The Potential Effect of Conferment *Jamu* "Pegal Linu" to Pharmacokinetics of Paracetamol in Wistar Male Rats (*Rattus norvegicus L.*)

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Abstract. Paracetamol (acetaminophen) is a ubiquitous drug usually prescribed for its analgesic and antipyretic effects. It is a readily available over-the-counter medication. Consumption of *jamu* and paracetamol causes pharmacokinetic interaction. This study aimed to determine how much the potential of *jamu* "pegal linu" affected pharmacokinetics profile of paracetamol. Paracetamol levels in rat plasma were analyzed by spectrophotometry UV-Vis using regression and residual methods to primary pharmacokinetic profile: constant absorption rate (Ka), distribution volume (Vd), maximum plasma concentration (Cpmax), Area Under Curve (AUC), and secondary parameter: constant elimination rate (Ke), half-life elimination ($t_{1/2}$), maximum concentration (Tmax). Animal subjects were grouped into two categories, group I or paracetamol group (positive control) and group II (paracetamol + jamu "pegal linu") with three Wistar male rats for each group. The result showed that if jamu "pegal linu" was given, the constant absorption rate (Ka) and elimination rate constant (Ke) were lower, but maximum plasma concentration (Cpmax) and Clearance (Cl) remained steady. Others pharmacokinetics, such as maximum concentration (Tmax), half-life elimination ($t_{1/2}$), distribution volume (Vd), and Area Under Curve (AUC), were higher. The bivariate analysis showed no major different ($p > 0,05$) for both groups. *Jamu* "pegal linu" co-administration has a potential effect to allegedly the plasma level of paracetamol.

Keywords: Jamu "Pegal Linu" · Paracetamol · Pharmacokinetic Profiles

1 Introduction

The use of herbal medicines has increased, particularly in the developing countries in Africa and Asia, especially in Indonesia [1]. Along with the public's high expectation of herbal treatment, many peoples in Indonesia consume traditional medication to provide a faster therapeutic effect [2]. *Jamu* (herbs) is one of the choices that most people choose because it has a potential effect of reducing pain [3]. The various ingredients in *jamu*, such as Zingeberis aromatica rhizome, Piper retrofractum fructus, Cyperi Rhizoma, and others contain multiple chemical components [4].

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Synthetic drugs are still an option to treat pain, fever, or headache. One of the most commonly used synthetic drugs is paracetamol. Paracetamol (acetaminophen) is a prevalent drug usually prescribed for its analgesic and antipyretic effects. It is a readily available over-the-counter medication [5].

Consumption of *jamu* and paracetamol at the same time can cause some interaction. A drug interaction occurs via three mechanisms, namely: pharmaceutical interaction, pharmacokinetic interaction, and pharmacodynamic interaction [6]. Pharmacokinetics interaction happens when a drug's plasma level might increase or decrease. As a result, drug toxicity and drug effectiveness might also increase or decrease [7].

According to the description above, the effect of conferment *jamu* allegedly can be affected to plasma level of paracetamol. This study aimed to determine how much the potential of *jamu* "pegal linu" affected to pharmacokinetics profile of paracetamol.

2 Material and Methods

2.1 Materials

The materials used in this study were Paracetamol (PT Brataco, Chemika) EDTA, Trichloroacetic acid (TCA) 10%, HCl, $NaNO_2$ 10%, Sulfamic Acid (H_3NSO_3) 10%, NaOH 10%, and samples of the existing herbal pain relief in the market.

2.1.1 Instrument

The instruments consisted of a sonde needle, 50 mL and 100 mL beaker glass (Pyrex), funnel, 100 mL Erlenmeyer (Pyrex), syringe (One Med), watch glass, 100 mL volumetric flask (Pyrex), freezer (Panasonic), adjustable micropipette 20−200 μL (DLAB), analytical balance (Ohaus), Centrifuge (DLAB), dropper pipette, multi-size measuring pipette (Pyrex), UV-Visible Spectrophotometer 1900, 1.5 mL blood tube (Eppendorf), test tube, test tube rack, and a capillary tube.

2.1.2 Animal Subject

The Wistar white male rats were aged 2–3 months from the pharmaceutical laboratory of STIKES Telogorejo Semarang. The rats weighed 150–200 g. The test animals were divided into two groups, namely group I or positive control (paracetamol group) and group II (paracetamol + *jamu* "pegal linu"). Each group consisted of three white male rats.

2.2 Methods

2.2.1 Dosing

The paracetamol dose set for the test subjects was 500 mg/kg with a conversion factor of 0.018 [8]. In this study, the samples of herbal medicine *tolak linu* were used to compare. The dose given to normal adult humans was 1–2 sachets @ 15 mL. Then, it was calculated according to the conversion factor and given with a 50 mg/mL volume. The dose was obtained using the formula as follows:

Do (mg) x conversion factors = yield x 50 mg/mL

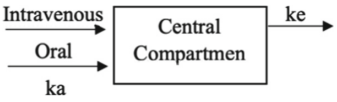

Fig. 1. One Compartment Model

2.2.2 Preparation of Standard Solution

The preparation of paracetamol standard solution was made by weighing 100 mg of paracetamol, then dissolved in ethanol to an exact volume of 100 mL and homogenized to obtain 1000 ppm of the paracetamol stock solution. The stock solution was diluted to 400 ppm. The 400-ppm stock solution was diluted into the various concentrations of paracetamol 2, 4, 6, 8, and 10 ppm, made in a 10 mL flask. The 10% NaOH (2.5 mL) and 6 N HCl (0.5 mL) were added.

2.2.3 Paracetamol Maximum Wavelength Determination (λmax)

The maximum determination of paracetamol wavelength on the UV-Vis Spectrometer was completed using a variation of 4 ppm paracetamol concentration. The wavelength range used was between 200–400 nm. The linearity was determined based on the regression equation $y = bx + a$.

2.2.4 Biosample Preparation

After the dose of paracetamol and *jamu* "pegal linu" for each subject was calculated, the drug was administered via the p.o route using a 1 mL probe. The positive control test was performed by taking 1000 μL through the orbital vein of the eye with a capillary tube at 0.25, 0.50, 1, 1.25, 1.50, 2, 2.50, 2.75, and 3 h. The blood sample was put into a centrifuge tube by adding a 10% TCA (1.0 mL) and 5% EDTA solution and then homogenized using a vortex for five minutes with a rotation speed of 4000 rpm. The supernatant (clear) was pipetted 1 mL into a test tube, then added 6 N HCl (0.5 mL) and 10% $NaNO_2$ (1.0 mL), homogenized again using a vortex, and added 15% sulfuric acid (1.0 mL) and 10% NaOH (2.5 mL) solutions carefully. The tube was left for three minutes in a container containing ice cubes.

3 Determination of Pharmacokinetic Profiles

The determination of the paracetamol pharmacokinetic profiles based on the data analysis on the drug levels in the blood obtained each time, Ln plasma concentration (Cp) curve versus time (t) was made using the residual method. The plasma concentration as the y-axis, and time as the x-axis. The formula equation used was the first formula order and the open compartment 1 as follows (Fig. 1).

The related equations for this model are [9]:

$$Cp = Cp^0.e^{-ke.t}(\text{intravenous route}) \quad (1)$$

$$Cp = B.e^{-ka.t} - A.e^{-a.t}(\text{oral}) \quad (2)$$

The First-order related equations:

$$dDb/dt = F.\,Ka.\,D_{GI} - KD_B \tag{3}$$

By calculating the maximum plasma concentration (Cpmax), therefore the value (Tmax) can be determined by the following equations:

$$Cpmax = Be^{-ke.tmax} - Ae^{-ka.tmax} \tag{4}$$

$$Ke^{-kt} = Kae^{-kat} \tag{5}$$

$$\begin{aligned} In\,K - kt &= In\,ka - kat \\ Tmax &= In\,ka - In\,k/ka - k = In\,(ka/k)/ka - k \\ Tmax &= 2,3\,log\,(ka/k)/ka - k \end{aligned}$$

The other pharmacokinetic profiles that were also analyzed in this study were the value of Ka (absorption), the distribution volume (Vd) calculated from the initial dose (Do) and the extrapolated (Cp0), the Area Under Curve (AUC), half-life elimination ($t_{1/2}$), the Ke (elimination), and the clearance (Cl). Furthermore, the data were analyzed using a bivariate independent t-test using the SPSS Statistics 20.0 program.

4 Results and Discussion

4.1 Weighing of the Experimental Subjects

In this experiment, the rats were weighed using a weighing scale that had been previously tamed with a 189 g container weight (Table 1).

4.2 Determination Dose and Volume Drug Administration

After the bodyweight was weighed, group I and II were given treatment according to the calculated dose and administration volume (Table 2).

Table 1. Animal Body Weight

Animal Subject	Rats Weight + Container (g)	Rats Weight (g)
Rat 1	376	187
Rat 2	343.5	154.5
Rat 3	350	161
Rat 4	353	164
Rat 5	351.5	162.5
Rat 6	343.5	153

Table 2. Dose dan Volume Drug Administration

Animal Subject	Rats Weight (g)	Dose of Paracetamol (mL)	Dose of *Jamu* "Pegal Linu" (mL)
Rat 1	187	0.1683	-
Rat 2	154.5	0.13905	-
Rat 3	161	0.1449	-
Rat 4	164	0.1476	0.2214
Rat 5	162.5	0.14625	0.43875
Rat 6	153	0.1377	0.6195

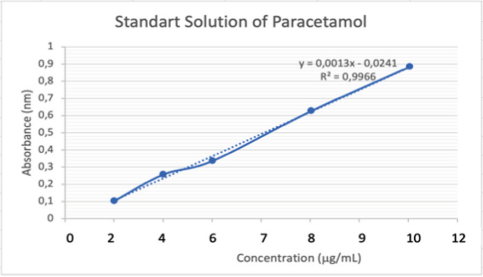

Fig. 2. Calibration Curve of Standard Paracetamol

4.3 Linearity

Figure 2 showed the equation obtained. The result is a regression value (r). The regression value of $r > 0.9966$ means a correlation between the level and the measured drug absorption [10]. The regression equation obtained is as follows $y = 0.001288x - 0.02414$ ($r = 0.9966$).

4.4 Determination Plasma Levels of Paracetamol

In determining the level of paracetamol in the blood, group I testing animals was used as positive controls, which was determined based on the analysis of blood samples from the three white male rats. The samples were observed from the results of blood samples analysis at 0.25, 0.50, 1, 1.25, 1.50, 2 2.50, 2.75, and 3 h. The blood sampling time orientation results for group I (positive control) can be seen in Fig. 3.

There was an increase in paracetamol levels from 0 to 30 min and reached the peak levels at 50 min. After reaching the peak levels, the drug levels in the blood decreased from the 60th minute to the 180th minute. The Ln content versus the time data was calculated to obtain an extrapolated value (Ln Cp^o) then anti-Ln was used to determine the paracetamol levels. Based on the sampling time, the average extrapolated value in rat 1 was 96.6309 g/mL, rat 2 was 147.0247 g/mL, and rat 3 was 280.9521 g/mL. The calculation results of half-life elimination ($t_{1/2}$) obtained 133.7093 min with the

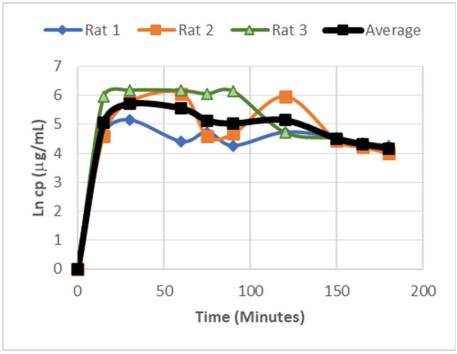

Fig. 3. Ln curve of blood levels (µg/mL) versus time (t) of paracetamol in each of group I experimental subjects (positive control)

condition that the steady-state time is 3–5 times the half-life, which is 401.1279 min; therefore, it can be said that the sampling time is still lacking [11].

The principle used in determining the level of paracetamol in the blood is the formation of a diazonium salt from the secondary aromatic amine group present in paracetamol. Diazonium salt formation occurs when the experimental temperature, sample, and reagent temperatures ranged from 5–15 °C. In this study, several reagents were needed, such as TCA, HCl, $NaNO_2$, Sulfamic Acid, and NaOH [12].

The widely used anticoagulants have the mechanism of converting calcium ions in the blood into Ca-EDTA complexes, which can prevent the coagulation process from occurring [13]. The blood sample is separated from the blood proteins using TCA, which functions to precipitate blood proteins; therefore, a clear liquid or supernatant is obtained in the following process [14].

The supernatant was added with HCl to hydrolyze the secondary amine group into the primary aromatic amine in paracetamol. The group will react with $NaNO_2$ to form a diazonium salt [15]. The addition of sulfamic acid aims to provide an intense acid atmosphere in the diazotation reaction consequently, the diazonium salt can be formed completely [16]. The addition of Sulfamic Acid can cause a foam formation reaction that needs to be dripped slowly, little by little, through the tube wall. Then, the 10% NaOH is added, which aims to neutralize the acid.

4.5 The Result of Conferment Jamu "Pegal Linu" to Plasma Levels of Paracetamol

The determination of paracetamol levels in the control group was then compared with the test animal group that were given intervention in the form of jamu "pegal linu". The primary pharmacokinetic parameters are the constant absorption rate (Ka), the volume of distribution (vd), the maximum plasma concentration (Cpmax), the AUC, and the secondary parameters are the constant elimination rate (Ke), the half-life elimination ($t_{1/2}$), and the maximum concentration (Tmax). Compared to Fig. 3, the mean Ka value of Group I is 3.357 min^{-1} versus group II 0.5678 min^{-1}. The Ka value describes the absorption rate, i.e., the drug entry into the systemic circulation from the digestive tract

Table 3. Pharmacokinetics Profile of Paracetamol in Both of Animal Subject Groups

Pharmacokinetics Profiles	Paracetamol (Mean ± SD)	Paracetamol-*Jamu* (Mean ± SD)
Absorption Constant Rate (Ka) (/minute^{-1})	3.357 ±	0.5678 ±
Maximum Concentration (Tmax) (minute)	54.9596 ±	85.5510 ±
maximum plasma concentration (Cpmax) (μg/ml)	3.6254	3.8924
Elimination Constant Rate (Ke) (/minute^{-1})	0,0110	0.0066
Half-life Elimination ($t_{1/2)}$ (minute)	85.5768	439.2827
Distribution Volume (Vd) (mL/gBB)	2.5545	6.2620
Clearance (Cl) (L/minute)	0.0118	0.0176
Area Under Curve (AUC) (μg.minute/L)	44685.80	69623.33

[17]. From the comparison of the Ka value in the two graphs, there was a decrease in the absorption value in group II after being given the *jamu* "pegal linu". It could be due to the concurrent administration of *Jamu* "pegal linu", which is suspected to affect the absorption of paracetamol in the gastrointestinal tract since paracetamol is almost completely absorbed in the small intestine that controlling entry and exit nutrients and xenobiotics [18]. The other factors that can influence are the sample of management techniques and the less precise sampling timings. The following primary pharmacokinetic parameter is the Distribution Volume which describes the dissolved drug volume. The distribution volume has an inverse relationship with the drug levels in plasma where and when a drug molecule is bound to the plasma proteins in large amounts or is in the blood vessels, the value of drug levels in plasma will be higher, resulting in a smaller Vd value [19] (Fig. 4).

Table 3 showed other primary pharmacokinetic parameters are Cpmax and AUC. The comparison of Cpmax values between the two groups of experimental subjects was insignificant, namely as 3.6254 g/mL versus 3.8924 g/mL after one hour of drug administration. It is not in line with the results of the previous studies, which stated that the administration of paracetamol and turmeric extract caused a significant increase in Cpmax of 70–90% after one hour of the oral route administration [2]. In contrast to the AUC value, there is a significant difference after giving *jamu* "pegal linu" to group II than group I that was 69623,33 μg.minute/L vs. 44685,80 μg.minute/L. The result was corresponding to the previous study by Pingli *et al.* (2015), giving herbal extract could increase the plasma level of paracetamol by inhibiting metabolic enzymes [20]. Flavonoid, especially quercetin, has a significant role as a CYP2E1 inhibitor, which has

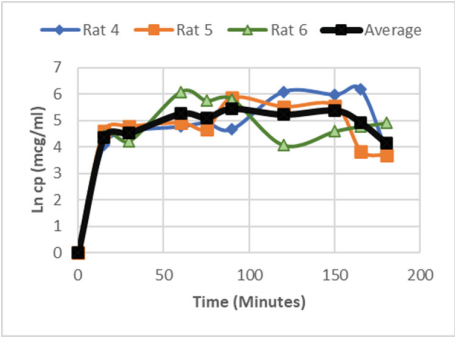

Fig. 4. Ln curve of blood levels (μg/mL) vs. time (t) of paracetamol in each of the experimental group II (Paracetamol + *Jamu* "pegal linu")

the function of forming N-acetyl-p-benzoquinone imine (NAPQI) in proses paracetamol bioactivation [20, 21].

According to secondary pharmacokinetic profiles, there was a difference among the two groups in elimination constant rate (Ke) and maximum concentration (Tmax) value. The given of *jamu* to group II can influence to elimination rate of paracetamol; in fact, that can be affected to Vd and clearance of the drug value. The elimination rate value was followed with longwise volume distribution and increased the clearance value duration [1, 22].

This study showed if *jamu* "pegal linu" was given, both the constant absorption rate (Ka) and constant elimination rate (Ke) were lower, but maximum plasma concentration (Cpmax) and Clearance (Cl) remained steady. Others pharmacokinetics, such as maximum concentration (Tmax), half-life elimination ($t_{1/2}$), distribution volume (Vd), and Area Under Curve (AUC), were higher. The bivariate analysis showed no significant difference ($p > 0{,}05$) for both groups. Co-administration *Jamu* "pegal linu" have a potential effect to allegedly plasma level of paracetamol.

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Authors' Contributions. GRA and O studied the concept, designed, and drafted the manuscript. O, R and AMS provided treatment to animal subjects and plasma handling. LI participated in chemical analysis with GRA and statistical analysis. All authors read and approved the final manuscript.

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