 2017. The path from mitochondrial ROS to aging runs through the mitochondrial permeability transition pore. Aging Cell, 16: 943-955.
33. Lim, M.L.R., T. Minamikawa and P. Nagley, 2001. The protonophore CCCP induces mitochondrial permeability transition without cytochrome c release in human osteosarcoma cells. FEBS Lett., 503: 69-74.
34. Zhou, S.F., B. Wang, L.P. Yang and J.P. Liu, 2010. Structure, function, regulation and polymorphism and the clinical significance of human cytochrome P450 1A2. Drug Metab. Rev., 42: 268-354.
35. Miners, J.O. and D.J. Birkett, 1998. Cytochrome P4502C9: An enzyme of major importance in human drug metabolism. Br. J. Clin. Pharmacol., 45: 525-538.
36. Martignoni, M., G.M.M. Groothuis and R. de Kanter, 2006. Species differences between mouse, rat, dog, monkey and human CYP-mediated drug metabolism, inhibition and induction. Expert Opin. Drug Metab. Toxicol., 2: 875-894.

37. Drozdzik, M., D. Busch, J. Lapczuk, J. Müller, M. Ostrowski, M. Kurzawski and S. Oswald, 2018. Protein abundance of clinically relevant drug-metabolizing enzymes in the human liver and intestine: A comparative analysis in paired tissue specimens. Clin. Pharmacol. Ther., 104: 515-524.
38. Bièche, I., C. Narjoz, T. Asselah, S. Vacher and P. Marcellin *et al*., 2007. Reverse transcriptase-PCR quantification of mRNA levels from cytochrome (CYP)1, CYP2 and CYP3 families in 22 different human tissues. Pharmacogenetics Genomics, 17: 731-742.
39. Bock, K.W., T. Eckle, M. Ouzzine and S. Fournel-Gigleux, 2000. Coordinate induction by antioxidants of UDP-glucuronosyltransferase UGT1A6 and the apical conjugate export pump MRP2 (multidrug resistance protein 2) in Caco-2 cells. Biochem. Pharmacol., 59: 467-470.
40. Husain, A., X. Zhang, M.A. Doll, J.C. States, D.F. Barker and D.W. Hein, 2007. Identification of N-acetyltransferase 2 (NAT2) transcription start sites and quantitation of NAT2-specific mRNA in human tissues. Drug Metab. Dispos., 35: 721-727.
41. Hein, D.W., 2002. Molecular genetics and function of NAT1 and NAT2: Role in aromatic amine metabolism and carcinogenesis. Mutat. Res. Fundam. Mol. Mech. Mutagen., 506-507: 65-77.
42. Kukongviriyapan, V., N. Phromsopha, W. Tassaneeyakul, U. Kukongviriyapan, B. Sripa, V. Hahnvajanawong and V. Bhudhisawasdi, 2006. Inhibitory effects of polyphenolic compounds on human arylamine N-acetyltransferase 1 and 2. Xenobiotica, 36: 15-28.
43. Chen, Y., P. Xiao, D.S. Ou-Yang, L. Fan and D. Guo *et al*., 2009. Simultaneous action of the flavonoid quercetin on cytochrome P450 (CYP) 1A2, CYP2A6, N-acetyltransferase and xanthine oxidase activity in healthy volunteers. Clin. Exp. Pharmacol., 36: 828-833.
44. Maubon, N., M.L. Vee, L. Fossati, M. Audry, E.L. Ferrec, S. Bolze and O. Fardel, 2007. Analysis of drug transporter expression in human intestinal Caco-2 cells by real-time PCR. Fundam. Clin. Pharmacol., 21: 659-663.
45. Wang, L. and D.H. Sweet, 2012. Potential for food–drug interactions by dietary phenolic acids on human organic anion transporters 1 (SLC22A6), 3 (SLC22A8) and 4 (SLC22A11). Biochem. Pharmacol., 84: 1088-1095.