



## Research article

## Single dose oral pharmacokinetic profile rubraxanthone in mice

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## ABSTRACT

Rubraxanthone is a main active constituent of kandis (*Garcinia cowa* Roxb), has showed many biological activity including antimicrobial, anti hypercholesterolemic, antiplatelet, antioxidant, cytotoxic, and anti-inflammatory properties. To the best of our knowledge, no reports on the pharmacokinetics (PK) of this rubraxanthone have been published. The PK of rubraxanthone in mice was examined after a single oral dose of 700 mg/kg rubraxanthone suspension in virgin coconut oil. Plasma samples were collected at different time points and further analyzed using validated chromatographic method. Pharmacokinetic parameters were calculated from observed plasma concentration-time profile. The maximum concentration of rubraxanthone in plasma was discovered in 1.5 h. The peak plasma concentration ( $C_{max}$ ) was 4.267  $\mu\text{g/mL}$ , and the area under the curve ( $\text{AUC}_{0-t \infty}$ ) was 560.99  $\mu\text{g h/L}$ , with a 6.72-hour terminal half-life ( $T_{1/2}$ ). The volume of distribution ( $V_d/F$ ) was 1200.19 mL/kg and 1123.88 mL/h/kg clearance ( $Cl/F$ ).

## 1. Introduction

*Garcinia cowa* Roxb, Guttiferae, is commonly used traditionally for the treatment of diarrhea, skin infections, wounds antiseptic and hyper cholesterol and blood circulation improvement [1]. Rubraxanthone (1,3,6, trihydroxy-8- geranyl-7-methoxy xanthone, Figure 1 is the main active xanthone compounds of *G cowa* Roxb (kandis) [2] and is present in the dried kandis stem bark extract at content up to 40 mg/g [3]. Previous studies showed that rubraxanthone has a variety of biological activity, including antimicrobial, antioxidant, cytotoxic and anti-inflammatory activity by inhibition of NO production and platelet activating factor (PAF) [4, 5, 6, 7]. *In vivo* studies reported, that administration of rubraxanthone 700 mg/kg bw was able to reduce total cholesterol, triglycerides and LDL cholesterol through the activation of the lipoprotein lipase enzyme [8].

Despite its widespread medicinal use, to the best of our knowledge, no reports on the pharmacokinetics (PK) of this rubraxanthone have been published. Pharmacokinetic studies were crucial in the development of novel medicines and in determining the disposition process so that the best route of administration and dosing regimen may be chosen. Many natural compounds exhibit promising activity have failed during clinical trials due to poor oral bioavailability, safety concerns, and lack of pharmacodynamic reproducibility in clinical trials [9]. The

pharmacokinetic profile of rubraxanthone in mice after oral administration was reported.

A number of techniques have been studied to identify and determine the content of rubraxanthone in *G. cowa* extract [3, 10, 11, 12]. In previous studies, we have developed a quantitative method of rubraxanthone in plasma by UHPLC-DAD with pretreatment analysis samples by protein precipitate [14]. For the first time, the pharmacokinetic profile of rubraxanthone in mice after oral administration has been characterized.

## 2. Material and methods

## 2.1. Chemicals

$\alpha$ -Mangostin as internal standard (IS) and rubraxanthone were previously isolated from *G. cowa* Roxb and the structure was characterized by spectral methods, including MS,  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra. The data were consistent with previous literatures [7] (Figure 1). Rubraxanthone and IS Purity (>98%). Acetonitrile and methanol for liquid chromatography were obtained from Merck. Distilled deionized water was taken from IKA water purification system. The other chemicals used were analytical grade.

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## 2.2. Instrumentation conditions

The UHPLC-DAD system of Agilent consisted of auto sampler. Column ZORBAX RRHD Eclipse Plus C18 (100 mm × 3.0 mm id, 1.8 μm) was used for stationary phase. Acetonitrile and 0.4% formic acid (3:1) were used as mobile phase. It was prefiltered through a Millipore 0.22 μm filter followed by sonication prior to use for analysis and pumped at a flow rate of 0.2 mL/min. The detector was set at a wavelength of 243 nm and the column temperature is kept at 20 ± 3 °C.

## 2.3. Preparation of standard solutions and standards calibration

The stock solutions of rubraxanthone and α-mangostin were prepared in methanol at a concentration of 1 mg/mL. The IS solution was diluted methanol to give the concentration of 100 μg/mL α-Mangostin was chosen as an IS since it has a similar physicochemical properties as rubraxanthone. The calibration standards were set by spiking an appropriate volume of the standard solutions into blank mice plasma samples (300 μL). The concentration of rubraxanthone in calibration curve was 0.128–5 μg/ml. For validation, quality control (QC) samples were prepared in the same way as the calibration standards at three concentrations (low concentration (LC) 0.768 μg/mL, medium concentration (MC) 2.067 μg/mL, and high concentration (HC) 3.750 μg/mL).

Plasma samples (300 μL) were admixed with 50 μL IS solution, and separated by being centrifuged for 10 min at 10000 rpm in room temperature. Three parts of acetonitrile (900 μL) were poured to the plasma, mixed for 2 min using vortex and separated by being centrifuged for 10 min at 10000 rpm. The top layer (900 μL) was put to another tube and dried using a rapid vap vertex evaporator (Lab Conco). The remain was reconstituted in 500 μL of acetonitrile, and separated by being centrifuged for 10 min at 10000 rpm. The supernatant solution was then put to autosampler flasks and 5 μL sample were injected into the UHPLC-DAD system.

## 2.4. Animal

A total of 90 mice (*Mus musculus*) aged about 7–8 weeks and 20 ± 5 g body weight was purchased from the Animal House Faculty of Pharmacy Universitas Andalas. Mice were dispensed with newly prepared of rubraxanthone suspension in 300 μL virgin coconut oil at a dose of 700 mg/kg. Rubraxanthone doses were given based body weight. The suspension of rubraxanthone was administrated orally to 84 mice and 6 mice were used as control. The mice were placed in cages and given a regular food and drink *ad libitum* before the test. The mice were kept in a 12 h/12 h light/dark cycle. The animal protocols were permitted by the Research Ethics committee of Faculty of Medicine Universitas Andalas no 392/KEP/FK/2017.

## 2.5. Pharmacokinetic study

Eighty-four mice were dispensed with new prepared suspension of rubraxanthone in virgin coconut oil at a dose of 700 mg/kg. The mice were anesthetized with isoflurane before blood was drawn from the mandibular veins. Blood of six mice was drawn on 0, 0.25, 0.75, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5.5, 7, 9, 12 and 24 h. Blood was kept in lithium-heparin covered vials and directly centrifuged for 15 min at 2000 x rpm at 4 °C. Plasma was put to a tube and kept at -80 °C until UHPLC-DAD analysis.

## 2.6. Data analysis

A non-compartmental technique was used for pharmacokinetic analysis. From zero to the latest recorded concentration, the area under the concentration versus time curve (AUC<sub>0-t</sub>) was computed using the trapezoidal rule. The non-compartmental model was based on statistical moment theory, with the following formulae calculating AUC and area under the first moment curve:

$$\begin{aligned} \text{Area under the zero moment curve} &= \int_{t=0}^{t=\infty} t^0 C_p dt \\ &= \int_{t=0}^{t=\infty} C_p dt = \text{AUC} \end{aligned}$$

$$\begin{aligned} \text{Area under the first moment curve} &= \int_{t=0}^{t=\infty} t^1 C_p dt \\ &= \int_{t=0}^{t=\infty} t C_p dt = \text{AUMC} \end{aligned}$$

The plasma concentration–time plots were used to determine pharmacokinetic characteristics such as maximum plasma concentration (C<sub>max</sub>) and time of maximum concentration (T<sub>max</sub>). The elimination rate constants (K<sub>e</sub>) were calculated using a linear regression analysis of the logarithmic transformation of the curve's last four points (3–5.5 h after administration).

The following equations were used to compute the area under the concentration versus time curve from zero to infinity (AUC<sub>0-∞</sub>), total clearance (CL), and volume of distribution (V<sub>d</sub>) of rubraxanthone:

$$\text{AUC}_{0-\infty} = \text{AUC}_{0-t} + C_t / k_c \quad (1)$$

$$\text{Cl} = \text{Dose} / \text{AUC}_{0-\infty} \quad (2)$$

$$\text{Vd} / F = \frac{K \cdot C_0}{(K_a - K)} \left( \frac{1}{\text{intercept}} \right) \quad (3)$$

where C is the drug's plasma concentration, CL is the drug's total body clearance from plasma, and C<sub>p0</sub> is the drug's blood concentration at time

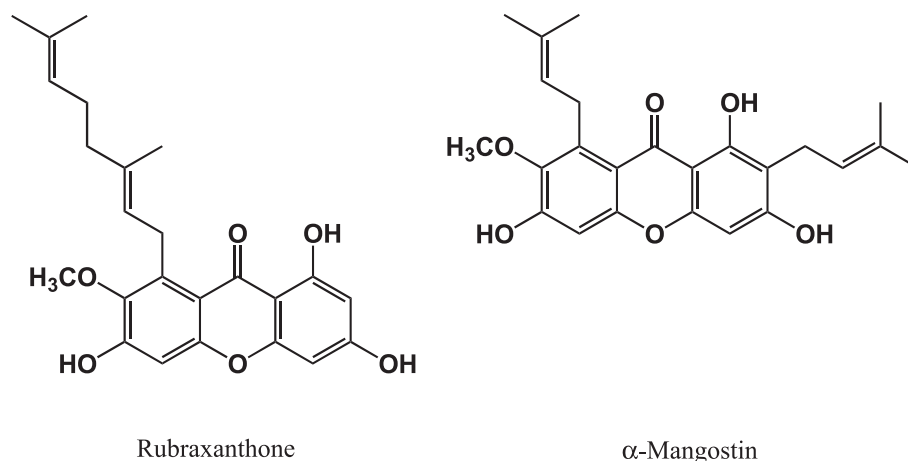
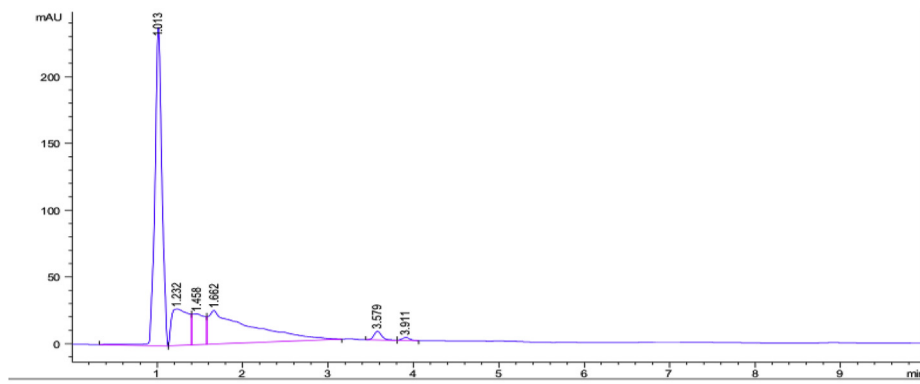
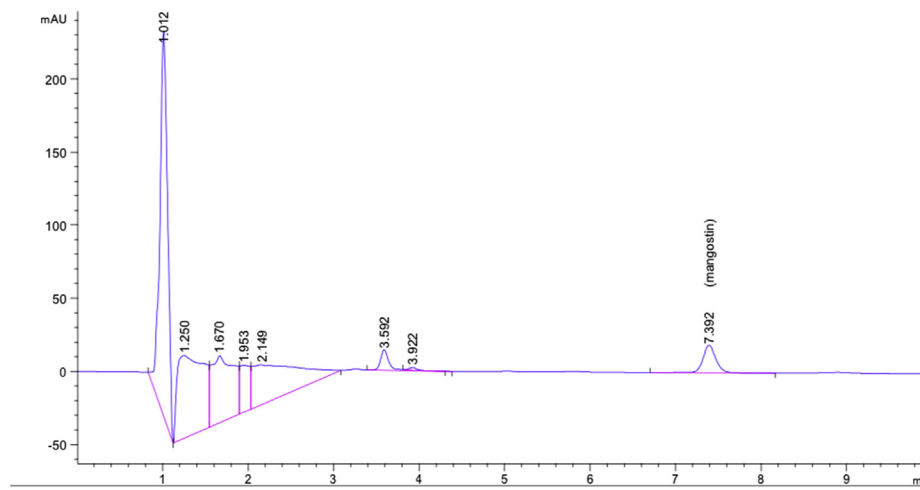


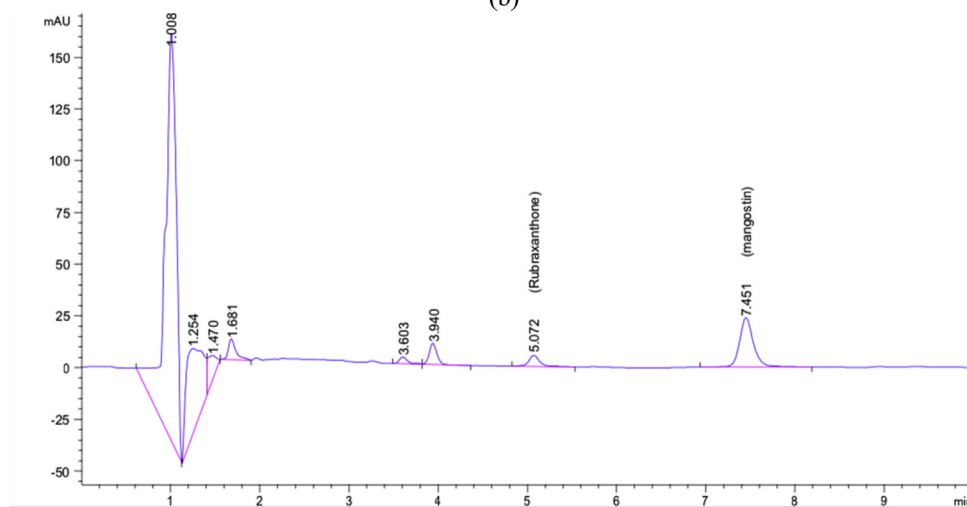
Figure 1. Chemical structure of rubraxanthone and α-mangostin



(a)



(b)



(c)

**Figure 2.** Chromatograms of [a] control mice plasma, [b] a control mice plasma spiked with  $\alpha$ - mangostin (IS) (Rt = 7.451 min) and (c) a sample in mice plasma found at 15 min following oral administration of rubraxanthone (700 mg/kg).

**Table 1.** Determination of intra- and inter-day precision and accuracy of rubraxanthone in mice plasma.

Concentration ( $\mu\text{g/mL}$ )	intra-day accuracy and precision (n = 3; 6 repetitions per batch)			intra-day accuracy and precision (n = 18; 6 repetitions per batch)		
	Conc. ( $\mu\text{g/mL}$ ) found Mean $\pm$ SD	Accuracy (%)	Precision (% CV)	Conc. ( $\mu\text{g/mL}$ ) found Mean $\pm$ SD	Accuracy (%)	Precision (% CV)
0.128	0.124 $\pm$ 0.02	-13.12–6.39	10.46	0.124 $\pm$ 0.01	-9.00–6.53	6.64
0.768	0.741 $\pm$ 0.04	-13.38–0.99	5.81	0.752 $\pm$ 0.04	-4.31–10.91	3.91
2.067	1.952 $\pm$ 0.14	-9.43–0.93	7.06	1.974 $\pm$ 0.09	-4.69–13.49	4.40
3.750	3.691 $\pm$ 0.15	-8.45–1.39	4.06	3.491 $\pm$ 0.12	-9.01–11.74	3.52

zero. The best fitting line was used to calculate the initial drug concentration, which was then projected to the y-axis. The starting drug concentration ( $C_{p0}$ ) was calculated using the y-intercept [14]. Microsoft Excel was used to calculate the figures.

### 3. Results and discussion

#### 3.1. Method development and validation

##### 3.1.1. UHPLC-DAD condition optimization

For the determination of rubraxanthone concentration in mouse plasma, an HPLC-DAD method was developed and validated. To achieve symmetric peak shapes and short run times of rubraxanthone and IS, the analytical column ZORBAX RRHD Eclipse Plus C18 (100 mm  $\times$  3.0 mm id, 1.8  $\mu\text{m}$ ) was used. Similar mobile elution system consisted of acetonitrile and 0.4% v/v formic acid in water (75:25) was used in the present method as previously described [13]. However, the flow rate programme was adjusted in 0.2 ml/min. It is well documented that better specificity can be achieved by reduction in the flow rate. There was no interference from endogenous components that were found in all samples at the same retention times as an analyte. Figure 2 showed the chromatograms of blank plasma, spiked plasma with IS (zero), suspension of rubraxanthone group plasma sample of 10-min time point.

##### 3.1.2. Validation of method

The standard curves showed very good linearity over a range of 0.128–5  $\mu\text{g/mL}$  for the analyte with coefficients of correlation ( $r$ ) more than 0.99, and the standard deviation of each point on calibration curve not more than  $\pm 3.8\%$ . The regression equation for the calibration curve were as follows:  $y = 0.049x + 0.0153$  ( $r = 0.996$ ). The LLOQ value for rubraxanthone was 0.128  $\mu\text{g/mL}$ . This value is better than the previous research that got 0.206  $\mu\text{g/mL}$  [12]. This method was appropriate for pharmacokinetic study and quantitative analysis of rubraxanthone in plasma. Table 1 showed the results of intra and inter-day accuracy and precision.

The average percentage of recovery from rubraxanthone at all QC levels were obtained to be  $95.74 \pm 3.5$ ,  $82.13 \pm 2.3$  and  $59.46 \pm 7.5\%$ , respectively. The average percentage recovery (mean  $\pm$  SD;  $n = 3$ ). There was no significant delayed between injections.

The freeze–thaw stability of rubraxanthone was assessed by performing a four-duplicate-investigation of MC, and HC samples. Rubraxanthone was remain stable after three freeze–thaw rounds as the % diff was 0.49, and 5.9% for MC and HC respectively. Eight duplicate examines of MC and HC samples were used to determine post-preparative and plasma consistency. Rubraxanthone was found to be stable since the ratio of area under curve of rubraxanthone to  $\alpha$ -mangostin in plasma kept at ambient temperature for 24 h before or after extraction was similar than plasma samples kept at  $-20^\circ\text{C}$  and extracted before analysis.

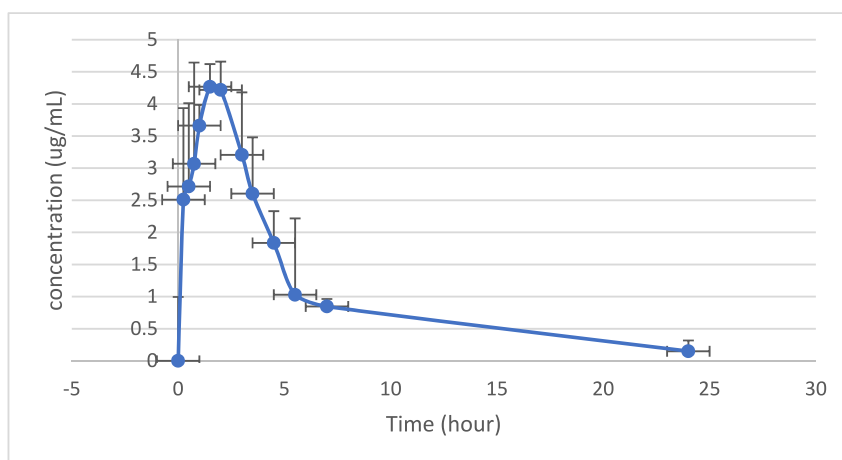
#### 3.2. PK study of rubraxanthone in mice

Pharmacokinetics had a valuable function in the biodistribution of compound at the place of action. The mice is the preferred replica for evaluating pharmacokinetics of the compounds. None of study has been carried out regarding to the pharmacokinetics of rubraxanthone after oral administration in mice. A precise and unique method with UHPLC-DAD was created for *in vivo* pharmacokinetics of rubraxanthone in plasma.

A dose of 700 mg/kg in mice was selected for this study. This dose that was chosen is nearer to the effective dose for lowering cholesterol levels in mice [15]. Virgin coconut oil (VCO) was chosen as the vehicle because it is known that VCO contains medium chain fatty acids which can help the absorption of lipophilic compounds in the digestive tract.

The mean plasma level of rubraxanthone against period after oral administration were displayed in Figure 3. Table 2 recapitulated the pharmacokinetic factors of rubraxanthone.

This substance was rapidly absorbed into the portal vein, with a stable absorption rate ( $K_a = 1.07\text{ h}^{-1}$ ). The fast absorption was related to the weak acid properties of rubraxanthone as one of the phenolic group compounds, the absorption of this compound was thought to occur through a passive diffusion mechanism in the gastrointestinal epithelial cell [9].

**Figure 3.** After an oral dosage of 700 mg/kg suspension in virgin coconut oil, the mean plasma concentration of rubraxanthone vs. time in mice plasma.

**Table 2.** Pharmacokinetic parameters of rubraxanthone after oral administration at 700 mg/kg.

Parameter	Mean $\pm$ SD (n = 6)
C <sub>max</sub> (ug/mL)	4.27 $\pm$ 1.43
T <sub>max</sub> (hour)	1.50 $\pm$ 0.99
T <sub>1/2</sub> (hour)	6.72 $\pm$ 1.29
K <sub>a</sub> (hour <sup>-1</sup> )	1.07 $\pm$ 0.87
K <sub>e</sub> (hour <sup>-1</sup> )	0.10 $\pm$ 0.49
VD/F (mL/kg)	1200 $\pm$ 139
Cl/F (mL/kg)	1124 $\pm$ 432
AUC t0-t24 (μg.h/mL)	560.57 $\pm$ 78
AUC t24-t ∞ (μg.h/mL)	0.42 $\pm$ 0.16
AUC t0-t ∞ (μg.h/mL)	560.99 $\pm$ 78.16

Values are expressed as mean  $\pm$  SD

The zone below the curve from 0-24 h (AUC<sub>0-24</sub>) and from 0-∞ (AUC<sub>0-∞</sub>) were projected and calculated by applying the trapezoidal law from time 0 to ∞ using equation (1) was 560.99 μg h/mL. The low value of AUC<sub>0-24</sub> rubraxanthone in plasma after oral administration at a dose of 700 mg/kg is thought to be caused by several things that may be experienced by this compound. Among them: 1) rubraxanthone undergoes First-pass-effect 2.) Rubraxanthone undergoes rapid metabolism by enzymes, thereby reducing the amount of circulating free drug.

From several reports of pharmacokinetic profiles of compounds from the xanthone group, it was known that in general, xanthone compounds have low bioavailability in plasma when administered orally. This was presumably because xanthenes undergo first-pass metabolism. Several studies specifically on xanthone metabolism have also reported that xanthenes were rapidly metabolized in the liver and small intestine. α – mangostin, for example, undergoes a rapid first-pass effect involving CYP (Phase I enzymes) [16, 17], and UGT (Phase II enzymes) enzymes after oral administration. The enzymatic reactions involved such as conjugation of glucuronide and bisglucuronate by UGTs (glucuronosyl transferase) enzymes as well as oxidation, methylation, hydrogenation, and dehydrogenation reactions involve CYP enzymes (cytochrome P450) [18]. Another report on xanthone metabolism also states that mangiferin (a xanthone glycoside) was metabolized by phase I and phase II enzymes rapidly after oral administration in the liver and also by intestinal flora [19]. The presence of hydroxyl and unsaturated carbon substituents in the xanthone skeleton which were considered important in xanthone metabolism by enzymes in the body.

Intensive research in an effort to increase the bioavailability of active plant metabolites has been carried out. One of them was by utilizing the new drug delivery system (DDS). Research in newer DDS is being carried out in liposomes, nanoparticles, niosomes, microencapsulation and polymers. This application has been researched and provides a significant improvement either to increase the solubility, pharmacological activity, or bioavailability of active secondary metabolites in the body [20].

The terminal half-life (T<sub>1/2</sub>) for oral administration of rubraxanthone was 6.72 h. The long elimination time was thought to be related to its lipophilic properties which allows rubraxanthone to be distributed to tissues, so that the elimination rate is small (K<sub>e</sub> = 0,10 h<sup>-1</sup>). The volume of distribution of rubraxanthone on extravascular administration (Vd/F) was determined using Eq. (3) and the value of Vd/F = 1200 mL/kg was obtained. Further work to characterize the pharmacokinetic properties of rubraxanthone were needed to optimize future dosing strategies.

#### 4. Conclusions

In conclusion, our work was the first to report the PK of rubraxanthone in mice after a 700 mg/kg oral dosage. The disposal half-life (t<sub>1/2</sub>) was 6.72  $\pm$  0.7 h. The long elimination time is thought to be related to its lipophilic properties which allows rubraxanthone to be distributed to

tissues. It is suggested to study the pharmacokinetic profile of rubraxanthone after intra-vena (IV) administration to determine its bioavailability.

#### Declarations

##### Author contribution statement

Meri Susanti: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Rizki Damayanti: Performed the experiments.

Yahdiana Harahap; Afrizal Itam: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Dachriyanus Hamidi: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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##### 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.

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