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HPLC Analysis Mangostin After Orally Administration in Rats

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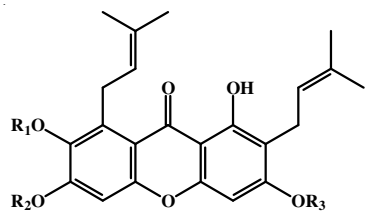
A simple sensitive isocratic method for the detection and quantification of mangostin in plasma has been developed. The assay consisted of reversed-phase HPLC with ultraviolet detection. Separation was achieved on a C_{18} reversed-phase column. The mobile phase consisting of methanol and water (95:5 % v/v) was delivered at a flow rate of 1.0 mL/min. The assay was shown to be linear over the range 4-100 µg/mL ($r \geq 0.9998$). The HPLC analysis has been successfully applied to pharmacokinetic studies of mangostin after oral administration at a dose 40 mg/kgbwt to rats. The main pharmacokinetic parameters were: $t_{1/2}$ 7.24 h; K el 0.058/h; t_{max} 62.99 min; C_{max} 4.79 µg/mL; AUC 702.45 µg min/mL, respectively.

Key Words: Mangostin HPLC pharmacokinetic studies, Rat.

INTRODUCTION

Garcinia parvifolia (Miq) Miq., has been widely used traditional medicine for the treatment of malaria and α-mangostin is one of an active compound[1]. Mangostin (Fig. 1), which was isolated from *G. mangostana* Linn to and its was found to have antiinflammatory[2], antioxidant[3], antimycobacterial[4], 5-hydroxytryptamine 2A receptor antagonist[5] and cytotoxic effect against patocelluler cell lines[6]. It was also reported to inhibit alveolar duct formation in a mouse mammary organ culture model and to supress the carcinogen induced formation crypt foci in a short-term colon carcinogen model[7].



α-mangostin: $R_1 = CH_3$, $R_2 = R_3 = H$
β-mangostin: $R_1 = R_3 = CH_3$, $R_2 = H$
γ-mangostin: $R_1 = R_2 = R_3 = H$

Fig. 1. Chemical structure of mangostin

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Various analytical methods to quantitative analysis of α-mangostin have been reported such as gas chromatography (GC) and high performance liquid chromatography (HPLC)[8,9]. However, there was no data of the pharmacokinetic of α-mangostin in male Sprague-Dawley rats. The present studies report the determination of α-mangostin in biological fluids and urine. A sensitive, simple, fast and reliable bioanalystical method is required in order to evaluate the pharmacokinetic disposition of α-mangostin.

EXPERIMENTAL

Mangostin used for this study was isolated from the stem barks of *G. parvifolia* (Miq) Miq as previously described[1]. α-Mangostin (98.5 % pure) for reference standard was ordered from the Chengdu Biopurify Phytochemicals Ltd., (Chengdu Sichuan China).

The HPLC-UV system consisted of HPLC Hewlett Packard® model 1100, Germany was used for the determination of mangostin. An Alltima® RP C-18 (5 μm, 4.6 mm × 250 mm) column and 5 % water in methanol were a stationary phase and mobile phase suitable for the separation. Peak and detection at the maximum UV absorption at 319 nm was performed over the rang of 25-125 μg/mL of mangostin.

Preparation of stock and calibration standard solutions: A stock solution of α-mangostin reference standard was prepared by dissolving an accurately weighed 10 mg of α-mangostin in 100 mL methanol in a volumetric flask. From this solution various concentrations of the standard solution were prepared in 10 mL of methanol in a volumetric flask to obtain final concentrations at 20, 16, 8, 4 and 2 μg/mL.

Animal study: The developed HPLC method was used in a pharmacokinetic disposition study after orally administration of α-mangostin to male Spraque-Dawley rats (6-7 weeks, 250 ± 12 g). Rats were anesthetized with single i.p. injection of sodium pentobarbital (60 mg kg^{-1}), cannulated *via* the right jugular vein one day prior to drug administration and fasted over-night. After a 1 day recovery period, α-mangostin dissolved in saline was orally at a dose of 40 mg/kg bwt. Venous blood samples were collected at 0.5, 10.0, 15.0, 30.0, 80.0, 90.0, 120.0 and 180.0 min post dose and collected in heparinized tubes. Blood samples were immediately centrifuged at 3000 g for 5 min and harvested serum samples were stored as -20 °C until analysis the volume of the serum samples used in the analysis was 50 μL.

Recovery: The recoveries of mangostin in rat plasma determined at the concentrations of 20 μg mL^{-1} was 58.45 % (n = 6).

Liniearity: Typical equation of the calibration curve were as follows $Y = 20.830X + 1.3 \times 10^{-4}$ ($R = 0.9998$, $n = 5$) for rat plasma samples. The linear range of mangostin in rat plasma was from 4-100 μg mL^{-1}.

Accuracy and precision: The accuracy and precision were determined with 5 determinations per concentration. Within- and between-day accuracy and precision values are given in Table-1.

TABLE-1
PRECISION AND ACCURACY OF HPLC-UV METHOD IN DETERMINING MANGOSTIN IN RAT PLASMA (n = 6)

Concentration (μg mL^{-1})	RSD (%)		RE (%)	
	Added	Found	Intra-day	Inter-day
80.78	80.06	2.8	7.3	0.0
85.37	85.57	4.9	4.9	2.4
81.03	80.64	3.6	8.2	-1.7

Application of the HPLC analysis and pharmacokinetic study: In a previous study, we studied the effect of mangostin (at three dosages 10, 20 and 40 mg/kg) against *P. berghei* of mice. The results indicated that the dose 40 mg/kg yielded significant antiplasmodial activity. Therefore to study the pharmacokinetic profile of mangostin, the mangostin at the dosage of 40 mg/kg was administered to rats by oral gavage. Venous blood samples were collected at 0.5, 10.0, 15.0, 30.0, 80.0, 90.0, 120.0 and 180.0 min post dose and collected in heparinized tubes. Blood samples were immediately centrifuged at 3000 g for 5 min and harvested plasma samples were stored as -20 °C until analysis the volume of the plasma samples used in the analysis was 50 μL. Mean plasma concentration profile of mangostin in rats is presented in Fig. 2.

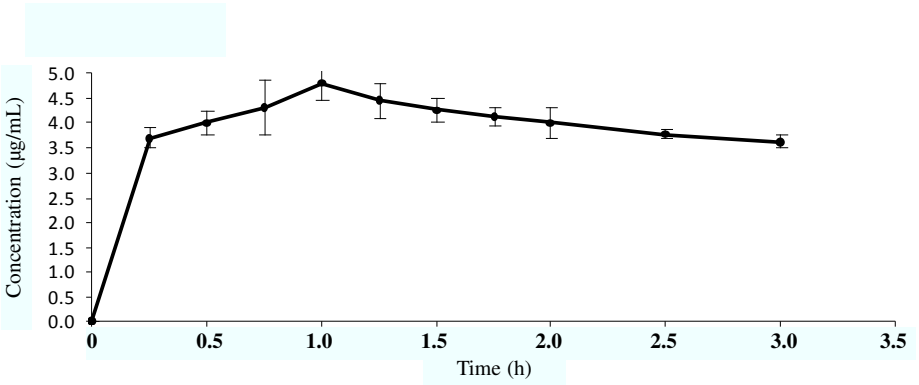


Fig. 2. Mean plasma concentration of mangostin in male SD rats after oral administration of mangostin at a dose 40 mg/kg

Table-2 summarizes the main pharmacokinetic parameters of mangostin in male SD rats after orally administration at dose of 40 mg/kg, the main pharmacokinetic parameters were: $t_{1/2}$ 7.24 h; K el 0.058/h; t_{max} 62.99 min; C_{max} 4.79 μg/mL; AUC 702.45 μg min/mL, respectively.

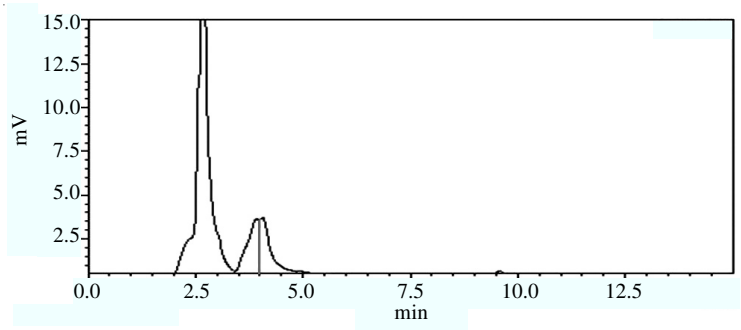
RESULTS AND DISCUSSION

The retention times for mangostin was 10.84 min, respectively. No interference from endogenous components or mangostin metabolites was observed in plasma.

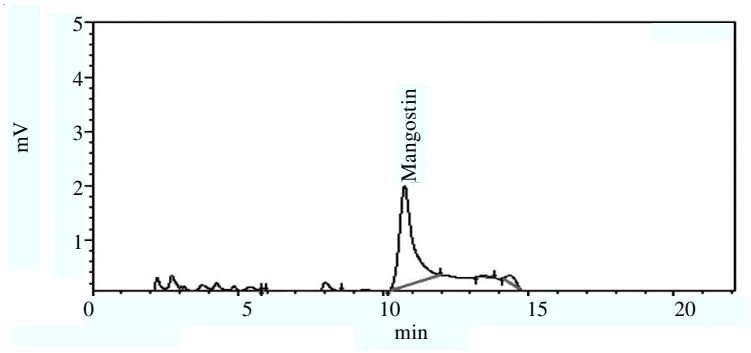
TABLE-2
PHARMACOKINETIC PARAMETERS OF MANGOSTIN IN MALE SD RATS FOLLOWING AN ORAL DETERMINATION

Parameters	Mangostin at dose 40 mg/kg
T_{max} (min)	62.99
C_{max} (µg mL^{-1})	4.79
AUC (µg min mL^{-1})	702.45
Half-life (h)	7.24

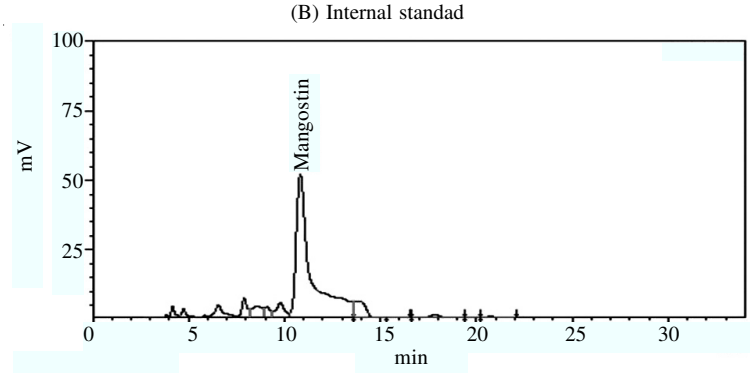
Male SD rats received 40 mg/kgbwt mangostin in corn oil orally.



(A) Blank rat plasma sample



(B) Internal standad



(C) Plasma from rat 1 h after oral administration of mangostin 40 mg/kg

Fig. 3. Chromatogram of mangostin of rat plasma blank (A) and rat

The baseline was relatively free from drift. Validation of the method consisted of two distinct phases: (a) the development phase, in which the assay was defined and (b) the application phase, in which the method was applied to the actual analysis of samples from a single 40 mg oral-dose mangostin pharmacokinetic study. Six concentrations (excluding blank values) defined the calibration curves. The linearity of the calibration curves was verified from 4-20 μg/L for mangostin in plasma. The correlation coefficients between the peak-area ratio of the drug to the IS and to concentration were > 0.999.

Fig. 3 shows the chromatogram of mangostin methanol and water (95:5) moving phase produced one peak. This shows the purity of the mangostin used in this research. Based on the mangostin chromatogram of the plasma sample, there are other peaks that could be from blood or metabolit of the mangostin. Fig. 2 shows the curve of mangostin content in the plasma based on time after the oral administration of 40 mg/kg. Table-2 presents the conclusions of pharmacokinetic parameters in the plasma when the mangostin doses of 40 mg/kg were administered orally. The pharmacokinetic parameters are: $t_{1/2}$ 7.24 h; K el 0.058/h; t_{max} 62.99 min; C_{max} 4.79 μg/mL; AUC 702.45 μg min/mL.

Conclusion

HPLC analysis can be used to identify the pharmacokinetic parameters of mangostin. The oral administration of mangostin doses of 40 mg/kg produced significantly different pharmacokinetic parameters in tmax, C_{max}, half life and AUC ($p < 0.05$).

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