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Pharmacokinetics of gallic acid following oral administration of Triphala formulation in rats

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Triphala formulation is one of the most common traditional medicines used for several health conditions. The study investigated the pharmacokinetics of gallic acid following an oral dose administration in rats. The pharmacokinetics of gallic acid was investigated in rats following a daily oral dose of 1,000 and 5,000 mg/kg body weight (28 days). Plasma concentrations of gallic acid were measured using liquid chromatography-mass spectrometry (LC-MS/MS). Non-compartmental pharmacokinetic analysis approach was applied for data analysis. The pharmacokinetics was linear without dose-dependent characteristics. Gallic acid was rapidly absorbed, reaching maximum concentration within 1 h and was also rapidly cleared from rat systemic circulation within 12 h of administration. The pharmacokinetics of gallic acid was linear with about 5-fold increase in C_{max} and systemic exposure $AUC_{0-\infty}$ when the dose was increased from 1,000 to 5,000 mg/kg body weight. The pharmacokinetics of gallic acid following both regimens was similar. Terminal phase elimination half-life ($t_{1/2z}$), apparent volume of distribution (V_z/F) and total clearance (CL/F) ranged from 0.7-1.7 h, 327-1,159 L and 195-607 L/h/kg. The pharmacokinetics of gallic acid obtained from the present study in rats provides preliminary information for designing proper pharmacokinetic studies in humans for further dose optimization of appropriate dosage regimens of Triphala formulation for treatment of various diseases or health conditions.

Key words: Triphala formulation, pharmacokinetics, gallic acid.

INTRODUCTION

The use of herbal products as complementary and alternative medicines for the prevention and treatment of various diseases has been increasing worldwide, including in Thailand (Welz et al., 2018; Peltzer and Pengpid, 2019). Triphala formulation is one of the most common Ayurvedic (King of Rasayana) and traditional Thai medicines used for several ailments and health conditions, such as chronic constipation, detoxification of the colon, food digestive problems, hypertension, hypercholesteremia, diabetes mellitus, upper respiratory

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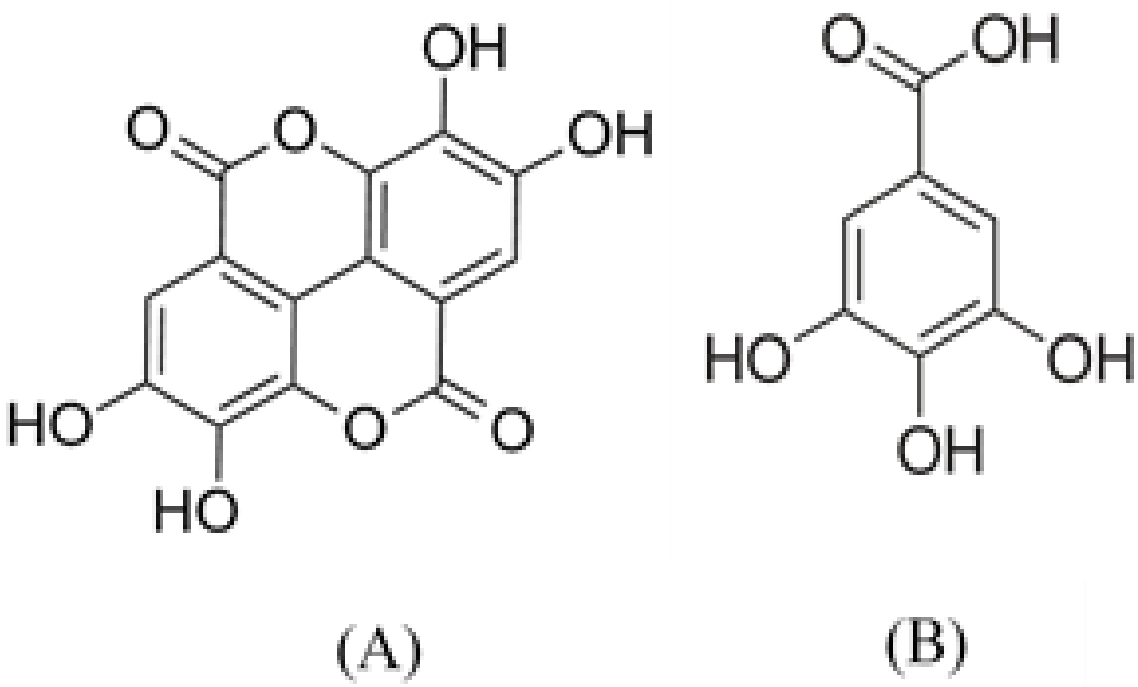

Figure 1. Chemical structures of the two major constituents of Triphala formulation: (A) ellagic acid, and (B) gallic acid.

diseases, peptic ulcers, mouth ulcers, anaemia, abdominal pain, skin wounds, obesity, and cancer (Mukherjee et al., 2008; Sabu and Kuttan, 2002; Takauji et al., 2016; Wongnoppavich et al., 2009; Phetkate et al., 2019). The formulation consists of dried pericarps of the fruits from three plants, that is, *Terminalia chebula* Retz., *Terminalia belliric* (Gaertn) Roxb., and *Emblica officinalis Gaertn.* Mixed in equal proportions (1:1:1) (Baliga, 2010). The primary chemical constituents are the two polyhydroxy phenolic compounds gallic acid (7-15%) and ellagic acid (2.4%) (Figure 1A and B) (Naik et al., 2006; Phetkate et al., 2020). Gallic acid is also found in other natural products, such as black and green tea, grapes, wines, strawberries, bananas, and other fruits (Lall et al., 2015). Experimental studies have confirmed several pharmacological activities of Triphala formulation, including appetite stimulation, antioxidant, anti-inflammatory, antibacterial, antimutagenic, antineoplastic, chemoprotective, and lipid- and blood sugar-lowering activities (Baliga et al., 2012; Baliga, 2010; Peterson et al., 2017). No acute and subacute toxicity was observed in rats following 2,000 and 5,000 mg/kg body weight dose levels. Results of a clinical study in healthy Thai subjects suggested that Triphala formulation was safe and significantly modulated human cellular immune response, particularly cytotoxic T cells and natural killer (NK) cells, following a daily dose of the ethanol extract of 1,050 mg for 14 days (Phetkate et al., 2012). The present study aimed to investigate the pharmacokinetics of gallic acid following oral administration of Triphala formulation in rats.

METHODOLOGY

Plant extract

The Triphala capsule formulation (water extract) was prepared by the Pharmaceutical Chemistry and Natural Products Research Unit, Faculty of Pharmacy, Mahasarakham University, Thailand. The plant materials were dried at 60 °C for 48 h in the hot-air oven after thoroughly washed with tap water and remove seeds of each fruit. All dried materials were powdered using the electric blender and passed through sieve No.14. The powdered plant material (15 g) was extracted by boiling in 1,000 ml of distilled water for 1 h, filtered through Whatman no. 1 filter paper, and freeze-dried. The Microbial contamination was controlled using gamma-irradiation. The gallic acid content of Triphala formulation was 133.80 ± 2.94 mg/g (mean±SD) of extract.

Animals

The study protocol was prepared and ethical approval of the study conduct was obtained from the Ethics Committee for Animal Research, Thammasat University, Thailand (No. 019/2559). Males and females of Wistar rats, aged between 5-7 weeks, weighing 150 to 180 g, were used in the study (National Laboratory Animal Center of Thailand). The study was carried out in accordance with the Guidelines for the Care and Use of Laboratory Animals (US National Research Council, 2011). The animals were acclimatized one week before study initiation. The animals were housed in

stainless-steel cages at the Laboratory Animal Center, Thammasat University under controlled light of 12 h dark/light cycle at 22±2°C and relative humidity 30-70%. All were fed with the stock diet and water ad libitum. During drug administration period, all animals were observed daily for the changes in body weight, behaviors, and mortality.

Pharmacokinetic study

Animals (20 rats) were randomly allocated to two groups of 5 males and 5 females each. Triphala formulation extract was dissolved in distilled water and fed to each rat by intragastric gavage at a daily oral dose of 1,000 or 5,000 mg/kg body weight for 28 days. Blood samples (150-200 µl each) were collected from the tail vein of each rat at 0, 0.5, 2, 4, 8, 12 and 24 h, respectively. Plasma samples were separated through centrifugation at 1,000 x g for 10 min and stored at -80ºC until analysis.

Determination of gallic acid concentrations in plasma

The bioactive compound, gallic acid, was used as the marker to investigate the pharmacokinetics of Triphala formulation in rats. Plasma sample (100 µl) was spiked with the internal standard, apigenin (10 µl of 30 ng/ml), and the resultant mixture was extracted with a mixture of 2% formic acid in acetonitrile (500 µl). After centrifugation (13,800 xg, 10 min), the clear organic layer was evaporated to dryness under nitrogen flow. The dried residue was reconstituted with the mobile phase (100 µl) and an aliquot (5 µl) was injected onto the liquid chromatographic-mass spectrometry (LC-MS/MS) in the negative-ion mode (ESI). The system consisted of Agilent 1260 Separation Module (Agilent Technologies, CA, USA) solvent and sample delivery, an AB SCIEX QTRAP® 5500 mass-spectrometer (AB Sciex, Foster City, CA, USA) in the Scheduled Multiple Reactions Monitoring (MRM) mode as follows: -4,500V ion spray, 50 ion source gas 1 (GS1) and 50 gas 2 (GS2), 30 curtain gas (CUR), 5 collision gas (CAD), and 500 °C source temperature. Optimal mass parameters, that is, 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 apigenin, respectively. The full-scan mass spectra of each analyze were set to monitor the transitions of the precursors to the product ions were m/z 168.9 →125.1 for gallic acid and 269→117 for apigenin. The separation was performed on a ZORBAX SB-C18 (4.6 x 50 mm, 3.5 µm particle size) (Agilent Technologies, CA, USA) with the mobile phase [a mixture of water containing 0.1 % formic acid and 100 % acetonitrile with gradient elution 0 min (60:40%), 5 min (20:80%), and 6.5 min (60:40%)] running at a flow-rate of 0.3 ml/min.

Assay validation

***Calibration curve*:** Calibration curves of gallic acid were prepared by replicate analysis as described above, of seven plasma samples (100 µl each) spiked with gallic acid (50-2,000 ng/ml) and an internal standard, apigenin (30 ng/ml). Samples were analyzed as described above (CDER, 2018).

Precision: Within-day repeatability and day-to-day variation (reproducibility) were determined by replicate analysis of six sets of samples spiked with gallic acid (400, 800, and 1,600 ng/ml). Coefficient of variation (%CV) was determined from the ratio of standard deviation (SD) and mean (CDER, 2018).

Accuracy: The accuracy of the method was determined by replicate analysis of six sets of samples spiked with gallic acid (400, 800, and 1,600 ng/ml) and comparing the difference between spiked value and that actually found (theoretical value) (CDER, 2018).

Recovery: The analytical recovery of sample preparation procedures was estimated by comparing the peak heights obtained from the sample with that measured with post-extraction sample spiked with equivalent amounts of gallic acid (400, 800 and 1,600 ng/ml) (CDER, 2018).

Selectivity: The selectivity of the method was verified by checking for interference by commonly used drugs, that is paracetamol and dimenhydrinate (CDER, 2018).

Limit of quantification: The limit of quantification (LOQ) of the method was determined from the lowest concentration of gallic acid in spiked plasma that produced a peak height ten times of the baseline noise at - 0.2 A (CDER, 2018).

Stability: The stability of gallic acid in plasma samples was determined by storing spiked samples (400, 800 and 1,600 ng/ml, triplicate each) at -80 ºC freezer (Sanyo, Japan) for three months. Concentrations were measured on days 1, 15, and months 1 and 3 (CDER, 2018).

Quality control: Quality control (QC) samples for gallic acid were made up in plasma samples using a stock solution (400, 800 and 1,600 ng/ml, triplicate each). The samples were aliquoted and stored at -80 ºC for use with each analytical run (CDER, 2018).

Pharmacokinetic analysis

Plasma concentration-time profile of gallic acid was plotted and the pharmacokinetic parameters were determined using a model-independent analysis (Gibaldi, 2011). The maximum concentration (C_{max}) and time to maximum concentration (t_{max}) were obtained from the plasma concentration-time data. The area under the curve from zero time to twenty-four hours of dosing (AUC_{0-24h}) was calculated using linear trapezoidal rule (ascending data points) and log-trapezoidal rule (descending data points). The AUC from the zero time to infinity ($AUC_{0-\infty}$) was determined from the sum of the AUC_{0-24h} and the extrapolated AUC. The terminal elimination half-life ($t_{1/2z}$) was calculated from log-linear regression of at least four of the last plasma concentration-time data. The apparent total body clearance (CL/F) and apparent volume of distribution (V_z/F) were calculated as $CL/F=dose/AUC_{0-\infty}$ and $V_z/F=[CL/F]/\lambda_z$.

Statistical analysis

Data are presented as median (interquartile range) values. Comparison of quantitative variables between the two groups was performed using Mann-Whitney U test for data not conforming to the normal distribution (SPSS version 13). Optimal sample size for the pharmacokinetic study in rats was determined based on the primary outcome pharmacokinetic parameter $AUC0_{0-\infty}$ (Aarons and Ogungbenro, 2010). Statistical significance level was at $\alpha < 0.05$.

RESULTS

Assay validation

Precision: The intra-day assay (%CV) of gallic acid in

Table 1. Accuracy, precision and recovery of gallic acid in rat plasma samples.

Concentration (ng/ml)	Precision (%CV)[a]		Accuracy (%DMV)[b]		% Recovery
	Intra-day	Inter-Day	Intra-day	Inter-Day	
400	8.13	5.70	3.04	5.92	105.9
800	9.38	6.94	2.50	-7.17	92.8
1,600	8.41	6.84	5.46	-4.35	95.7
Mean ± SD	8.64±0.66	6.49±0.68	3.67±1.58	-1.87±6.89	98.13±6.88

[a]CV: Coefficient of variation (%); [b]DMV: Deviation of mean value from theoretical value (%).

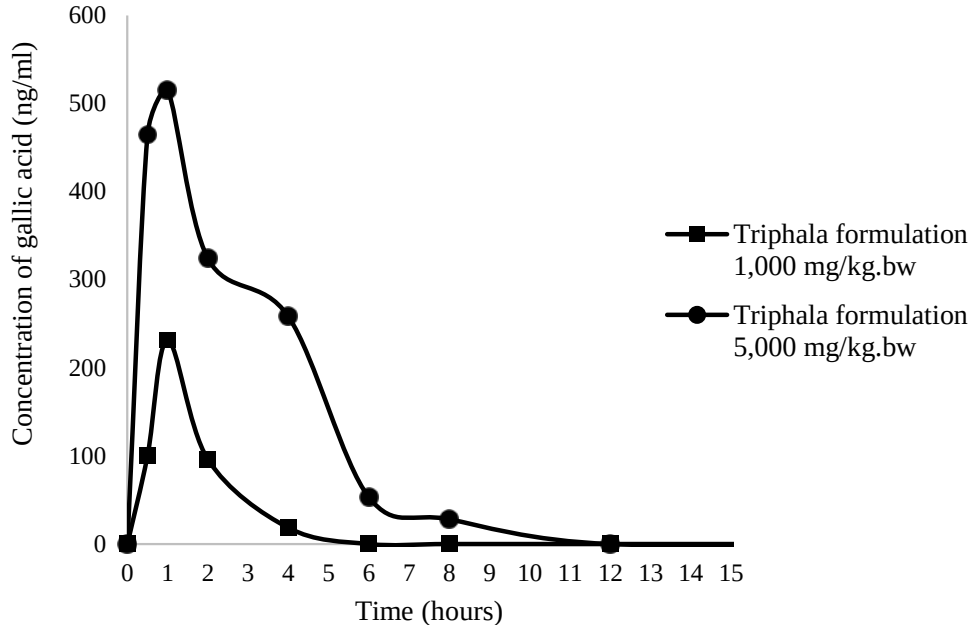

Figure 2. Median plasma concentration-time profiles of gallic acid in Wistar rats oral administration of multiple doses of 1,000 mg/kg and 5,000 mg/kg body weight of Triphala formulation.

plasma varied between 8.13% and 9.38 %. The inter-day assay (%CV) varied between 5.70% and 6.94% (Table 1). Results suggested acceptable precision of the assay method.

Accuracy: Good accuracy was observed from both the intra-day or inter-day assays. For intra-day assay validation, deviation of mean value from theoretical value (%DMV) varied between 2.50% and 5.46 %. The inter-day assay (%MDV) varied between -7.17% and 5.92% (Table1).

Recovery: The mean recoveries of gallic acid at 400, 800 and 1,600 ng/ml were 105.9%, 92.8% and 95.7%, respectively (Table 1). Results suggested high recovery of gallic acid from the spiked plasma samples.

Limit of quantification: The LOQ of gallic acid in plasma was accepted as 2.5 ng/ml using 100 µl blood.

Stability: The analysis of gallic in plasma samples was found to be stable following storage at -80 °C for a minimum of three months without significant decomposition of the drug.

Pharmacokinetics of gallic acid

Triphala formulation was well tolerated in all rats receiving both dose levels of Triphala formulation with no abnormal signs and symptoms and body weight changes. Gallic acid in both groups of Triphala formulation was rapidly absorbed after oral dose administration with a median t_{max} of 0.5-1 h that received a dose of 5,000 mg/kg body weight and 1,000 mg of Triphala formulation, respectively (Figure 2). It disappeared after that from

Table 2. The pharmacokinetic parameters in Wistar rats following oral administration of multiple doses of 1,000 mg (group 1, n=10), and 5,000 mg (group 2, n=10) Triphala formulation.

Pharmacokinetic parameter	Median (interquartile range)		P-value [a]
	Group 1	Group 2	
C_{max} (ng/ml)	194.7 (112.2-294.7)	866.2 (400.1-1204.6)	0.0001*
t_{max} (h)	1 (0.5-2.0)	0.5 (0.5-1.25)	0.481
AUC (ng*h/ml)	383.2 (198.7-566.4)	1910.6 (1075.07-2565.64)	0.0001*
$AUC_{0-\infty}$ (ng*h/ml)	421.8 (216.2-634.72)	1917.9 (1135.56-3356.13)	0.0001*
$t_{1/2z}$ (h)	1 (0.7-1.7)	1.3 (1.1-1.5)	0.299
V_z/F (l)	357.9 (327.7-622.8)	455.1 (414.1-1159.6)	0.113
CL/F (l/h/kg)	251.90 (195.3-550.7)	447.0 (247.4-607.9)	0.351

*Statistical significant difference between Group 1 and 2 *(*Mann-Whitney U test*)*.

systemic circulation within 12 h. The median C_{max} increased from 194.7 ng/ml to 866.2 ng/ml and the AUC_{0-24h} and $AUC_{0-\infty}$ increased from 383.2 ng*h/ml to 1,910.6 ng*h/ml and 421.8 ng*h/ml to 1,917.9 ng*h/ml, respectively. It was found that almost 5-times in the group receiving 1,000 mg/kg body weight compared with 5,000 mg/kg body weight, while other pharmacokinetic parameters (CL/F, V_z/F, and $t_{1/2z}$) were comparable **(**Table 2).

DISCUSSION

The pharmacokinetics of gallic acid has previously been reported in rats following oral administration of gallic acid or other herbal extracts, but not Triphala formulation (Ferruzzi et al., 2009; Konishi et al., 2004; Ma et al., 2015; Song et al., 2010). Characterization of the pharmacokinetic profile of gallic acid in animals following the clinically used formulation (Triphala) is essential to provide preliminary information for human study. In the current study, the pharmacokinetics of gallic acid was investigated in rats following 1,000 and 5,000 mg/kg body weight Triphala formulation, which corresponds to the minimally and maximally safe dose levels of Triphala formulation reported in rats (Phetkate et al., 2019). Gallic acid from both dose levels of Triphala formulation was rapidly absorbed, reaching its maximum plasma concentration within 1 h. The compound was rapidly cleared from blood circulation within 12 h of administration. The pharmacokinetics of gallic acid was linear with about 5-fold increase in C_{max} and systemic exposure $AUC_{0-\infty}$ when the dose was increased from 1,000 to 5,000 mg/kg body weight. This pharmacokinetic profile was in general, in agreement with those reported following the administration of gallic acid and other herbal extracts (Ferruzzi et al., 2009; Konishi et al., 2004; Ma et al., 2015; Song et al., 2010). Gallic acid at the dose of 100 µmol/kg body weight (17 mg/kg) was shown to be rapidly absorbed with C_{max} of 0.71 µmol/l (120.78 ng/ml) and AUC of 42.6 µmol*min/l) (120.78 ng*h/ml) (Konishi et al., 2004). In another study, after oral Polygonum capitatum extract containing 4-24 mg/kg gallic acid, C_{max} of gallic acid was 246-806 ng/ml. AUC_{0-t} and $AUC_{0-\infty}$ were 695-1,832 and 896-3,403 ng/ml*h, respectively (Ma et al., 2015). Following the administration of 50, 100, and 150 mg grape seed polyphenolic extract, C_{max} and AUC_{0-8h} were 239-323 ng/ml and 512-707 ng*h/ml (Ferruzzi et al., 2009). The variation of the reported C_{max} and AUC of gallic acid is explained by the variability of sources of gallic acid and species of plants under investigation.

Conclusion

The pharmacokinetics of gallic acid obtained from the present study in rats provides preliminary information for designing proper pharmacokinetic studies in humans for further dose optimization of appropriate dosage regimens of Triphala formulation for treatment of various diseases or health conditions.

CONFLICT OF INTERESTS

The authors have not declared any conflicts of interests.

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