Journal of Applied Pharmaceutical Science Vol. 7 (01), pp. 156-159, January, 2017
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2017.70121
ISSN 2231-3354

Chromatogram profiles of andrographolide in A23187-induced New Zealand rabbit's urine and faeces

Jutti Levita[1*], Tanti Juwita[1], Selma Ramadhani[1], Nyi Mekar Saptarini[2], Mutakin Mutakin[2]

[1]Department of Pharmacology and Clinical Pharmacy Faculty of Pharmacy Universitas Padjadjaran Jl. Raya Bandung-Sumedang km.21 Jatinangor West Java Indonesia. [2]Department of Pharmaceutical Analysis and Medicinal Chemistry Faculty of Pharmacy Universitas Padjadjaran Jl. Raya Bandung-Sumedang km. 21 Jatinangor West Java, Indonesia.

ARTICLE INFO

Article history:
Received on: 03/11/2016
Revised on: 20/11/2016
Accepted on: 10/12/2016
Available online: 31/01/2017

Key words:
Antiinflammation, calcium ionophore, conjugation, cyclooxygenase, drug metabolism, inflammation, pharmacokinetics.

ABSTRACT

Andrographolide is the main bioactive component of *Andrographis paniculata* (Burm. F.) Nees) which has been traditionally used as pain reducer in Indonesia. Our previous study showed that boiled water of *Andrographis paniculata* herbs, calculated as andrographolide, administered in healthy rabbits, was fastly absorbed from the stomach (t_{max} 1 hour), distributed in the circulation system (t_{max} 1.5 hours) and metabolized in the liver (t_{max} 2 hours), in subsequent process. In this work, we investigated the chromatogram profile of andrographolide in A23187 induced-New Zealand rabbits' urine and faeces. Prior to be treated, the animals were given orally 40 mg of andrographolide. Urine and faeces were collected during 2 x 24 hours then were extracted using a mixture of ethyl acetate-water (1:1). The water extract was further analyzed using reversed-phase HPLC with methanol-water (55:45) as mobile phase, flow rate 1 mL/minute for faeces and 0.5 mL/minute for urine. Detection was set at λ = 227 nm. HPLC chromatogram showed that andrographolide was not detected in the urine while compounds with higher polarity were observed at 3.0 to 6.0 minutes of elution. Andrographolide was still detected in faeces along with a more nonpolar compound. It could be concluded that andrographolide showed a good bioavailability in rabbit. This compound was metabolized and excreted in the form of more polar compounds in urine and a more nonpolar compound in faeces.

INTRODUCTION

Andrographolide is the main bioactive component of *Andrographis paniculata* (Burm. F.) Nees) which has been traditionally used as pain reducer in Indonesia. This compound has been proven in exerting anti-inflammatory activity (Levita *et al*, 2010; Lim *et al*, 2012), hepato-protector (Maiti *et al*, 2009), anti-hyperglycemia (Zhang *et al*, 2009), anti-diare (Hui *et al*, 2013), anti-oxidant (Guan *et al*, 2013), anti-HIV (Reddy *et al*, 2005), and anti-cancer (Song *et al*, 2015). Andrographolide and its derivates reduced the production of inflammation mediator due to the inhibitory of NF-kB signaling pathway (Lim *et al*, 2012). Furthermore, Tao Zhu and colleagues concluded that there was a decreasing of inflammation in LPS-induced rats due to inhibition of NF-kB activity (Zhu *et al*, 2013). A few investigations of the pharmacokinetics have been reported after oral administration of andrographolide. Six new andrographolide metabolites were isolated from urine, faeces, and small intestine contents of rats, as reported by He and colleagues (He *et al*, 2003), while four new metabolites in healthy human urine were reported by Cui, who concluded that four of them were characterized as sulfate and one was cysteine-S-conjugate (Cui *et al*, 2005). A comparative investigation of andrographolide's *in vitro* metabolic pathways in human, dog, and rat liver microsomes was carried out by Zhao and teamwork. They concluded that significant species differences indicated a more cautious strategy for further pharmacokinetics research of andrographolide in animal models (Zhao *et al*, 2013).

* *Corresponding Author*
Jutti Levita, Department of Pharmacology and Clinical Pharmacy Faculty of Pharmacy Universitas Padjadjaran Jl. Raya Bandung-Sumedang km.21 Jatinangor West Java Indonesia.
Email: jutti.levita @ unpad.ac.id

Our previous study showed that boiled water of *Andrographis paniculata* herbs, calculated as andrographolide, administered in healthy rabbits, was fastly absorbed from the stomach (C_{max} 0.5549 µg/mL; AUC 1.7451 µg.h/mL; t_{max} 1 h), distributed in the circulation system (C_{max} 0.2136 µg/mL; AUC 0.434 µg.h/mL; t_{max} 1.5 h), and metabolized in the liver (C_{max} 0.0051 µg/mL; 0.0038 µg.h/mL; t_{max} 2 h), in subsequent process. *Andrographis paniculata* herbs infusion showed good bioavailability in rabbit (Levita *et al*, 2014).

In this work, we investigated the chromatogram profile of andrographolide in A23187 induced-New Zealand rabbit's urine and faeces. A23187 was chosen as inducer, as it was reported by Rao and colleagues that this compound could affect the changes of intracellular calcium and induced inflammation in rats (Rao, *et al*, 1994). Other researchers concluded that A23187 stimulated prostaglandin synthase type 2 (PTGS2) and suppressed 15-hydroxyprostaglandin dehydrogenase (PGDH) expression (Casciani *et al*, 2008). A23187 also activated IkappaB kinase 2 in mast cells and NF-kB as reported by Hosokawa, hence this compound played a role in degranulation and cytokine production in activated mast cells through independent mechanism of PKCβ (Hosokawa *et al*, 2013).

MATERIALS AND METHODS

Materials

Chemicals

Andrographolide CAT 365645 (Sigma Aldrich), A23187 (Sigma Aldrich), ethanol technical grade 95% (Bratachem), ethyl acetate technical grade (Bratachem), chloroform technical grade (Bratachem), methanol technical grade (Bratachem), double-distilled water (PT IPHA Laboratorium), methanol for HPLC (Merck).

Instruments:

pH meter (Mettler Toledo), waterbath (Memmert), rotavapor (IKA® HB 10 digital), centrifugator (Hettich Zentrifugen EBA 20), spectrophotometer ultraviolet-visible (Specord 200, Analytik Jenna), RP-HPLC (HPLC Thermoscientific Ultimate 3000 pump) with UV detector and manual sample injector valve for Ultimate 3000.

Animals used in this project were New Zealand strain rabbits (*Oryctolagus cuniculus*) (4 males and 2 females), purchased from Roemah Kelinci Sumedang, West Java, Indonesia.

Methods

The New Zealand rabbits (four males and two females; 2-2.5 kg of body weight) were acclimatized for 7 days and treated according to the ethical standards of Universitas Padjadjaran Health Research Ethics Committee No. 771/UN6.C1.3.2/KEPK/PN/2015. Standard feed (a mixture of soy bean curd, spinach, and carrot) and drink were given *ad libitum*. No vitamins were added in their feed. At the 8th day, the rabbits were inflammation-induced by injecting 0.5 mL of A23187 (10 mg of A23187 in 10 mL of 0.5 M phosphate buffer pH 7.4) into their ear marginal vein 30 minutes prior to be treated with andrographolide dose 40 mg/rabbit regardless its body weight. Urine and faeces were collected during 2 x 24 hours then were extracted using a mixture of ethyl acetate-water (1:1). Validation of the method was performed according to ICH Q2A only for the LOD and LOQ, by using 10 mL of rabbit plasma, with the following procedure: Into 10 centrifuge tubes was put @ 1 mL of plasma and added by 5 mL of methanol. The mixture was vortex-shaken for 2 minutes, followed by centrifugation for 30 minutes at 3000 rpm, and was used to dilute standard solutions of andrographolide until various concentrations of 0.5; 1.0; 2.0; 4.0; 8.0; 10.0 µg/mL were obtained. The solutions were Millipore-filtered. 200 µL of the filtrates was injected into Acclaim® PolarAdvantage C18 column, 250 mm in length, 5-µm particle size, and 120 Å pore size. A mixture of methanol-water (55:45) was used as mobile phase, whereas flow rate was set at 1 mL/min. Detection was set at λ = 227 nm which is the wavelength where maxima of andrographolide occurs. This step was repeated 3x for LOD and LOQ calculation. 200 µL water extract was further analyzed using RP-HPLC with the same condition, that were methanol-water (55:45) as mobile phase, flow rate 0.5 mL/minute for urine and 1.0 mL/min for faeces.

RESULTS AND DISCUSSION

The rabbits were examined their health prior treatment and the result is given in Table 1.

Table 1: Baseline data of the rabbits.

No	Sex	Weight (kg)	Body temperature (°C)	Respiration (exhale/minute)
1	Male	2.3	39.6 ± 0.2	30
2	Male	2.3	39.6 ± 0.2	38
3	Male	1.9	40.1 ± 0.2	49
4	Male	2.5	39.8 ± 0.2	45
5	Female	2.0	39.7 ± 0.2	35
6	Female	2.2	38.8 ± 0.2	34

Inducing the rabbits with A23187 resulted in a slight elevation of the temperature, whereas andrographolide treatment lowered it (but not all, only 66.67%, all in male animals), as showed in Fig.1.

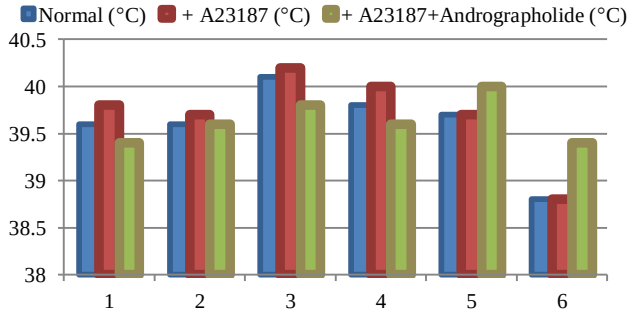

Fig. 1: Body temperature (°C) of each rabbit: normal (blue); after induced with A23187 (orange); after treated with andrographolide (grey).

A fever was observed in animals No.5 and No.6 which both were females, which probably due to hormonal factors. Rao and colleagues observed that intraperitoneal injection of A23187 (20 µg/mouse) to mice could elicite marked and transient increases in immunoreactive levels of 6-ketoprostaglandin-F2 alpha, leukotrienes B4, C4, D4, E4, and F4 (Rao *et al*, 1993). LOD and LOQ calculations were showed in Table 2.

Tabel 2: AUC measurements for LOD and LOQ calculations.

C (µg/mL)	AUC1	AUC2	AUC3	AUC ± SD
10	20.929	23.631	23.280	22.6134 ± 1.469
8	16.885	18.311	18.999	18.0653 ± 1.078
6	13.498	14.115	14.312	13.9751 ± 0.424
4	9.767	9.568	9.748	9.6941 ± 0.110
2	5.275	4.867	5.132	5.0913 ± 0.207
1	0.963	0.959	0.942	0.9549 ± 0.011
0.5	0.433	0.472	0.405	0.4367 ± 0.034

$$S_{y/x} = \sqrt{\frac{(y - yi)^2}{n-2}}$$

$$S_{y/x} = \sqrt{\frac{(20{,}9288-22{,}61337)^2+(23{,}6312-22{,}61337)^2+\cdots+(5{,}1324-5{,}0913)^2}{5}}$$

$$= 1.193$$

$$\text{LOD} = \frac{2b + 3Sy/x}{a} = \frac{2x\ 0.404 + 3x\ 1.193}{2.338} = 1.87\ \mu g/mL$$

$$\text{LOQ} = \frac{2b + 10Sy/x}{a} = \frac{2x\ 0.404 + 10x\ 1.193}{2.338} = 5.45\ \mu g/mL$$

Overlayed HPLC chromatograms of urine extract and andrographolide standard with the same condition (flow rate 0.5 mL/minute) (Fig.2) showed that andrographolide was not detected in the urine (t_R = 11.4 minutes), while compounds with higher polarity (blue peaks) were observed at 3.0 to 6.0 minutes. Andrographolide standard, in various concentrations (darker peaks), was eluted at 11.4 minutes.

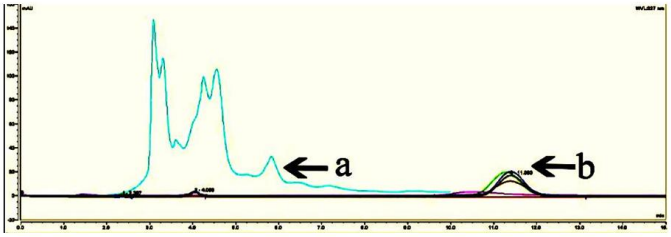

Fig. 2: Overlayed HPLC chromatograms of urine (a) and andrographolide standard (b) Andrographolide (Rt = 11.56 minutes) was still detected in faeces along with a more nonpolar compound (Fig.2).

Polar metabolites of andrographolide isolated in urine had been reported (Cui *et al*, 2004; Cui *et al*, 2005; Zhao *et al*, 2013). Different metabolic profiles of human, dog, and rat, were also reported (Zhao *et al*, 2013).

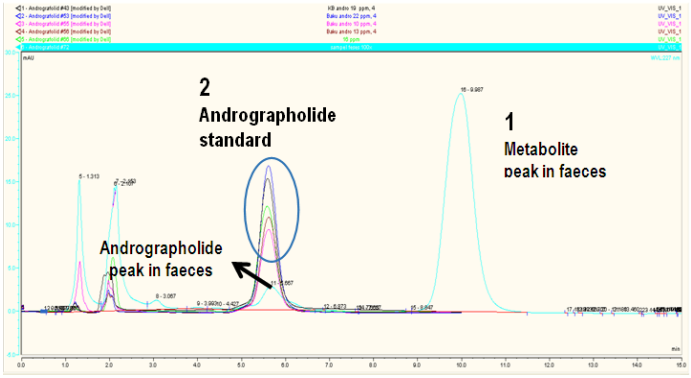

Fig. 3: Overlayed HPLC chromatograms of faeces (1) and andrographolide standard (2).

Overlayed HPLC chromatograms of faeces extract and andrographolide standard with the same condition (flow rate 1 mL/minute) (Fig.3) showed that andrographolide was still detected in the faeces (t_R = 5.5 minutes), while one peak of compound with lower polarity (blue peak) was observed at 10 minutes. Andrographolide standard, in various concentrations (colourful peaks), was eluted at 5.5 minutes.

IV. CONCLUSION

In six A23187 induced-New Zealand strain rabbits (*Oryctolagus cuniculus*) (4 males and 2 females), bred in Roemah Kelinci Sumedang, West Java, Indonesia, which were orally treated with 40 mg of andrographolide, this compound was metabolized and excreted in the form of more polar compounds in the urine and a more nonpolar compound in the faeces.

ACKNOWLEDGEMENT

Financial support and sponsorship: This project was funded by the Ministry of Research, Technology, and Higher Education of the Republic of Indonesia via Competence Grant No. 431/UN6.R/PL/2015.

Conflict of Interests: There are no conflicts of interest.

REFERENCES

Casciani V, Premyslova M, Luo D, Marinoni E, Di Iorio R, Challis JR. Effect of calcium ionophore A23187 on prostaglandin synthase type 2 and 15-hydroxy-prostaglandin dehydrogenase expression in human chorion trophoblast cells. *Am J Obstet Gynecol*, 2008; 199: 554.

Cui L, Qiu F, Yao X. Isolation and identification of seven glucuronide conjugates of andrographolide in human urine. *Drug Metabolism and Disposition*, 2005; 33: 555-562.

Guan SP, Tee W, Ng DSW, Chan TK, Peh HY, Ho WE, Cheng C,
Mak JC, Wong WS. Andrographolide protects against cigarette smoke-induced oxidative lung injury via augmentation of Nrf2 activity. *British Journal of Pharmacology*, 2013; 168: 1707–1718.

He X, Li J, Gao H, Qiu F, Cui X, Yao X. Six new andrographolide metabolites in rats. *Chem. Pharm. Bull*, 2003; 51: 586-589.

Hosokawa J, Suzuki K, Nakagomi D, Tamachi T, Takatori H, Suto A, Nakajima H. Role of calcium ionophore A23187-induced

activation of IkappaB kinase 2 in mast cells. *Int Arch Allergy Immunology*, 2013; 161: 37-43.

Hui H, Chen M, Li G, Feng H, Zang L, Xi Y, Dong T, Zhou X. Andrographolide attenuates senna- and castor oil-induced diarrhea in mice. *Latin American Journal of Pharmacy* (formerly *Acta Farmacéutica Bonaerense)*, 2013; 32: 1113-1117.

Kumaran S, Thirugnanasambantham P, Viswanathan S, Murthy MSR. An HPLC method for the estimation of andrographolide in rabbit serum. *Indian Journal of Pharmacology*, 2002; 35: 109-112.

Levita J, Himawati H, Lukman RV, Afdila M, Holik HA, Saptarini NM, Hasanah AN, Nawawi A, Mutalib A, Ibrahim S. Bioavailability study of sambiloto (*Andrographis paniculata*) herbs infusion in rabbit. *Indonesian J. Pharm*, 2014; 25: 138 – 144.

Lim JCW, Chan TK, Ng DSW, Sagineedu SR, Stanslas J, Wong WSF. Andrographolide and its analogues: versatile bioactive molecules for combating inflammation and cancer. *Impact Factor*, 2012; 39: 300-310.

Maiti K, Mukherjee K, Murugan V, Saha BP, Mukherjee PK. Enhancing bioavailability and hepatoprotective activity of andrographolide from *Andrographis paniculata*, a well-known medicinal food, through its herbosome. *Journal of the Science of Food and Agriculture*, 2010; 1: 43-51.

Rao TS, Shaffer AF, Currie JL, Isakson PC. Role of mast cells in calcium ionophore (A23187)-induced peritoneal inflammation in mice. *Inflammation*, 1994; 18:187-92.

Reddy VL, Reddy SM, Ravikanth V, Krishnaiah P, Goud TV, Rao TP, Ram TS, Gonnade RG, Bhadbhade M, Venkateswarlu Y. A new bis-andrographolide ether from *Andrographis paniculata* Nees and evaluation of anti-HIV activity. *Nat Prod Res*, 2005; 19: 223-230.

Song Y, Xin Z, Wan Y, Li J, Ye B, Xue X. Synthesis and anticancer activity of some novel indolo[3,2-b]andrographolide derivatives as apoptosis-inducing agents. *European Journal of Medicinal Chemistry*, 2015; 90: 695–706.

Zhang Z, Jiang J, Yu P, Zeng X, Larrick JW, Wang Y. Hypoglycemic and beta cell protective effects of andrographolide analogue for diabetes treatment. *Journal of Translational Medicine*, 2009; **7**:

Zhao HY, Hu H, Wang YT. Comparative metabolism and stability of andrographolide in liver microsomes from humans, dogs, and rats, using ultra-performance liquid chromatography coupled with triple-quadrupole and Fourier Transform ion cyclotron resonance mass spectrometry. *Rapid Commun Mass Spectrom*, 2013; 27: 1385-1392.

Zhu T, Wang D-X, Zhang W, Liao X-Q, Guan X, Bo H, Sun J-Y, Huang N-W, He J, Zhang Y-K, Tong J, Li CY. Andrographolide Protects against LPS-Induced Acute Lung Injury by Inactivation of NF-kB. *PLUS ONE*, 2013; 8: e56407 1-12.

How to cite this article:

Levita J, Juwita T, Ramadhani S, Saptarini NM, Mutakin M. Chromatogram profiles of andrographolide in A23187-induced New Zealand rabbit's urine and faeces. J App Pharm Sci, 2017; 7 (01): 156-159.