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Research Article
Dill Shows Potential for Herb-Drug Interactions via Up-Regulation of *CYP1A2*, *CYP2C19*, *SULT1A1*, *NAT2* and *ABCB1* in Caco-2 Cells

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Abstract

Background and Objective: Dill (*Anethum graveolens* L.) has the potential to develop as a new alternative medicine due to its pharmacological activities. However, studies into its safety regarding herb-drug interactions have been neglected. This study investigated the risk of dill-induced herb-drug interactions (HDI) by examining its effect on the expression of phase I and II drug-metabolizing enzyme and transporter genes in Caco-2 cells. **Materials and Methods:** Caco-2 cells (5×10^5 cells/well) were treated with 10 μM ketoconazole, 20 μM rifampicin or dill extract (60-240 μg mL^{-1}) for 72 hrs. Cell viability was assessed using the resazurin assay and reactive oxygen species (ROS) content was determined with 2 ,7 -dichlorofluorescein diacetate. Aspartate (AST) and alanine aminotransferase (ALT) levels were measured using L-aspartate and L-alanine with α-ketoglutarate as substrate. Expression of phase I (*CYP1A2*, *CYP2C19*, *CYP2D6*, *CYP2E1* and *CYP3A4*) and II (*UGT1A6*, *SULT1A1*, *NAT1*, *NAT2* and *GSTA1/2*) metabolizing genes and transporters (*ABCB1*, *ABCC2*, *ABCG2* and *SLCO1B1*) were determined by RT/qPCR. **Results:** All tested concentrations of dill did not affect cell viability or AST and ALT levels. The highest concentration of dill extract (240 μg mL^{-1}) significantly lowered the ROS level. Expression of *CYP1A2*, *CYP2C19*, *SULT1A1*, *NAT2* and *ABCB1* mRNA was significantly up-regulated by dill extract. **Conclusion:** Dill extract did not directly damage Caco-2 cells but prolonged use of dill may increase the risk of HDI via the up-regulation of the drug-metabolizing genes *CYP1A2*, *CYP2C19*, *SULT1A1*, *NAT2* and the transporter *ABCB1*.

Key words: Dill, reactive oxygen species, herb-drug interaction, phase I metabolism, phase II metabolism, transporter

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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

Dill (*Anethum graveolens* L. or Phak Chee Lao in Thai) is an annual herb in the Apiaceae or Umbelliferae family found widely in Europe, North America and Asia. Dill has been extensively used as a food in sauces, vinegar and pastries and as a carminative and herbal medicine for several ailments. Dill is a basic ingredient in Phikatthianthang 5, 7 and 9, Yahom and several other Thai traditional herbal formulas found in the National essential herbal drug list of Thailand and Tamra Kanpaetthaidoem[1]. Dill has significant biological activities such as anti-microbial, anti-inflammatory, analgesic, mucosal protective, antisecretory, antioxidant and hypolipidemic effects[2]. The pharmacological activity of dill has been thoroughly studied. For example, anti-hypercholesterolemia and insulin sensitization activities[3] have been studied in clinical trials, hepatoprotective and antioxidant activities have been studied in *in vivo* models[4] and anti-microbial activity has been investigated *in vitro*[5]. However, there have been only limited studies of dill's safety concerning herb-drug interactions (HDIs). One study reported that carvone, a main essential oil of dill, may interfere with the regulatory metabolism of phase I and II enzymes, which could lead to HDIs[6]. Therefore, to avoid dill-induced HDIs, it is worth understanding the regulatory metabolism of dill.

Metabolism is a crucial process to eradicate drugs, xenobiotics and harmful compounds from the body. The major site of metabolism is the liver, which contains several phase I (cytochrome P450 1A2 (*CYP1A2*), *CYP2C9*, *CYP2C19*, *CYP2D6*, *CYP2E1* and *CYP3A4*) and phase II (UDP-glucuronosyltransferase 1A6 (*UGT1A6*), *N*-acetyltransferase 2 (*NAT2*), glutathione *S*-transferase alpha 1 (*GSTA1*) and *(GSTA2*) metabolizing enzymes and transporters (ATP-binding cassette C2 (ABCC2) and solute carrier organic anion 1B1 (SLCO1B1)). Some essential metabolizing enzymes and transporters are primarily expressed in the intestine, including sulfotransferases 1A1 (*SULT1A1*), *NAT1*, *ABCB1* and *ABCG2*[7]. Hence, the liver and intestine should be investigated for an entire understanding of metabolism pathways.

Caco-2 cell is a human colon adenocarcinoma cell line that has a similar structure and drug absorption characteristics to the human jejunum and is, therefore, an excellent *in vitro* model for intestinal drug absorption and transporter affinity or inhibitory evaluation. Furthermore, many drug-metabolizing genes are expressed at detectable levels in Caco-2 cells[8,9]. Therefore, Caco-2 cells were employed to be a surrogate for the human intestinal cell to investigate the safety and metabolic pathways of dill.

MATERIALS AND METHODS

Study area: The study was performed at the Research Group for Pharmaceutical Activities of Natural Products using Pharmaceutical Biotechnology, Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen 40002, Thailand between July, 2020 and May, 2021.

Chemicals: Dulbecco's modified Eagle medium (DMEM; cat no. 11885-084) and fetal bovine serum (FBS) were purchased from Gibco® (New York, USA). Resazurin, 2,4-dinitrophenylhydrazine (DNPH), 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA), rifampicin and ketoconazole were obtained from Sigma-Aldrich (St. Louis, Missouri, USA). L-aspartate, L-alanine and α-ketoglutarate were purchased from Sigma-Aldrich Chemical. Forward and reverse primers of *CYP1A2*, *CYP2C19*, *CYP2D6*, *CYP2E1*, *CYP3A4, UGT1A6*, *SULT1A1*, *NAT1*, *NAT2*, *GSTA1/2*, *ABCB1*, *ABCC2*, *ABCG2*, *SLCO1B1* and *GAPDH* genes were synthesized by Bio Basic, Inc. (Markham, Ontario, Canada). Other chemicals were of the highest purity from suppliers.

Sample preparation: Aerial parts of dill were dried, ground and macerated in 95% ethanol for 72 hrs. Then, the mixture was evaporated and freeze-dried to yield the dill extract (Thai petty patent No. 16714). The content of chlorogenic acid, a major constituent in dill extract, was quantified by reverse phase-HPLC consisting of a Phenomenex Luna C18 column (250×4.6 mm; Phenomenex®, Torrance, CA, USA) with gradient elution of 0.1% orthophosphoric acid in water and acetonitrile from 90:10 at 0-5 min, 85:15 at 5-10 min, 80:20 at 10-15 min, 70:30 at 15-25 min, 80:20 at 25-30 min and 90:10 at 30-40 min at a flow rate of 1 mL min^{-1} and a wavelength of 245 nm. The dill extract contained chlorogenic acid of 0.689% dry weight.

Cell culture: Caco-2 cells (Riken Cell Bank RBRC-RBC0988, Wako, Saitama, Japan) were seeded at 5×10^5 cells/well in 24-wells plates and incubated at 37°C with 5% CO_2 and 95% relative humidity under sterile condition for 48 hrs. Then, the cells were treated with 0.2% dimethyl sulfoxide (DMSO) as control, 10 mM ketoconazole or 20 mM rifampicin as typical modifiers or 60-240 mg mL^{-1} of the dill extract for 72 hrs. The medium was collected for the determination of cell viability, reactive oxygen species (ROS), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels. The cells were

harvested for extraction of total RNA and determination of mRNA expression using reverse transcription and real-time polymerase chain reaction (RT/qPCR).

Assessment of cell viability: Cell viability was assessed by resazurin assay which examined cellular enzyme capacities of transforming reduced non-fluorescent blue resazurin to fluorescent pink resorufin. Briefly, the medium was mixed with 1 mM resazurin (medium: resazurin, 10: 1) and incubated in 5% CO_2 at 37°C for 1 hr. Then, the fluorescence intensity of resorufin was measured at an excitation of 530 nm and emissions of 590 nm and calculated for a percentage of cell viability[10].

Determination of ROS level: ROS level was determined using the 2,7-dichlorofluorescein diacetate (DCFH-DA) method. DCFH-DA reacts with ROS to produce fluorescent dichlorofluorescein (DCF)[11]. Briefly, the medium was mixed with 0.06 μM DCFH-DA before incubation at 25°C for 40 min in the dark. The fluorescence intensity of DCF was measured at an excitation of 484 nm and emission of 530 nm and ROS level was calculated by comparison with a hydrogen peroxide standard curve (ranged 25-1,000 μM).

Determination of AST and ALT levels: AST (L-aspartate and α-ketoglutarate) or ALT (L-alanine and α-ketoglutarate) substrates were used to determine AST and ALT levels[12]. The medium was mixed with AST (for AST level) and ALT (for ALT level) substrates at 37°C for 30 or 20 min, respectively. Then, 2,4-dinitrophenylhydrazine (DNPH) was added to the medium for 20 min before the addition of 4 N NaOH. AST and ALT levels were calculated by comparison of absorbance at a wavelength of 505 nm with a sodium pyruvate standard curve (ranged 100-500 μM).

Quantitative determination of mRNA expression by reverse transcription/real-time polymerase chain reaction (RT/qPCR): Total RNA was extracted from Caco-2 cells by guanidinium thiocyanate-phenol-chloroform method[13]. The purity and concentration were determined at 260/280 and

Table 1: Forward and reverse primer sequences

Genes	Primers (5′-3′)®	$T_{Annealing}$ (°C)	Product size (bp)
CYP1A2	*F* ACAAGGGACACAACGCTGAA	60	160
	R AGGGCTTGTTAATGGCAGTG		
CYP2C19	*F* GGATTGTAAGCACCCCCTG	60	174
	R TAAAGTCCCGAGGGTTGTTG		
CYP2D6	*F* AGCTTTCTGGTGACCCCATC	61.1	135
	R GGACCCGAGTTGGAACTACC		
CYP2E1	*F* AATGGACCTACCTGGAAGGAC	60	96
	R CCTCTGGATCCGGCTCTCATT		
CYP3A4	*F* CTTCATCCAATGGACTGCATAAA	55	87
	R TCCCAAGTATAACACTCTACACACACA		
UGT1A6	*F* AGCCCAGACCCTGTGTCCTA	58.2	76
	R CCACTCGTTGGGAAAAAGTCA		
SULT1A1	*F* GTCACCGAGCTCCCATCTTC	60	76
	R GTCTCCATCCCTGAGGGAATC		
NAT1	*F* GAATTCAAGCCAGGAAGAAGCA	60	151
	R TCCAAGTCCAATTTGTTCCTAGACT		
NAT2	*F* ACGTCTCCAACATCTTCATTTATAACC	60	161
	R TCAACCTCTTCCTCAGTGAGAGTTTTA		
GSTA1/2	*F* AGCCGGGCTGACATTCATCT	60	230
	R TGGCCTCCATGACTGCGTTA		
ABCB1 (P-glycoprotein)	*F* GGGATGGTCAGTGTTGATGGA	60	110
	R GCTATCGTGGTGGCAAACAATA		
ABCC2 (MRP2)	*F* ATATAAGAAGGCATTGACCC	60	144
	R ATCTGTAGAACACTTGACC		
ABCG2 (BCRP)	*F* ACGATATGGATTTACGGCTTT	60	138
	R TCGATGCCCTGCTTTACCAA		
SLCO1B1 (OATP1B1)	*F* GAATGCCCAAGAGATGATGCTT	60	154
	R AACCCAGTGCAAGTGATTTCAAT		
GAPDH	*F* CACCATCTTCCAGGAGCGAG	61.1	72
	R GACTCCACGACGTACTCAGC		

260/230 nm using a NanoDrop 2000c UV-Vis Spectrophotometer (NanoDrop Technologies, Inc., Thermo Fisher Scientific, MA, USA). The integrity was examined by 1.25% agarose gel electrophoresis before transcribing total RNA to cDNA using ReverTra Ace® (Toyobo Co., Ltd., Osaka, Japan) at 25°C for 10 min, 42°C for 60 min and 95°C for 5 min. cDNA was amplified to determine mRNA expression of phase I (i.e., *CYP1A2*, *CYP2C19*, *CYP2D6*, *CYP2E1* and *CYP3A4*) and II (i.e., *UGT1A6*, *SULT1A1*, *NAT1*, *NAT2* and *GSTA1/2*) metabolizing genes and transporters (i.e., *ABCB1*, *ABCC2*, *ABCG2* and *SLCO1B1*) with specific forward and reverse primers of each gene in Table 1. The mRNA expression was normalized to a reference gene *GAPDH* and expressed as a relative fold expression using ΔΔCt method[14], by which ΔCt is the difference in Ct values of the target gene and *GAPDH* for a given sample and ΔΔCt is the difference between the ΔCt values of each treatment and the control. Hence, a relative fold difference equals $2^{-\Delta\Delta Ct}$.

Statistical analysis: The results are reported as Mean± standard deviation (SD). Statistical analysis was performed by One-Way ANOVA with Tukey's statistical *post hoc* test at a significance level of $p<0.05$ using SPSS version 26.0 (Armonk, New York, USA).

RESULTS

Effects of dill extract on cell viability and ROS, AST and ALT levels in Caco-2 cells: The tested dill extracts and modifiers did not affect the viability of Caco-2 cells. The percentage viabilities of Caco-2 cells treated with 60, 120 and 240 μg mL^{-1} dill extract were 92.0±16.6% (80-113%), 87.8±6.6% (81-95%) and 86.3±9.3% (78-94%), respectively and 115.0±14.3% (99-117%) and 73.8±13.4% (62-84%) for cells treated with 10 μM ketoconazole and 20 μM rifampicin, respectively in Fig. 1a. The ROS level in Caco-2 cells was not changed by treatment with either ketoconazole, rifampicin or 60 and

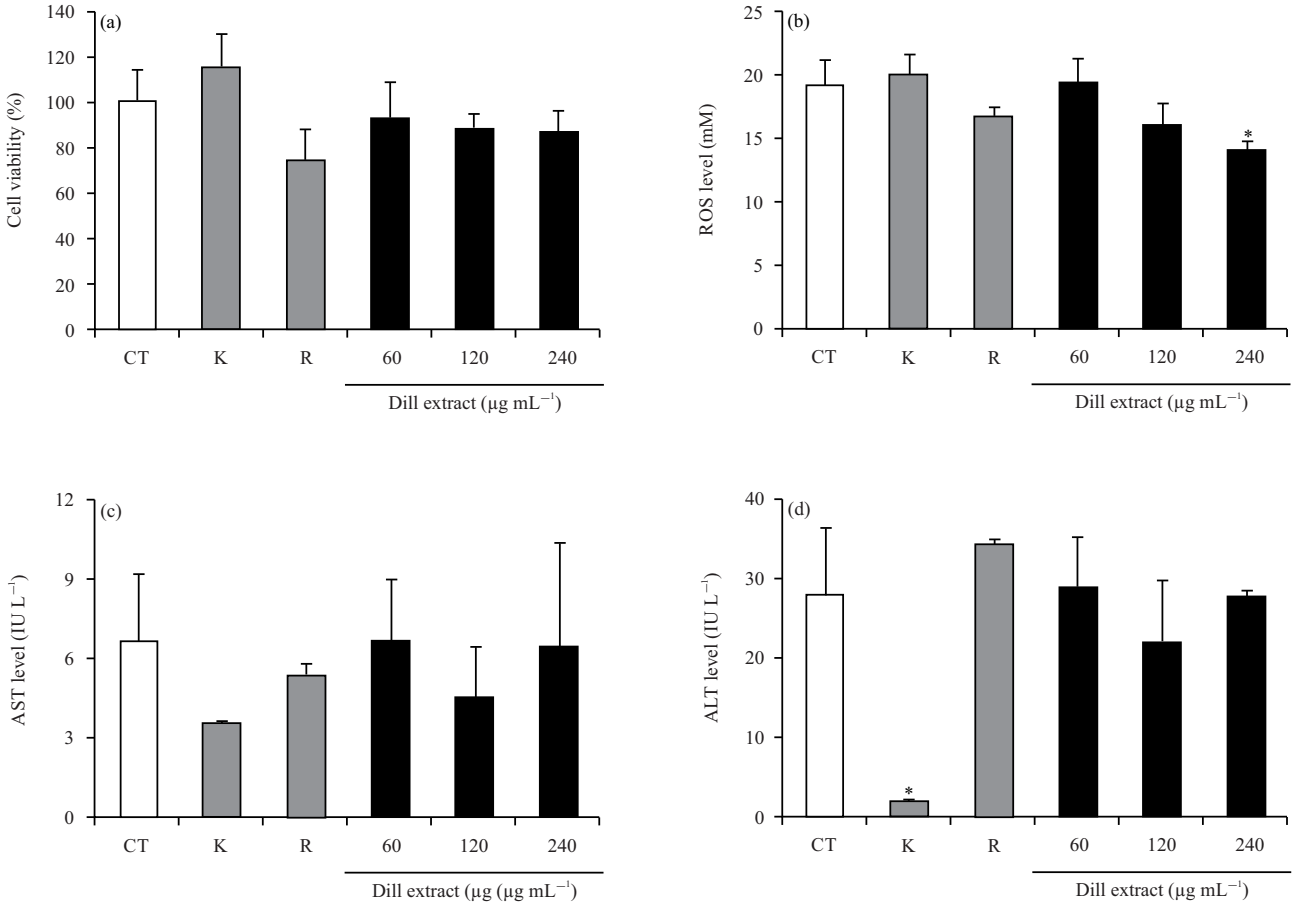

Fig. 1(a-d): Effects of dill extracts, (a) Cell viability, (b) ROS, (c) AST and (d) ALT level in Caco-2 cells
CT: 0.2% DMSO, K: 10 mM ketoconazole, R: 20 mM rifampicin, n = 4, *p<0.05 vs. CT

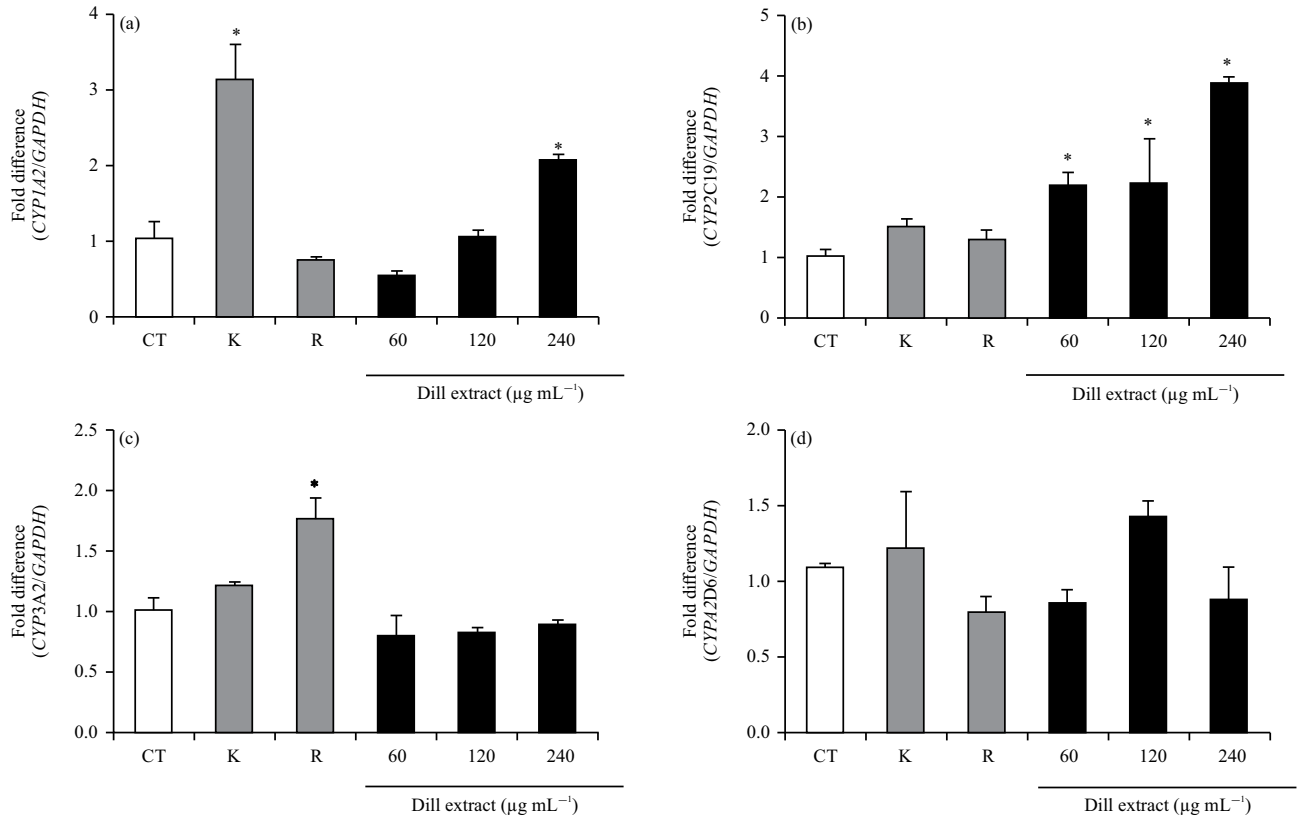

Fig. 2(a-d): Effects of dill extracts on expressions of phase I metabolizing genes, (a) *CYP1A2*, (b) *CYP2C19*, (c) *CYP3A4* and (d) *CYP2D6* mRNAs in Caco-2 cells

Figure depicts relative fold differences between the expression of target genes in phase 1 compared to the reference gene *GAPDH* (in Y-axis) and each treatment (in X-axis), CT: 0.2% DMSO, K: 10 mM ketoconazole, R: 20 mM rifampicin, n = 4, *p<0.05 vs. CT

120 μg mL^{-1} dill extract (ROS levels of 20.0±1.5, 16.7±0.7, 19.3±1.9 and 16.0±1.7 mM, respectively) compared to the control (19.1±2.0 mM), but cells treated with the highest concentration of dill extract (240 mg mL^{-1}) showed a significantly reduced ROS level (14.0±0.7 mM) in Fig. 1b. Other than a decrease in ALT level induced by ketoconazole, no significant changes in AST and ALT levels were observed by all treatments in Fig. 1c and d. Average AST levels were 6.6±2.6, 3.6±0.1, 5.4±0.4, 6.7±2.3, 4.5±2.0 and 6.5±3.9 IU L^{-1} and average ALT levels were 27.9±8.4, 1.6±1.0 ($p < 0.05$ compared to control), 34.3±0.6, 29.0±6.2, 21.7±8.0 and 27.7±1.6 IU L^{-1} for the control, ketoconazole, rifampicin and dill extract (60, 120 and 240 μg mL^{-1}) treated cells, respectively. Hence, these concentrations were employed for the examination of expression profiles of phase I and II metabolizing genes and transporters in Caco-2 cells.

Effects of dill extract on mRNA expression of phase I metabolizing genes in Caco-2 cells: Expression of *CYP1A2* was significantly increased by the highest concentration of dill extract (240 mg mL^{-1}, 2.06-fold) and ketoconazole (3.12-fold) in Caco-2 cells ($p<0.05$), but not by rifampicin (0.75-fold) or 60 and 120 μg mL^{-1} dill extract (0.52 and 1.05-fold, respectively) in Fig. 2a. Dill extracts significantly elevated expression of *CYP2C19* in a dose-dependent pattern (from 2.20-3.93-fold; $p<0.05$) in Fig. 2b and *CYP3A4* expression was only induced by rifampicin (1.77-fold increase, $p<0.05$) in Fig. 2c. In contrast, *CYP2D6* expression was not modified by any dill extracts (60, 120 and 240 mg mL^{-1} for 0.85, 1.43 and 0.88-fold, respectively) or by ketoconazole (1.22-fold) and rifampicin (0.80-fold) in Fig. 2d and *CYP3A4* expression was not changed by ketoconazole (1.21-fold) or 60, 120 and 240 mg mL^{-1} dill extract (0.79, 0.82 and 0.89-fold, respectively). Neither ketoconazole nor rifampicin changed the expression of *CYP2C19* (1.51 and 1.31-fold) respectively and *CYP2D6* mRNA (1.22 and 0.80-fold) respectively. Expression of *CYP2E1* mRNA was undetectable in Caco-2 cells (data not shown). These observations suggested that dill extract up-regulated *CYP1A2* and *CYP2C19* expression in Caco-2 cells in a dose-dependent pattern.

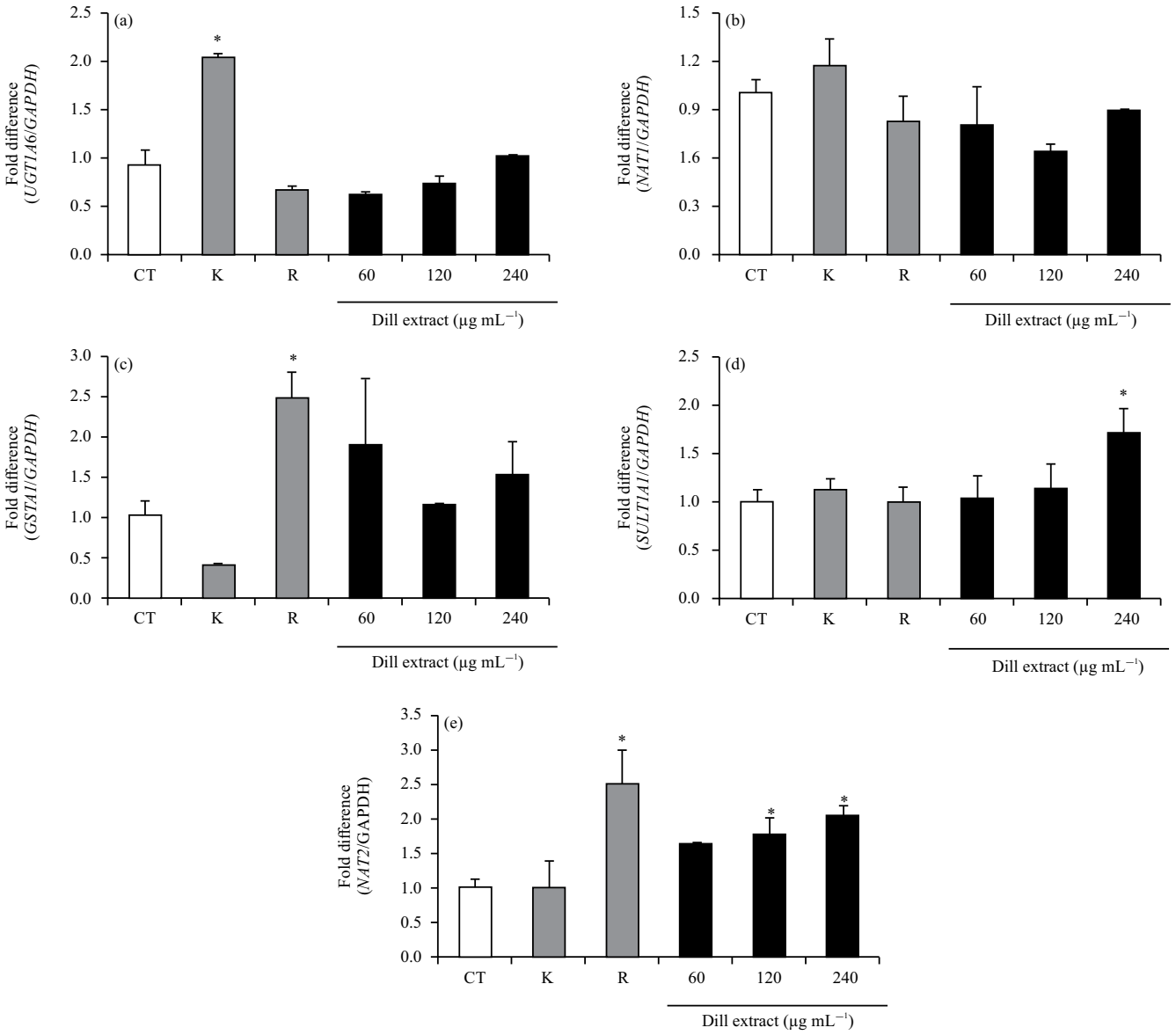

Fig. 3(a-e): Effects of dill extracts on expressions of phase II metabolizing genes, (a) *UGT1A6*, (b) *NAT1*, (c) *GSTA1/2*, (d) *SULT1A1* and (e) *NAT2* mRNAs in Caco-2 cells

Figure depicts relative fold differences between the expression of target genes in phase 2 compared to the reference gene *GAPDH* (in Y-axis) and each treatment (in X-axis), CT: 0.2% DMSO, K: 10 mM ketoconazole, R: 20 mM rifampicin, n = 4, *p<0.05 vs. CT

Effects of dill extract on mRNA expression of phase II metabolizing genes in Caco-2 cells: Dill extract (60, 120 and 240 µg mL^{-1}) did not modify the expression of *UGT1A6* (0.61, 0.73 and 1.01-fold, respectively in Fig. 3a, *NAT1* (0.80, 0.64 and 0.89-fold, respectively) in Fig. 3b and *GSTA1/2* (1.92, 1.17 and 1.53-fold, respectively) in Fig. 3c-mRNAs. Rifampicin did not change *UGT1A6* (0.66-fold) and *NAT1* (0.82-fold) mRNAs (Fig. 3a and b) and ketoconazole did not induce *NAT1* (1.17-fold) and *GSTA1/2* (0.41-fold) mRNAs (Fig. 3b and c) in Caco-2 cells. However, ketoconazole and rifampicin significantly induced *UGT1A6* and *GSTA1/2* expression by 2.02 and 2.48-fold ($p<0.05$), respectively. Expression of *SULT1A1* mRNA was significantly induced (1.7-fold; $p<0.05$) by dill extract at 240 µg mL^{-1}, but not by ketoconazole (1.11-fold) or rifampicin (1.00-fold), or 60 and 120 µg mL^{-1} dill extract (1.02 and 1.14-fold, respectively in Fig. 3d. Expression of *NAT2* mRNA was induced by rifampicin and dill extracts at 120 and 240 µg mL^{-1} (for 2.49, 1.78 and 2.04-fold,

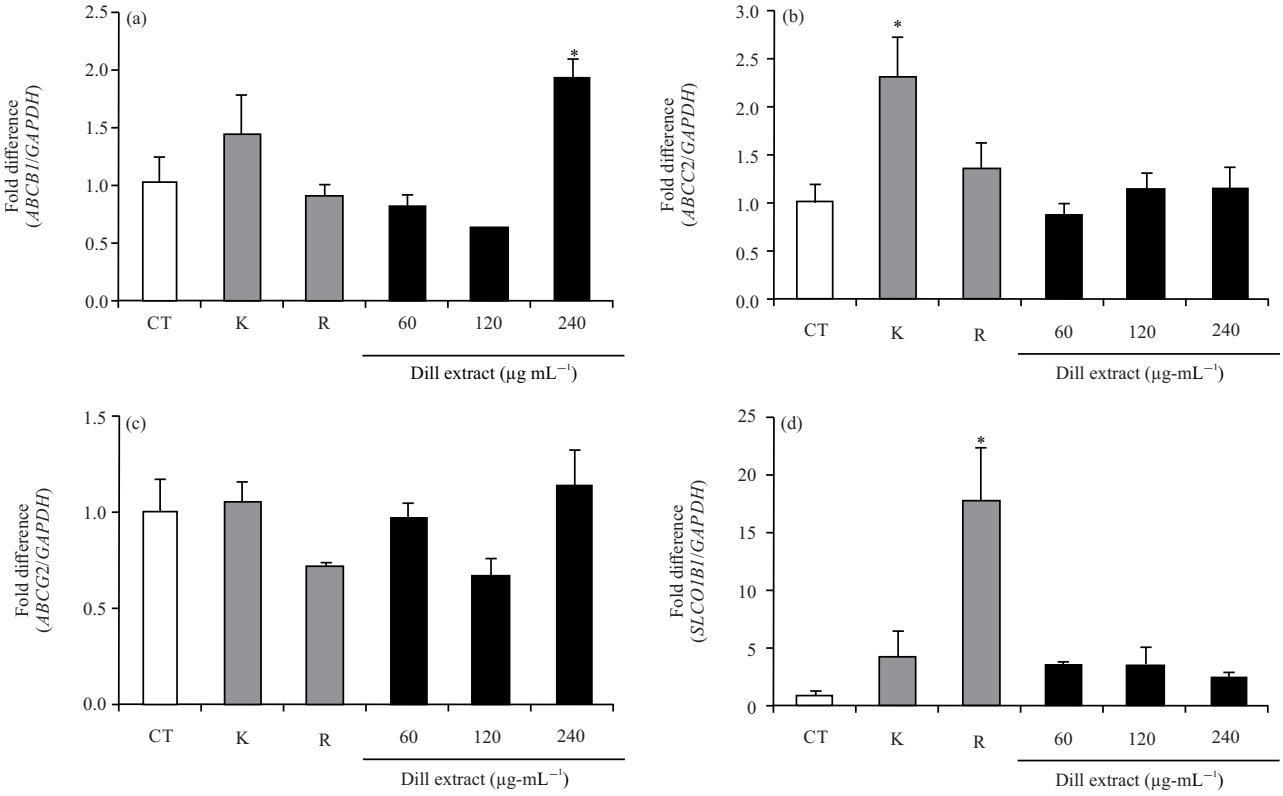

Fig. 4(a-d): Effects of dill extracts on different expression of transporter genes, (a) *ABCB1*, (b)*ABCC2*, (c) *ABCG2* and (d) *SLCO1B1* mRNAs in Caco-2 cells

Figure depicts relative fold differences between the expression of target genes compared to the reference gene *GAPDH* (in Y-axis) and each treatment (in X-axis), CT: 0.2% DMSO, K: 10 mM ketoconazole, R: 20 mM rifampicin, n = 4, *p<0.05 vs. CT

respectively; p<0.05), but not by ketoconazole (0.98-fold) or 60 μg mL^{-1} dill extract (1.62-fold) in Fig. 3e. In addition to up-regulation of *CYP1A2* and *CYP2C19* expression, dill extracts up-regulated *SULT1A1* and *NAT2* expression in Caco-2 cells in a dose-dependent pattern.

Effects of dill extract on mRNA expression of transporter genes in Caco-2 cells: Expression of *ABCB1* mRNA in Caco-2 cells was significantly elevated 1.93-fold by dill extract at 240 μg mL^{-1} by (p<0.05), but not by ketoconazole (1.45-fold), rifampicin (0.90-fold) or 60 and 120 μg mL^{-1} dill extract (0.81 and 0.63-fold, respectively in Fig. 4a. All tested concentrations of dill extract (60, 120 and 240 μg mL^{-1}) did not alter expression of *ABCC2* (0.87, 1.13 and 1.15-fold, respectively in Fig. 4b, *ABCG2* (0.97, 0.68 and 1.14-fold, respectively in Fig. 4c or *SLCO1B1* (3.49, 3.50 and 2.36-fold, respectively) Fig. 4d mRNAs. Ketoconazole induced expression of *ABCC2* by 2.31-fold (p<0.05) (Fig. 4b) and rifampicin induced expression of *SLCO1B1* by 17.79-fold (p<0.05) (Fig. 4d), but ketoconazole did not modify *ABCG2* (1.06-fold; Fig. 4c) or *SLCO1B1* (4.20-fold; Fig. 4d) mRNAs and rifampicin did not modify *ABCC2* (1.36-fold; Fig. 4b) or *ABCG2* (0.72-fold; Fig. 4c) mRN As. These findings suggested that only *ABCB1* expression was meaningfully elevated by dill extract in Caco-2 cells.

DISCUSSION

Though dill possesses several pharmacological activities and has been used as a herbal medicine for a long time, its safety information is limited, particularly concerning potential HDIs. Dill extract (60-240 μg mL^{-1}) did not adversely affect Caco-2 cell viability or AST or ALT levels after 72 hrs treatment. Indeed, the 240 mg mL^{-1} concentration of dill extract demonstrated antioxidant activity via a significant decrease in ROS level in Caco-2 cells. This corresponds to a previous study that showed the antioxidant activity of ethanolic extract of dill leaves (50-100 mg kg^{-1}) through lowered hepatic lipid peroxidation in healthy female Wister rats[15]. Taher *et al.*[16] also

showed that the volatile oil of dill seeds (500-1,000 µL kg^{-1}) decreased AST and ALT activities 24 hrs after injection in rats. In the present study, dill extract did not affect AST and ALT levels and this might be due to various factors including the cell-type selected and the pathological condition of the cells as well as the season and age of harvesting of the dill, the method of extraction and the concentration/period of treatment. Interestingly, ketoconazole significantly reduced ALT level in Caco-2 cells after 72 hrs treatment while several studies have noted that ketoconazole is potentially hepatotoxic and its use could increase AST and ALT levels in rats and humans[17,18]. However, Akhtar *et al.*[19] have noted that ketoconazole's inhibitory and antagonistic effects against CYPs and nuclear receptors could interfere with the metabolism or bioactivation of xenobiotics, resulting in either improvement or impairment of xenobiotic effects. For example, ketoconazole inhibited the activation of the hepatic injury inducer carbon tetrachloride (CCl_4), reducing serum ALT level in CCl_4-induced liver fibrosis in male C57BL/6 mice.

Ketoconazole is well-known as an enzyme inhibitor due to its antagonistic effects against constitutive androstane receptor (CAR), farnesoid X receptor (FXR), glucocorticoid receptor, liver X receptor (LXR) and peroxisome proliferator-activated receptor-gamma, which results in a decrease in phase I enzyme activities[20]. Conversely, ketoconazole possesses aryl hydrocarbon receptor (AHR) and partial pregnane X receptor (PXR) agonistic effects that induce *CYP1A2, UGT1A6* and *ABCC2* expression. Novotna *et al.*[21] reported that induction of *CYP1A2* mRNA expression and activity in primary hepatocytes by ketoconazole was through the AhR regulatory pathway. Additionally, it has been reported that ketoconazole induces *UGT1A6* through the AhR and PXR transcriptional pathway[22,23] and *ABCC2* via PXR activation[24].

Rifampicin has been shown to induce the expression of drug-metabolizing enzymes and transporters by activating the CAR and PXR transcription factors[25]. The present observations support that rifampicin elevates *CYP3A4* expression via PXR and CAR activation[26] and *GSTA1/2* expression through the PXR transcriptional pathway[27]. In contrast, the transcriptional pathway of *SLCO1B1* is complicated and it is difficult to describe how rifampicin extremely induced *SLCO1B1* expression in the present study. According to Schwabedissen *et al.*[28], PXR (rifampicin) and CAR (6-(4-chlorophenyl) imidazo[2,1-*b*][1,3]thiazole-5-carbaldehyde O-(3,4-dichlorobenzene)oxime; CITCO) agonists did not induce *SLCO1B1* mRNA expression in Huh-7 cells but LXRα (TO-901317) and FXR (chenodeoxycholic acid) agonists did. Nevertheless, PXR is mentioned as a target of the SLCO superfamily including *SLCO1A2, SLCO1B1, SLCO1B3* and *SLC22A5*[29], whilst PXR and CAR play a crucial role in transcriptional regulation of SLC proteins[30]. As ketoconazole and rifampicin can induce multiple pharmacologically important genes, they were suitable as positive controls in this study.

Dill interfered with drug and xenobiotic-metabolizing and transport pathways via a significant up-regulation of mRNA expression of the pharmacologically important genes *CYP1A2, CYP2C19*, *SULT1A1, NAT2* and *ABCB1* in Caco-2 cells. Therefore, dill could cause an HDI with co-administrated drugs that are metabolized by these enzymes due to the accelerated rate of biotransformation and consequential lowering of their effectiveness. The drugs at risk of reduced effectiveness with co-administration of dill include: *CYP1A2* substrates such as non-steroidal anti-inflammatory drugs (e.g. naproxen), antidepressants (e.g. clozapine and olanzapine), cardiovascular drugs (e.g. propranolol, verapamil and warfarin), oral contraceptives (e.g. ethinyl estradiol), sedatives (e.g. zolpidem) and bronchodilators (e.g. theophylline); *CYP2C19* substrates such as antidepressants (e.g. amitriptyline and fluoxetine), neurological drugs (e.g. phenytoin), oral contraceptives (e.g. ethinyl estradiol and progesterone), proton pump inhibitors (e.g. omeprazole) and sedatives (e.g. diazepam)[31]; *SULT1A1* substrates such as hormones (e.g. iodothyronines and ethinyl estradiol) and small phenolic drugs[32]; *NAT2* substrates such as antihypertensive drugs (e.g. hydralazine) and antituberculosis agents (e.g. isoniazid)[33] and *ABCB1* substrates such as antiarrhythmic drugs (e.g. digoxin), anticancer drugs (e.g. doxorubicin, paclitaxel, vinblastine and vincristine), antihypertensive drugs (e.g. prazosin and temocapril), antiparasitic agents (e.g. ivermectin), immunosuppressants (cyclosporine, methotrexate and tacrolimus) and steroids (e.g. hydrocortisone)[34]. On the other hand, clinical drugs that require activation by these enzymes, such as flutamide and retinoic acid by *CYP1A2*, clopidogrel by *CYP2C19*[26] and tamoxifen by *SULT1A1*[35], might result in increased biotransformation. Since *CYP2E1* is normally not expressed in the intestine, it was undetectable in Caco-2 cells[7].

Apart from any potential HDIs arising from the use of dill, the up-regulation of clinically important genes could produce other undesirable effects. The CYP1 family that is inducible by the AhR transcriptional factor regulates the bioactivation of procarcinogens to active carcinogens[36]. Likewise, *SULT1A1* and *NAT2* are involved in carcinogenesis, particularly in breast and bladder cancers, respectively[37,38]. Additionally, *ABCB1* participates in the development of

chemoresistance in cancer cells[39]. Consequently, up-regulation of *CYP1A2, SULT1A1, NAT2* and *ABCB1* mRNA expression by dill might promote progression of cancers and/or multidrug resistance.

Though Caco-2 cells are suitable for the study of *in vitro* drug permeability and affinity to transporters, the expression of some metabolizing genes is different to normal human intestinal cells due to dissimilar manifestations of the transcriptional regulator(s). Brück *et al*.[40] and Janssen *et al*.[41] noted that expression of FXR and PXR mRNAs was lower in Caco-2 cells than primary human intestinal cells, while the effect on AhR and CAR mRNAs was controversial. Hence, the responses of Caco-2 cells to dill and the typical modifiers employed in this study might deviate from normal human intestinal cells. Therefore *in vivo* and clinical studies are worth pursuing to confirm these findings and to further unravel the regulatory pathways of dill behind its pharmacological activities.

CONCLUSION

The cell viability and oxidant-antioxidant status of Caco-2 cells were not disturbed by dill extract after 72 hrs incubation. Nevertheless, high concentrations (over 120 μg mL^{-1}) of dill up-regulated expression of clinically important genes, namely *CYP1A2, CYP2C19, SULT1A1, NAT2* and *ABCB1* in Caco-2 cells, could result in HDI events. To avoid this, the risk-benefit of concurrent treatment of clinical drugs with dill at a high dose over extended periods should be considered for individuals.

SIGNIFICANCE STATEMENT

This study discovered that dill (*Anethum graveolens* L.) did not adversely affect Caco-2 cell viability or aspartate and alanine aminotransferase levels, did show potential as an antioxidant by decreasing reactive oxygen species levels and did not interfere with the expression of *CYP2D6, CYP3A4, UGT1A6, NAT1, GSTA1/2, ABCC2, ABCG2* and *SLCO1B1*. However, the consumption of high amounts and/or prolonged use of dill could lead to herb-drug interactions via transcriptional up-regulation of the *CYP1A2, CYP2C19, SULT1A1* and *NAT2* drug-metabolizing enzymes and the *ABCB1* transporter. This study will provide a basis for *in vivo* and clinical studies to confirm these findings and cell studies to unravel the regulatory pathways behind dill's pharmacological activities.

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