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Original Article

PHARMACOKINETIC PROFILE OF TETRAPRENYLTOLUQUINONE AFTER SINGLE-DOSE ORAL ADMINISTRATION IN MALE MICE

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ABSTRACT

Objective: The aim of this study to investigate pharmacokinetic profile of tetraprenyltoluquinone (TPTQ) in male mice's blood plasma.

Methods: A single dose of 800 mg/kg carried by Virgin Coconut Oil (VCO) was given orally where VCO only also administered as a control. Bloods were collected from vena jugularis after 0, ½, ¾, 1, 1½, 2, 3, 4, 6, 8, 12, and 24 h. The TPTQ levels in plasma were analyzed using High-Performance Liquid Chromatography (HPLC) following pre-treatment to induce protein precipitation.

Results: The formed pharmacokinetic profile follows the two-compartment model where TPTQ levels increase during the absorption phase and form a biphasic pattern after it decrease. The results showed the pharmacokinetic parameters had C_{max} value of 154.92±19.55 µg/ml at t_{max} of 1.117 h with $AUC_{0-\infty}$ of 1067.59 µg. h/ml. Other parameters were also obtained such as ka = 1.448±0.17 h^{-1}, α = 0.511±0.07 h^{-1}, ke = 0.057±0.02 h^{-1}, $t_{½}$ absorption = 0.483±0.05 h, $t_{½}$ elimination = 12.131±0.55 h, Vd/F = 5284.79±629.49 ml, dan Cl/F = 751.84±53.85 ml/h.

Conclusion: The pharmacokinetic profile of TPTQ administered orally show that TPTQ absorbed rapidly, eliminated slowly, and also distributed to peripheral tissues.

Keywords: Tetraprenyltoluquinone (TPTQ), Pharmacokinetic, Oral, Blood plasma, High performance liquid chromatography (HPLC)

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INTRODUCTION

Natural product has an important role in the discovery of new drugs, especially for cancer and infection diseases. Government has sought various chemotherapy treatment in dealing with cancer. But currently available anticancer drugs are mostly chemically synthesized. On the other hand, this kind of drugs give an adverse side effect on normal cells that undergo rapid grow, such as hair and blood. Therefore, research on cancer drug continues to be carried out and natural product become an ideal alternative for cancer treatment because of its smaller side effects than synthetic drugs [1]. Many studies exhibit promising activity by natural compounds which could trigger a selective immune response against cancer cell [2]. One of them is *Garcinia cowa* Roxb., known as asam kandis, widely used as a traditional medicine that spread in Indonesia [3]. Tetraprenyltoluquinone (TPTQ) is one of the metabolite isolated from the hexane fraction of *G. cowa*'s stem bark [4].

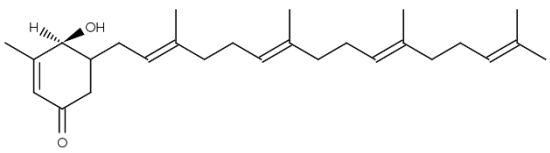

Fig. 1: Chemical structure of TPTQ

TPTQ (fig. 1) reported had a selective cytotoxic activity against small lung cancer [5]. The only *in vivo* study reported an antitumor activity by inhibiting tumor growth in H-460 tumor xenografted nude mice with dose at 800 mg/kg [6]. This compound also had a strong anti-inflammatory activity againts NO [7], immunomodulatory, and safety against RAW 264.7 cell and leukocyte cell [8]. However, until now there has been no report regarding the pharmacokinetics of TPTQ in animals. Pharmacokinetic study plays a crucial role in the early stage of the development of new drugs in choosing the best dosing regimen and route of administration [9]. Despite of its promising *in vitro* studies, a lot of natural compounds have failed during preclinical trails due to poor pharmacokinetic properties, such as low bioavailability, low metabolic stability, and lack of pharmacodynamic reproducibility [10]

In most cases, drug compounds have small amounts in the blood. Therefore, research related to the pharmacokinetic profile of TPTQ compounds requires a simple and sensitive assay method using High Performance Liquid Chromatography (HPLC) to measure the levels of drug compounds in the blood. This refers to the quantitative method that has been developed and validated *in vitro* analysis of TPTQ compounds in blood plasma [11]. In the present study, pharmacokinetics profile of TPTQ after single oral administration in male mice were investigated, which may led as a reference on the future development of TPTQ as a drug candidate.

MATERIALS AND METHODS

Chemicals

TPTQ (purity>98%) was previously isolated from *G. cowa* at Research Laboratory, Faculty of Pharmacy, Universitas Andalas. Fenofibrate as internal standard (IS). Virgin Coconut Oil (VCO) was purchased from Herbal Mumtaz. Methanol, formic acid, and acetonitril at HPLC grade were obtained from Merck. Distilled deionized water was prepared using IKA water purification system. The other chemicals and reagents were analytical grade.

Animal experiments

A total of 48 male white mice (*Mus musculus*) age between 8-12 w old were obtained from Animal House, Faculty of Pharmacy, Universitas Andalas. The mice were isolated in cages with a uniform cycle of 12 hour of light and 12 hour of dark given a regular pellet and water *ad libitum* before the test. All the experiments were done according to the protocols approved by the Research Ethics Committee of the Faculty of Medicine, Universitas Andalas no. 676/UN.16.2/KEP-FK/2022.

TPTQ was administered orally to twelve groups of three mice at dose of 800 mg/kg with VCO as a carrier. The leftover of twelve mice were given a VCO only as a control subject. The number of samples according to Federer's formula follows that this sample requires 4 replications by each sampling time, so it needs 3 replication for the treatment group and 1 for control group as it only contain VCO.

The process of taking blood from mice was carried out in the jugular veins. Blood was taken from the mice at 0, ½, ¾, 1, 1½, 2, 3, 4, 6, 8, 12, and 24 h with four mice in each treatment. Blood was accommodated in a tube filled with EDTA as an anticoagulant and centrifuged at 4000 rpm for 10 min to form a blood plasma separation. Plasma is stored in a freezer if it is not analyzed immediately.

Preparation of standard samples

A standard stock solution of TPTQ was prepared by dissolving it in 200 ml methanol. The calibration standard and quality control (QC) were set by diluting an appropriate volume based on concentration into blank mice plasma samples (200 µl). Calibration standard sample were prepared at concentrations of 0.58 µg/ml, 1.16 µg/ml, 2.32 µg/ml, 4.64 µg/ml, 5.80 µg/ml, and 9.280 µg/ml also QC solutions at Low Limit of Quantification (LLOQ), low, medium, and high concentrations (0.58 µg/ml, 1.74 µg/ml, 4.35 µg/ml, and 6.96 µg/ml) by dilluting the standard stock solution. Fenofibrate was used as IS since it has a similar physicochemical property as TPTQ. The IS solution (500 µg/ml) was prepared by dissolving fenofibrate (100 mg) in 100 ml methanol and diluting it to the needed concentration.

Instrumentation conditions

The HPLC system of Agilent LC-20 consisted of autosampler with UV-Vis M-20A Diode Array Detector. Column Agilent Eclipse Plus C18 RRHD (2.1 x 1000 mm, 1.8 µm) was used for the stationary phase. Acetonitril and 0.4 formic acid (87:13) was used for mobile phase pumped at flow rate of 0.2 ml/min that was already prefiltered through a milipore 0.22 µm followed by sinication prior. The detector was set at a wavelength of 230 nm with column temperature kept at 20±3 °C.

Determination of TPTQ in plasma

A 200 µl aliquots of plasma sample were solubilized with 50 µl IS solution inside the microtube then vortex-mixed for 30 sec. After adding 800 µl of acetonitril to precipitate the protein, the samples were vortex-mixed again for 2 min and centrifuged at 15.000 rpm for 30 min. The supernatant solution was then evaporated under nitrogen gas and the residue was added by acetonitril until reach 0.5 ml of volume. A 5 µl sample solution were injected into the HPLC system.

Pharmacokinetic analysis

The pharmacokinetic profile was formed based on a one-point curve between plasma drug concentration and sample acquisition time. Pharmacokinetic parameters were determined based on the plasma drug concentration curve with the sample acquisition time. The analysis was carried out with the assumption of a two-compartment open extravascular model using manual calculations with formulas. Pharmacokinetic parameters include the highest drug concentration in the blood after a dose given (Cmax), time of highest drug concentration (tmax), absorption and elimination half-life (t½), Area Under Curve (AUC), volume of distribution of drug (Vd), Peripheral distribution volume (Vp), clearance (Cl), absorption rate constant (ka), distribution rate constant (α), drug distribution rate constant in central and peripheral (k12, k21), and elimination rate constant (ke).

RESULTS AND DISCUSSION

HPLC examination

Under the analytical condition, the representative HPLC chromatograms of plasma sample are shown in fig. 2. There are no interfering peak was observed at the retention times of TPTQ and IS. The calibration curves showed good linearity in ranges of 0,58-9,28 µg/ml with the regression equation as follows: y = 0,0381x-0,0117 (r=0,993). The analysis of precision and accuracy of the method indicated that all coefficients of variation (CV) were <11% and the relative errors were<15%. In term of precision and accuracy, the validity of this method was sufficiently sensitive to measure the sample concentration and acceptable.

Pharmacokinetics study of TPTQ in male mice

In determining the pharmacokinetic profile of TPTQ after oral administration, a dose of 800 mg/kg was used. This dose refers to a previous study about the higher dose of TPTQ that can be tolerated inside the mice, known as Maximum Tolerated Dose (MTD) [6]. The small amount of TPTQ to be given orally makes it should be diluted into 80 mg/ml solution. Based on its, this compound should be formulated with an oil base or emulsion dosage form. In this case, VCO was chosen due to its ability to dissolve the TPTQ. VCO also contains Medium Chain Fatty Acid (MCFA) that is highly soluble in water to help the absorption of fatty acid. In pharmacokinetic studies, VCO known to increase drug metabolism in the body [12]. Therefore, VCO as a control not affecting any peak of TPTQ or IS as shown in fig. 2. The analyte chromatogram also showed that there were several low peaks outside the retention time of TPTQ and IS due to hemolysis during blood sampling. If that peak not interfering another analyte peak, the quantification results are still acceptable [13]. TPTQ's analyte also showed as a wide peak based from its optimization before [11].

The mean plasma concentrations-time curve of TPTQ in male mice's plasma after oral administration are show in fig. 3. The pharmacokinetic parameters calculated by residual method by Shargel's equation [14] are shown in table 1. TPTQ reached maximum levels after 1.5 h and then declined rapidly before 6 h. A slow decrease in levels to 24 h indicates that TPTQ concentrations in plasma are eliminated quite slowly in the body. All the concentrations were not detected lower than LLOQ.

The profile above forms a biphasic pattern in the distribution and elimination phases indicating that TPTQ kinetics after oral administration follows a two-compartment extravascular model which is clearly shown from the gradient of the elimination phase which is smaller than the distribution phase. The plasma concentration from the results of this study were reported in a general equation so that the C_{max} of the TPTQ was 154.92±19.55 µg/ml at t_{max} of 1.12±0.03 h but the C_{max} observed was 178.53±2.81 µg/ml at 1.5 h. This differentiation happen since the value of C_{max} was influenced by the frequency of the sampling scheme and the magnitude of the assay errors [15]. Thus, it needs to be studied more at sampling time which near the t_{max}. The entire pharmacokinetic process was manifested from below the curve from 0-∞ by applyng the trapezoidal equation with $AUC_{0-\infty}$ 1067.59±74.14 µg. h/ml. theoretically, this parameter is highly dependent on the administered dose.

Table 1: The estimated mean pharmacokinetic parameters of TPTQ after oral administrations at 800 mg/kg

Parameters	Value±SD*
C_{max} (µg/ml)	154.92±19.55
t_{max} (h)	1.12±0.03
ka (h^{-1})	1.45±0.17
α (h^{-1})	0.51±0.07
ke (h^{-1})	0.06±0.00
k_{21} (h^{-1})	0.21±0.06
k_{12} (h^{-1})	0.13±0.03
$t_{½}$ absorption (h)	0.48±0.05
$t_{½}$ elimination (h)	12.13±0.55
$AUC_{0-\infty}$ (µg. h/ml)	1067.59±74.14
Vd/F (ml)	5284.79±629.49
Vp (ml)	3277.64±305.60
Cl/F (ml/h)	751.84±53.85

*Data are expressed as mean±standard deviation from 3 mice at different time points (n = 3).

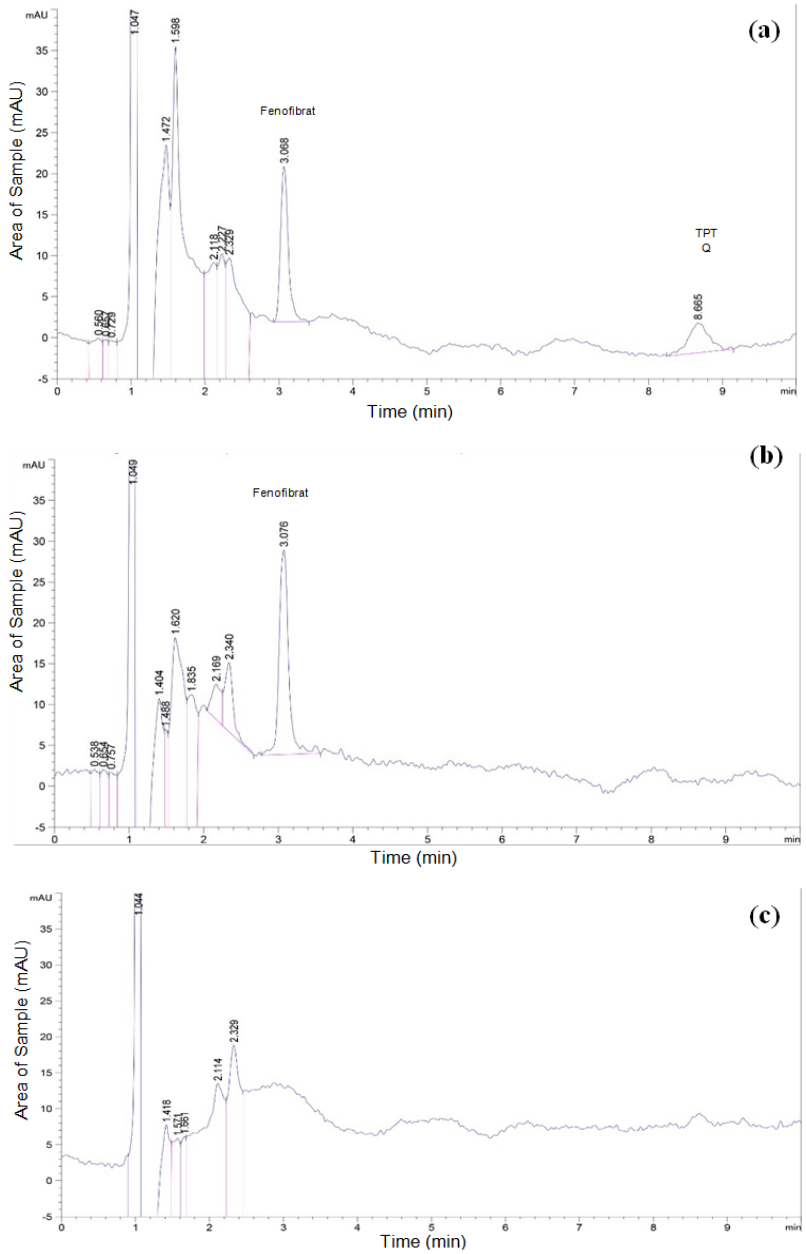

Fig. 2: Chromatogram profile of (a) plasma sample with TPTQ and IS (b) plasma control with VCO only (c) blank plasma

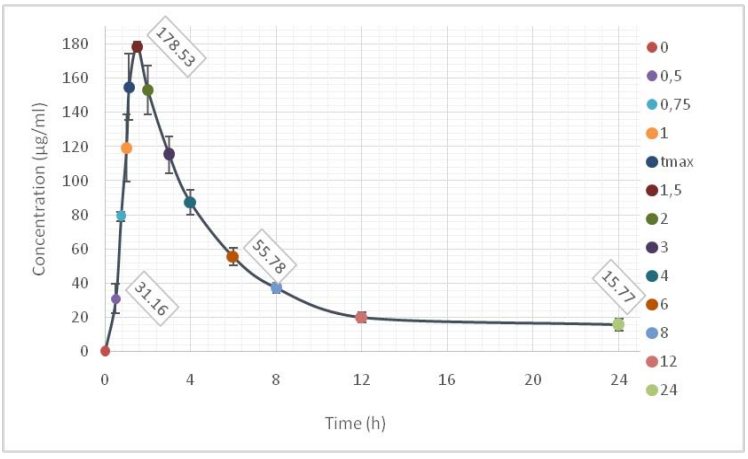

Fig. 3: The mean plasma concentration-time curve of TPTQ administered orally at a dose of 800 mg/kg to male mice

ADME discussion

TPTQ was shown to have rapidly absorption in the gastrointestinal tract with the absorption half-life parameter at 0.48±0.05 h and absorption rate constant (ka) of 1.448±0.17 h^{-1}. The value of ka describes the amount of drug that moves from the drug absorbed to the extravascular administration expressed per unit time [14] Viewed from its structure, TPTQ has a weak acid tructure characterized by the presence of a phenolic group, where absorption occurs through a passive diffusion mechanism in epithelial cells [9]. Drugs that are weakly acidic (pKa 3–7.5) in acidic stomach will be distributed in non-ionized form, so they are easily soluble in fat and easily penetrate the gastric membrane [16]. Not only that, TPTQ is lipophilic and formulated in the form of oil which increases its solubility to be absorbed in the stomach to deep into the small intestine by the limphatic system [17].

Overall, the distribution of TPTQ in the body has a rate constant (α) of 0.51±0.07 h^{-1}. The value of the volume of distribution is related to the bioavailability (F) therefore, the value is expressed in units of the absorbed drug fraction [10]. In the two-compartment open pharmacokinetics model, the distribution of the drug in tissues and organs is expressed by the rate constant for drug distribution to the peripheral compartment (k_{12}) and the rate constant for drug distribution out of the peripheral compartment (k_{21}). This is confirmed by the sum of the values of k_{21} and $k_{12,}$ which are less than twenty times the value of k. This value means that the distribution is slower than its elimination [18]. The volume of TPTQ was also more widely distributed in the central compartment as much as 5.284±0.63 l than in the peripheral compartment.

Research related to the effect of absorption, distribution, metabolism, and elimination of TPTQ on organs so far has not been reported. Menaquinone-4 as a quinone group compound that has a similar structure to TPTQ is reported to undergo absorption in the lymphatic system involving bile acids by the portal vein. Consumption of food before oral administration is highly recommended because it can increase the bioavailability of menaquinone-4 [19]. The metabolic pathway begins with a demethylation reaction in the side chain of this compound which is followed by an oxidation reaction to produce acetate metabolites to become vitamin K acids 1 and 2. Products that gone through phase I reactions continue to phase II metabolism, which involves enzymatic reaction to produce more polar metabolites such as gulcuronide and sulfate [20]. Spread of menaquinone-4 accumulated more in bone and adipose tissue than in blood plasma. The concentration of drugs left in the tissue exerts a pharmacological effect on osteoporosis [21].

TPTQ might have a long elimination time in the body. One of the parameters is the elimination half-life, which is the time it takes for the drug in the blood plasma to decrease to half of it starting dose in the body. The value of t½ elimination is inversely proportional to the elimination constant (ke), where the longer or higher the elimination time, the lower the value of ke. The elimination half-life of the TPTQ compound was at 12.131±0.55 h and it takes four to five half-lives for the drug to be eliminated by 94-97% [22]. It is suspected that this is related to its lipophilic structure which allows TPTQ to be distributed to tissues outside the plasma; this causes its elimination rate to be slow, namely 0.057±0.0 h^{-1} [9].

CONCLUSION

For the first time, we reported the pharmacokinetic profle of TPTQ in male mice after a single dose of 800 mg/kg oral dosage. TPTQ has rapidly absorbed dan slowly eliminated in the body due to its lipophilic properties. We hypothesized that TPTQ also had a potential effect to peripheral tissues. These findings suggest to study the pharmacokinetic profile of TPTQ after intravena (IV) administration to measure the oral bioavailability.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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