Heliyon 8 (2022) e09416

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Clinical safety and pharmacokinetic evaluation of aqueous extract of *Cocculus hirsutus,* an anti-viral phytopharmacetical drug as a potential for the treatment of dengue and COVID-19

Sajad Khaliq Dar [a,*], Sudershan Kumar [a], Sovan Maiti [a], Shilpi Dhawan [a], Sadhna Joglekar [a], Upasana Arora [a], Rinku Kalra [a], Sumit Madan [a], Altaf A. Lal [a], Venugopal Singamaneni [b], Prasoon Gupta [b], Utpal Nandi [b], Deepika Singh [b], Arshad H. Khuroo [a]

[a] *Sun Pharmaceutical Industries Limited, India*
[b] *Indian Institute of Integrative Medicine (CSIR), India*

ARTICLE INFO

Keywords:
Sinococuline
Tolerability
Steady state
Simulation
SAS
Phoenix
LC-MS/MS

ABSTRACT

Background and aim: Dengue a worldwide concern for public health has no effective vaccine or drug available for its prevention or treatment. There are billions of people who are at risk of contracting the dengue virus (DENV) infections with only anti-mosquito strategies to combat this disease. Based on the reports, particularly in vitro studies and small animal studies showing anti-viral activity of aqueous extract of *Cocculus hirsutus* (AQCH), studies were conducted on AQCH tablets as a potential for the treatment of dengue and COVID-19 infections. The current study was part of the research on AQCH tablet formulation and was aimed to evaluate safety and pharmacokinetics in healthy human subjects.

Materials and methods: Sixty healthy adult human subjects were divided into 5 groups (cohorts: I to V; n = 12 per cohort) and randomized in the ratio of 3:1 to receive active treatment or placebo in a blinded manner. Five doses 100 mg, 200 mg, 400 mg, 600 mg and 800 mg tablets were administered three times daily at an interval of 8 h for days 01–09 under fasting conditions and a single dose in morning on day 10. Safety assessment was based on monitoring the occurrence, pattern, intensity, and severity of adverse events during study period. Blood samples were collected for measurement of the bio-active marker Sinococuline concentrations by a validated LC-MS/MS method followed by pharmacokinetic evaluation.

Results and conclusion: The test formulation was well tolerated in all cohorts. Sinococuline peak plasma concentration (C_{max}) and total exposure of plasma concentration (AUC) demonstrated linearity up to 600 mg and saturation kinetics at 800 mg dose. There was no difference observed in elimination half-life for all the cohorts, suggesting absence of saturation in rate of elimination. Dose accumulation was observed and steady state was achieved within 3 days. The information on human pharmacokinetics of AQCH tablets would assist in further dose optimization with defined pharmacokinetic-pharmacodynamic relationship.

1. Introduction

AQCH is an aqueous extract of stem of *Cocculus hirsutus* (family: Menispermaceae), a climbing shrub distributed widely in India and Africa [1] *Cocculus hirsustus* (commonly known as Jal-Jamni in India) has been used from centuries in traditional Indian medicine (Ayurveda) which is one of the most ancient yet living traditions with sound philosophical and experimental basis to cure various diseases and ailments. In Ayurveda various extracts from different parts of this plant (*Cocculus hirsustus*) are being used for their medicinal properties and is becoming increasingly popular, with many chronic conditions responding well to it [2, 4]. Medicinal properties of *Cocculus hirsutus* have been attributed to phytochemicals like *β*-sitosterol, trilobine, isotrilobine, syringaresional, protoquercitol, ginnol and related glycosides. The stem contains mainly cyclopeptide alkaloids and isoquinoline alkaloids. The plant contains Coclaurine, Sinococuline, Magnoflorine, *β*-Sitosterol, Ginnol and Monomethyl ether of Inositol [5, 6, 7, 8].

* Corresponding author.
E-mail address: sajadkhaliq.dar@sunpharma.com (S.K. Dar).

https://doi.org/10.1016/j.heliyon.2022.e09416
Received 20 January 2022; Received in revised form 18 March 2022; Accepted 10 May 2022

Ethanol extract of the leaves and stem have been reported to have anticancer and hypotensive activities associated with the alkaloid rich fraction which contains bisbenzylisoquinoline alkaloids (including pendulin and cocsulin). Aqueous extract of *Cocculus hirsutus* has shown high flavonoid content as compared to ethanol extract [9, 10, 11, 12, 13, 14]. The roots and leaves of *Cocculus hirsutus* have great medicinal value and are used both, internally and externally. Aqueous extract of *Cocculus hirsutus* is reported to have anti hyperglycaemic and antibacterial activities [15, 16, 17]. Based on in-vitro and/or in-vivo studies, we have shown the aqueous extract of this plant to have potent anti-dengue [18] and anti-COVID-19 activity.

In the case of dengue, AQCH has demonstrated potent activity against all four serotypes of dengue virus in in-vitro studies. It has been found to be protective against dengue in small animal studies (in primary infected AG129 mice and in secondary DENV infected AG129 mice models when administered QID and TID at dose of 25 mg/kg body weight); it significantly reduced small intestinal viral load, cytokine storm and vascular leakage. Its toxicological evaluation through acute and repeated dose toxicity studies has been completed in rodent and non-rodent species and No-Observed-adverse-effect-level (NOAEL) has been established. The drug was also not been found to be mutagenic and clastogenic [18].

With the establishment of pre-clinical safety and efficacy, the present study was undertaken to investigate human safety and pharmacokinetics of the drug as part of overall program aimed at development of this phyto-pharmaceutical drug against dengue and COVID-19 [18]. For pharmacokinetic evaluation, Sinococuline was used, which is one of the bioactive entities among five characterised chemical marker compounds of AQCH. The other four chemical marker compounds of AQCH were not used for pharmacokinetic evaluation because their quantifiable plasma concentrations were not sufficient to characterize pharmacokinetic parameters.

2. Methods

2.1. Participants and study design

This was a randomized, phase-I, double blind, placebo-controlled, multiple cohorts, and multiple dose (dose-escalation) study. The study protocol was approved by the Institutional Ethics Committee of Riddhi Medical Nursing Home. Written informed consent was received from all the study subjects prior to participation in the study. Sixty healthy adult human subjects, aged between 18 and 50 years who were non-smokers were recruited in the study. Other criteria for subject inclusion were (i) absence of acute or chronic diseases that could affect vital organ functions, (ii) subject should not have undergone any surgery within the past six months, (ii) no history of drugs or herbal products related hypersensitivity reactions or idiosyncratic reactions to, (iv) no history or concurrent administration of herbal products or drugs (except antipyretic or anti-emetic drugs) within the past two weeks, (v) no history or current drug abuse, (vi) study participants should be able to communicate (read, write, and speak) effectively, and (vii) willing to give informed consent for study participation. Exclusion criteria were (i) during physical examination any clinically significant abnormality, (ii) clinically significant abnormality of electrocardiograms (ECG) or chest x-ray, (iii) lactation or pregnancy, (iv) positive blood tests for HBsAg, HCV, or HIV, (v) history or concurrent use of antiplatelet medicine or anticoagulants or abnormality in blood coagulation (vi) blood donation or participation in any other study during past 90 days.

After obtaining written informed consents, clinical and laboratory investigations which included physical examination, electrocardiogram (ECG) monitoring, chest X-ray test, and laboratory investigations (urinalysis, haematology, serum biochemistry, serology, and pregnancy status) were carried out to confirm the eligibility of study participants. Screening and recruitment of study participants continued until the required number was achieved. All the recruited subjects were admitted in the clinical pharmacology unit well before time to meet the 10 h fasting condition before dosing.

Table 1. Summary of the study design and drug administration.

Cohort	Population	Sample size			Dose	Frequency
		Total	Active	Placebo		
I	Normal Healthy Subjects	12	9	3	100 mg	Three times a day
II	Normal Healthy Subjects	12	9	3	200 mg (100 mg x2)	Three times a day
III	Normal Healthy Subjects	12	9	3	400 mg (100 mg + 300 mg)	Three times a day
IV	Normal Healthy Subjects	12	9	3	600 mg (300 mg x 2)	Three times a day
V	Normal Healthy Subjects	12	9	3	800 mg (500 mg + 300 mg)	Three times a day

2.2. Drug administration

Sixty subjects were divided into 5 groups (cohorts: I to V; n = 12 per cohort) and randomized in the ratio of 3:1 to receive active treatment or placebo in a blinded manner. From each group 9 subjects received AQCH tablets and 3 received placebo. Five doses 100 mg, 200 mg, 400 mg, 600 mg and 800 mg tablets were tested in this study in a sequential manner. Each dose was administered to the subjects of a particular cohort three times daily at an interval of 8 h from Day 01 to Day 09, under fasting conditions. On Day 10, subjects received only the morning dose. The drug was administered to subjects in sitting position with 240 mL of water. The summary of the study design and drug administration is presented in Table 1. For the morning dose the subjects were made to fast for 8 h pre-dose and 2 h post dose in all the cohorts (I to V). For the afternoon dose, 2 h of pre dose and 2 h of post dose fasting and for the night dose pre dose fasting of 2 h was ensured. Water was restricted for 1 h before and after dosing, except for 240 mL of water given at the time of drug administration.

2.3. Clinical safety assessment

Clinical safety was assessed from the screening period till the end of the study through clinical examination, vital signs assessment, oral/axillary body temperature, 12-lead electrocardiogram (ECG) recording, blood glucose level (mg/dL) measurement, chest X-ray (posterior-anterior view) recording, orthostatic hypotension measurement, clinical laboratory parameters (e.g. biochemistry, haematology, immunology and urine analysis), subjective symptomatology and monitoring of adverse events.

Table 2. The summary of mean ± SD of age, height, weight and BMI of study subjects.

Parameter (Units)	Mean ± SD	
	N = 60 (Dosed Subjects)	N = 57 (Subjects who completed the clinical phase of the study)
Age (years)	32.6 ± 6.46	32.7 ± 6.56
Height (cm)	166.60 ± 6.406	166.41 ± 6.457
Weight (kg)	64.373 ± 9.4032	64.098 ± 9.5664
BMI (kg/m^2)	23.161 ± 2.8445	23.113 ± 2.8981

Table 3. List of adverse events following active treatment of AQCH tablet and placebo in healthy adult human subjects under fasting condition [Cohort I (100 mg), II (200 mg), IV (600 mg) & V (800 mg)].

Cohort (Dose)	Adverse Event	Active treatment	Placebo
		Number AEs (%)	Number AEs (%)
		N = 9	N = 3
I (100 mg)	White blood cell count increase	1 (11.11)	1 (33.33)
	Oropharyngeal pain	0	1 (33.33)
	Transaminases increase	2 (22.22)	0
	Alanine aminotransferase increase	1 (11.11)	0
	Pain in extremity	1 (11.11)	0
	Tonsillitis	1 (11.11)	0
	Pain		
II (200 mg)	Transaminases increase	2 (22.22)	0
IV (600 mg)	Alanine aminotransferase increase	1 (11.11)	0
V (800 mg)	Transaminases increase	0	1 (33.33)
	Alanine aminotransferase increase	1 (11.11)	0
	Diarrhoea	1 (11.11)	0
	Nausea	1 (11.11)	0

Safety evaluation was based on clinical and laboratory assessments during follow-up, according to Common Toxicity Criteria (CTC) Grading System for adverse events and Naranjo algorithm [19, 20]. At different intervals the occurrence, pattern, intensity, and severity of adverse events (clinical assessments, vital signs, and ECGs, along with clinical laboratory parameters) during the study period were monitored. 12-lead ECG was obtained in each cohort at screening, after check-in, on Day 01: before first dosing (within 60 min) and at 0.5, 1, 2, 4, 6, 8 & 12 h post-morning dose, on Days 02–09: before first dosing (within 15 min) and at 1–2 h post-morning dose, on Day 10: before dosing (within 15 min) and at 0.5, 1, 2, 4, 6, 8, 12 h post-dose and on Day 11: end of the study (before check-out).

Adverse events that were likely to be related to the test formulation were assessed using the Naranjo algorithm. Haematology, serum biochemistry, and urinalysis were monitored on Days 01 and 10 for all the groups. Any abnormal laboratory result was followed up with repeat checks until it returned to normal. Laboratory abnormalities (outside the normal ranges) that first occurred or increased in intensity during follow-up were evaluated. Total 57 out of 60 subjects completed the study while 3 subjects were withdrawn from study due to adverse events (2 from Cohort I and 1 from Cohort V).

2.4. Pharmacokinetic evaluation

2.4.1. Blood sample collection

Venous blood samples were collected through an indwelling intravenous cannula inserted in the forearm of the subjects during 24 h period of frequent blood sampling. The patency of cannula was maintained with 5 IU/mL of heparin in normal saline solution. Blood samples were collected in pre-chilled K_3EDTA vacutainers before drug administration (on Day 01) and at 0.250, 0.500, 1.000, 1.500, 2.000, 2.500, 3.000, 3.500, 4.000, 5.000, 6.000, 7.000, 8.000, 8.250, 8.500, 9.000, 9.500, 10.000, 10.500, 11.000, 11.500, 12.000, 16.000, 20.000 and 24.000 h following drug administration on Day 01 and Day 10 for all the groups. On Days 03, 05, 08 and 09, only pre-dose samples were collected. Plasma was harvested (within 1.5 h of blood collection) and stored at or below -50 °*C prior* to analysis.

2.4.2. Determination of Sinococuline concentration

Sinococuline was extracted from the human plasma using solid phase extraction technique. Chromatographic separation using Shimadzu Nexera X2 HPLC system (Shimadzu Corporation; Kyoto, Japan) was achieved on a reversed phase C18 analytical column (Luna® Omega 1.6 µm Polar C18 100A, 100 × 2.1 mm). Isocratic solvent system of 10 mM ammonium formate and methanol 40:60, with 0.1% formic acid v/v at flow rate of 0.250 mL/min was run for a total run time of 3.500 min. A triple quadruple mass spectrometer, MDS Sciex API-4000 (MDS Sciex, Toronto, Ontario, Canada) equipped with electrospray ionization (LC-ESI-MS/MS) was tuned for detection in positive ionization mode. *m/z* transitions observed in multiple reaction monitoring (MRM) mode were *m/z* 334.2 → 229.1 and *m/z* 342.2 → 324.5 for Sinococuline and Naltrexone (internal standard) respectively. Linear concentration ranges used for Sinococuline were 1.50–103.75 ng/mL for (Cohort I to III) and 1.50–122.49 ng/mL for (Cohort IV to V). Analysis for Sinococuline was performed on 2940 plasma samples, which included 2850 samples from the 57 subjects who completed the study and 90 samples from 3 subjects who were withdrawn from the study due to adverse events.

2.4.3. Pharmacokinetic analysis

The pharmacokinetic parameters were estimated from the plasma concentrations obtained from subjects of each cohort by non-compartmental model using Phoenix® WinNonlin® 8.1 Pharsight, Inc. USA. Scheduled sample collection time was used for pharmacokinetic analysis. The maximum concentration observed (referred as C_{max}) and

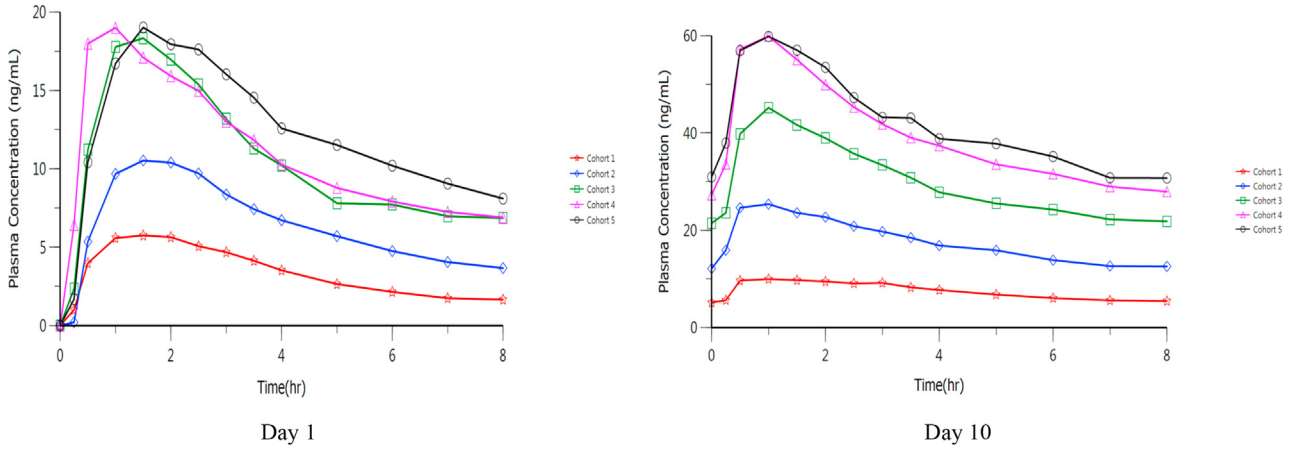

Figure 1. Linear Plot of mean plasma concentration of Sinococuline Day 1 & Day 10 following multiple ascending oral dose of AQCH tablet in healthy adult human subjects under fasting condition [Cohort I (100 mg), II (200 mg), III (400 mg), IV (600 mg) & V (800 mg)].

Table 4. Summary of PK parameters on day 01 following oral administration of AQCH tablet in healthy adults human subjects under fasting condition [Cohort I (100 mg), II (200 mg), III (400 mg), IV (600 mg) & V (800 mg)].

Day 01	Cohort-I	Cohort-II	Cohort-III	Cohort-IV	Cohort-V
N	9	9	9	9	9
*Dose (mg):	100	200	400	600	800
#T_{max} (hours)	1.5	1.5	1.5	1	1.5
C_{max}[a] (ng/mL)	7.23 (2.75–16.22)	11.27 (6.83–14.64)	20.18 (12.81–31.08)	21.26 (14.64–27.46)	21.06 (13.14–34.05)
(range[b])cv[c]	51.84	21.87	30.26	22.9	32.63
AUC_{0-8}[a] (hr*ng/mL)	27.11 (6.79–42.18)	51.49 (33.75–65.10)	84.83 (62.03–115.62)	89.36 (70.27–116.50)	99.53 (64.16–142.75)
(range[b])cv[c]	35.21	18.08	24.84	18.17	24.24
AUC_{0-24}[a] (hr*ng/mL)	69.62 (29.19–88.67)	159.38 (126.45–192.87)	258.98 (193.13–362.58)	314.46 (263.39–366.59)	339.80 (226.93–485.30)
(range[b])cv[c])	26.16	21.87	24.59	12.41	22.97
@$T_{1/2}$ (hr)	3.6	4.8	5.19	5.79	5.79

* TID Dose was administered to the subjects three times daily at an interval of 8 ± 1 h, under fasting conditions., # Median T_{max}, a (mean), b (range), c (%CV), @ Calculated based on 0–8 h concentration profile after 1st dose on Day 1.

Table 5. Summary of PK parameters on day 10 following oral administration of AQCH tablet in healthy adults human subjects under fasting condition [Cohort I (100 mg), II (200 mg), III (400 mg), IV (600 mg) & V (800 mg)].

Day 10	Cohort-I	Cohort-II	Cohort-III	Cohort-IV	Cohort-V
N	7	9	9	9	8
$Dose (mg):	100	200	400	600	800
#T_{max} (hour)	1	1	1	1	1
C_{max}[a] (ng/mL)	10.84 (7.41–14.00)	27.80 (19.58–45.18)	45.42 (29.44–76.95)	63.28 (29.21–93.80)	62.06 (35.05–83.44)
(range[b])cv[c]	23.11	29.59	36.91	28.63	30.73
AUC_{0-8}[a] (hr*ng/mL)	60.43 (44.23–75.43)	141.36 (108.65–186.13)	240.06 (173.20–388.58)	314.93 (186.15–397.28)	334.92 (226.38–448.64)
(range[b])cv[c]	18.96	18.94	28.65	19.06	24.63
AUC_{0-24}[a] (hr*ng/mL)	125.34 (96.31–168.03)	289.99 (212.53–386.09)	486.80 (359.86–782.68)	627.34 (393.47–801.52)	672.39 (470.44–882.40)
(range[b])cv[c]	21.71	23.14	27.74	19	23.87
$T_{1/2}$ (hr)	20.5	17.8	16.6	18.1	15.1

$ On day 10, subjects received only the morning dose, # Median T_{max}, a (mean), b (range), c (%CV).

Table 6. Mean trough concentration (C_{trough}) of Sinococuline in Cohorts I (100 mg), II (200 mg), III (400 mg), IV (600 mg) & V (800 mg).

Cohort	Dose	Day 01	Day 03	Day 05	Day 08	Day 09	Day 10
	mg	(ng/mL)	(ng/mL)	(ng/mL)	(ng/mL)	(ng/mL)	(ng/mL)
		Mean (range)	Mean (range)	Mean (range)	Mean (range)	Mean (range)	Mean (range)
I	100	0.00 (0.00–0.00)	4.33 (3.39–6.02)	5.27 (4.24–7.03)	4.98 (3.94–6.03)	4.99 (4.23–6.96)	5.13 (3.77–7.13)
II	200	0.00 (0.00–0.00)	10.76 (8.62–14.78)	11.93 (8.52–15.32)	13.21 (7.64–18.35)	13.30 (9.99–16.76)	12.11 (9.03–14.28)
III	400	0.00 (0.00–0.00)	17.80 (11.50–22.09)	21.48 (17.05–30.37)	21.27 (14.74–33.41)	21.07 (16.92–30.93)	21.44 (17.24–30.81)
IV	600	0.00 (0.00–0.00)	23.71 (15.82–31.11)	26.24 (15.62–32.79)	24.69 (15.48–31.03)	26.75 (19.44–31.82)	27.26 (18.98–33.34)
V	800	0.00 (0.00–0.00)	29.00 (17.89–39.19)	31.20 (20.79–48.12)	28.76 (17.80–38.78)	29.61 (21.69–36.52)	30.96 (21.60–42.79)

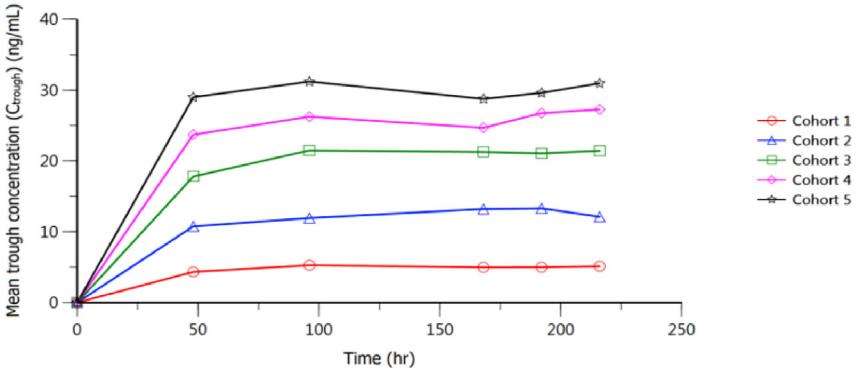

Figure 2. Linear Plot of mean trough concentration (Ctrough) of Sinococuline.

Table 7. Mean pharmacokinetic parameters of Sinococuline (0–8 h) on day 01 and day 10 in Cohorts I (100 mg), II (200 mg), III (400 mg), IV (600 mg) & V (800 mg).

Dose	N	Median T_{max} (hr)		C_{max} (ng/mL)		Accumulation (C_{max})	AUC_{0-8} (hr*ng/mL)		Accumulation (AUC_{0-8})
		Day 01	Day 10	Day 01	Day 10	(Day 10/Day 01)	Day 01	Day 10	(Day 10/Day 01)
100 mg	9**	1.500	1.000	7.228	10.844	1.500	27.109	60.428	2.229
200 mg	9	1.500	1.000	11.272	27.798	2.466	51.487	141.361	2.746
400 mg	9	1.500	1.000	20.184	45.416	2.250	84.834	240.060	2.830
600 mg	9	1.000	1.000	21.264	63.282	2.976	89.356	314.928	3.524
800 mg	9*	1.500	1.000	21.063	62.063	2.947	99.528	334.922	3.365

** N = 7 (2 subjects withdrawn due to AEs), *N = 8 (1 subjects withdrawn due to AEs).

time at which maximum concentrations occurred (referred as T_{max}) were obtained directly from the plasma concentration-time data. Terminal elimination half-life ($t_{1/2}$) was calculated using the plasma concentration-time data and area under the curve from zero time to the last observed time (AUC_{0-t}) was calculated using the linear trapezoidal rule. The pharmacokinetic parameters C_{max}, AUC_{0-8}, T_{max}, k_{el} and $t_{1/2}$ were determined from plasma concentrations and $t_{1/2}$.

2.4.4. Statistical analysis

Statistical analysis was performed using SAS® system for Windows, release 9.4 (SAS Institute Inc., USA) and Phoenix® WinNonlin® 8.1. Arithmetic means, standard deviations and coefficients of variation were calculated for all the pharmacokinetic parameters. Steady state analysis was performed by repeated measure analysis technique on pre-dose concentrations (C_{trough}) observed on Days 01, 03, 05, 08, 09 and 10, using a PROC GLM, SAS procedure. The p-values were tested at 5% level of significance for Time effect and Formulation* Time effect on log-transformed data. Dose linearity (0–8 h) was calculated on Days 01 and 10 in all the cohorts for C_{max} and AUC using linear regression analysis.

2.4.5. Simulation models

Pharmacokinetic simulation was performed to predict the pharmacokinetic profile of Sinococuline on Days 03, 05, 08 and 09 at 200, 400, 600 and 800 mg TID doses of AQCH. Individual plasma concentration data of Sinococuline at 100 mg dose was used for model development of pharmacokinetic simulation. The simulation was done using Phoenix Modelling by Phoenix® WinNonlin® Version 8.2 using compartmental modelling approach. A total of 1000 iterations were used during prediction of pharmacokinetic profile for different dose levels.

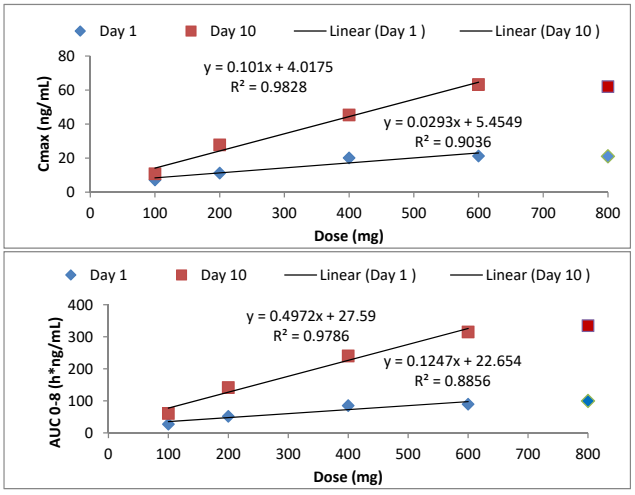

Figure 3. Linearity plot of Sinococuline of C_{max} and AUC_{0-8} on Days 01 and 10.

3. Results

3.1. Demographics

Demographic and baseline laboratory data of all 60 volunteers included in all the 5 cohorts (Cohort I to Cohort V) are summarized in Table 2. There was no significant difference in any of the parameters among participants allocated to the 5 cohorts as well as in each cohort between AQCH and placebo-treated subjects. All research participants were healthy as verified by results of clinical (physical examination, vital signs, chest X-ray, and ECG) and laboratory assessments. All the laboratory parameters (haematology and serum biochemistry), vital signs and ECG were within normal ranges.

3.2. Safety and tolerability following drug administration

The list of adverse events following active treatment of AQCH tablets or placebo is listed in Table 3. AQCH tablets were well tolerated in all the 5 cohorts. There were no clinically significant findings in the vital signs assessment, 12-lead ECG recording or the laboratory tests in any of the subjects in the study. No subject had a maximum on-treatment QTcB interval >450 ms while receiving AQCH or Placebo. There were no significant changes in QTc intervals after the dosing. The analysis of adverse events was carried out treatment-wise. There were no deaths or serious adverse events during the conduct of the study.

Ten (10) subjects reported a total of fifteen (15) adverse events (AE) during the conduct of the study, out of which nine (09) adverse events were reported in Cohort-I, one (01) adverse event in Cohort-II, one (01) adverse event in Cohort-IV and four (04) adverse events were reported in Cohort-V of the study. Seven (07) subjects from active treatment group reported ten (10) adverse events and three (03) subjects from the Placebo treatment group reported five (05) adverse events. Out of fifteen (15) adverse events reported, the causality assessment was judged as possible for thirteen (13) adverse events and as unlikely for two (02) adverse events.

The most frequently reported adverse event was increase in transaminases for both treatment groups combined (active treatment; 03

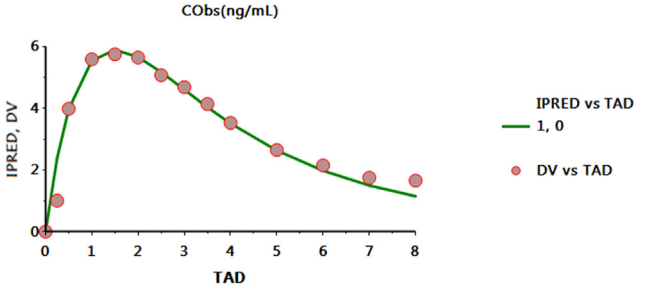

Figure 4. Simulation model showing the predicted data (Solid line) vs observed data (Circle).

Table 8. Predicted C_{max} for 200, 400, 600 and 800 mg doses on days 01, 03, 05, 08, 09 and 10 and predicted AUC_{0-8} for 200, 400, 600 and 800 mg doses on days 01, 03, 05, 08, 09 and 10 through simulations.

Predicted C_{max} (ng/mL)								Predicted-AUC_{0-8} (hr*ng/mL)					
Cohort	Dose (mg)	Day 01	Day 03	Day 05	Day 08	Day 09	Day 10	Day 01	Day 03	Day 05	Day 08	Day 09	Day 10
II	200 mg	11.785	15.635	17.611	19.695	20.213	20.663	53.789	80.079	95.654	112.072	116.157	119.35
III	400 mg	23.569	31.2695	35.222	39.39	40.426	41.325	107.578	160.157	191.309	224.144	232.314	238.699
IV	600 mg	35.354	46.904	52.834	59.084	60.64	61.988	161.367	240.236	286.963	336.217	348.472	358.049
V	800 mg	47.139	62.539	70.445	78.78	80.853	82.651	215.156	320.315	382.617	448.289	464.629	477.399

subjects, 6.67% and placebo treatment; 02 subjects, 4.45%). The reported adverse events were treated appropriately and followed up until resolution. Causality assessments were also done on the adverse events observed in the subjects withdrawn from the study. Tonsillitis was assessed as unlikely and rest of the adverse events was assessed as possible.

3.3. Pharmacokinetics

The linear plot of mean plasma concentration of Sinococuline on Days 01 and 10 following multiple ascending oral dose of AQCH tablets in healthy adult human subjects under fasting condition [Cohort 1(100 mg), 2 (200 mg), 3 (400 mg), 4 (600 mg) & 5 (800 mg) is presented in Figure 1. The C_{max} and AUC ranged between 7-21 ng/ml and 69–339 h*ng/ml respectively on Day 01. C_{max} and AUC ranged between 10-62 ng/ml and 125–672 h*ng/ml respectively on Day 10. Tables 4 and 5 summarize the mean pharmacokinetic parameters of Sinococuline on Days 01 and 10. Table 6 presents the mean trough concentration of Sinococuline on different days and the linear plot of mean trough concentration (C_{trough}) of Sinococuline is presented in Figure 2. Accumulation of Sinococuline was observed at all dosage levels and C_{max} and AUC increased by ~ 1.5–3.5 times on Day 10 compared to Day 01 (Table 7).

3.4. Statistics

The p-values of time effect and formulation*time effect were 0.932 and 0.999 respectively for steady state analysis, confirming that the subjects reached steady state within 03 days at all the dosage levels. The descriptive statistics shows inter-subject CV of 21–51% for C_{max}, 18–35% for AUC_{0-8}, and 12–26% for AUC_{0-24} on Day 01. The inter-subject CV observed on Day 10 is 23–36% for C_{max}, 18–28% for AUC_{0-8} and 19–27% for AUC_{0-24}. Dose linearity evaluation using linear regression analysis shows R^2 value of 0.98 for C_{max} and 0.97 for AUC on Day 10. Linearity plots of C_{max} and AUC_{0-8} on Days 01 and 10 are presented in Figure 3.

3.5. Simulation results

The optimized compartment model using observed plasma concentration of 100 mg dose along with predicted data points is presented in Figure 4. Pharmacokinetic parameter estimates were calculated from the predicted profiles using non-compartment approach. The C_{max} ranges from ~11 to 82 ng/ml and AUC_{0-8} ranges between ~53 to 477 h*ng/mL for 200 mg–800 mg dose. Table 8 presents the predicted C_{max} and AUC_{0-8} values for 200, 400, 600 and 800 mg doses on Days 01, 03, 05, 08, 09 and 10. Predicted profile for doses 200 mg, 400 mg, 600 mg and 800 mg through simulation model is presented in Figure 5.

4. Discussion

This is the first study evaluating safety and pharmacokinetics of AQCH tablets in humans. The test formulation was well tolerated in all healthy participants of each cohort, which is verified by the absence of significant clinical as well as laboratory-related adverse events/adverse reactions. The study revealed no correlation between dose and adverse events (AE) as number of adverse events observed with higher doses were lesser compared to lower doses. The data further confirms absence of correlation between systemic Sinococuline concentrations and adverse events.

In recent years, many pharmacokinetic studies of herbal medicines have been conducted. However, unlike allopathic medicines, the pharmacological actions of herbal medicine are thought to be due to the synergistic effects of multiple components, and multi-targets/multi-pathways [21]. Therefore, the pharmacokinetics of herbal medicine is

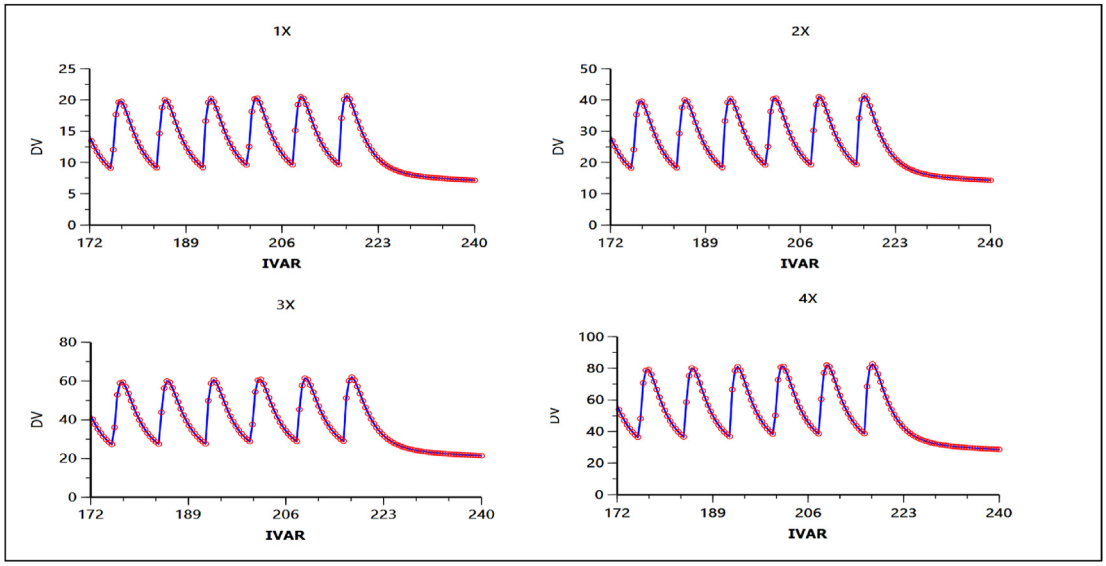

Figure 5. The predicted profile at 200(1X), 400(2X), 600(3X) and 800 (4X) mg doses for days 01–10 using simulation model.

relatively more complex than synthetic drugs. Information on the pharmacokinetic profile of a drug helps to understand the relationship between intensity and time course of pharmacological and toxicological effects of phytochemicals in the human body. We have characterised Sinococulin and four additional chemical entities in the drug ACQH, which are Magnoflorine, Makisterone-A, 20-hydroxyecdysone and Coniferyl alcohol. All these compounds were tried for estimation in collected plasma; however, except Sinococuline quantifiable concentrations of other compounds were negligible and not enough to characterize the pharmacokinetic parameters.

Pharmacokinetic investigation of Sinococuline showed linear increase in C_{max} and AUC with ascending doses up to 600 mg and upon further increase in dose up to 800 mg, no increase was observed in both rate and extent of absorption. R^2 value is 0.98 for C_{max} and 0.97 for AUC on Day 10, which indicates C_{max} and AUC are increasing in linear fashion up to 600 mg dose range. However, linear increase in C_{max} and AUC is not observed for 800 mg dose level, confirming saturation in absorption beyond 600 mg dose.

The lack of significant difference in terminal half-life between all the doses (100 mg–800 mg) indicates elimination rate is not saturated. The saturation in absorption phase could be due to saturation in solubility or carrier mediated absorption process that limits the bioavailability at higher doses. However, this needs further investigation to understand the exact reason for non-linearity above 600 mg dose. The T_{max} values of 1.00–1.500 h on Day 01 and 1.000 h on Day 10 confirm rapid absorption of Sinococuline and absence of saturation in rate of absorption at all the dose levels.

It was observed that steady state is achieved within the first 3 days which could be confirmed by the pre-dose concentrations (C_{trough} values). Even though sufficient sampling time points were ensured on Days 01 and 10, it was not possible to collect samples for complete PK profiles on all days. Pre-dose morning samples collected on Days 03, 05, 08 and 09 were used for steady state evaluations. But to evaluate C_{max} and AUC for all these days, pharmacokinetic simulation was done with model based analysis using Phoenix® WinNonlin® Version 8.2. The simulations drawn for all the dosage regimens between Days 01 and 10 were correlated with the actual results. The predicted pharmacokinetic parameters were found closer to observed values, which validate the robustness of pharmacokinetic model used to predict the concentrations till 600 mg dose. However, model could not predict expected pharmacokinetics at 800 mg dose due to saturation kinetics.

5. Conclusion

This study was a randomized, double blind, placebo-controlled, multiple cohorts, and multiple dose (dose-escalation) study, wherein 100 mg–800 mg drug (TID) were administered for ten days. The safety data and findings from the study suggest AQCH tablets are well tolerated in healthy human subjects across all doses with no QTcB prolongation or any other ECG abnormality. The pharmacokinetics of Sinococuline shows linear increase in C_{max} and AUC with ascending doses up to 600 mg and upon further increase in dose to 800 mg, linearity is not observed for both rate and extent of absorption. Consistent pre-dose levels observed on Days 03, 05, 08, 09 and 10 confirm that steady state is attained at each dose level within 3 days. There is no correlation between dose and adverse events (AEs) and data also indicates no correlation between Sinococuline concentrations and frequency or severity of adverse events (AEs). This study provides rationale and optimism for conducting efficacy studies of AQCH for treatment of dengue and COVID-19.

6. Ethics considerations

The study protocol was approved by Riddhi Medical Nursing Home -Institutional Ethics Committee (RMNH IEC) A/101, Jalaram Plaza, Jawahar Chowk, Maninagar, Ahmedabad-380008, Gujarat, India. Written consent was obtained from all the study participants prior to initiation of the research.

Declarations

Author contribution statement

Sajad Khaliq Dar: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Sudershan Kumar; Sovan Maiti; Shilpi Dhawan; Sadhna Joglekar; Upasana Arora; Rinku Kalra; Sumit Madan; Altaf A. Lal; Arshad H. Khuroo: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Venugopal Singamaneni; Prasoon Gupta; Utpal Nandi; Deepika Singh: Conceived and designed the experiments; Performed the experiments.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgements

All the authors wish to acknowledge the support and facilities received from Sun Pharmaceutical Industries Ltd. India and International Centre for Genetic Engineering and Biotechnology, New Delhi, India for carrying out this work. We would like to thank Dr. Ram Vishwakarma, Dr. Azadar Khan, Dr. Mohan Prasad, Mr. Raj Philip, Dr. Romi Singh, Dr. Kohinoor Das, Dr. Gaurav Sahal, Dr. Maulik Doshi, Mr. Narendra Lakkad, for their support and guidance.

References

[1] R. Logesh, N. Das, A.A. Devkota, H.P. Devkota, Cocculus hirsutus (L.) W.Theob. (Menispermaceae): a review on traditional uses, phytochemistry and pharmacological activities, Medicines 7 (2020) 69.

[2] B. Sangameswaran, B. Jayakar, Anti-diabetic and spermatogenic activity of Cocculus hirsutus (L) Diels, Afr. J. Biotechnol. 6 (10) (16 May 2007) 1212–1216.

[4] S. Kumar, G.J. Dobos, T. Rampp, The significance of ayurvedic medicinal plants, J. Evid-Based Compl. Alternat. Med. 22 (3) (2017 Jul) 494–501.

[5] B.H. Marya, S. Bothara, Ethnopharmacological properties of *Cocculus hirsutus* (L.) diels- a review, Int. J. Pharmaceut. Sci. Rev. Res. 7 (2011 Mar 1) 108–112.

[6] A. Das, S.L. Bondya, Cocculus hirsutus (L.) Diels : diverse uses by the tribal population of Dumka, The Biobrio 3 (3&4) (2016) 190–193 [Internet].

[7] S. Ganapaty, G.K. Dash, T. Subburaju, P. Suresh, Diuretic, laxative and toxicity studies of *Cocculus hirsutus* aerial parts, Fitoterapia 73 (2002) 28–31.

[8] P. Abiramasundari, V. Priya, G.P. Jeyanthi, et al., Evaluation of the antibacterial activity of *Cocculus hirsutus*, Hygeia J. Drugs Med. 3 (2011) 26–31.

[9] K. Jethva, D. Bhatt, B. Dhru, S. Patel, M. Zaveri, Phyto pharmacognostical evaluation of leaf of *Cocculus hirsutus*, Int. J. Pharmaceut. Sci. Rev. Res. 38 (2016 Jan 1) 165–170.

[10] H. Itokawa, S. Tsuruoka, K. Takeya, N. Mori, T. Sonobe, S. Kosemurab, T. Hamanaka, An antitumor morohinane alkaloid, sinococuline, from *Cocculus trilobus*, Chem. Pharm. Bull. 35 (1987) 1660–1662.

[11] Y. Hitotsuyanagi, K. Nishimura, H. Ikuta, K. Takeya, H. Itokawa, Syntheses of antitumor morphinane alkaloids, sinococuline and 6-epi-, 7-epi-, and 6-epi-7-epi-Sinococuline from sinomenine, J. Org. Chem. 60 (14) (1995) 4549–4558.
[12] W.K. Liu, X.K. Wang, C.T. Che, Cytotoxic effects of sinococuline, Cancer Lett. 99 (2) (1996) 217–224.
[13] J. Bero, M. Frederich, J.Q. Leclercq, Antimalarial compounds isolated from plants used in traditional medicine, J. Pharm. Pharmacol. 61 (2009) 1401–1433.
[14] C.P. Khare, in: C.P. Khare (Ed.), Indian Medicinal Plants - an Illustrated Dictionary, Springer-Verlag, Berlin/Heidelberg, 2007, p. 162.
[15] D. Rao, V. Rao, P. Murthy, G. Mohan Narasimha Rao, Y. Venkateswara, Some ethno medicinal plants of parnasala sacred grove area eastern Ghats of Khammam District, Telangana, India, J. Pharmaceut. Sci. Res. 7 (2015 Jan 1) 210.
[16] S. Badole, N. Patel, S. Bodhankar, B. Jain, S. Bhardwaj, Antihyperglycemic activity of aqueous extract of leaves of *Cocculus hirsutus* (L.) Diels in alloxan-induced diabetic mice, Indian J. Pharmacol. 38 (2006).
[17] R. Jeyachandran, A. Mahesh, L. Cindrella, X. Baskaran, Screening antibacterial activity of root extract of *Cocculus hirsutus* (L.) diels. Used in Folklore Remedies in South India, J. Plant Sci. 3 (2008) 194–198.
[18] R. Shukla, R.K. Rajpoot, et al., Cocculus hirsutus-derived phytopharmaceutical drug has potent anti-dengue activity, J. Front. Microbiol. 12 (2021 Nov 29).
[19] C.A. Naranjo, U. Busto, et al., A method for estimating the probability of adverse drug reactions, J. Clin. Pharmacol. Therapeut. 30 (2) (1981 Aug) 239–245.
[20] C.A. Naranjo, U. Busto, E.M. Sellers, Comparison of two recently published algorithms for assessing the probability of adverse drug reactions, Br. J. Pharmacol. 13 (2) (1982 Feb) 223–227.
[21] K. Na-Bangchang, I. Kulma, T. Plengsuriyakarn, et al., Phase I clinical trial to evaluate the safety and pharmacokinetics of capsule formulation of the standardized extract of Atractylodes lancea, J. Tradit. Compl. Med. 11 (2021).