The Pharmacokinetic Drug-Drug Interactions of *Andrographis paniculata* and Ibuprofen in the Plasma of Healthy *Oryctolagus cuniculus* Rabbits

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**Abstract**

An HPLC method was developed and validated for the pharmacokinetic drug-drug interaction between *Andrographis paniculata* and ibuprofen in the plasma of *Oryctolagus cuniculus* rabbits after a single oral administration of the mixture. Nine healthy rabbits (6 males and 3 females, weight 1.68-2.42 kg) were acclimatized for 7 days and were randomly divided into 3 groups. At day-8th the rabbits were group (1) treated with a single oral administration of ibuprofen (dose of 28 mg/kg BW); group (2) treated with a single oral administration of *Andrographis paniculata* infusion (7.04 mL/kg BW); group (3) treated with a single oral administration of a mixture of *Andrographis paniculata* (7.04 mL/kg BW) infusion and ibuprofen (dose of 28 mg/kg BW). Plasma samples were prepared by collecting the blood from the marginal ear vein at 0, 30, 60, 90, and 120 minutes after the mixture administration, followed by centrifuging it for 30 minutes 3000 rpm. Chromatographic separation was performed on a LiChrosorb RP-18 with methanol and double-distilled water (70:30) as the mobile phase, flow rate 1 mL/minute. UV detection was set at 227 nm. The absorption and distribution of ibuprofen were fast (Tmax = 30 min; Cmax = 4.02962 mcg/mL), however, interestingly this drug could improve the absorption and distribution of andrographolide in *Oryctolagus cuniculus* rabbits.

**Keywords:** *Andrographis paniculata*, andrographolide, antiinflammation, ibuprofen, NSAIDs

**Introduction**

The bioavailability of a drug is defined as its amount and rate in the systemic circulation. Once entering the blood, the concentration of a drug will denote its total concentration in the circulatory system.¹ The bioavailability of a drug can be interfered with by another drug when both drugs are administered at the same time due to the occurrence of pharmacokinetic drug-drug interactions (DDIs). Many pharmacokinetic DDIs studies in humans have been reported.

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underlying the mechanisms for DDIs of different degrees.\textsuperscript{2-6}

Andrographolide has been reported in possessing anti-inflammatory activity among its other pharmacological activities.\textsuperscript{7-11} A previous bioavailability study of sambiloto (\textit{Andrographis paniculata}) infusion, determined as andrographolide (Figure 1a), reported that this lactone diterpene compound was absorbed and quantified in the plasma of New Zealand male rabbits at 1.5h (Cmax = 0.2136 µg/mL) after a single oral administration.\textsuperscript{12} A bioavailability of andrographolide in a mixture of ethanolic extracts of sambiloto and turmeric in rabbits has been reported. In this study, andrographolide was absorbed and distributed in blood within 60-90 minutes (Cmax = 3.06 - 4.41 ppm).\textsuperscript{13} However, there is a very limited report of its pharmacokinetic DDIs study with NSAIDs. This work aimed to study the pharmacokinetic DDIs of \textit{Andrographis paniculata} infusion and ibuprofen (Figure 1b) in the plasma of healthy \textit{Oryctolagus cuniculus} rabbits.

\section*{Methods}

\textit{Chemicals and Plants}

Andrographolide 98\% 500 mg CAS 5508-58-7 for R & D use (Aldrich) (Figure 1a), ibuprofen pharmaceutical grade (CV Agung Menara Abadi, Bandung) (Figure 1b), chloroform (Merck), double-distilled water for HPLC (PT IPHA), methanol for HPLC (JT Baker), dried sambiloto (\textit{Andrographis paniculata}) herbs and the fresh plants were obtained from Kebun Percobaan Manoko Lembang. The plants were taxonomically identified at Taxonomy Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran (Letter No. 419/HB/05/2016).

\textit{Identification of Ibuprofen and Andrographolide}

Each 2-5 mg of ibuprofen and andrographolide was dispersed in 195-198 mg of potassium bromide crystals. The pellets were measured by using the FT-IR instrument (Shimadzu IR Prestige-21) in the range of 1781-1683 cm\textsuperscript{-1} (Figure 2).

\textit{Determination of the Maximum Absorption of Ibuprofen and Andrographolide}

Each 10 mg of ibuprofen and andrographolide was dissolved separately in analytical grade ethanol (for ibuprofen) and methanol (for andrographolide) to obtain 25 mcg/mL. The solutions were scanned (Figure 3) in a UV spectrophotometer (Specord 200, Analitik Jena).

\textit{Optimization of the HPLC System}

20 mcg/mL of andrographolide solution was injected into a C-18 column with a mixture

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure1.png}
\caption{2D structure of (a) andrographolide and (b) ibuprofen}
\end{figure}
of methanol: double distilled water (55:45, 65:35, and 70:30) as the mobile phase. The flow rate was 1 mL/minute and detection was set at 227 nm. Parameters observed were resolution, time of retention, and tailing factor.

Validation of Bioanalytical Method
Validation was performed by following the method proposed by Levita et al., 2013.12

Preparation of Andrographis paniculata Infusion
The preparation of Andrographis paniculata infusion was carried out by following the method proposed by Levita and co-workers.12

Animals
This research has been approved by The Ethics Research Committee of Universitas Padjadjaran (Document No. 1047/UN6/C1.3.2/KEP/PN/2016). Nine healthy New Zealand rabbits (6 males and 3 females, weight 1.68-2.42 kg) were purchased from Peternakan, Budidaya, dan Wirausaha Rajawali Farm. The rabbits were randomly divided into 3 groups and were acclimatized for 7 days. Standard pellet feeds (@ 75 g/rabbit) were given twice at 08.00 and 16.00 daily.

The pharmacokinetic DDIs of Andrographis paniculata infusion and ibuprofen in the plasma of healthy Oryctolagus cuniculus rabbits
At day-8th the rabbits were observed for their heart rate (HR), respiration rate, body temperature, and body weight at day-8th (Table 1). At the same day, the rabbits were hematology-analyzed (Table 1) and were treated with: group (1) a single oral administration of ibuprofen (dose of 28 mg/kg BW); group (2) a single oral administration of Andrographis paniculata infusion (7.04 mL/kg BW); group (3) a single oral administration of a mixture of Andrographis paniculata (7.04 mL/kg BW) infusion and ibuprofen (dose of 28 mg/kg BW). Plasma samples were prepared by collecting the blood from the marginal ear vein (Figure 5) at 0, 30, 60, 90, and 120 minutes after the mixture administration, followed by centrifuging it for 30 minutes 3000 rpm. Chromatographic separation was performed on a LiChrosorb RP-18 with methanol and double-distilled water (70:30) as the mobile phase, flow rate 1 mL/minute. UV detection was set at 227 nm.

Results and Discussion
Infrared and Ultraviolet Spectrophotometry
The ultraviolet spectrum of andrographolide showed one maximum at 227 nm, which is caused by the pi -> pi* transition of two double bonds (C8-C9 and C11-C12). The peak of ibuprofen occurs at 225 nm due to the aromatic transition.
The IR spectrum of andrographolide (Figure 3a) reveals the presence of O-H (3398 cm\(^{-1}\)), alkyls (2969 cm\(^{-1}\), 2929 cm\(^{-1}\) and 2847.92 cm\(^{-1}\)), carbonyl (1726 cm\(^{-1}\)), alkene -C=C- (1674 cm\(^{-1}\)), while ibuprofen (Figure 3b) shows aromatics (1438.10 cm\(^{-1}\)), carbonyl of carboxylic acid (1714.75 cm\(^{-1}\)), and overlapped O-H with alkyl (2940.53 cm\(^{-1}\)).

**Optimization of the HPLC System**
The optimization of the HPLC system indicated that methanol: double distilled water (70:30) is the best condition (resolution 1.39; retention factor 0.94; tailing factor 1.14; retention time 1.58 minutes). The chromatogram of andrographolide (Figure 4a) indicates a distinct peak at 1.58 minutes, while ibuprofen elutes at 6.88 minutes.

**Validation of Bioanalytical Method**
Validation of the bioanalytical method resulted in a good linearity \(y = 0.5349x + 0.9888; R^2 = 0.9971\), % recovery 93.85-107.53%; RSD = 3.976%; LLOD = 0.11304 mcg/mL and LLOQ = 0.376799 mcg/mL.

**The pharmacokinetic DDIs of Andrographis paniculata infusion and ibuprofen in the plasma of healthy Oryctolagus cuniculus rabbits**
During 7 days of acclimatization, all rabbits were in healthy condition as proven by their physiological status (Table 1) and biochemical parameters (Table 2).

Rabbits are defined as suffering an abnormal condition when its heart rate exceeds 300
ibuprofen as proven by its occurrence in the rabbit’s blood at 60 minutes (Figure 6: green curve). These data confirmed that there is a pharmacokinetic DDIs between Andrographis paniculata (measured as andrographolide) and ibuprofen when these compounds are taken together at the same time.

Conclusion
A pharmacokinetic DDIs between Andrographis paniculata (measured as andrographolide) and ibuprofen when these compounds are taken together at the same time.

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Table 1. Health condition of the rabbits at day-8th

| Rabbit | BW (kg) | Heart rate per minute | Respiration rate per minute | Body temperature (°C) |
|--------|---------|-----------------------|-----------------------------|-----------------------|
|        |         | Experiment | Reference | Experiment | Reference | Experiment | Reference |
| 1      | 1.80    | 131        |           | 60        |           | 37.33      |           |
| 2      | 2.08    | 124        |           | 55        |           | 38.44      |           |
| 3      | 2.04    | 147        |           | 68        |           | 38.01      |           |
| 4      | 1.96    | 147        |           | 58        |           | 38.29      |           |
| 5      | 1.68    | 122        | 130-300   | 64        | 30-60     | 37.76      | 38-40     |
| 6      | 2.20    | 139        |           | 62        |           | 38.71      |           |
| 7      | 2.40    | 129        |           | 65        |           | 38.46      |           |
| 8      | 2.42    | 134        |           | 62        |           | 38.41      |           |
| 9      | 2.08    | 145        |           | 62        |           | 38.34      |           |

Reference is cited from River et al.15
Table 2. Health condition of the rabbits at day-8th

| Parameter                              | R1     | R2     | R3     | R4     | R5     | R6     | R7     | R8     | R9     |
|----------------------------------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| Leucocyte (10^3/µl)                    | 10.76  | 9.03   | 11.91  | -      | 5.25   | 15.84  | 8.69   | 6.01   | 7.68   |
| Lymphocyte (10^3/µl)                   | 4.02   | 3.45   | 2.67   | -      | 2.36   | 9.13   | 1.11   | 2.83   | 5.31   |
| Monocyte (10^3/µl)                     | 1.88   | 1.08   | 2.25   | -      | 2.09   | 2.64   | 2.27   | 0.59   | 0.78   |
| Granulocyte (10^3/µl)                  | 4.85   | 4.51   | 6.99   | -      | 0.80   | 4.07   | 5.30   | 2.59   | 1.59   |
| Lymphocyte (%)                         | 37.40  | 38.20  | 22.40  | -      | 45.00  | 57.60  | 12.80  | 47.10  | 69.20  |
| Monocyte (%)                           | 17.50  | 12.00  | 18.90  | -      | 39.80  | 16.70  | 26.20  | 9.80   | 10.10  |
| Granulocyte (%)                        | 45.10  | 49.90  | 58.70  | -      | 15.20  | 25.70  | 61.00  | 43.10  | 20.70  |
| Erythrocyte (10^6/µl)                  | 5.37   | 6.49   | 5.63   | -      | 4.96   | 5.51   | 4.32   | 5.35   | 4.73   |
| Hb (g/dl)                              | 11.60  | 14.70  | 13.00  | -      | 12.20  | 12.40  | 10.80  | 12.30  | 11.40  |
| Hematocrit (%)                         | 28.92  | 38.00  | 32.32  | -      | 31.62  | 33.23  | 27.27  | 31.80  | 29.94  |
| Mean corpuscular volume (fl)           | 54     | 599    | 57     | -      | 64     | 60     | 63     | 59     | 63     |
| Mean corpuscular hemoglobin (pg)       | 21.6   | 22.6   | 23.2   | -      | 24.7   | 22.5   | 25.1   | 22.9   | 24.2   |
| Mean corpuscular hemoglobin concentration (g/dl) | 40.2   | 38.6   | 40.4   | -      | 38.7   | 37.3   | 39.8   | 38.6   | 38.2   |
| Red cell distribution width (%)        | 18.3   | 16.6   | 16.1   | -      | 15.5   | 16.4   | 18.4   | 16.4   | 16.2   |
| Platelet (10^3/µl)                     | 254    | 417    | 151    | -      | 105    | 342    | 232    | 195    | 223    |
| Plateletcrit (%)                       | 0.14   | 0.13   | 0.04   | -      | 0.03   | 0.17   | 0.12   | 0.03   | 0.12   |
| Mean platelet volume (fl)              | 5.6    | 3.2    | 2.7    | -      | 3.2    | 5      | 5.3    | 1.6    | 5.2    |
| Platelet distribution width %          | 33.80  | 33     | 30.2   | -      | 29.5   | 33.8   | 33.8   | 28.5   | 32.3   |

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