P-ISSN 2586-9000
E-ISSN 2586-9027
Homepage : https://tci-thaijo.org/index.php/SciTechAsia
Science & Technology Asia
Vol.27 No.1 January - March 2022
Page: [57-66]
Original research article

Pharmacokinetics of Gallic Acid Following Single-Dose Administration of Triphala Formulation in Healthy Thai Subjects

Piracha Jumpa-ngern[1], Somboon Kietinun[1], Kusuma Sriyakul[1], Parunkul Tungsukruthai[1], Wiratchanee Mahavorasirikul[2], Kesara Na-Bangchang[1,2,3,*]

[1]*Chulabhorn International College of Medicine, Thammasat University, Pathum Thani 12120, Thailand*
[2]*Drug Discovery and Development Center, Office of Advanced Science and Technology, Thammasat University, Pathum Thani 12120, Thailand*
[3]*Center of Excellence in Pharmacology and Molecular Biology of Malaria and Cholangiocarcinoma, Thammasat University, Pathum Thani 12120, Thailand*

Received 4 March 2021; Received in revised form 20 May 2021
Accepted 22 July 2021; Available online 28 March 2022

ABSTRACT

Triphala is commonly used in Thai traditional medicine for the treatment of various health conditions. The present study investigated the pharmacokinetics of a single oral dose (2,000 and 4,000 mg) of an aqueous extract Triphala formulation in 32 healthy Thai subject. Venous blood samples were collected from each subject at specific time points and concentrations of gallic acid, the major active component of the formulation, were measured using LC-MS/MS. Plasma concentration-time profiles were analyzed by the model-independent approach. Triphala formulations at both dose levels were well tolerated in all subjects. The pharmacokinetics of gallic acid was dose-independent. The median value for maximum concentration (C_{max}), area under the curve from zero time to 48 hours after dosing (AUC_{0-48hr}) and total AUC ($AUC_{0-\infty}$) following 4,000 mg dose of Triphala were about twice that of the 2,000 mg dose (C_{max}: 70.81 *vs.* 41.84 ng/mL, AUC_{0-48hr}: 150.12 *vs.* 88.46 ng.hr/ml, and $AUC_{0-\infty}$: 151.87 *vs.* 91.78 ng·hr/ml, respectively). The median time to maximum concentration (t_{max}) and the terminal elimination half-life ($t_{1/2z}$) was 1 hour for both dose levels. The study provides preliminary information on the pharmacokinetics of gallic acid in humans for further dose optimization of Triphala formulation in treating various health conditions.

Keywords: Gallic acid; Pharmacokinetics; *Phyllanthus emblica*; *Terminalia chebula*; *Terminalia bellerica*; Triphala

***Corresponding author:** kesaratmu@yahoo.com

1. Introduction

Triphala is one of the most commonly used Ayurvedic and Thai traditional medicine formulations [1-2]. The formulation consists of the dried fruit of three herbal plants in equal proportions, these being *Phyllanthus emblica, Terminalia chebula* and *Terminalia bellerica*. Gallic acid is one of the major phytoconstituents of Triphala [3-4]. It is also found in other natural products such as black and green tea, grapes, wines, strawberries, and bananas [5]. A wide range of health benefits from gallic acid has been reported, including anticancer, anti -inflammatory, antidiabetic and cardioprotective properties [6]. Previous studies have demonstrated that Triphala is potentially effective for appetite stimulation, as well as for providing antioxidant, antibacterial, antimutagenic, anti-inflammatory, antineoplastic, and chemoprotective effects [7-9]. In Thailand, Triphala is a widely prescribed drug for balancing the body elements in summer and is usually prescribed with other herbal medicines to relieve a cough, sore throat and constipation . It shows significant immunostimulatory effects on the human cellular immune response, particularly cytotoxic T cells and natural killer (NK) cells [10]. Moreover, it has been shown to significantly reduce high-density lipoprotein cholesterol (HDL-C) and blood sugar levels [11]. Acute and subacute toxicity testing in animals at doses of 1,000, 3,000 and 5,000 mg/kg body weight revealed the absence of toxic signs and symptoms in all animals [12]. This finding supported the safety profile in a clinical study in healthy volunteers, following a daily dose of the ethanol extract of 1,050 mg for 14 days, and a daily dose of the aqueous extract of 2,500 mg for 28 days [10-11]. The potential clinical uses and satisfactory safety profile of Triphala signify the value of investigating its pharmacokinetics. The aim of the present study was to preliminarily investigate the pharmacokinetic characteristics of Triphala formulation in healthy Thai subjects for further dose optimization in various disease treatments or health conditions.

2. Materials and Methods

2.1 Chemicals

The standardized Triphala formulation capsules were prepared by Pharmaceutical Chemistry and Natural Products, Faculty of Pharmacy, Mahasarakham University, Thailand. The standard powder of gallic acid and apigenin were purchased from Sigma-Aldrich (St. Louis, MO, USA). Water, acetonitrile, methanol, and formic acid were purchased from Fisher Scientific (Waltham, MA, USA).

2.2 Preparation of the standardized triphala formulation capsules

Plant materials, i.e., fresh fruits of *Terminalia chebula*, *Terminalia bellerica*, and *Phyllanthus emblica*, were collected from Doi Saket district, Chiang Mai Province, Thailand. The voucher specimens of *Terminalia chebula* (MSU.PH-COM-TC01), *Terminalia bellerica* (MSU.PH-COM-TB01), and *Phyllanthus emblica* (MSU.PH-EUP-PE01) were deposited at the Faculty of Pharmacy, Mahasarakham University, Maha Sarakham, Thailand. The plant materials were rinsed thoroughly with tap water to remove extraneous contaminants and seeds of each fruit, then hot air oven-dried at 60°C for 48 hours. All materials were ground into powder using a blender and sieved through no.14 mesh. The powdered plant material (15 g for each plant) was extracted by boiling in 1,000 ml of distilled water for 1 hour. The extracted solvent was separated and filtered through Whatman no. 1 filter paper. The extracts were evaporated using the freeze-drying technique and irradiated with gamma-rays for the elimination of microbial contamination.

The Triphala formulation capsules were prepared by the Pharmaceutical Chemistry and Natural Products Research Units, Faculty of Pharmacy, Mahasarakham

University, Thailand, and characterized according to the USP Standard (weight variation, content uniformity, disintegration, and dissolution), as well as hardness, thickness, and friability. Each 500 mg capsule of Triphala (water extract) contained 67 mg of gallic acid.

2.3 Study subjects

The study protocol was approved by the Human Ethics Committee of the Faculty of Medicine, Thammasat University, Thailand. Thirty-two healthy Thai subjects (16 males and 16 females), aged between 20 and 45 years, with body mass indexes (BMI) between 20 and 25 kg *per* m^2 participated in the study. Inclusion criteria were: (i) absence of underlying or history of significant clinical conditions or diseases, particularly liver, kidney, and cardiovascular diseases, peripheral neuropathy or allergic conditions, (ii) non-lactating and non-pregnant (females), (iii) no current use of medicines including herbal medicines, (iv) no previous consumption of food containing components of Triphala formulation (vegetables and fruits containing gallic acid such as soybeans, bean sprouts, eggplants, olives, cauliflower, apples, grapes, bananas, blueberries, cherries, and pomegranates) within two weeks, and (v) no history of alcoholism, cigarette smoking or unlikely to refrain from excessive alcohol consumption or smoking during the study period. A subject was excluded from the study if he/she had a significantly abnormal routine laboratory test (blood chemistry, hematology, or urinalysis) during screening. Written informed consent for participation was obtained from each volunteer before study initiation.

2.4 Drug administration

The subjects were divided into two groups (8 males and 8 females in each group) and received a single oral dose of 2,000 mg (group 1) or 4,000 mg (group 2) of Triphala formulation. Drug administration was done under supervision, taken with 150 ml of water. No food was allowed until 2 hours after Triphala administration. Subjects were admitted to a ward at the Thai Traditional and Alternative Medicine Hospital, Bangkok, one day before and on the day of the pharmacokinetic study. No other medications, herbal drugs, smoking, and alcohol consumption were allowed throughout the study period. Food without vegetables or fruits containing gallic acid was provided to each subject during the investigation period. Adverse events that occurred after drug administration were assessed on the basis of non-suggestive questioning by the study investigators.

2.5 Collection of blood samples

Venous blood samples were collected into sterile heparinized plastic tubes taken from the forearm through an inserted catheter before dosing and at 0.5, 1, 2, 4, 6, 8, 10, 12, 16 and 24 hours, then by venipuncture at 48 hours after drug administration. Blood samples were centrifuged at 3,000 rpm for 10 minutes to separate the plasma, then stored at -80 ^{o}C until analysis.

2.6 Determination of gallic acid in plasma samples

Plasma samples (200 µl) were spiked with internal standard (10 µl), and the resultant mixture was extracted with a mixture of 2% formic acid in acetonitrile (1 ml). After centrifugation at 12,000 *rpm* for 10 minutes, the supernatant layer was evaporated to dryness under a nitrogen stream. The residue was dissolved in 200 µl of the mobile phase, and an aliquot of 5 µl was injected into the LC-MS/MS system.

Gallic acid concentrations in plasma samples were analyzed by LC-MS/MS using Agilent 1260 Separation Module (Agilent Technologies, CA, US) Solvent and Sample Delivery, an AB SCIEX QTRAP® 5500 mass spectrometer (AB Sciex, Foster City, CA, USA) in Scheduled Multiple Reactions Monitoring (MRM) mode. The

chromatographic separation was performed on a ZORBAX SB-C18 (4.6 x 50 mm, 3.5 µm particle size) (Agilent Technologies, CA, USA). The mobile phase consisted of a mixture of (A) water containing 0.1% formic acid and (B) 100% acetonitrile with gradient elution 0 min (60%:40%), 5 min (20%:80%) and 6.5 min (60%:40%), at a flow-rate of 0.3 ml/min. The mass spectrometric analysis was performed in the negative electrospray ionization (ESI) mode. The following sets of source and gas parameters were used: ion spray voltage (IS) -4,500 V, source temperature TEM 500 °C, curtain gas (CUR) 30, ion source gas 1 (GS1) and ion source gas 2 (GS2) 50, and collision gas (CDA) medium. Optimal mass parameters including declustering potential (DP), entrance potential (EP), collision energy (CE), and cell exit potential (CXP) were 50, 6, 17 and 11 psi for gallic acid, and 67, 6, 42, and 8 psi for IS, respectively. The mass transitions of the precursors to the product ions were 168.9→125.1 for gallic acid and 269→117 for IS. Analysis methods met the acceptance criteria of the US FDA [13].

Plasma concentration of gallic acid was quantified using the ratio of the peak area of gallic acid to that of the internal standard. The LC-MS/MS method for the analysis of gallic acid in Triphala formulation was calibrated using 200 µl of plasma samples with various concentrations of gallic acid (2, 5, 10, 20, 50, 100, and 200 ng/ml). The internal standard was used at a concentration of 3 ng/ml. The correlation coefficient for all calibration curves (*r*) was higher than 0.999. Quality control (QC) samples for gallic acid were prepared in plasma using a stock solution separated from that used to prepare the calibration curves, at the concentrations of 25 (low), 75 (medium), and 150 ng/ml (high) of gallic acid, and 30 ng/ml internal standard. The intra-day assay coefficients of variation (CV) of gallic acid in plasma samples varied between 4.09% and 10.31%. The inter-day assay CV varied between 2.79% and 7.16%. For the intra-day assay accuracy, the mean deviation from the theoretical values (MDV) varied between -3.57% and -12.29%. The inter-day assay MDV varied between -8.52% and -10.95%. The mean recoveries for gallic acid at 25, 75 and 150 ng/ml were 89.1, 92.3 and 91.5%, respectively. The results reflect essentially high recovery for gallic acid from the spiked plasma. The lower limit of quantification (LLOQ) for gallic acid was accepted as 2 ng/ml.

2.7 Pharmacokinetic and statistical analysis

The plasma concentration-time profile of gallic acid for each subject was plotted, and the pharmacokinetic analysis was performed using a model-independent analysis approach. Maximum concentration (C_{max}) and time to maximum concentration (t_{max}) were obtained directly from the concentration-time profile of each individual. The area under the curve from zero time to 48 hours of dosing ($AUC_{0\text{-}48hr}$) and total AUC ($AUC_{0\text{-}\infty}$) were estimated using the linear trapezoidal rule for ascending data points and the log-linear trapezoidal rule for descending data points . The terminal elimination half-life ($t_{1/2z}$) was calculated as $0.693/\lambda_z$. All data are presented as median (interquartile range) values. Comparison of the pharmacokinetic parameters obtained from subjects following administration of the two-dose regimens of Triphala formulation was performed using the Mann-Whitney U test at a statistical significance level of $\alpha = 0.05$.

3. Results

3.1 Subject characteristics

Thirty-two healthy Thai subjects (16 males and 16 females), aged between 22 and 37 years, weighing 45 to 77 kg, with BMI between 20 and 25 kg *per* m^2, were included in the study. The flowchart of the study procedures is shown in Fig. 1. All were healthy as verified by laboratory results, physical examination, and vital sign

monitoring. There were no complications in any subjects. The normal baseline demographic characteristics (age, body weight, height, and BMI) and laboratory investigations (hematology, biochemistry, and urinalysis) of the groups were comparable to each other (Table 1).

3.2 Assessment of safety

Triphala formulation at the dose levels of 2,000 and 4,000 mg were well-tolerated. No adverse drug reactions were observed.

3.3 Pharmacokinetics of gallic acid

Plasma concentration-time profiles (median values) of gallic acid following the administration of the two dose regimen of Triphala formulation, in both groups of subjects, are shown in Fig. 2.

The pharmacokinetic parameters of gallic acid in the Triphala formulation calculated based on a model- independent approach are summarized in Table 2. Following oral administration of Triphala at both dose levels, gallic acid was rapidly absorbed with a median t_{max} of 1 hr. Plasma concentrations in most cases were maintained above the limit of quantification for 10 hours. The median C_{max}, $AUC_{0\text{-}48hr}$ and $AUC_{0\text{-}\infty}$ were found to be about twice as much in the group receiving 4,000 mg compared with the 2,000 mg dose group C_{max}: 70.81 (46.26-90.88) *vs.* 41. 84 (32. 29- 59. 54) ng/ ml; $AUC_{0\text{-}48hr}$: 150. 12 (113. 90- 177. 23) *vs.* 88. 46 (77. 51-122. 82) ng.hr/ml; $AUC_{0\text{-}\infty}$: 151.87 (118. 53-178. 27) *vs.* 91.78 (79.96-126.13) ng.hr/ml, respectively. The terminal phase elimination half- life ($t_{1/2z}$) was 1 hour for both dose levels.

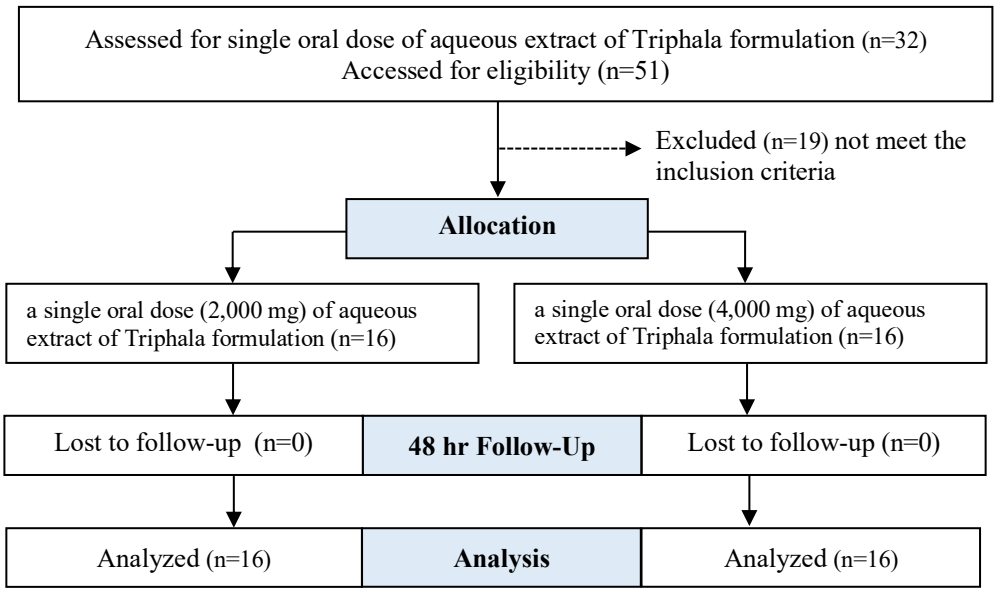

Fig. 1. Flowchart of study participants.

Table 1. Baseline characteristics of healthy subjects in group 1 (2,000 mg Triphala formulation) and group 2 (4,000 mg Triphala formulation). Data are presented as median (interquartile range) values.

Demographic data	Group 1	Group 2
Age (years)	26.5 (24.00-30.75)	25.5 (23.25-31.00)
Weight (kg)	62.5 (53.50-66.75)	58.0 (54.63-67.50)
Height (cm)	166.5 (160.00-172.00)	167.0 (159.75-172.00)
BMI (kg/m^2)	21.8 (20.94-23.14)	21.1 (20.20-24.54)
Hematology		
White blood cell ($x10^3/\mu l$)	6.03 (5.84-6.95)	6.00 (4.95-6.25)
Neutrophil (%)	53.30 (43.10-58.43)	51.85 (46.03-59.58)
Lymphocyte (%)	39.50 (35.15-43.93)	37.95 (32.03-43.30)
Monocyte (%)	3.80 (3.33-4.78)	4.20 (3.43-4.80)
Eosinophil (%)	2.55 (1.43-4.68)	2.75 (2.00-4.68)
Basophil (%)	0.35 (0.23-0.40)	0.40 (0.30-0.60)
Red blood cells ($x10^6/\mu l$)	5.01 (4.68-5.58)	4.93 (4.53-5.45)
Hemoglobin (gm/dl)	14.65 (12.38-15.58)	14.10 (11.95-15.45)
Hematocrit (%)	42.30 (36.38-45.98)	41.00 (35.73-45.88)
Platelets ($x\ 10^3/\mu l$)	256.50 (232.50-291.50)	245.00 (207.75-283.75)
Biochemistry		
Glucose (mg/dl)	80.50 (78.00-87.25)	84.00 (81.00-87.00)
Blood urea nitrogen (mg/dl)	11.80 (9.70-12.60)	11.20 (9.30-13.10)
Creatinine (mg/dl)	0.79 (0.67-1.01)	0.81 (0.66-0.93)
HDL-cholesterol (mg/dl)	63.50 (56.00-70.50)	63.00 (56.00-71.75)
Total cholesterol (mg/dl)	196.00 (170.00-207.00)	185.50 (165.75-208.00)
LDL-cholesterol (mg/dl)	109.00 (103.50-124.00)	114.00 (90.00-130.00)
Triglyceride (mg/dl)	67.50 (41.25-108.50)	59.00 (43.25-64.25)
Total protein (g/dl)	8.20 (7.83-8.30)	7.75 (7.60-8.28)
Albumin (g/dl)	4.20 (3.95-4.38)	4.00 (3.90-4.18)
Globulin (g/dl)	3.95 (3.65-4.28)	3.85 (3.70-4.18)
Total bilirubin (mg/dl)	0.55 (0.43-0.70)	0.70 (0.40-0.95)
Conjugated bilirubin (mg/dl)	0.10 (0.10-0.20)	0.15 (0.10-0.20)
Aspartate aminotransferase (U/l)	14.00 (12.00-17.25)	16.50 (10.75-23.00)
Alanine aminotransferase (U/l)	25.50 (16.75-34.25)	26.50 (19.25-41.00)
Alkaline Phosphatase (U/l)	63.50 (49.25-73.00)	58.00 (45.00-70.50)

Table 2. Pharmacokinetic parameters of gallic acid following a single oral dose of 2,000 mg (group 1, n=16) and 4,000 mg (group 2, n=16) Triphala formulation in Thai healthy subjects. Data are presented as median (interquartile range) values.

Pharmacokinetic parameters	Triphala (2,000 mg)	Triphala (4,000 mg)	*P-value* [a]
C_{max} (ng/ml)	41.84 (32.29-59.54)	70.81 (46.26-90.88)	0.004*
t_{max} (hr)	1 (1.0-1.0)	1 (1.0-1.0)	0.184
AUC_{0-48} (ng.hr/ml)	88.46 (77.51-122.82)	150.12 (113.90-177.23)	0.001*
$AUC_{0-\infty}$ (ng.hr/ml)	91.78 (79.96-126.13)	151.87 (118.53-178.27)	0.002*
$t_{½z}$ (hr)	1.03 (0.85-1.72)	1.05 (0.87-1.21)	0.515
Vz/F (l/kg)	75.85 (54.51-105.01)	88.29 (59.39-122.44)	0.367
CL/F (l/h/kg)	43.29 (31.72-61.40)	63.77 (44.24-87.25)	0.029*

[a] *Mann-Whitney U Test* * *Statistically significantly different between group 1 and group 2*

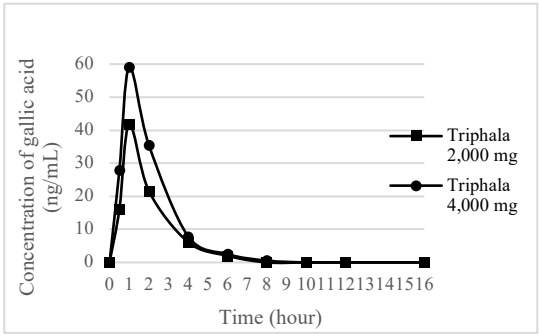

Fig. 2. Plasma concentration- time profiles of gallic acid following a single oral dose of 2,000 (Group 1, n= 16) and 4,000 mg (Group 2, n= 16) Triphala formulation. Data are presented as median values.

4. Discussion

The pharmacokinetics of gallic acid, when given as purified compound or as herbal extracts, was previously investigated in rats [14-16] and healthy human subjects [17]. However, there has been no study reporting on the pharmacokinetics of gallic acid as a major active compound in the Triphala formulation. This study is the first investigation of the pharmacokinetics of gallic acid following the administration of a single oral dose of Triphala formulation in healthy Thai subjects. Both dose levels (2,000 mg and 4,000 mg) were well-tolerated in all subjects. C_{max}, $AUC_{0\text{-}48hr}$ and $AUC_{0\text{-}\infty}$ of gallic acid increased proportionally with dose size. The oral absorption of gallic acid in Triphala was rapid with a t_{max} of 1 hr. Systemic bioavailability was sustained up until 10 hours, with a median $t_{1/2z}$ of approximately 1 hour. The results were generally in broad agreement with a previous report on single oral dose administration of Acidum gallicum tablets or Assam black tea (each containing 50 mg gallic acid). The mean t_{max} of gallic acid from both tablet and tea preparations were 1.3 and 1.4 hr, respectively, and the mean $t_{1/2z}$ values were 1.2 and 1.1 hr, respectively. It was noted, however, for the remarkably lower C_{max} and $AUC_{0\text{-}12hr}$ following both preparations. The C_{max} and $AUC_{0\text{-}12hr}$ following administration of the tablet formulation containing 50 mg gallic acid were 1.8 µmol/l (306.22 ng/ml) and 4.3 µmol.hr/l (731.52 ng.hr/ml), respectively. The corresponding values for the tea preparation were 2.1 µmol/l (357.25 ng/ml) and 4.5 µmol.hr/l (765.54 ng.hr/ml), respectively [17]. The bioavailability of gallic acid from Triphala formulation is markedly different from Acidum gallicum tablets or Assam black tea. This can be explained by the difference in the pharmaceutical formulations or nutritional sources containing gallic acid. In the previous studies, gallic acid from concord grape juice and a polyphenol-rich juice drink was not detected in plasma [18,19], but was in ileal fluid [18]. However, its metabolites such as 4-methyl gallic acid and 3-*O* methyl gallic acid were detectable [19]. In addition, a study on the bioaccessibility of Padma Hepaten (Triphala) using an *in vitro* dynamic model simulating gastrointestinal digestion [20] demonstrated that gallic acid was mainly achieved during the gastric phase (95% recovery) with a slight further release during intestinal digestion. This may explain the reduction in the amount of gallic acid removed from the portal circulation for metabolism.

In rats, gallic acid has been reported to be mainly distributed in kidneys, followed by lungs, liver, and heart after oral administration of aqueous extract of *Polygonum capitatum* at a single dose of 12 mg/kg body weight. There is no report of accumulation in brain tissue, suggesting an absence, or low level, of permeation across the blood-brain-barrier [21-22]. The compound was excreted in urine as unchanged form. The amount of gallic acid excreted in urine during the 48-hour period after administration was about 14.6% of the administered dose [21]. In humans, unchanged gallic acid and the metabolite 4-*O*-methyl gallic acid were detected in the urine after the intake of 50 mg gallic acid in the form of Acidum gallicum tablet and tea

preparations. The urinary excretion was 36% and 40% after, respectively [17].

Gallic acid has been reported to significantly enhance the bioavailability of drugs such as diltiazem, metoprolol, and nifedipine in animal models. The mechanism involved works through the inhibition of CYP3A4 and/or CYP2D6-mediated metabolism as well as the transport protein P-glycoprotein (P-gp) in the intestine and/or liver [23-27]. Administration of Triphala or herbal preparations containing gallic acid should be done with caution to avoid any clinically relevant pharmacokinetic drug interactions in patients given concomitant drug administration, especially involving drugs which are substrates of CYP3A4, CYP2D6, P-gp and possibly other drug-metabolizing enzymes.

5. Conclusion

This study provides preliminary information on the absorption and disposition characteristics of gallic acid following the administration of a single oral dose of the aqueous extract of Triphala in humans. This constitutes key information for further simulation of multiple-dose pharmacokinetics and dose-optimization of Triphala formulations for the control or treatment of various diseases or health conditions.

Acknowledgements

This study was financed by The Thai Traditional Medical Knowledge Fund, Thailand. The authors gratefully acknowledge Asst. Prof. Prasoborn Rinthong, Mahasarakham University, for providing Triphala capsule formulation. We would like to thank the staff of Thai Traditional and Alternative Medicine Hospital, Bangkok and The Drug Discovery and Development Center, Thammasat University, for their kind help and technical support during the study.

References

[1] Takauji Y, Miki K, Mita J, et al. Triphala, a formulation of traditional Ayurvedic medicine, shows protective effect against X-radiation in HeLa cells. J Biosci 2016;41(4):569-75.

[2] Wongnoppavich A, Kanjana J, Seewaboon S. Triphala: The Thai traditional herbal formulation for cancer treatment. Songklanakarin J Sci Technol 2009;31(2): 139-49.

[3] Mukherjee PK, Rai S, Bhattacharya S, et al. Marker analysis of polyherbal formulation, Triphala - a well known Indian traditional. Article. Indian J tradit Know 2008;7(3):379-83.

[4] Kaur S, Michael H, Arora S, et al. The in vitro cytotoxic and apoptotic activity of Triphala - an Indian herbal drug. J Ethnopharmacol 2005;97(1):15-20.

[5] Lall RK, Syed DN, Adhami VM, et al. Dietary polyphenols in prevention and treatment of prostate cancer. Int J Mol Sci 2015;16(2):3350-76.

[6] Mansouri MT, Soltani M, Naghizadeh B, et al. A possible mechanism for the anxiolytic-like effect of gallic acid in the rat elevated plus maze. Pharmacol Biochem Behav 2014;117:40-6.

[7] Baliga MS. Triphala, Ayurvedic formulation for treating and preventing cancer: a review. J Altern Complement Med 2010;16(12):1301-8.

[8] Baliga MS, Meera S, Mathai B, et al. Scientific validation of the ethnomedicinal properties of the Ayurvedic drug Triphala: a review. Chin J Integr Med 2012;18(12): 946-54.

[9] Peterson CT, Denniston K, Chopra D. Therapeutic Uses of Triphala in Ayurvedic Medicine. J Altern Complement Med. 2017;23(8):607-14.

[10] Phetkate P, Kummalue T, U-pratya Y, et al. Significant increase in cytotoxic T

lymphocytes and natural killer cells by triphala: a clinical phase I study. Evid Based Complement Alternat Med. 2012; 239856.

[11] Phetkate P, Kummalue T, Rinthong P, et al. Study of the safety of oral Triphala aqueous extract on healthy volunteers. J Integr Med 2020;18(1):35-40.

[12] Phetkate P, Plengsuriyakarn T, Bangchang K, Rinthong P, Kietinun S, Sriyakul K: Acute and subacute oral toxicity evaluation of the water extract of triphala formulation in rats. Int J Biol Pharm Allied Sci 2019;8(4):779-92.

[13] US Department of Health and Human Services Food and Drug Administration, Center for Drug Evaluation and Research, and Center for Veterinary Medicine. Bioanalytical Method Validation Guidance for Industry [Internet]. [cited 2018 Oct 19]. Available from: https://www.fda.gov/files/drugs/published/Bioanalytical-Method-Validation-Guidance-for-Industry.pdf

[14] Ma F, Gong X, Zhou X, et al. An UHPLC-MS/MS method for simultaneous quantification of gallic acid and protocatechuic acid in rat plasma after oral administration of Polygonum capitatum extract and its application to pharmacokinetics. J Ethnopharmacol 2015;162:377-83.

[15] Zhu H, Liu X, Zhu TT, et al. UHPLC-MS/MS method for the simultaneous quantitation of five anthraquinones and gallic acid in rat plasma after oral administration of prepared rhubarb decoction and its application to a pharmacokinetic study in normal and acute blood stasis rats. J Sep Sci 2017;40(11): 2382-9.

[16] Xu C, Yu Y, Ling L, et al. A C8-Modified Graphene@mSiO2 Composites Based Method for Quantification of Gallic Acid in Rat Plasma after Oral Administration of Changtai Granule and Its Application to Pharmacokinetics. Biol Pharm Bull 2017; 40(7):1021-8.

[17] Shahrzad S, Aoyagi K, Winter A, et al. Pharmacokinetics of gallic acid and its relative bioavailability from tea in healthy humans. J Nutr. Apr 2001;131(4):1207-10.

[18] Stalmach A, Edwards CA, Wightman JD, et al. Gastrointestinal stability and bioavailability of (poly)phenolic compounds following ingestion of Concord grape juice by humans. Mol Nutr Food Res 2012;56(3):497-509.

[19] Mullen W, Borges G, Lean ME, et al. Identification of metabolites in human plasma and urine after consumption of a polyphenol-rich juice drink. J Agric Food Chem 2010;58(4):2586-95.

[20] Olennikov DN, Kashchenko NI, Chirikova NK. In Vitro Bioaccessibility, Human Gut Microbiota Metabolites and Hepatoprotective Potential of Chebulic Ellagitannins: A Case of Padma Hepaten® Formulation. Nutrients 2015;7(10):8456-77.

[21] Margalef M, Pons Z, Bravo FI, et al. Tissue distribution of rat flavanol metabolites at different doses. The Journal of Nutritional Biochemistry 2015;26(10):987-95.

[22] Ma FW, Deng QF, Zhou X, et al. The Tissue Distribution and Urinary Excretion Study of Gallic Acid and Protocatechuic Acid after Oral Administration of Polygonum Capitatum Extract in Rats. Molecules 2016;21(4):399.

[23] Athukuri BL, Neerati P. Enhanced oral bioavailability of metoprolol with gallic acid and ellagic acid in male Wistar rats: involvement of CYP2D6 inhibition. Drug Metab Pers Ther 2016;31(4):229-34.

[24] Athukuri BL, Neerati P. Enhanced Oral Bioavailability of Diltiazem by the Influence of Gallic Acid and Ellagic Acid in Male Wistar Rats: Involvement of

CYP3A and P-gp Inhibition. Phytother Res 2017;31(9):1441-8.

[25] Chieli E, Romiti N, Rodeiro I, et al. In vitro modulation of ABCB1/P-glycoprotein expression by polyphenols from Mangifera indica. Chem Biol Interact. 2010;186(3):287-94.

[26] Okuda T, Yoshida T, Hatano T. Hydrolyzable tannins and related polyphenols. Fortschr Chem Org Naturst 1995;66:1-117.

[27] Stupans L, Tan HW, Kirlich A, et al. Inhibition of CYP3A-mediated oxidation in human hepatic microsomes by the dietary derived complex phenol, gallic acid. J Pharm Pharmacol 2002;54(2):269-75.