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Phase I clinical trial to evaluate the safety and pharmacokinetics of capsule formulation of the standardized extract of *Atractylodes lancea*

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ABSTRACT

Background and aim: *Atractylodes lancea* (AL) has been demonstrated in a series of studies to be a potential candidate for the treatment of cholangiocarcinoma. The aim of the current study was to evaluate the safety and pharmacokinetics of the capsule formulation of the standardized AL extract in healthy Thai participants.

Experimental procedure: Forty-eight healthy Thai participants who fulfilled the inclusion and had none of the exclusion criteria were allocated to two study groups. The *group 1* participants were randomized to receive a single oral dose of 1,000 mg of AL or placebo (20:4 participants). The *group 2* participants were randomized to receive daily oral doses of 1,000 mg AL or placebo daily for 21 days (20:4 participants). Safety and tolerability of the two AL regimens were monitored. Blood samples were collected for measurement of atractylodin concentrations by HPLC and pharmacokinetic analysis was performed using model-dependent and model-independent analysis.

Results and conclusion: The AL extract was well tolerated in both groups. Atractylodin was rapidly absorbed but with low systemic exposure and residence time. There was no difference in the pharmacokinetic parameters of atractylodin following a single or multiple dosing, suggesting the absence of accumulation and dose-dependency in human plasma after continuous dosing for 21 days. The information on human pharmacokinetics of AL, when given as capsule formulation of the standardized extract, would assist in further dose optimization in cholangiocarcinoma patients with the defined pharmacokinetic-pharmacodynamic relationship.

1. Introduction

Cholangiocarcinoma (CCA), the bile duct cancer, is an extremely aggressive cancer with increasing worldwide incidence and mortality rate, particularly in Northeastern Thailand. It accounts for approximately 15% of liver cancer worldwide. The global incidence rate of CCA shows substantial geographical variation ranging from 0.3 to 85 cases *per* 100,000 population.[1] The age-standardized incidence rate (ASR) in Thailand during the period 1988–2012 was between 53.4 and 94.8 *per* 100,000 for males and 18.5 and 39.4 *per* 100,000 for females.[2] The significant risk of CCA in most countries is primary sclerosing cholangitis. For Southeast Asian countries including Thailand, the primary risk factor is the consumption of improperly cooked cyprinoid fish which contains infective metacercaria of the liver fluke *Opisthorchis viverrini*, *O. felines*, or *Clonorchis sinensis*, together with dimethylnitrosamine

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Lists of abbreviation

AIC	Akaike information criterion
AL	*Atractylodes lancea* (Thunb.) DC
AUC_{0-t}	Area under plasma concentration-time curve from zero to the last observed time
$AUC_{0-\infty}$	Area under plasma concentration-time curve from zero to infinity
$AUMC_{0-\infty}$	Area under the first moment curve
BMI	Body mass index
CAFs	Cholangiocarcinoma-associated fibroblasts
CCA	Cholangiocarcinoma
CL/F	Total clearance (corrected with bioavailability)
C_{max}	Maximum plasma concentration
CNS	Central nervous system
COX-1	Cyclooxygenase 1
CPK	Creatinine phosphokinase
CTC	Common Toxicity Criteria
DMN	Dimethylnitrosamine
ECG	Electrocardiogram
CMC	Chemisty Manufacturing and Control
HDL	High-density lipoprotein
HED	Human Equivalent Dose
HPLC	High-performance liquid chromatography
IC_{50}	Fifty percent inhibitory concentration
IFNγ	Interferon-gamma
IL	Interleukin
INR	International Normalized Ratio
λ_z	Elimination rate constant
LC-MS/MS	Liquid chromatography mass-spectrometry
LDL	Low-density lipoprotein
LOQ	Limit of quantitation
5-LOX	5-Lipoxygenase
MRSD	Maximum Recommended Starting Dose
MRT	Mean residence time
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NMJ	Neuromuscular junction
NOAEL	No observed adverse effect level
p38MAPK	p38 mitogen-activated protein kinases
P13K/AKT/mTOR	Phosphatidylinositol 3-kinase/Protein kinase B/Mammalian target of the rapamycin
PT	Prothrombin time
PTT	Partial thromboplastin time
QC	Quality control
t_{max}	Time to maximum plasma concentration
TXA_2	Thromboxane A2
V_z/F	Apparent volume of distribution associated with terminal phase elimination half-life (corrected with bioavailability)

(DMN) from fermented meat.[3] Lack of early diagnostic tool and effective chemotherapeutics are the major constraints for controlling this type of cancer. Clinical efficacy of the standard chemotherapeutic drugs 5-fluorouracil (5-FU), cisplatin, and gemcitabine given as single drugs or combinations remains unsatisfactory. Less than 5% of the advanced stage patients survive for up to five years.[4] Research and development of effective alternative medicines, particularly those from natural sources for the treatment and control of CCA is challenging.

In the past few years, we have performed a series of studies applying the reverse pharmacology approach to support the development of *Atractylodes lancea* (Thunb.) DC. (AL) as a potential chemotherapeutic for CCA.[5] The dried rhizome of AL is used in Chinese ("Cang Zhu"), Japanese ("So-jutsu"), and Thai ("Khod-Kha-Mao") traditional medicines for various pharmacological properties including anticancer, anti-inflammatory, antimicrobial activities, and activities on central nervous, cardiovascular, and gastrointestinal systems. These pharmacological properties explain the traditional uses of AL in eliminating dampness, strengthening the spleen, expelling wind-cold from the superficial parts of the body, and relieving the common cold. In Thai traditional medicine, the primary use of AL is for treatment of fever and the common cold.[6] We have demonstrated that AL extract (crude ethanolic extract and standardized extract), as well as its major bioactive compounds atractylodin and β-eudesmol, are potential candidates for CCA.[5] The supporting pieces of evidence are based on a series of nonclinical investigations, *i.e.,* anti-CCA activity (*in vitro* and *in vivo*), phytochemistry, pharmacological activity (*in vivo*), pharmacokinetics and potential of metabolic drug interactions (*in vitro* and *in vivo*), toxicity (*in vitro* and *in vivo*), and potential molecular targets of action against CCA.[5] CCA is a typical inflammatory tumor characterized by intensive infiltration of cholangiocarcinoma-associated fibroblasts (CAFs) and production of the proinflammatory cytokines particularly interleukin 6 (IL6).[7] These processes have been shown to impact on CCA progression *via* affecting autophagy.[7] Therapeutic potential of autophagy modulation in CCA is being an area of research focus to search for effective compounds or plant-derived compounds for CCA control.[8] The polyphenolic compound resveratrol (from grapes, blueberries, and cranberries, *etc.*) as well as xantohumol (from *Humulus lupulus*, also known as hops), were demonstrated to restore autophagy and thus reducing CCA cell invasiveness and improving chemosensitivity.[7,9,10] More recently, atractylodin has been shown to inhibit the proliferation and induce autophagy of the CCA cell *via* regulating PI3K/AKT/mTOR and p38MAPK signaling pathways (our unpublished data). Furthermore, the *ex vivo* study in healthy subjects suggests the immunomodulatory activity of AL through decreasing the levels of the pro-inflammatory cytokines IFNγ, IL6, IL10 and IL17A, as well as increasing the number of B cells, NK cells, $CD4^+$ cells, and $CD8^+$ cells (Kulma et al, BMC Complementary Medicine and Therapies, in press). The effects of AL or its active components on CAFs associated autophagy remain to be explored.

Based on these reports, particularly the *in vitro* synergistic interaction between components of AL extract, it was decided that further development of AL should be as an oral formulation of the standardized crude AL extract. Large scale production of the capsule pharmaceutical formulation of the standardized AL extract (CMC: Chemistry Manufacturing and Control formulation) was completed for preclinical (acute, subacute, and chronic toxicity tests), and clinical (phase I and phase II clinical trials) evaluations.[11] The aim of the current study was to evaluate the safety and pharmacokinetics of the capsule formulation (CMC) of the standardized AL extract in healthy Thai participants. Atractylodin was used as the marker compound for pharmacokinetic investigation. The pharmacokinetics of atractylodin following oral doses of *Atactylodis rhizoma* (dried root and stem of *A. lancea* or *Atractylodes chinensis* (DC) Koidz) was investigated in rats in two studies.[12,13] In the first study,[12] atractylodin pharmacokinetics was conducted following oral administration of 30 g/kg body weight *A. rhizoma* and intravenous administration of 2 mg/kg body weight of the active compound atractylodin. For oral administration, mean maximum plasma concentration (C_{max}), time to C_{max} (t_{max}), and terminal

elimination half-life ($t_{1/2}$) were 1,930 ng/ml, 2.25 h and 8 h, respectively. The $t_{1/2}$, central volume of distribution (Vc), and total clearance (CL) following intravenous administration were 3.3 h, 31.7 l/kg, and 6.59 l/h/kg, respectively. In another study,[13] the pharmacokinetics of atractylodin after oral administration of 40 g/kg body weight crude and processed *A. rhizoma* were investigated in rats. For the crude *A. rhizoma*, mean C_{max} of 625 ng/ml was achieved at 2.2 h. The $t_{1/2z}$, mean residence time (MRT) and CL/F were 2.38 h, 4.8 h, and 6.67 l/kg/h. For the processed *A. rhizoma*, mean C_{max}, t_{max}, $t_{1/2z}$, MRT and CL/F were 2,299 ng/ml, 0.33 h, 5.13 h, 7.06 h, and 2.56 l/kg/h, respectively. It was noted for double peaks of atractylodin in rat plasma after oral administration of *A. rhizoma* in both studies (approximately 2 h and 5 h).

2. Methods

2.1. Participants and study design

The study was an open, randomized, placebo-controlled design. Approval of the study protocol was obtained from the Ethics Committee, Thammasat University. Written informed consent was obtained from all research participants. Forty-eight healthy Thai participants (24 males and 24 females), aged between 20 and 45 years with body mass index (BMI) between 20 and 25 kg/m^2, who were non-smokers and non-alcohol drinkers and were residents of Bangkok or suburb areas, were recruited into the study. Additional inclusion criteria were (i) absence of acute or chronic diseases that could affect vital organ functions, (ii) no history of surgery within the past six months, (ii) no history of hypersensitivity reactions or idiosyncratic reactions to drugs or herbal products, (iv) no concurrent or history of administration of drugs or herbal products within the past two weeks (except antipyretic or anti-emetic drugs), (v) no history or current drug abuse, (vi) ability to communicate (reading, writing, and speaking) effectively, and (vii) willing to give informed consent for study participation. Exclusion criteria were those with (i) clinical significant abnormality of physical examination, (ii) clinical significant abnormality of electrocardiograms (ECG) or chest x-ray, (iii) pregnancy or lactation, (iv) blood tests positive for HBsAg, HCV, or HIV, (v) abnormality in blood coagulation or history or concurrent use of anticoagulants or antiplatelets, or (vi) participation in any other study in the past three months.

After obtaining written informed consents, clinical and laboratory investigations were carried out to confirm the eligibility of the research participants. These included physical examination, electrocardiogram (ECG) monitoring, chest X-ray test, and laboratory investigations (hematology, serum biochemistry, blood coagulation, urinalysis, serology, and pregnancy status). The recruitment continued until reaching the number of eligible research participants required, *i.e.,* 24 females and 24 males. Eligible participants were admitted to the ward at the Clinical Research Center, Faculty of Medicine, Thammasat University during the first two days of the pharmacokinetic study and returned for drug administration and follow up daily until 12 or 21 days, depending on the allocated drug regimens.

2.2. CMC (Chemistry, Manufacturing, and control) capsule formulation of the standardized extract of Atractylodes lancea (Thunb.) DC

The crude standardized ethanolic extract of *Atractylodes lancea* dried rhizomes (AL, consisting of 4.84% and 9.17% of atractylodin and β-eudesmol, respectively) was formulated in one capsule (No. 00) with lactose (water-soluble filler), sodium lauryl sulfate (surfactant), and talcum (glidant) at the ratio of 3:1: 0.0005:0.1. One capsule contained 2.45 mg and 4.06 mg atractylodin and β-eudesmol, respectively. The pharmaceutical properties of the AL capsule were evaluated according to the standard procedures.[11] Results showed acceptable properties of the formulation (bulk density, solubility, tapped density, Hausner ratio, compressibility index, angle of repose, flowability, weight variation, disintegration, and dissolution). Cytotoxic activity of capsule formulation of the standardized AL extract against CL-6 cells was confirmed using MTT assay, and results showed comparable activity of the crude ethanolic extract with mean ± SD IC_{50} (concentration that inhibits cell growth by 50%) of 29.60 ± 2.24 μg/ml).[11]

2.3. Drug administration

Study participants (24 males and 24 females) were allocated to two groups (12 males and 12 females for each group) as follows:

Group 1. Participants were randomized (using a randomization table) to receive a single oral dose of either 1,000 mg of capsule formulation of the standardized AL extract (9 capsules, 112.5 mg each, Kaolaor Laboratories Co. Ltd.) or placebo at the ratio of 20:4 participants.

Group 2. Participants were randomized to receive multiple oral doses of either 1,000 mg of capsule formulation of the standardized AL extract or placebo daily for 21 days at the ratio of 20:4 participants.

All capsules were taken at once with 200 ml drinking water. No food was consumed, although alcohol-free and xanthine-free fluids were permissible the night before the study. Participants were fasted for 2 h after drug administration to avoid any interaction between food and drugs. No other drugs, except analgesic, antipyretic and anti-emetic drugs were allowed during the study period.

The starting dose of the AL capsules used in the study was 1,000 mg which is about 50% of the maximum recommended starting dose (MRSD).[14] This 1,000 mg AL extract dose is equivalent to 48.4 mg atractylodin.

The MRSD of capsule formulation of the standardized AL extract was estimated as follow:

$$\text{MRSD} = \frac{\text{HED}}{\text{Safety factor}}$$

Where HED (human equivalent dose) is the ratio between NOAEL (no observed adverse effect level) and surface area conversion factor for mice (12.3). The NOAEL is the dose of capsule formulation of the standardized AL extract that did not cause any abnormal sign or symptom in mice was 5,000 mg/kg body weight.[15,16] Applying the safety factor of 10, the estimated MRSD was 40.65 mg/kg human body weight or 2,400 mg of the standardized AL extract for the average body weight of 60 kg. To ensure safety and avoid unwanted effects, the starting dose was, however, lower down to 1,000 mg (equivalent to 48.4 mg atractylodin).

2.4. Assessments of safety and tolerability

Safety and tolerability of the two drug regimens were evaluated based on clinical and laboratory assessments during follow-up, according to NIH/NCI Common Toxicity Criteria (CTC) Grading System for Adverse Events.[17] The occurrence, pattern, intensity, and severity of adverse events (clinical assessments, vital signs, and ECGs, together with clinical laboratory parameters) were monitored at intervals during the study period. Adverse events that were likely to relate to AL were assessed using the Naranjo algorithm.[18] Hematology, serum biochemistry, and urinalysis were monitored

on days 4 and 12 for *group 1* and on days 4 and 21 for *group 2* participants. Blood coagulation tests were performed on days 1, 4 and 14 for *group 1* and on days 1, 4, 5, 7, 9, 11, 14, 16, 18, 20 and 23 for *group 2* participants. Any abnormal laboratory result was followed up with repeat checks every week until it returned to normal. Laboratory abnormalities (outside the normal ranges) that first occurred or increased in intensity during follow-up were evaluated.

2.5. Pharmacokinetic investigation

2.5.1. Blood sample collection

Serial venous blood samples were collected through an indwelling intravenous Teflon™ catheter, inserted into a forearm vein of the subject during the 24 h of frequent blood sampling; patency was maintained with sodium-heparinized saline. Blood sampling after 24 h was obtained by direct venipuncture. In *group 1*, a total of 18 venous blood samples (3 ml each) were collected into heparin-coated plastic tubes before drug administration at 0 h (day 1), and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 16, 24 (day 2), 36, and 48 (day 3) hours after drug administration. In *group 2*, a total of 13 blood samples (3 ml each) were collected on day 1 at 0, 0.5, 1, 2, 4, 6, 8, 10, 12, 16, 24, 36 and 48 h after drug administration, and on days 5, 7, 9, 11, 14, 16, 18, and 20 (1 h after drug administration on each day) of drug administration. On day 21, 13 blood samples were collected at 0, 0.5, 1, 2, 4, 6, 8, 10, 12, 16, 24, 36 and 48 h of drug administration.

2.5.2. Determination of atractylodin concentrations

Atractylodin concentrations in plasma samples collected from volunteers who received capsule formulation of the standardized AL extract (20 participants for each group) were measured using high-performance liquid chromatography according to the previously described method[19] with modifications. The chromatographic system consisted of the elution solvent delivery (SpectraSystem P4000 Quaternary Solvent Delivery/Controller: Thermo Fisher Scientific, CA, USA), equipped with solvent degasser (SpectraSystem SCM1000 Solvent Degasser: Thermo Fisher Scientific, CA, USA), an auto-sampler (SpectraSystem AS3500: Thermo Fisher Scientific, CA, USA) and a UV detector (SpectraSystem UV/Vis 3000: Thermo Fisher Scientific, CA, USA). The UV wavelength was set at 340 nm. The separation was carried out on a reversed-phase column (Thermo Hypersil Gold C18, 250 mm × 2.1 mm i.d., 5 μm: Thermo Scientific, CA, USA). The elution solvent consisted of acetonitrile and distilled water at the ratio of 70:30 (v:v). The chromatographic analysis was operated at 25 °C. Aliquots of 200 μl samples or standard solutions were injected onto the column with an elution buffer at a flow rate of 1.0 ml/min.

Plasma samples were prepared using protein precipitation followed by liquid-liquid extraction. To 1 ml plasma, 20 μl of the internal standard (1,8-dihydroxyanthraquinone) (250 ng/ml working solution) was added. After thoroughly mixing, 2 ml of acetonitrile was added. The mixture was vortexed for 30 s and centrifuged at 3,000×*g* for 10 min. The supernatant was transferred to a 15 ml test tube and extracted with 4 ml of dichloromethane for 30 min. The organic phase (upper layer) was separated through centrifugation at 3,000×*g* (4 °C) for 10 min. The organic phase was transferred to a new polypropylene tube and evaporated to dryness under the nitrogen stream at 40 °C. The residue was reconstituted with 100 μl of the mobile phase and filtered through a 0.22 μm nylon filter membrane, and an aliquot of 40 μl was injected onto the column.

The calibration curves were linear over the concentration range of 2.5–500 ng/ml with correlation coefficients (*r*) of 0.999 or better. Accuracy and precision were assessed by analyzing six aliquots of low (25 ng/ml), medium (100 ng/ml), and high (500 ng/ml) spiked samples with atractylodin. The mean deviation from the theoretical values (accuracy) varied between −0.26% and 7.21%. Low variation of atractylodin assay in plasma samples was observed; coefficients of variation (CV) values were all below 5%. The analytical recovery of sample preparation procedure for atractylodin in plasma samples ranged from 75.6% to 77.4%. Stability analysis showed no significant sample loss over 6 h at room temperature (25 °C), and three freeze-thaw cycles. The selectivity of the chromatographic separation was demonstrated by the absence of interferences from endogenous peaks and commonly used drugs in plasma. The limit of quantification (LOQ) in human plasma samples for atractylodin was accepted as 2.5 ng/ml using 1 ml plasma.

Quality control (QC) samples for atractylodin were made up in plasma samples using a stock solution separate from that used to prepare the calibration curve, at the concentrations 25, 100 and 500 ng/ml (triplicate each) and stored at −80 °C for use with each analytical run. The results of the QC samples provided the basis of accepting or rejecting the run. At least four of every six QC samples had to be within ±20% of their respective nominal value. Two of the six QC samples could be outside the ±20% of their respective nominal values, but not at the same concentration. Results of the assay validation are summarized in the Supplementary document.

2.5.3. Pharmacokinetic analysis

The appropriate pharmacokinetic parameters were estimated from the obtained plasma concentration-time profiles of atractylodin using model-dependent and model-independent analysis approaches[20] using Phoenix/WinNonlin version 8.3 (Pharsight Corporation. 2016, Cary, North Carolina, USA).

For model-independent analysis, the time at which maximum concentrations occurred (t_{max}) and the maximum concentration (C_{max}) were obtained directly from the plasma concentration-time data. The terminal elimination half-life ($t_{1/2z}$) was calculated from log-linear regression of at least three of the last plasma concentration-time data. The area under the curve from zero time to the last observed time ($AUC_{0\text{-}t}$) was calculated using the linear trapezoidal rule for ascending data points, and the log trapezoidal rule for descending data points. The area under the curve, extrapolated from the last data point to infinity, was estimated by dividing the concentration at the last time point by the elimination rate constant (λ_z). The extrapolations contributed to less than 5% of the areas. The AUC from the zero time to infinity ($AUC_{0\text{-}\infty}$) and the area under the first moment curve of the plasma concentration-time profile from time zero to infinity ($AUMC_{0\text{-}\infty}$) were determined. The apparent total body clearance (CL/F) and apparent volume of distribution (V_z/F) associated with the terminal phase were calculated as $CL/F = dose/AUC_{0\text{-}\infty}$ and $V_z/F = [CL/F]/\lambda_z$. The mean residence time (MRT) was calculated from the ratio of $AUMC_{0\text{-}\infty}$ and $AUC_{0\text{-}\infty}$.

For the purpose of future stimulation and prediction as well as pharmacokinetic/pharmacodynamic modelling, the compartment open model (one- or two-) with first-order absorption and elimination with absorption lag-time was fitted to the data by iterative, weighted non-linear regression. The observed concentrations of atractylodin were weighted as the reciprocal of the analytical variance. The adequacy of the pharmacokinetic models chosen was based on statistical methods for assessing the validity of the models for describing the concentration-time data, *i.e.,* F-ratio test, Akaike information criterion (AIC), Schwartz and Imbimbo criteria.

2.6. Statistical analysis

Statistical analysis was performed using SPSS for Windows Software version 12 (IBM, New York, USA). Nonparametric analysis approach was applied for data not conforming to normal distribution. Quantitative data are summarized as median (range) and

qualitative data are summarized as number and percentage values. Comparison of two independent quantitative variables was performed using Mann-Whitney *U* test and comparison of two dependent quantitative variables was performed using Wilcoxon Signed Rank test. Statistical significance level was set at $\alpha = 0.05$ for all tests.

3. Results

3.1. Demographic and baseline clinical and laboratory data

Demographic and baseline laboratory data of all 48 volunteers included in *group 1*, and *group 2* participants (AL and placebo) are summarized in Table 1. There was no significant difference between all parameters in participants allocated to both groups as well as in each group between AL and placebo-treated groups.

All research participants were healthy as verified by results of clinical (physical examination, vital signs, chest X-ray, and ECG) and laboratory assessments (Tables 2–4). Almost all of the laboratory parameters (hematology and serum biochemistry), vital signs and ECG were within normal ranges. Total cholesterol, LDL (low-density lipoprotein), HDL (high-density lipoprotein), and CPK (creatinine phosphokinase) in 10 (41.7%), 10 (41.7%), 12 (50%), and 16 (66.67%) participants were higher than the upper limits, without clinical signs and symptoms. Significant changes in RBC count, hemoglobin and hematocrit on days 4 and 7 were found in participants in *group 2* compared with baseline levels. All of these values lied within normal ranges and returned to baseline levels within one week.

3.2. Safety and tolerability assessments following drug administration

There was no adverse event reported in any research participants in neither group, (AL nor placebo). The AL extract was well tolerated in all healthy participants in both groups, which was verified by the absence of significant clinical as well as laboratory-associated adverse events/adverse reactions. There was no changes in ECGs, no prolongation of QTc interval. No significant clinical signs and symptoms were reported in participants who had total cholesterol, LDL, HDL and CPK (about 50% of the participants) higher than the upper limits at baseline and during the study period.

3.3. Pharmacokinetics

Model-independent analysis: Median (range) plasma concentration-time profile of atractylodin on day 1 (10 males and 10 females) in *group 1* following a single oral dose of 1,000 mg capsule formulation of the standardized AL extract is presented in Fig. 1A, and the pharmacokinetic parameters analyzed by model-independent approach are summarized in Table 3. Median (range) plasma concentration-time profiles of atractylodin on days 1 and 21 (10 males and 10 females) in *group 2* following daily oral doses of 1,000 mg capsule formulation of standardized AL extract are presented in Fig. 1B, and the pharmacokinetic parameters analyzed by model-independent approach are summarized in Table 5. Fig. 2 presents plasma concentrations of atractylodin (1 h after dosing) during days 5–20 in 20 healthy participants in *group 2* participants following daily oral doses of 1,000 mg capsule formulation of the standardized AL extract. Plasma atractylodin concentrations at each time point of blood collection in both groups varied between 39.5% and 102.2% during 0–6 h of drug administration. After 6 h, atractylodin was undetectable in the plasma of all participants. Following a single oral dose (*group 1*), atractylodin was detected at the first time of blood sampling (0.25 h) in 10 participants (50%), with the levels varying between 1.5 ng/ml and 11.2 ng/ml. In most cases, concentrations were measurable until 4 h of drug administration, with levels varying between 3.5 ng/ml and 20.5 ng/ml. Following daily oral doses (*group 2*), atractylodin was detected at the first time of blood sampling (0.5 h) of days 1 and 21 in 17 participants (85%) and 19 participants (95%), respectively, with the levels varying between 1.5 ng/ml and 11.2 ng/ml. In most cases, concentrations were measurable until 4 h of drug administration (2.5–18.2 ng/ml). Interindividual variation was observed for most pharmacokinetic parameters, ranging from 19.6% to 49.1%. There was no significan difference in the pharmacokinetic parameters of atractylodin following a single or multiple dosing. Plasma concentrations of atractylodin at 1 h of dosing on days 5, 7, 9, 11, 14, 16, 18, and 20 in participants receiving multiple dosing of AL were comparable to the C_{max} observed on days 1 and 21 of dosing**.** Males and females showed a significant difference only in CL/F.

Model-dependent analysis: The observed and predicted (median) plasma concentration-time profiles of atractylodin on day 1 (10 males and 10 females) in *group 1* participants following a single oral dose of 1,000 mg capsule formulation of the standardized AL extract is presented in Fig. 3 and the pharmacokinetic parameters analyzed by model-dependent approach (1-compartment open model with absorption lag time and first-order absorption and elimination) are summarized in Table 6. The observed and predicted (median) plasma concentration-time profiles of atractylodin on day 1 and day 21 (10 males and 10 females) in *group 2* participants are presented in Fig. 4 and the pharmacokinetic parameters analyzed by model-independent approach (1-compartment open model with absorption lag time and first-order absorption and elimination) are summarized in Table 6. There was no significant difference in the pharmacokinetic parameters of atractylodin

Table 1
Demographics and baseline vital signs of 40 healthy Thai study participants (20 males, 20 females) allocated to *group 1* (a single dose of 1,000 mg capsule formulation of the standardized AL extract) and *group 2* (daily doses of 1,000 mg capsule formulation of the standardized AL extract for 21 days). Data are presented as number or median (range) values.

	Group 1		Group 2	
	AL	Placebo	AL	Placebo
Male: Female (n)	10, 10	2, 2	10, 10	2, 2
Age (years)	22.0 (21.0–27.0)	23.2 (20.9–27.5)	25.0 (21.0–29.0)	23.9 (20.8–28.3)
Body weight (kg)	62.8 (54.9–65.5)	63.4 (55.5–62.8)	61.0 (56.7–66.2)	62.2 (56.2–64.7)
Height (cm)	163.0 (158.0–170.5)	164.8 (159.5–172.5)	165.0 (161.0–170.5)	164.5 (159.0–169.5)
BMI	23.07 (21.1–24.6)	22.9 (22.2–24.4)	22.41 (20.74–24.17)	23.40 (21.55–24.89)
Systolic blood pressure (mmHg)	120 (110–130)	120 (102–130)	121 (116–129)	122 (108–126)
Diastolic blood pressure (mmHg)	67 (60–74)	66 (60–72)	69 (62–78)	66 (62–74)
Heart rate (/min)	69 (61–74)	70 (64–76)	72 (65–78)	71 (62–74)
Respiratory rate (/min)	22 (20–22)	22 (20–22)	22 (21–22)	21 (10–22)
Body temperature (°C)	36.5 (36.4–36.7)	36.6 (36.2–37.0)	36.6 (36.5–36.8)	36.3 (36.4–36.9)

Table 2
Laboratory data of 40 healthy Thai study participants (20 males, 20 females) allocated to *group 1* (a single dose of 1,000 mg capsule formulation of the standardized AL extract) and *group 2* (daily doses of 1,000 mg capsule formulation of the standardized AL extract for 21 days). Data are presented as median (range) or percentage (%) values.

A. Hematology

Parameters	Normal range	Group 1				Group 2			
		Placebo (n = 4) Day 0	AL (n = 20)			Placebo (n = 4) Day 0	AL (n = 20)		
			Day 0	Day 4	Day 14		Day 0	Day 4	Day 21
WBC (x10^3/μl)	4.0–11.0	6.02 (5.05–7.23)	6.31 (5.55–7.30)	5.99 (5.70–7.30)	5.97 (4.92–7.54)	6.02 (5.11–7.09)	6.25 (5.05–7.24)	5.99 (4.91–6.44)	6.16 (4.53–6.68)
Platelets (x10^3/μl)	150-400	249.5 (215–286)	252.5 (217–279)	251.5 (216–279)	255 (241–306)	266.0 (245–282)	261.0 (221–279)	262.5 (251–293)	270 (245–314)
RBC (x10^6/μl)[a]	4.5–6.0	5.02 (4.30–5.48)	5.10 (4.47–5.52)	4.91 (4.43–5.30)	4.94 (4.08–5.52)	4.80 (4.60–5.03)	4.99 (4.69–5.23)	4.82 (4.64–4.92)	4.70 (4.33–5.10)
Hemoglobin (mg/dL)[b]	Male:14.0–18.0 Female: 12.0–16.0	13.9 (12.9–14.8)	13.8 (13.1–14.6)	13.65 (12.0–14.3)	13.5 (11.9–14.4)	14.2 (13.0–14.9)	14.4 (13.0–14.9)	13.5 (12.4–14.3)	13.1 (12.2–14.6)
Hematocrit (%)[c]	Male:39.0–57.0 Female: 36.0–48.0	42.22 (38.8–44.9)	42.15 (39.6–44.2)	41.6 (39.1–45.4)	42.5 (36.8–44.7)	42.12 (39.2–45.8)	43.25 (39.6–46.0)	41.1 (38.9–44.2)	41.5 (38.5–45.3)
Neutrophil (%)	45-75	55.9 (52.5–58.0)	54.4 (52.5–58.0)	54.7 (47.6–59.8)	57.0 (51.9–62.3)	54.2 (50.8–56.6)	55.2 (52.4–59.9)	54.75 (51.7–57.6)	53.30 (48.9–57.3)
Lymphocyte (%)	20-45	37.3 (33.9–40.6)	37.5 (34.1–41.3)	37.2 (33.2–41.7)	34.6 (31.6–39.7)	37.55 (33.2–40.1)	37.35 (32.2–41.7)	38.65 (35.1–41.0)	38.00 (35.4–40.7)
Monocyte (%)	2.0–10.0	3.50 (3.20–4.20)	3.80 (3.30–4.60)	3.60 (3.0–4.3)	3.6 (2.9–4.5)	3.20 (3.2–4.3)	3.30 (3.0–4.1)	3.25 (3.2–4.7)	3.10 (3.2–4.9)
Eosinophil (%)	4.0–6.0	2.55 (1.70–5.20)	2.75 (1.60–5.50)	3.65 (2.0–6.1)	2.7 (2.1–5.5)	2.89 (2.5–3.8)	3.00 (2.3–4.0)	2.85 (2.5–4.0)	3.70 (2.9–3.7)
Basophil (%)	0.0–1.0	0.4 (0.3–0.5)	0.4 (0.4–0.5)	0.4 (0.3–0.5)	0.4 (0.3–0.5)	0.35 (0.3–0.5)	0.35 (0.3–0.4)	0.35 (0.3–0.5)	0.40 (0.3–0.5)
PT (sec)	10.2–12.6	11.25 (11.0–12.2)	11.85 (11.3–12.0)	11.45 (11.1–11.8)	11.5 (11.3–11.7)	11.16 (10.8–11.5)	11.2 (11.0–11.9)	11.15 (10.9–11.7)	11.0 (10.6–11.6)
INR (sec)	2-3	1.002 (0.93–1.01	1.005 (0.95–1.02)	0.965 (0.94–1.00)	0.97 (0.95–0.99)	0.94 (0.91–1.02)	0.945 (0.93–1.01)	0.945 (0.92–0.99)	0.93 (0.89–0.98)
PTT (sec)	22.2–28.3	26.1 (25.5–28.6)	26.4 (25.9–29.1)	26.2 (24.9–27.5)	26.0 (24.4–27.5)	25.4 (24.2–27.5)	25.9 (24.3–27.9)	25.5 (24.0–27.8)	24.0 (22.6–26.2)

B. Biochemistry

Parameters	Normal range	Group 1				Group 2			
		Placebo (n = 4) Day 0	AL (N = 20)			Placebo (n = 4) Day 0	AL (n = 20)		
			Day 0	Day 4	Day 14		Day 0	Day 4	Day 21
Creatinine (mg/dl)	0.67-1.17	0.85 (0.70–0.99)	0.84 (0.73–1.03)	0.88 (0.73–0.97)	0.87 (0.74–0.97)	0.84 (0.75–1.06)	0.82 (0.73–0.99)	0.83 (0.74–0.90)	0.87 (0.79–1.07)
BUN (mg/dl)	7-18	12.40 (10.5–13.3)	12.55 (10.8–12.3)	13.00 (10.5–14.9)	12.90 (9.8–15.1)	12.46 (9.5–13.0)	12.5 (9.9–13.6)	12.45 (9.9–13.5)	12.4 (9.9–13.0)
Uric acid (mg/dl)	3.7–7.2	4.70 (3.70–5.50)	4.80 (3.80–5.70)	4.65 (3.70–5.60)	4.50 (3.80–6.10)	4.80 (3.82–5.70)	4.85 (3.80–5.90)	4.80 (3.80–5.80)	4.80 (3.80–6.10)
AST (U/l)	15-37	16.5 (15.5–20.0)	16.0 (15.0–18.0)	16.5 (15.0–22.0)	15.0 (16.0–20.0)	14.5 (15.0–18.0)	14.0 (15.0–18.0)	15.5 (16.0–19.0)	17.0 (16.0–21.0)
ALT (U/l)	16-63	27.0 (18.0–35.0)	30.0 (20.0–39.0)	27.5 (20.0–35.5)	25.0 (20.0–33.0)	28.0 (18.5–34.0)	28.5 (19.0–33.0)	30.0 (22.0–35.0)	29.0 (24.0–37.0)
Direct Bilirubin (mg/dl)	0.0–0.20	0.18 (0.20–0.20)	0.20 (0.20–0.20)	0.20 (0.10–0.20)	0.18 (0.10–0.20)	0.10 (0.10–0.20)	0.10 (0.10–0.20)	0.10 (0.10–0.20)	0.10 (0.10–0.18)
Total Bilirubin (mg/dl)	0.2-1.00	0.65 (0.50–0.80)	0.65 (0.60–0.90)	0.60 (0.40–0.60)	0.60 (0.40–0.90)	0.52 (0.43–0.65)	0.55 (0.40–0.70)	0.50 (0.40–0.60)	0.50 (0.40–0.60)
Total Protein (mg/dl)	6.4–8.2	7.65 (7.60–8.30)	7.95 (7.70–8.20)	7.70 (7.50–8.20)	7.70 (7.60–7.90)	7.80 (7.40–8.10)	7.90 (7.50–8.30)	7.80 (7.30–8.10)	7.70 (7.50–8.00)
Albumin (mg/dl)	3.5-4.0	4.15 (3.90–4.26)	4.20 (4.00–4.30)	4.10 (3.90–4.30)	4.10 (3.90–4.30)	4.10 (4.00–4.50)	4.20 (4.00–4.40)	4.00 (3.90–4.30)	4.00 (3.80–4.40)
LDH (U/l)	207-414	310.5 (299–330)	317 (300–336)	311.5 (298–362)	309 (295–364)	300.5 (282–332)	297.5 (274–332)	302.0 (278–323)	292.0 (268–316)
ALP (U/l)	46-116	58.0 (50.0–78.0)	60.5 (53.0–71.0)	57.5 (49.0–70.0)	61.0 (48.0–70.0)	58.5 (50.5–70.0)	58.0 (50.0–73.0)	58.5 (49.0–73.0)	59.0 (49.0–70.0)
CPK (U/l)	38-174	139.5 (101.0–227.0)	140.5 (89.0–227.0)	140.5 (93.0–296.0)	139.0 (103.0–296.0)	132.5 (90.0–169.0)	130.0 (89.0–165.0)	133.5 (83.0–168.0)	135.0 (86.0–177.0)
Phosphorus (mg/dl)	2.5-4.9	4.0 (3.8–4.2)	4.0 (3.7–4.1)	Not applicable	Not applicable	3.80 (3.70–4.10)	3.80 (3.70–4.30)	Not applicable	Not applicable
Calcium (mEq/l)	8.5–10.1	9.60 (9.40–9.50)	9.70 (9.60–10.00)	Not applicable	Not applicable	9.70 (9.20–10.00)	9.75 (9.50–10.00)	Not applicable	Not applicable
Total cholesterol (mg/dl)	0-200	200 (170–220)	203 (177–231)	202 (171–217)	210 (165–221)	203 (182–220)	203.5 (192–211)	196.5 (185–213)	207.0 (193–242)
Triglycerides (mg/dl)	0-150	63.0 (40.0–70.0)	62.0 (43.0–72.0)	60.5 (44.0–79.0)	65.0 (46.0–92.0)	63.5 (43.5–90.5)	64.0 (43.0–93.0)	64.0 (48.0–95.0)	67.0 (47.0–105.0)
LDL (mg/dl)	0-100	127.0 (112.0–155.0)	127.0 (112.0–155.0)	128.5 (115.0–142.0)	127.0 (103.0–152.0)	124.0 (114.0–140.0)	124.5 (118.0–139.0)	123.5 (112.0–145.0)	130.0 (115.0–145.0)
HDL (mg/dl)	40-60								

Table 2 (*continued*)

B. Biochemistry									
Parameters	Normal range	Group 1				Group 2			
		Placebo (n = 4) Day 0	AL (N = 20)			Placebo (n = 4) Day 0	AL (n = 20)		
			Day 0	Day 4	Day 14		Day 0	Day 4	Day 21
			61.0 (55.0 −73.0)	61.5 (51.0 −68.0)	60.0 (52.0 −69.0)	60.5 (53.9 −67.9)	60.5 (52.0 −68.0)	59.0 (55.0 −65.0)	61.0 (54.0 −67.0)

C. Urinalysis									
Parameters	Normal range	Group 1				Group 2			
		Placebo (n = 4) Day 0	AL (n = 20)			Placebo (n = 4) Day 0	AL (n = 20)		
			Day 0	Day 4	Day 14		Day 0	Day 4	Day 21
Specific gravity	1.003-1.029	1.018 (1.015 −1.024)	1.020 (1.016 −1.025)	1.020 (1.015 −1.025)	1.015 (1.010 −1.020)	1.020 (1.018 −1.026)	1.020 (1.020 −1.025)	1.020 (1.015 −1.025)	1.020 (1.015 −1.023)
pH	4.5–7.8	6.3 (6.0–7.0)	6.5 (6.0–7.0)	6.0 (6.0–7.0)	6.0 (5.5–6.5)	6.3 (6.0–7.0)	6.5 (6.0–7.0)	6.5 (6.0–7.0)	6.5 (6.0–7.0)
Protein:	Negative/	100%	100%	35	74	90	95	90	74
Negative (%)	Trace	0	0	64	26	10	5	10	25
Trace (%)		0	0	0	0	0	0	0	0
1+ (%)		0	0	0	0	0	0	0	0
2+ (%)									
Glucose:	Negative	100	100	100	100	100	100	100	100
Negative (%)									
Ketones:	Negative	100	100	100	100	100	100	100	100
Negative (%)		0	0	0	0	0	0	0	0
Trace (%)		0	0	0	0	0	0	0	0
1+ (%)		0	0	0	0	0	0	0	0
2+ (%)									

a Statistical significant difference: Group 2 between Day 4 vs. Day 0 (p = 0.01) and Day 21 vs. Day 0 (p = 0.02, Wilconxon Sign-Rank test).
b Statistical significant difference: Group 2 between Day 4 vs. Day 0 (p = 0.01) and Day 21 vs. Day 0 (0.02, Wilcoxon Signed Rank test).
c Statistical significant difference: Group 2 between Day 4 vs Day 0 (p = 0.03)and Day 21 vs. Day 0 (p = 0.019, Wilcoxon Signed Rank test).

Table 3
ECG data of 40 healthy Thai study participants (20 males, 20 females) allocated to group 1 (a single dose of 1,000 mg capsule formulation of the standardized AL extract) and group 2 (daily doses of 1,000 mg capsule formulation of the standardized AL extract for 21 days). Data are presented as median (range) or percentage (%) values.

	Normal range	Palcebo (n = 4) Day 0 H 0	AL (n = 20)						
			Day 0				Day 5	Day 7	Day 14
			H 0	H 2	H 6	H 12			
Ventricular rate (bpm)	60-100	64 (62–76)	63 (60–72)	63.5 (62–76)	63 (59–68)	63 (64–68)	65 (62–70)	63 (62–68)	64.5 (62–72)
RR interval (msec)	600-1200	940 (870 −1040)	938 (846 −1043)	931 (908 −1014)	949 (874 −1010)	949 (887 −1012)	914 (852 −1049)	973 (879 −1059)	998 (830 −1300)
PR interval (msec)	120-200	156 (140–174)	152 (136–162)	155 (146–170)	151 (138–164)	156 (144–166)	150 (144–164)	150 (140–166)	156 (128–166)
QRS interval (msec)	80-100	88 (80–90)	90 (88–94)	90 (80–94)	89 (84–96)	89 (84–94)	90 (86–96)	91 (88–96)	90 (88–96)
QTc interval (msec)	<430	420 (390–410)	419 (396–430)	420.5 (404 −430)	415 (403–430)	413 (404–423)	419 (417–430)	414.5 (402 −429)	414 (381–438)

observed following a single or multiple dosing. Males and females showed significant difference in C_{max} (day 1), $AUC_{0-\infty}$ (days 1 and 21), and CL/F (day 21).

4. Discussion

To our knowledge, the study is the first that evaluated safety and pharmacokinetics of atractylodin in humans after oral dose administration of CMC capsule formulation of the standardized AL rhizome extract. Previous clinical studies were conducted in patients with different diseases/symptoms or healthy participants using AL as a component of the herbal formulations.[21–25] The herbal extract was well tolerated in all healthy participants in both groups, which was verified by the absence of significant clinical as well as laboratory-related adverse events/adverse reactions. High level of the CPK (creatinine phosphokinase) enzyme found in some participants could be due to regular and intensive exercises in some participants who were sport science students. Precautionary use of AL has been suggested in individuals with bleeding disorders due to the possibility of AL to increase the risk of bleeding. The suggestion is based on the inhibitory effects of AL on 5-lipoxygenase (5-LOX) and cyclooxygenase (COX) enzymes[26] and on collagen-induced platelet aggregation observed in the *in vitro* model.[27] In platelets, arachidonic acid is metabolized by COX-1 to prostaglandins PGG_2 and PGH_2, which is further metabolized by thromboxane synthase to thromboxane A2 (TXA_2), a potent activator of platelet aggregation. It is possible that the signalling pathway that might be the target of AL on the inhibition of platelet aggregation is through the activation of phospholipase A2 (PLA_2) and generation of TXA_2.[27] In this study, no clinical sign nor significant changes in laboratory parameters (prolongation of PT, PTT and INR) was found. Nevertheless, monitoring of blood coagulation profiles in individuals with abnormal blood coagulation who will receive AL is suggested, particularly CCA patients with signs and symptoms of liver failure. In animal studies in rats and mice, standardized AL extract, as well as the current CMC capsule formulation of the standardized AL

Table 4
ECG data of 20 healthy 40 healthy Thai study participants (20 males, 20 females) allocated to *group 1* (a single dose of 1,000 mg capsule formulation of the standardized AL extract) and *group 2* (daily doses of 1,000 mg capsule formulation of the standardized AL extract for 21 days). Data are presented as median (range) or percentage (%) values. Data are presented as median (range) or percentage (%) values.

Parameters	Normal range	Placebo (n = 4) Day 0 H 0	AL (n = 20) Day 0				Day 1	Day 2	Day 5
			H0	H2	H6	H12			
Ventricular rate (bpm)	60-100	67.0 (64–78)	67.5 (66–75)	65.5 (67–71)	66 (65–73)	65 (62–68)	63 (64–70)	67.5 (62–74)	73.5 (63–79)
RR interval (msec)	600-1200	910.0 (890–1020)	876.5 (789–984)	937 (839–1041)	898.5 (812–1005)	919 (872–984)	942 (877–1042)	865 (799–959)	813 (753–951)
PR interval (msec)	120-200	144.2 (138–156)	144.5 (140–154)	149 (140–162)	148 (144–160)	146 (140–154)	146 (142–158)	148 (140–162)	145 (140–160)
QRS interval (msec)	80-100	90 (89–92)	94 (86–96)	89 (84–96)	92 (88–98)	91 (84–100)	93 (84–98)	95 (86–100)	93 (88–101)
QTc interval (msec)	<430	414 (410–428)	414 (410–428)	408 (412–422)	408.5 (412–426)	408 (410–427)	419[a] (407–443)	406.5 (410–431)	407 (409–429)

	Normal range	Day 7	Day 9	Day 11	Day 14	Day 18	Day 20
Ventricular rate (bpm)	60–100	69 (62–76)	66.5 (62–77)	68 (62–75)	69 (63–77)	68 (63–76)	72 (64–80)
RR interval (msec)	600–1200	866 (782–982)	895 (779–961)	881 (796–996)	865 (773–944)	875 (783–974)	825 (745–976)
PR interval (msec)	120–200	152 (144–166)	148 (134–158)	150 (140–162)	148 (138–160)	154 (136–162)	152 (144–162)
QRS interval (msec)	80–100	98 (88–104)	94 (88–100)	94 (88–100)	96 (90–98)	96 (92–98)	94 (86–100)
QTc interval (msec)	<430	414 (412–423)	410 (401–421)	409 (407–417)	410 (405–413)	411 (401–419)	412 (404–416)

	Normal range	Day 21				Day 22	Day 23
		H0	H2	H6	H12		
Ventricular rate (bpm)	60–100	72 (65–76)	63 (58–69)	66 (59–74)	65 (60–71)	61 (59–72)	65 (61–78)
RR interval (msec)	600–1200	828 (783–921)	940 (867–1031)	903 (805–1013)	914 (840–991)	968 (826–1006)	918 (763–981)
PR interval (msec)	120–200	150 (140–164)	152 (140–164)	150 (138–164)	150 (140–156)	150 (138–164)	150 (138–160)
QRS interval (msec)	80–100	96 (88–100)	90 (88–100)	94 (88–98)	90 (88–98)	96 (88–100)	96 (88–102)
QTc interval (msec)	<400	408 (402–420)	408 (401–420)	408 (402–417)	404 (402–421)	414 (402–421)	403 (402–417)

extract, were shown to be non-toxic at the highest dose level of 5,000 mg/kg body weight in both acute and subacute toxicity tests.[5,15] No significant signs and symptoms were observed except stomach irritation and general CNS depressant signs (reduced alertness and locomotion, and decreased response to touch and balance) which occurred at the highest 5,000 mg/kg body weight dose level.[15] All of these symptoms occurred within 1 h and subsided within 2 h of administration. The muscle relaxant activity of β-eudesmol on the post-synaptic neuromuscular junction (NMJ) was shown to be through an inhibitory effect on nicotinic acetylcholine receptor.[28–30] The effect was more prominent in diabetes patients.[28,31] Although the side effects of AL on the nervous system have not been demonstrated in humans, the clinical use of AL in individuals with nervous system-related signs/symptoms should be with caution, particularly in diabetes patients.

Pharmacokinetic study of herbal medicine has, in recent years becomes one of the important research interests. However, unlike western medicines, the pharmacological actions of herbal medicine are thought to result from the synergistic integration of multi-components, and multi-targets/multi-pathways. Therefore, the pharmacokinetics of herbal medicine is relatively more complicated than synthetic drugs. Information on the pharmacokinetic profile of a drug helps to understand the relationship between intensity and time courses of pharmacological and toxicological effects of phytochemicals in the human body. Several major constituents have been isolated and identified in AL rhizomes with various pharmacological activities.[32] These include atractylodin, atractylone, atractylenolide I, atractylenolide II, atractylenolide III, β-eudesmol, β-sitosterol, hinesol, and stigmasterol. Among these, only atractylodin and β-eudesmol have been demonstrated in the bioassay-guided activity to exert potent anti-CCA activities with comparable potency (IC_{50} 20–25 μg/ml).[5] In the present study, the pharmacokinetics of the CMC capsule formulation of AL was characterized using the bioactive marker atractylodin. The compound was also used as an analytical marker for quality control of the AL extract.[11] Due to the instability nature of β-eudesmol (9–10% of total extract), development of the analytical method in human plasma with adequate sensitivity was not possible. In a previous study, β-eudesmol was quantified in rat plasma using LC-MS/MS (limit of quantification = 3 ng/ml) following the administration of the pure compound, *i.e.*, intravenous bolus and intragastric doses of β-eudesmol at 2 and 50 mg/kg body weight, respectively.[33] The analytical method for the determination of AL components in human plasma remains challenging; it may be wise to consider for the alternatives. Both atractylodin and β-eudesmol have been shown to act synergistically when given in combination with hinesol, the component with weak activity.[34] This implies that further pharmacokinetic study of AL could be based on the measurement of total bioactivity of all components of the AL extract rather than a single compound. This is another area to explore and confirm in future studies.

Marked variability in plasma concentration-time profiles and pharmacokinetic parameters of atractylodin was observed in both groups (*group 1* and *2*) of participants following a single oral dose of 1,000 mg of capsule formulation of the standardized AL extract and daily oral doses of 1,000 mg AL extract for 21 days (each dose is equivalent to 2.45 mg atractylodin). The model-independent analysis revealed that oral absorption of atractylodin was rapid but variable, with the observed t_{max} and C_{max} ranging from 0.5 to 2 h and 7.1–152.7 ng/ml, respectively. Atractylodin was cleared in plasma of most participants within 4 h of administration. The systemic bioavailability reflected by $AUC_{0-\infty}$ was relatively low due to the rapid systemic clearance (CL/F: 2.87–14.82 l/kg/h) and large apparent volume of distribution (V_z/F: 3.58–31.55 l/kg). Besides, the low oral bioavailability of atractylodin could be explained by the relatively low content of the compound in the extract and the formulation. Physicochemical property of atractylodin of being low water solubility could also be another factor that limits the

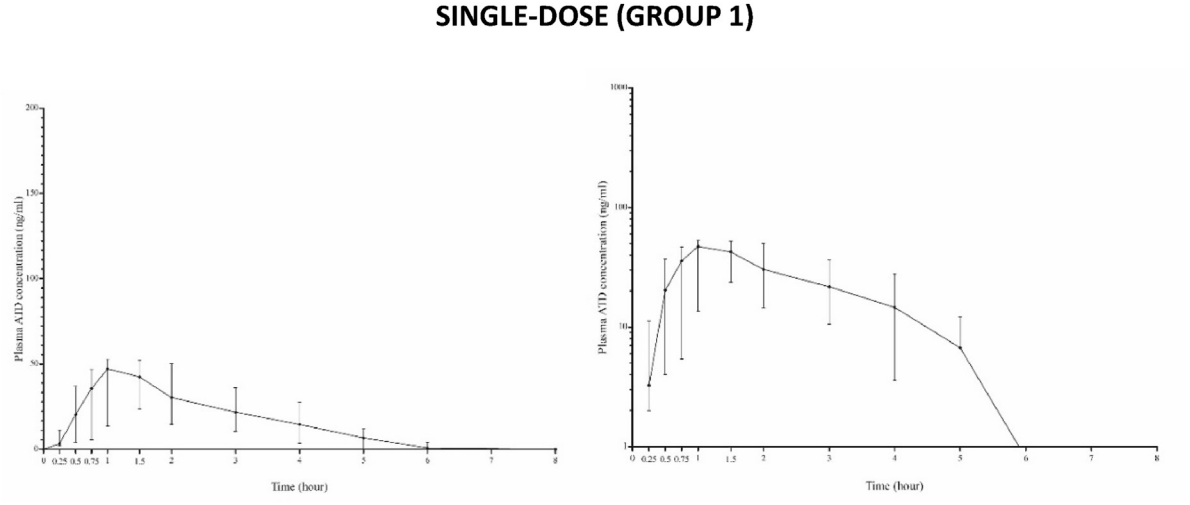

Fig. 1. Median (range) plasma concentration-time profiles of atractylodin (ATD) in 20 healthy subjects on day 1 following a single oral dose of 1,000 mg in *group 1* (A) and on day 1 and 21 following daily oral doses of 1,000 mg in *group 2* (B) of capsule formulation of the standardized AL extract. Plasma ATD concentrations are presented in normal and semi-log scales.

Table 5
Pharmacokinetic parameters of atractylodin by model-independent analysis following a single dose of 1,000 mg (group 1) and daily doses of 1,000 mg for 21 days (group 2) of the capsule formulation of standardized AL extract.

Pharmacokinetic parameters	Group 1	Group 2	
		Day 1	Day 21
All			
N	10 M, 10 F	10 M, 10 F	10 M, 10 F
C_{max} (ng/ml)	50.35 (13.8–52.90)	47.10 (7.10–129.90)	46.95 (20.10–152.70)
t_{max} (h)	1 (0.75–1.5)	1.0 (0.5–2.0)	1.0 (0.5–2.0)
AUC_{0-t} (ng.h/ml)	118.27 (72.47–169.90)	93.06 (12.13–276.28)	106.67 (40.42–259.81)
$AUC_{0-\infty}$ **(ng.h/ml)**	123.70 (75.13–176.96)	112.2 (20.11–306.76)	117.75 (48.73-269-21)
λ (/h)	0.62 (0.47–0.89)	0.60 (0.19–1.11)	0.61 (0.32–0.87)
$t_{1/2z}$ (h)	1.13 (0.78–1.48)	1.16 (0.63–3.75)	1.14 (0.80–2.17)
CL/F (l/kg/h)	6.68 (4.46–13.70)	7.33 (2.87–33.43)	6.80 (2.95–14.82)
V_z/F (l/kg))	11.45 (7.06–20.17)	12.75 (4.44–61.56)	12.85 (3.58–31.55)
MRT (h)	2.42 (1.74–3.11)	2.60 (1.58–5.17)	2.64 (1.58–4.54)
Male			
N	10 M, 10 F	10 M, 10 F	10 M, 10 F
C_{max} (ng/ml)	59.20 (41.80.50–99.30)	41.90 (7.10–67.20)	44.0 (22.0–107.0)
t_{max} (h)	1.0 (0.75–2.0)	1.0 (1.0–2.0)	1.0 (1.0–2.0)
AUC_{0-t} (ng.h/ml)	142.14 (83.48–199.75)	82.88 (12.13–181.08)	75.38 (40.42–259.81)
$AUC_{0-\infty}$ **(ng.h/ml)**	146.88 (86.09–226.53)	92.40 (20.11–210.23)	91.67 (48.73-269-21)
λ (/h)	0.85 (0.29–1.10)	0.64 (0.19–1.11)	0.64 (0.42–0.82)
$t_{1/2z}$ (h)	1.23 (0.13–2.77)	1.09 (0.63–3.75)	1.08 (0.84–1.65)
CL/F (l/kg/h)	6.29 (2.80–8.92)	8.38 (3.77–33.43)	7.90 (2.95–14.82)
V_z/F (l/kg))	11.49 (8.35–56.42)[a]	13.96 (6.73–61.56)	13.79 (3.58–22.13)
MRT (h)	2.42 (1.71–2.94)	2.47 (1.58–5.17)	2.64 (1.95–3.08)
Female			
N	10 M, 10 F	10 M, 10 F	10 M, 10 F
C_{max} (ng/ml)	58.65 (42.50–96.80)	54.35 (16.10–129.90)	52.55 (20.10–152.70)
t_{max} (h)	1.0 (0.75–1.0)	1.0 (0–0.5)	1.0 (0.5–2.0)
AUC_{0-t} (ng.h/ml)	122.67 (72.47–192.85)	99.67 (42.88–276.28)	118.05 (42.97–212.09)
$AUC_{0-\infty}$ **(ng.h/ml)**	128.13 (75.13–199.88)	139.56 (70.21–248.22)	145.54 (67.49–232.33)
λ (/h)	0.71 (0.37–0.92)	0.52 (0.40–0.82)	0.54 (0.32–0.87)
$t_{1/2z}$ (h)	1.18 (0.72–2.19)	1.36 (0.85–1.75)	1.28 (0.80–2.17)
CL/F (l/kg/h)	6.87 (4.48–13.70)	6.43 (2.87–15.00)	5.93 (3.79–11.38)
V_z/F (l/kg))	12.11 (6.62–20.17)	11.97 (4.44–37.88)	12.12 (5.11–31.55)
MRT (h)	2.21 (1.70–3.74)	2.66 (1.70–3.99)	2.61 (1.58–4.54)

[a] Statistically significant difference from females (p 0.013, Mann-Whitney *U* test).

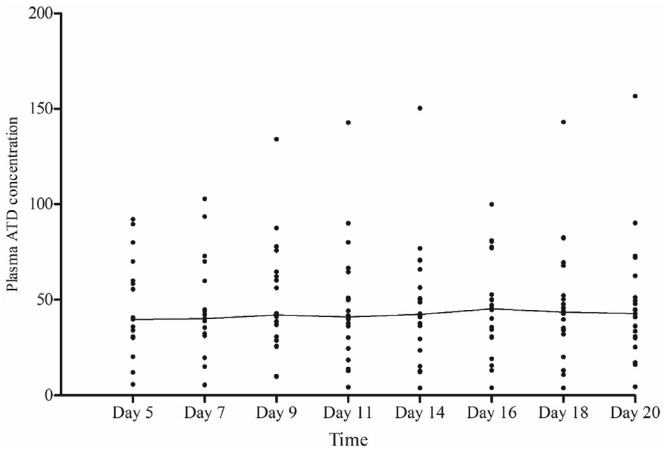

Fig. 2. Plasma concentrations of atractylodin (ATD) during days 5–20 (1 h after dosing) in 20 healthy subjects in group 2 following daily oral doses of 1,000 mg capsule formulation of standardized AL extract.

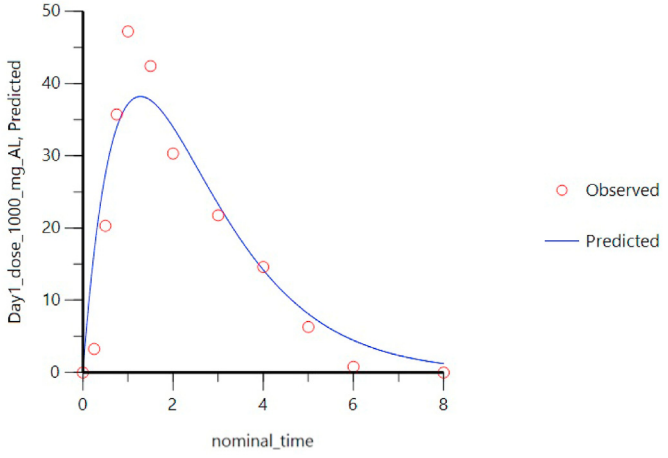

Fig. 3. Observed and predicted plasma concentration-time profiles (median values) of atractylodin (ATD) analyzed by 1-compartment model in healthy subjects in *group 1* following a single oral dose of 1,000 mg capsule formulation of the standardized AL extract (n = 20) on day 1.

bioavailability of this compound.[9] High sensitivity of the analytical assay is required for determination of atractylodin in plasma, which is the limitation of the currently available assay methods. Atractylodin exerted no accumulation and dose-dependency characteristics in human plasma after multiple oral dosing for 21 days. The pharmacokinetics of atractylodin following a single and multiple dosing was comparable. Besides, plasma concentrations at 1 h (the average t_{max}) of dosing on days 5, 7, 9, 11, 14, 16, 18 and 20 in the group receiving multiple dosing (*group 2*) were also comparable to those of day 1 and day 21. This suggests the absence of auto-induction or auto-inhibition of metabolism/biotransformation of

Table 6
Pharmacokinetic parameters of atractylodin by model-dependent analysis (1-compartment model) following a single dose of 1,000 mg (group 1) and daily doses of 1,000 mg for 21 days (group 2) of the capsule formulation of standardized AL extract.

Pharmacokinetic parameters	Group 1	Group 2	
		Day 1	Day 21
All			
N	10 M, 10 F	10 M, 10 F	10 M, 10 F
C_{max} (ng/ml)	29.1 (17.1–42.51)	32.11 (4.9–117.8)	31.84 (9.38–97.64)
t_{max} (h)	1.41 (0.94–2.3)	1.18 (0.17–2.34)	1.32 (0.24–9.44)
t_{lag} (h)	0.13 (0.0–0.5)	0 (0–0.5)	0 (0–0.5)
K_{01} (/h)	0.68 (0.43–1.00)	0.83 (0.43–22.67)	0.74 (0.10–1.92)
$t_{1/2a}$ (h)	0.97 (0.41–1.58)	0.84 (0.03–1.63)	0.94 (0.36–6.61)
$AUC_{0-\infty}$ **(ng.h/ml)**	105.07 (65.93–168.90)	103.7 (16.5–315.4)	123.18 (49.22–419.61)
k_{10} (/h)	0.71 (0.44–1.71)	0.71 (0.43–1.25)	0.73 (0.11–15.31)
$t_{1/2z}$ (h)	0.88 (0.37–1.74)	0.84 (0.03–1.63)	0.94 (0.05–6.49)
CL/F (l/kg/h)	7.28 (4.78–15.62)	8.11 (2.73–40.75)	7.10 (1.83–14.67)
V_z/F (L/kg)	10.18 (6.85–19.53)	12.74 (2.687–47.03)	9.66 (0.47–26.32)
Male			
C_{max} (ng/ml)	31.05 (17.86–51.36)	23.77 (4.97–49.75)[a]	26.03 (9.39–97.64)
t_{max} (h)	1.48 (1.05–2.29)	1.25 (0.81–1.73)	1.24 (1.0–1.94)
t_{lag} (h)	0.25 (0–0.5)	0.0 (0.0–0.5)	0 (0.0–0.0)
K_{01} (/h)	0.68 (0.44–0.95)	0.78 (0.57–1.89)	0.83 (0.52–1.57)
$t_{1/2a}$ (h)	1.02 (0.73–1.58)	0.89 (0.37–1.21)	0.84 (0.44–1.34)
$AUC_{0-\infty}$ **(ng.h/ml)**	118.85 (74.25–189.98)	91.88 (16.5–109.93)[b]	87.18 (49.22–292.77)[c]
K_{10} (/h)	0.67 (0.43–0.96)	0.72 (0.24–1.25)	0.71 (0.42–0.99)
$t_{1/2z}$ (h)	1.02 (0.73–1.57)	0.89 (0.37–1.21)	0.99 (0.70–1.67)
CL/F (l/kg/h)	6.35 (3.34–10.33)	8.75 (5.8–40.75)	8.16 (2.70–14.67)[d]
V_z/F (l/kg)	8.41 (5.14–18.70)	15.52 (6.43–47.03)	13.32 (3.05–26.32)
Female			
C_{max} (ng/ml)	31.04 (19.29–49.21)	42.26 (8.41–117.80)	39.49 (11.40–91.24)
t_{max} (h)	1.33 (0.89–1.70)	1.11 (0.17–2.34)	1.36 (0.24–9.45)
t_{lag} (h)	0.0 (0.0–0.25)	0.0 (0.-0.5)	0 (0–0.5)
K_{01} (/h)	0.75 (0.59–1.48)	0.96 (0.43–22.67)	0.72 (0.11–1.92)
$t_{1/2a}$ (h)	0.92 (0.47–1.19)	0.73 (0.03–1.63)	0.97 (0.36–6.61)
$AUC_{0-\infty}$ **(ng.h/ml)**	108.19 (65.93–186.59)	130.62 (52.04–315.40)	144.4 (69.1–420.3)
K_{10} (/h)	0.76 (0.59–1.16)	0.63 (0.43–1.19)	0.74 (0.11–15.31)
$t_{1/2z}$ (h)	0.92 (0.47–1.19)	0.73 (0.03–1.63)	0.94 (0.05–6.49)
CL/F (l/kg/h)	8.06 (4.80–15.62)	6.50 (2.73–14.76)	5.91 (1.83–11.11)
Vz/F (l/kg)	11.81 (5.65–19.53)	10.09 (2.68–33.56)	8.18 (0.47–25.06)

[a] Statistically significant difference from females (p 0.023, Mann-Whitney *U* test).
[b] Statistically significant difference from females (p 0.034, Mann-Whitney *U* test).
[c] Statistically significant difference from females (p 0.049, Mann-Whitney *U* test).
[d] Statistically significant difference from females (p 0.049, Mann-Whitney *U* test).

atractylodin. The findings suggest that AL extract can be safe in long-term use. In the animal study, the AL extract could control the growth of the CCA as long as the administration of the extract was continued,[11,12] suggesting that the extract may have to be used for a long period. There appeared to be no significant influence of gender on the pharmacokinetics of atractylodin and similar dosage regimens can be used in both males and females. The model-dependent analysis showed that atractylodin pharmacokinetics followed 1-compartment model. It was noted for the relatively low (60–70%) C_{max} of atractylodin when the model-dependent analysis was applied compared with model-independent analysis. Other pharmacokinetic parameters were comparable. The pharmacokinetics of atractylodin in humans appeared to be markedly different from rats. The observation of double peaks reported in animal studies[12,13] was not found in humans in the current study and the study in healthy Japanese subjects following the oral doses (2.5, 5.0, and 7.5 g) of the rikkunshito (Japanese Kampo consisting of AL).[35] The relatively larger apparent volume of distribution and lower clearance resulted in more prolonged elimination half-life and mean residence time of atractylodin in humans compared with rats.

5. Conclusions

The daily dose of 1,000 mg AL extract was well tolerated in healthy research participants. The information on human pharmacokinetics of AL, when given as capsule formulation of the standardized extract, would assist in further dose optimization in CCA patients with the defined pharmacokinetic-pharmacodynamic relationship. The half-life is short and accumulation is not expected in long-term use. The 1,000 mg daily doses can be used as a safe starting dose in the escalating dose study to evaluate the clinical efficacy and safety of AL in CCA patients. The activity of AL or its active components on modulating CAFs and IL6 associated autophagy need to be investigated.

Ethics considerations

The study protocol was approved by the Ethics Committee of Thammasat University (TU-MED 2018-021, dated 23 May 2018). We have obtained written consent for study participation from all volunteers.

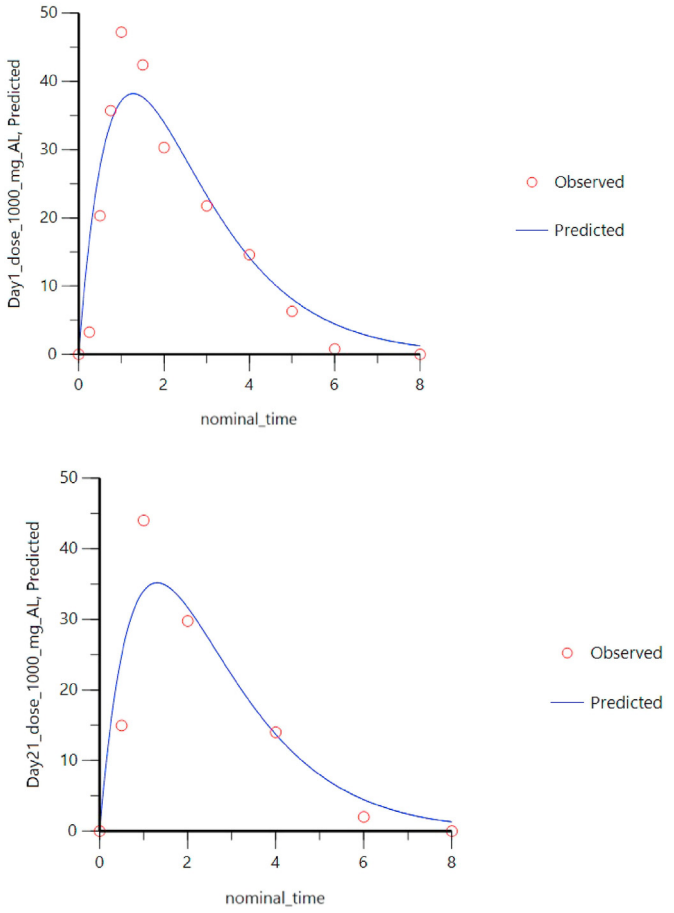

Fig. 4. Predicted plasma concentration-time profile (by 1-compartment model analysis) of atractylodin on day 1 (a) and 21 (b) in healthy subjects in *group 2* following daily oral dose of 1,000 mg capsule formulation of standardized AL extract (n = 20).

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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