RESEARCH ARTICLE

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Cytotoxicity Activity of Graviola Fruit Extract with Carbamazepine and Valproic Acid Show Antagonistic and Indifferent Effects

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Abstract

Objective: Graviola is a tropical fruit with medicinal properties, used for treating various diseases such as inflammation, diabetes, and cancer. Histone deacetylase inhibitors (HDACIs), including carbamazepine (CBZ) and valproic acid (VPA), have been proven strong inhibitors against cancer cell growth. This study investigated the effect of Graviola fruit extract (GFE) on CBZ in healthy rat plasma using high-performance liquid chromatography (HPLC). In addition, the effect of GFE in combination with CBZ and VPA on two human cancer cell lines (PC3 and MCF-7) was explored. **Methods:** The CBZ levels were analyzed using a simple validated HPLC method. The linearity was achieved at a 0.9998 coefficient of determination over a range of 75–5000 ng/mL CBZ. The MTT assay was used to quantify the percentage of viable cells. **Result:** The maximum plasma concentration (C_{max}) and area under the curve (AUC) for CBZ alone were 4,631 ng/mL and 49,225 ng. h/mL, respectively. However, in the presence of GFE, the values reduced significantly to 2,994 ng/mL and 26,587 ng. h/mL, while the p-value was <0.05. The 3-(4,5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT) assay results for VPA showed a weak cytotoxicity activity on PC3 and MCF-7 cell lines. **Conclusion:** A simple and validated HPLC method was used to determine CBZ levels in rats' plasma. The plasma CBZ levels (C_{max}) were significantly reduced in the presence of GFE, indicating the importance of drug-herb interactions. For in vitro studies, two human cancer cell lines, MCF-7 (breast cancer cells) and PC3 (prostate cancer cells), were used to screen the cytotoxicity activity of GFE, CBZ, and VPA. We observed an antagonism effect for GFE and CBZ combination in both cell lines with FIC values > 4. On the contrary, the combination of GFE and VPA showed an additive or indifferent effect.

Keywords: Cancer- Pharmacokinetics- cell lines

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Introduction

Complementary and alternative medicines (CAMs) are a diverse community of health care approaches focusing on health and disease philosophies, distinct from traditional, scientific, and pharmacy systems. Herbal medicine (HM) is termed a "holistic" healing method with a different tactic for the diagnosis and treatment of a broad range of conditions (Al-Mamoori et al., 2018). The pharmacological activity of a drug can alter in the presence of another drug, food, HM, or drink, which is considered a drug interaction. The risk of potential drug interaction could lead to a decrease or increase in the drug's efficacy or adverse reactions (Malhotra et al., 2002). The herb-drug interactions may pose a higher risk than drug-drug interactions because drugs are typically single chemical entities, whereas most HMs contain at least 100 active ingredients (Izzo, 2005). Graviola plant is often used as a medicine for various diseases such as insomnia, inflammation, headache, infections, diabetes, and cancer. Graviola (also known as Annona muricata L.) is a tropical fruit that belongs to the Annonaceae family (Moghadamtousi et al., 2015; Ioannis et al., 2015). The plant is widely grown in tropical and subtropical areas such as South and North America, India, Malaysia, Nigeria, and the rain forests of Africa (Adedeji, 2014;

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Rady et al., 2018; Daddiouaissa et al., 2019). Flavonoids are the main bioactive compounds extracted from various parts of Graviola (Wahab et al., 2018; El-Khashab and Aniss, 2018). These compounds were found to have long-chain fatty acids (C35 or C3) derived from the polyketide pathway with significant anti-tumorous characteristics and toxicity against cancer cells and multi-drug-resistant tumor cell lines (Sun et al., 2014).

Carbamazepine (CBZ) is an antiepileptic drug approved by the FDA that belongs to the dibenzazepine class. It has prophylactic and antimanic effects that are widely considered for treating manic-depressive disorders and first-line treatment for trigeminal neuralgia or tic douloureux (Akbarzadeh et al., 2016; Sohaib and Ezhilarasan, 2020). CBZ is erratically and slowly absorbed after oral administration, and approximately 75% binds to plasma proteins. It is entirely metabolized in the liver, with only about 5% of the drug excreted unchanged without metabolism. The main reaction of CBZ metabolism is its transformation to its active metabolite carbamazepine 10.11-epoxide (CBZ-E), primarily catalyzed by cytochrome p (CYP), CYP3A4 and CYP3A5, while CYP2C8, CYP2E1, CYP2B6, CYP2A6, and CYP1A2 play a minor role (Salemaldajeh et al., 2015; Emad et al., 2018).

Valproic acid (VPA) is a potential neuroprotective drug and short-chain fatty acid that has gained interest as an anticonvulsant for many human epilepsy forms and is commonly used for treating mood disorders (Ximenes et al., 2013).

CBZ and VPA are histone deacetylase inhibitors (HDACIs), which in the past few years have been proven to be potent inducers of cancer cell growth arrest (including drug-resistant subtypes), differentiation, and apoptotic cell death of transformed cells (Akbarzadeh et al., 2016; Qi et al., 2019; Sohaib and Ezhilarasan, 2020)

The aim of this study was investigating the effect of Graviola fruit extract (GFE) on the pharmacokinetic profile of CBZ in rat plasma and screen the cytotoxicity activity of CBZ and VPA in combination with GFE on human cancer cell lines.

Materials and Methods

Chemicals and reagents

Nanopure deionized water, methanol, and acetonitrile (advanced gradient grade) were supplied by Fisher Scientific. Orthophosphoric acid (85%) and triethylamine were purchased from GPR RECTAPUR and TEDIA, respectively. Rats were donated by the animal house from the Applied Science Private University. CBZ and VPA raw materials were generously donated by the Dar Al Dawa pharmaceutical company. Metronidazole benzoate was obtained from Jordan Pharmaceuticals Manufacturing (JPM) Co. (Amman-Jordan). Graviola fruit extract (84%; DER 10:1) was purchased from Fairvital B.V. (Netherland 6370 AC Landgraaf). Human prostate cancer cells (PC3) and human breast cancer cells (MCF-7) were procured from the American Type Culture Collection (ATCC, Rockville, MD, USA). Dulbecco's minimum essential high-glucose medium (DMEM), Roswell Park Memorial Institute (RPMI-1640), dimethylsulfoxide (DMSO), and fetal bovine serum (FBS) from purchased from Capricorn Scientific GmbH (Ebsdorfergrund, Germany). Penicillin, streptomycin, amphotericin B, L-glutamine, and trypsin-ethylenediaminetetraacetic acid (EDTA) were supplied by Euroclone S.p.A. (Via Figino 20/22-0016 Pero MI, Italy). 3-(4,5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT) was purchased from Sigma.

In vivo studies

Preparation of standard solutions and quality control samples

To prepare the internal standard (IS) stock solution, metronidazole benzoate was dissolved in acetonitrile to get a 1 mg/mL concentration. The stock solution was then diluted using acetonitrile to obtain a working solution of 8 µg/mL. The CBZ stock solution of 200 µg/mL was prepared by dissolving it in methanol and stored at -20 ℃ till further usage. Subsequently, different working solutions of CBZ (3, 6, 12, 30, 60, 120, 200 µg/mL) and quality control (QC) (10, 80, and 160 µg/mL) were prepared by diluting the stock solution in methanol. The concentrations for the calibration curve (75, 150, 300, 750, 1,500, 3,000, and 5,000 ng/mL) were obtained by taking 25 µL from each working solution spiked in 1,000 µL of plasma. The concentrations of QC low (QCL), QC mid (QCM), and QC high (QCH) were 250, 2,000, and 4,000 ng/mL, respectively.

Sample preparation

The following protocol was used for sample extraction: First, 100 µL of each test sample (blank, standards, QCL, QCM, QCH, and plasma samples) was transferred to an Eppendorf. Second, 150 µL of the IS (from the working stock prepared above) was added to the test samples, followed by vortexing for 1 min, and then centrifugation at 14000 rpm for 15 min at 25°C. Finally, the clear supernatant was transferred to a flat bottom insert, and 20 µL was injected into the high-performance liquid chromatography (HPLC) column. Analysis were performed in duplicate.

Instrumentation and analytical conditions for HPLC

The analysis was carried out at the instrumental lab at the Pharmaceutical Center of Petra University employing HPLC (Finnigan Surveyor) equipped with a UV-Vis plus detector (ChromQuest software 4.2.34), a solvent delivery systems pump (LC Pump Plus), and an automatic sampling system (autosampler Plus). For product separation, a 150 mm × 4.6 mm (i.d.) reverse-phase C18 (Thermo Electron Corporation, San Jose, CA, USA) BDS column with an average particle size of 5 µm was used. The wavelength was adjusted to 285 nm, and a computer equipped with Windows XP SP3 operating system was used to analyze the chromatographic data. Conditions for chromatographic analysis of CBZ in rat plasma are illustrated in Table 1.

Method validation

A validation method was applied according to EMA guidelines to demonstrate the reliability of the present

HPLC method for determining CBZ concentration in rat plasma (European Medicines Agency, 1922). The process was validated for its sensitivity, accuracy, precision, and linearity.

Accuracy and Precision

The accuracy and precision of the HPLC method were determined at the lower limit of quantitation (LLOQ; 70 ng/mL) on two different days by analyzing three replicates of QC samples, viz. QCL, QCM, and QCH at concentrations of 250, 2,000, and 4,000 ng/mL, respectively.

Sensitivity

The lowest concentration, which could be determined depending on acceptable precision and accuracy criteria, is expressed as the method's sensitivity. The accepted standard of sensitivity is 20% (European Medicines Agency, 1922).

Linearity

The linearity of the method was evaluated by plotting the peak area ratio (PAR) of the drug to IS against the drug concentration (C; 75, 150, 300, 750, 1,500, 3,000, and 5000 ng/mL), taking the mean of seven calibration curves.

Pharmacokinetic study

The protocol used for the pharmacokinetic study was approved by the Ethical Committee of the High Research Council, Faculty of Pharmacy and Medical Science, University of Petra, Amman, Jordan (1A/9/2020). The rats (male and female) used in this study were around 200 g belonging to the Sprague Dawley species. Air conditioners were used to keep the rats at 20–25°C under a photoperiod cycle of "12 h light/12 h dark" daily. In addition, rats were subjected to fasting for 24 h before conducting the experiments.

The rats' tails were uniquely marked for identification and were classified into two groups of seven rats each after being weighed. The first group was considered a control group with 10 mg\kg of CBZ administered through oral gavage, while the second group was assigned a combination of GFE and CBZ. Three days before the experiment, 30 mg/kg of GFE was administered through oral gavage and was given again on the day of the experiment half an hour before CBZ administration. The optical veins of rats were the source of taking blood samples at the following time intervals: 0, 0.25, 0.5, 1, 2.0, 3.5, 4.5, 6.5, 8, and 24 h; the blood samples were placed into EDTA-containing tubes. The plasma was separated by centrifugation for 10 min at 5,000 rpm and stored at –80°C until analysis.

In vitro studies

Cell culture

PC3 cells were cultured in RPMI-1640 medium, while MCF-7 cells were cultured in DMEM medium supplemented with 10% heat-inactivated FBS and 1% penicillin/streptomycin, L-glutamine, and amphotericin-B each. The cells were cultured using 96-well culture plates at a density of 2×10^4 cells per well in a final 100 µL volume and then incubated in a 95% humidified incubator with 5% CO_2 at 37°C until the confluency reached approximately 70%.

Cell treatment

The cells were treated with CBZ and VPA starting at 1 mg/mL and GFE at 2.5 mg/mL and consecutively with their dilutions till 8×. Furthermore, the combinations of GFE with VPA and CBZ were applied, as shown in Figure 1. The cells, treated with various concentrations of the three compounds, were then incubated for 48 h at 37°C in a 5% CO_2 incubator.

Cell Viability Assay (MTT assay)

The MTT assay was used to quantify the percentage of viable cells. A volume of 20 µL of 5 mg/mL MTT solution was added per well, and the samples were then incubated for 4 h at 25°C. The color intensity of the formazan solution, which reflects the number of cells under specific growth conditions, was determined by measuring the absorbance at 570 nm using an ELISA plate reader.

Results

Statistical analyses were performed using Minitab 16.

HPLC method validation used for in vivo studies

Accuracy and Precision

The precision (coefficient of variation, CV%) and accuracy of the method used for analyzing QC samples of CBZ are illustrated in Table 2. Figure 2 shows the chromatograms of QCH and QCM, respectively. According to the accepted percentage in EMA guidelines (European Medicines Agency, 1922) for accuracy (85%–115%) and precision (15%), all the results were within the acceptance criteria.

Sensitivity

The accuracy obtained for LLOQ was between 99 and 107%, which is within the required criteria (Table 2). However, the average accuracy for LLOQ was approximately 104%. The precision (CV%) did not exceed 20% for LLOQ, which again proves the closeness of the measurements (Medicines Agency, 1922).

Linearity

The correlation coefficient (R^2) obtained after plotting the PAR to IS ratio against drug concentration was 0.9998, indicating that our method is linear over the specified concentration range. The regression equation is shown in Figure 3.

Pharmacokinetic parameters

The plasma concentration of CBZ alone and CBZ in combination with GFE as a function of time is shown in Figure 4, and the pharmacokinetic parameters with p-values are shown in Table 3. For CBZ, the area under the curve (AUC) for 0-24 h was 49,225.30 ng. h/mL, and the elimination rate constant (Kel) and half-life were observed to be 0.18 hr-1 and 3.8 h, respectively. While for CBZ with GFE, AUC for 0–24 h was 26,587 ng. h/mL with Kel and half-life of 0.13 hr-1 and 5.2 h, respectively.

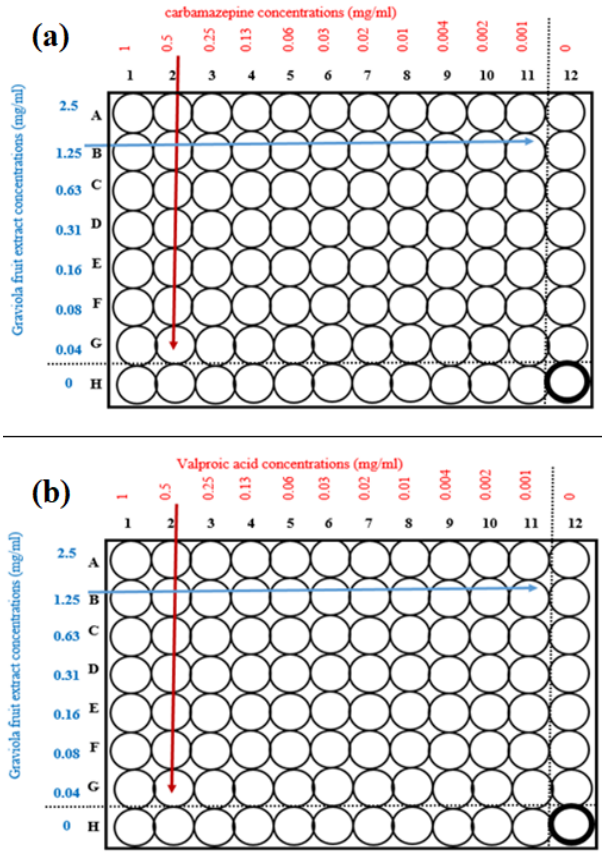

Figure 1. The Combination of (a) Graviola Fruit Extract (GFE) with Carbamazepine (CBZ) and (b) GFE with Valproic Acid (VPA).

Cell culture studies

The killing percentage and the inhibitory concentrations for the three compounds alone (CBZ, VPA, and GFE) were assessed by MTT assay after 48 h of incubation. The inhibition percentage of PC3 and MCF-7 cancer cells treated with each compound or drug is presented in Table 4 and Figure 5. VPA showed weak cytotoxicity activity on both cell lines; however, CBZ and GFE exhibited robust cytotoxicity activity. Furthermore, Table 5 shows the IC_{50} values of the three compounds against the two cell lines. The IC_{50} was calculated using a graph in which growth inhibition was plotted against log drug concentration using GraphPad Prism software.

The MTT assay was also used for the combinations of the compounds (GFE with CBZ and GFE with VPA) to determine the killing percentage, and the fractional inhibitory concentration (FIC) index was used to quantify the interactions between the compounds being tested according to the following equation 1 (Lorian, 2005).

Antagonism between GFE and CBZ on both cell lines could be observed because the FIC values were more than 4 (Table 6). However, the combination of GFE and VPA showed an additive effect or was indifferent.

Table 1. Chromatographic Conditions (Salemaldajeh et al., 2015)

Mobile Phase Composition	55% Water (with 1 mL triethylamine per Liter)
	45% Acetonitrile
	pH=6.00, adjusted with H_3PO_4
Column Type	Hypers Thermo Electron Corporation, BDS C18 (150mm × 4.6 mm, 5µm)
HPLC Condition	
Wavelength	285 nm
Pump Flow Rate	1.0 mL/min
Auto-Sampler Injection Volume	20 µL
Auto-Sampler Temperature	10 °C
Column Oven Temperature	25 °C
Expected Retention Time (Min)	
Carbamazepine	2.8
Metronidazole Benzoate (IS)	3.7

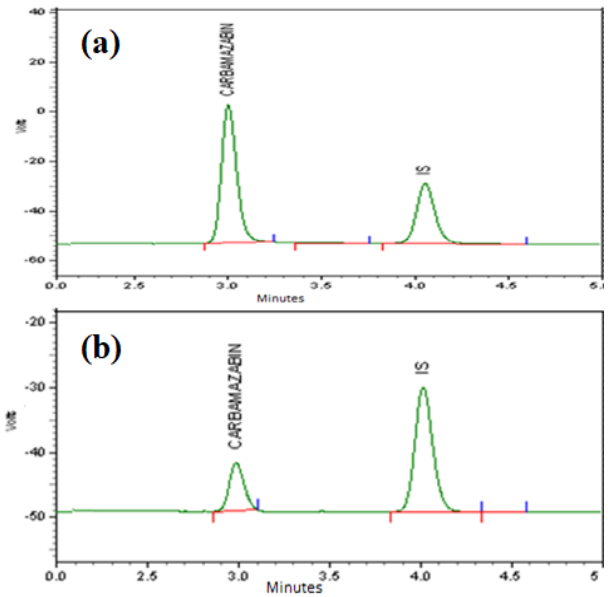

Figure 2. Chromatograms of (a) QC high (QCH) and (b) QC medium (QCM) samples were detected at 285 nm.

Discussion

During the pharmacokinetic analysis, CBZ alone reached its maximum plasma concentration (C_{max}) of

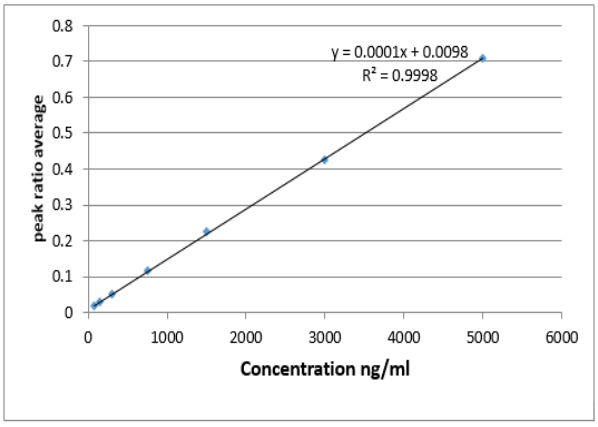

Figure 3. The Linearity Plot for Validation of the HPLC Method Using the Mean of Seven Calibration Curves.

Table 2. Average Accuracy and Precision (%) based on the Mean Concentration of each QC and the Lower Limit of Quantitation (LLOQ) Over Two Different Days

	Day one	Day two				
	Average measured concentration		Mean concentration	STD	CV (%)	Average accuracy (%)
QCL (250 ng/mL)	256.9	258	257.4	5.6	2.2	103
QCM (2000 ng/mL)	2019.4	2043.2	2031.3	36.9	1.8	101.6
QCH (4000 ng/mL)	4076.3	4092.6	4084.5	85.3	2.1	102.1
LLOQ (70 ng/mL)	73.6	71.6	72.6	2.2	3	103.7

QC low, (QCL); QC mid, (QCM); QC high, (QCH)

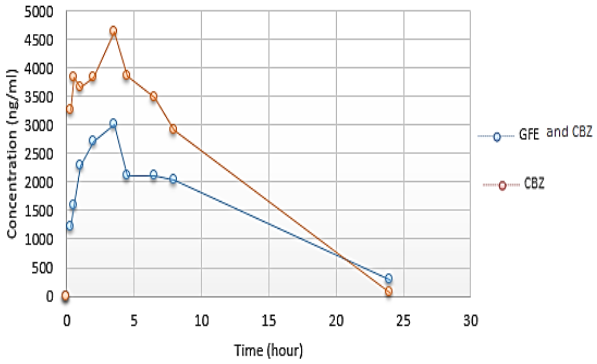

Figure 4. The Concentration of Carbamazepine (CBZ) Alone and CBZ with Graviola Fruit Extract (GFE) in Rat's Plasma as a Function of Time.

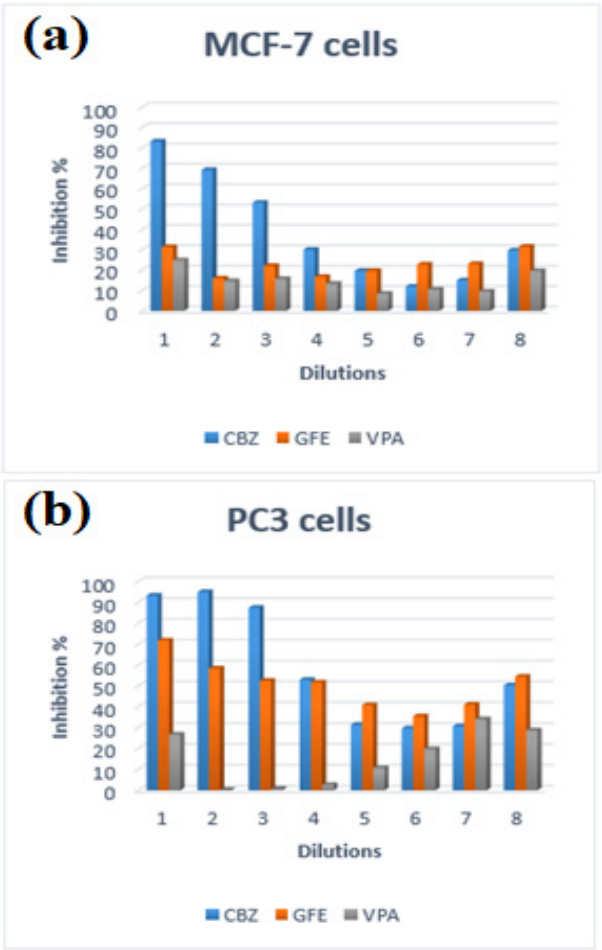

Figure 5. Inhibition Percentage of (a) MCF-7 and (b) PC3 cells in response to eight dilutions of the cytotoxicity compounds (carbamazepine (CBZ), Graviola fruit extract (GFE), and valproic acid (VPA)).

Table 3. Comparison of Major Pharmacokinetic Parameters between CBZ Alone and CBZ with GFE

	CBZ alone	CBZ+GFE	
Parameters	Mean	Mean	P-value
AUC (0–24 h)	49225	26587	0.004
T_{max} (h)	3.5	3.5	0.999
C_{max} (ng/mL)	4631	2994	0
K_{el} (h^{-1})	0.18	0.13	0.75
Half-life (h)	3.78	5.2	0.67

4,631 ng/mL after 3.5 h of administration. Further, CBZ concentration was gradually decreased to achieve its minimum concentration of 65.2 ng/mL after 24 h (Figure 4). Concomitantly, CBZ combined with GFE reached its C_{max} (2,994 ng/mL) after 3.5 h of administration and then gradually decreased to its minimum concentration. The result is consistent with a previous study that determined the levels of CBZ in rat

Table 4. Inhibition Percentage of MCF-7 and PC3 Cells in Response to Different Concentrations of GFE, CBZ, and VPA. The CVs were within 15%.

	Concentration (mg/mL)	Inhibition (%) of MCF-7 Cells	Inhibition (%) of PC3 Cells
GFE	2.5	31.39	71.83
	1.25	16	58.42
	0.63	22.14	52.54
	0.31	16.78	51.73
	0.16	19.69	40.9
	0.08	22.87	35.52
	0.04	23.13	41.09
CBZ	1	83.3	93.36
	0.5	69.31	95.1
	0.25	53.18	87.52
	0.13	30.12	52.99
	0.06	19.68	31.38
	0.03	11.94	29.69
VPA	1	24.94	26.71
	0.5	14.68	0.24
	0.25	15.72	0.74
	0.13	13.39	2.62
	0.06	8.5	10.68
	0.03	10.57	19.83

Table 5. IC_{50} Values of GFE, CBZ, and VPA on MCF-7 and PC3 Cell Lines were Expressed as mean ± SD.

Compounds	IC_{50} µg/mL on MCF-7	IC_{50} µg/mL on PC3
GFE	>2500	470 ± 38
CBZ	250 ± 44	130 ± 27
VPA	>1000	>1000
Doxorubicin	0.64 ± 0.04	0.62 ± 0.06
Cisplatin	14.6 ± 2.4	8.6 ± 1.5

plasma with graviola juice (Salemaldajeh et al., 2015); C_{max} (4,130 ng/mL) for CBZ alone was attained after 1 h of administration, followed by its gradual decline to reach minimum concentration. However, when combined with graviola juice, CBZ achieved its C_{max} (2,886 ng/mL) after 30 min of administration and gradually decreased to reach its minimum concentration. Therefore, comparing CBZ alone and CBZ with GFE, the plasma levels of CBZ were reduced in the presence of GFE (Figure 4). Additionally, C_{max} (2,994.27 ng/mL) and AUC (26,587.114 ng. hr/mL) also decreased significantly. The probable reason for this could be the induction of cytochrome P450 3A4 (CYP3A4) in the presence of GFE. Yang et al., (2015) have reported that flavonoids are one of the main active ingredients of Graviola fruit with antioxidant efficacy, and the metabolites of flavonoids have been proven to induce polyglycoprotein expression while activating drug-metabolizing enzymes such as CYP3A4. Moreover, Salemaldajeh et al., (2015) suggested that any modulation in CYP3A4 could change the pharmacokinetics of its substrate CBZ. In the future, further elaboration is required for the same.

Our study aims to investigate the cytotoxicity activity of GFE in combination with HDACIs, CBZ and VPA. Therefore, their interaction should be critically examined to achieve the best outcomes, such as providing more significant benefits to patients and avoiding adverse side effects. Synergistic, additive, and antagonistic effects may be observed during the interaction between herbal products and conventional drugs or biochemical compounds (Pezzani et al., 2019; Hackman et al., 2020). El-Khashab and Aniss (2018) reported that Caco-2 cells treated with Graviola and/or Cranberry showed significantly increased malondialdehyde (MDA) levels and significantly decreased levels of reduced glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) compared to normal Caco-2 cells. They suggested that HDACIs generate reactive oxygen species (ROS) in leukemia cells based on a previous study (Martirosyan et al., 2006). ROS can influence mitochondrial function, mediating the elevation of intracellular Ca^{2+} and leading to the activation of caspase cascade. In addition, oxidative stress reduces Na+ channel availability, which could explain the drastic increase in MDA levels in Caco-2 cells treated with Graviola and/or Cranberry. Furthermore, Martirosyan et al., (2006) investigated the functions of a histone deacetylase inhibitor NSC3852 (5-nitroso-8-quinolinol), linking ROS formation to cell differentiation and apoptosis in MCF-7 human mammary tumor cells. In another study, Yiallouris et al., (2018) demonstrated the antiproliferative and cytotoxicity effects of Graviola leaf extract in various cancer cell lines with limited toxicity effects on non-transformed cells. Additionally, they revealed that known inhibitors of Na/K and sarcoplasmic reticulum ATPase pumps could also promote cell death in several cancer cell lines. This could explain the antagonistic effects of GFE on CBZ in our study when used in combination.

Table 6. FIC Values of the Tested Compounds were Expressed as mean ± SD.

Compounds	FIC value on MCF-7 cells	FIC value on PC3 cells
GFE and CBZ	4.25 ± 0.40	4.33 ± 0.34
GFE and VPA	2.74 ± 0.19	2.00 ± 0.12

This manuscript followed the MIRDA standard reporting recommendation (Ahmad et al., 2022)

Abbreviations

HDACIs, histone deacetylase inhibitors; CBZ, carbamazepine; VPA, valproic acid; GFE, Graviola fruit extract; HPLC, high-performance liquid chromatography; C_{max}; maximum plasma concentration; AUC, area under the curve; MTT, 3-(4,5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide; FIC, fractional inhibitory concentration; CAMs, complementary and alternative medicines; HM, herbal medicine; ATCC, American Type Culture Collection; DMEM, Dulbecco's minimum essential high-glucose medium; RPMI, Roswell Park Memorial Institute; DMSO, dimethylsulfoxide; FBS, fetal bovine serum; EDTA, ethylenediaminetetraacetic acid; LLOQ; lower limit of quantitation; CV%; coefficient of variation; PAR, peak area ratio; IC50, half-maximal inhibitory concentration; CYP, cytochrome P450; GSH, reduced glutathione; SOD, superoxide dismutase; CAT, catalase; MDA, malondialdehyde; ROS, reactive oxygen species

Author Contribution Statement

All authors contributed in results' interpretation and discussion. Analysis: Asma Abdelqader Abu Soukhon; Supervision, conceptual idea, manuscript revision: Luay Abu-Qatouseh; participating in clinical part, Methodology: Kenza Mansoor; Preclinical study: Feras Darwish El-hajji; Statistical analysis: Mohammad Al-Najjar; first draft writing: Shady Awwad; Statistical analysis: Riad Awad; Verifying, revising, writing: Khaled W. Omari; Supervision, conceptual idea, manuscript revision: Eyad Mallah. The authors would like to acknowledge the efforts of Mr. Salem Alshawabkeh from the Applied Science Private University.

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Ethical approval

The research aspects were approved by the Ethical Committee of the High Research Council, Faculty of Pharmacy and Medical Science, University of Petra, Amman, Jordan (1A/9/2020).

Conflict of Interest

The authors declare that there are no conflicts of interest.

References

Ahmad R, Riaz M, Aldholmi M, et al (2022). A. Development of a Critical Appraisal Tool (AIMRDA) for the Peer-Review of Studies Assessing the Anticancer Activity of Natural Products: A Step towards Reproducibility. *Asian Pac J Cancer Prev*, **22**, 3735-40.

Ajayi AA, Peter-Albert CF, Adedeji OM (2014). Modification of cell wall degrading enzymes from soursop (Annona muricata) fruit deterioration for improved commercial development of clarified soursop juice (a review). *Med Aromat Plants*, **4**.

Akbarzadeh L, Zanjani TM, Sabetkasaei M (2016). Comparison of anticancer effects of carbamazepine and valproic acid. Iran. *Red Crescent Med J*, **18**.

Al-Mamoori F, Mansoor K, Mallah E, Elhajji F (2018). Pharmacodynamic and pharmacokinetic interaction of warfarin in the presence of some commonly used complementary and alternative medicines (CAMs) in rat plasma by using HPLC. *Int J Pharm Sci Rev Res*, **53**, 77–87.

Daddiouaissa D, Amid A, Kabbashi NA, et al (2019). Antiproliferative activity of ionic liquid-graviola fruit extract against human breast cancer (MCF-7) cell lines using flow cytometry techniques. *J Ethnopharmacol*, **236**, 466–73.

El-Khashab IH, Aniss NND (2018). Anti-tumor effect of graviola and/or cranberry as a natural antiproliferative agent on colorectal cell lines (caco-2). *Eur J Biomed Pharm Sci*, **5**, 496-508.

Emad R, Mallah E, Shahin F, et al (2018). Determination of carbamazepine in rat plasma by using high performance liquid chromatography (HPLC) in presence of some traditional beverages (tamarind, mango, sugarcane, red bull) and its pharmacokinetic applications. *Int J Bio Pharm Allied Sci*, **7**, 1908–21.

European Medicines Agency (1922). Guideline on bioanalytical method validation. https://www.ema.europa.eu/en/ich-m10-bioanalytical-method-validation.

Hackman GL, Collins M, Lu X, et al (2020). Predicting and quantifying antagonistic effects of natural compounds given with chemotherapeutic agents: applications for high-throughput screening. *Cancers*, **12**, 3714.

Ioannis P, Anastasis S, Andreas Y (2015). Graviola: A systematic review on its anticancer properties. *Am J Cancer Prev*, **3**, 128–31.

Izzo AA (2005). Herb-drug interactions: an overview of the clinical evidence. *Fundam Clin Pharmacol*, **19**, 1–16.

Lorian V (2005). Antibiotics in laboratory medicine, ed. Lippincott Williams & Wilkins.

Malhotra S, Dixit RK, Garg SK (2002). Effect of an acidic beverage (Coca-Cola) on the pharmacokinetics of carbamazepine in healthy volunteers. *Methods Find Exp Clin Pharmacol*, **24**, 31–6.

Martirosyan A, Leonard S, Shi X (2006). Actions of a histone deacetylase inhibitor NSC3852 (5-nitroso-8-quinolinol) link reactive oxygen species to cell differentiation and apoptosis in MCF-7 human mammary tumor cells. *J Pharmacol Exp Ther*, **317**, 546–52.

Moghadamtousi SZ, Fadaeinasab M, Nikzad S, et al (2015). Annona muricata (Annonaceae): a review of its traditional uses, isolated acetogenins and biological activities. *Int J Mol Sci*, **16**, 15625–58.

Pezzani R, Salehi B, Vitalini S, et al (2019). Synergistic effects of plant derivatives and conventional chemotherapeutic agents: An update on the cancer perspective. *Medicina*, **55**, 110.

Qi G, Lu G, Yu J, Zhao Y (2019). Up-regulation of TIF1γ by valproic acid inhibits the epithelial mesenchymal transition in prostate carcinoma through TGF-β/Smad signaling pathway. *Eur J Pharmacol*, **860**, 172551.

Rady I, Bloch MB, Chamcheu RCN, et al (2018). Anticancer properties of graviola (Annona muricata): a comprehensive mechanistic review. *Oxid Med Cell Longev*, **2018**.

Salemaldajeh M, Mallah E, Riad A, et al (2015). Determination of carbamazepine in rat plasma in the presence of licorice juice by using HPLC and its pharmacokinetic applications. *J Chem Pharmcol Res*, **7**, 116–26.

Sohaib M, Ezhilarasan D (2020). Carbamazepine, a histone deacetylase inhibitor induces apoptosis in human colon adenocarcinoma cell line HT-29. *J Gastrointest Cancer*, **51**, 564–70.

Sun S, Liu J, Kadouh H, Sun X , Zhou K (2014). Three new anti-proliferative Annonaceous acetogenins with mono-tetrahydrofuran ring from graviola fruit (Annona muricata). *Bioorganic Med Chem Lett*, **24**, 2773–6.

Wahab SMA, Jantan I, Haque MA, Arshad L (2018). Exploring the leaves of Annona muricata L. as a source of potential anti-inflammatory and anticancer agents. *Front Pharmacol*, **9**.

Ximenes JCM, de Oliveira Gonçalves D, Siqueira RMP, et al (2013). Valproic acid: An anticonvulsant drug with potent antinociceptive and anti-inflammatory properties. *Naunyn Schmiedebergs Arch Pharmacol*, **386**, 575–87.

Yang C, Gundala SR, Mukkavilli R, et al (2015). Synergistic interactions among flavonoids and acetogenins in graviola (Annona muricata) leaves confer protection against prostate cancer. *Carcinogenesis*, **36**, 656–65.

Yiallouris A, Patrikios I, Johnson EO, et al (2018). Annonacin promotes selective cancer cell death via NKA-dependent and SERCA-dependent pathways. *Cell Death Dis*, **9**, 1–13.