PENGARUH PEMBERIAN EKSTRAK *Apium Graveolens* L. TERHADAP FARMAKOKINETIK KAPTOPRIL TIKUS

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ABSTRAK

Apium graveolens L. (seledri) merupakan herba yang biasa dikonsumsi dan berkhasiat sebagai antihipertensi. Penelitian terdahulu melaporkan bahwa pemberian ekstrak seledri satu jam sebelum kaptopril, dapat mempengaruhi farmakokinetik kaptopril. Penelitian ini bertujuan untuk membuktikan pengaruh ekstrak seledri pada farmakokinetik kaptopril jika diberikan bersamaan tanpa jeda waktu. Tikus galur *Spraque-Dawley* sebanyak 12 ekor dibagi menjadi 2 kelompok. Kelompok I diberikan kaptopril tunggal dosis 2,5 mg/kg bb secara oral. Kelompok II diberikan campuran kaptopril dosis 2,5 mg/kg bb dan ekstrak seledri 40 mg/kg bb. Sampel darah diambil pada rentang waktu tertentu selama 7 jam dan dilakukan pengukuran dengan metode kromatografi cair kinerja ultra tinggi tandem spektrofotometri massa (*LC MS/MS*). Hasil uji farmakokinetik menunjukkan bahwa ekstrak seledri menurunkan parameter farmakokinetik kaptopril pada kelompok II yaitu C_{max} (70,43%), K_e (47,47%), dan AUC (46,84%), serta meningkatkan $T_{1/2}$ (104,97%) dan T_{max} (100%) dibandingkan dengan kelompok I. Kesimpulan dari penelitian ini adalah ekstrak seledri dapat mengubah farmakokinetik kaptopril jika diberikan secara bersamaan. Campuran kaptopril dan ekstrak seledri ketika diberikan secara bersamaan tanpa jeda waktu, tidak memberikan manfaat pada pengobatan hipertensi, karena ekstrak seledri dapat menurunkan konsentrasi kaptopril pada plasma, yang dapat menurunkan efikasi kaptopril.

Kata Kunci: *Apium graveolens*, farmakokinetik, interaksi herbal dan obat, kaptopril, ekstrak seledri

ABSTRACT

Celery (*Apium graveolens* L.) is an edible herb usually used as an antihypertensive. In the previous research, there was evidence that celery extract influenced pharmacokinetics of captopril when given one hour before captopril administration. The research aim is to study the effect of celery extract on pharmacokinetics captopril when presented at the same time. Twelve Spraque-Dawley strain rats was divided into two groups. Group I was given captopril (2,5 mg/kg Body Weight) orally, while group II was given the mixture of captopril and celery extract (40 mg/kg Body Weight), orally. The blood sample was collected at the various time for 7 hours after the drug administration. The influence of celery extract on captopril pharmacokinetics was studied by liquid chromatography-mass spectrometry (LC-MS/MS) method. The pharmacokinetics studies show that celery extract decrease C_{max} (70.43%), K_e (47.47%), and AUC (46.84%), increased $T_{1/2}$ (104.97%) and T_{max} (100%) of captopril in Group II (combination captopril and celery extract) compare with group I. It was concluded that celery extract could alter the

pharmacokinetics of captopril when given in combination. It seems that the mix, when presented at the same time, did not provide excellent benefits for the treatment of hypertension, as celery extract causes a decrease in the plasma level of captopril, which can reduce the efficacy.

Keyword: *Apium graveolens*, captopril, celery extracts, herb drug interaction, pharmacokinetics

INTRODUCTION

In many developing countries, herbal and traditional medicines are applied in treating many diseases, including experimental hypertension, diabetes (Asdaq & Inamdar, 2010). *Apium graveolens* L. (celery) is a plant, belonging to the parsley descent (Umbelliferae), an herbaceous, biennial, and branched stem plant, with a height of 20 to 60 cm (Nasri, Ramazani & Yasa, 2009). Celery contains different chemical compound such as apiin, apigenin, isoquercitrin, and sesquiterpene (Kooti et al., 2015, Al-Snafi, 2014). It has been widely used in traditional medicine for treatment of various disorder including hypertension (Kooti et al., 2015).

Herbal medicines are mixtures of more than one active ingredient. The multitude of pharmacologically active compounds naturally increases the likelihood of interaction taking place (Izzo, 2005). An important safety concern associated with the use of herbal medicines is the risk of interaction with prescription medication (Izzo, Di Carlo, Borrelli & Ernst, 2005). Recent examination has indicated that as many as 16% of prescription drug users consume herbal supplement (Kaufman, 2002). Other research has shown that there was evidence that 71.4% of patients in the public community health center have combined celery herbs with captopril, finding that this combination can reduce blood pressure better than captopril alone (Gusmira, 2012). Captopril is a sulfhydryl, is an orally active inhibitor of angiotensin-converting enzyme (ACE) and widely used in the treatment of hypertension and congestive heart failure (Weir, Hanes, Klassen & Wasser, 2015).

Herbs and drugs may interact with either pharmacokinetically or pharmacodynamically (Izzo, 2005, Setiawati, 2007). In the previous research, there was evidence that celery extract could alter the pharmacokinetics of captopril when given in combination. The results showed that oral administration of the celery extract one hour before captopril, increased C_{max} (38.67%), $T_{1/2}$ (37.84%), and *AUC* (58.10%) and decreased K_e (27.45%) of captopril (Siska, Munim, Bahtiar & Suyatna, 2018). According to that result, we aimed to study the effect of celery extract on pharmacokinetics captopril when given at the same time using rats as the experimental animal model.

MATERIALS AND METHODS

Experimental Animals

This study was conducted experimentally using white male Sprague-Dawley rats that were obtained from the Bogor Agricultural University (IPB), Bogor, West Java, Indonesia; weighing 200–250 g. The rats were kept in standard polycarbonate cages, carpeted with wood chips and the temperatures around 25 ± 5 °C in a well-ventilated animal house under a 12:12 h light/dark cycle. The rats had free access to food and water. The study protocol has been approved by the Health Research Ethics Committee of the Faculty of Medicine, Universitas Indonesia, Central Jakarta, Indonesia. The reference number for approval was 666/UN2.F1/ETHICS/2016.

Preparation of Celery Ethanol Extract

Celery herb was purchased from the local market. The plant was identified at The Research Center for Biology, Indonesian Institute of Sciences (LIPI), Cibinong, West Java, Indonesia; with the determination specimen number of 1777/IPH.1.01/if.07/VIII/2016 (10). The herbs were rinsed and dried in the oven and powders were stored in a refrigerator (4^0C) until extraction. Celery herb powder was mixed in 50% ethanol in a macerator apparatus for three days. Then, the mixture was filtered with a filter paper and was concentrated in a rotary vacuum evaporator (Buchi, Darmstadt, Germany). The concentrated extract was stored at 4^0C until use (Siska, Munim, Bahtiar & Suyatna, 2018).

Chromatography Condition

C18 column Acquity (Waters, Milford, CT, USA) (100 mm × 2.1 mm) used as the chromatography, with a particle size of 1.7 µm at a temperature of 40 °C. A mixture of 0.1% formic acid and acetonitrile (60:40 *v*/*v*), with a flow rate of 0.3 mL/second used as the gradient system of the mobile phase composition. For mass detection, we used a Waters Xevo Triple Quadrupole (Waters) equipped with electrospray ionization (ESI) source in positive ions in multiple reaction monitoring (MRM) modes.

Calibration Standard and Quality Control Sample Preparation

Prepared a stock solution of captopril with a concentration of 100 µg/mL by dissolving 40 mg in 100 mL of water. Propranolol (1 mg/mL) and apigenin (1 mg/mL) stock solution ware prepared by dissolving 40 mg in 100 mL of methanol. Calibration standards and the Quality

Control (QC) sample were prepared by diluting the stock solution with Sprague-Dawley rat plasma to form the calibration standards of captopril in the presence of apigenin (3, 6, 12, 25, 50, and 100 ng/mL) and QC sample (9, 40, and 80 ng/mL). Prepared a stock solution of 2,4-dibromo acetophenone with a concentration of 520 µg/mL by dissolving 52 mL in 100 mL of methanol.

Plasma Sample Preparation

Pure protein precipitation procedure was applied to clean up the plasma sample before use (Donáth-Nagy, Vancea & Imre, 2011). A total of 180 µL of plasma containing the specific concentration of captopril and apigenin was added to 20 µL of 2,4-dibromo acetophenone working solution and 5% ammonia solution, vortexed for 30 s and stored at 25°C for 30 min, before adding 20 µL of 15% formic acid, propranolol (1 µL/mL), and 600 µL of acetonitrile. The solution was vortexed for 30 s and centrifuged at 12.000 rpm for 5 min. The supernatant was transferred to an autosampler vial before 5 µL was injected into the liquid chromatography–mass spectrometry (LC-MS/MS) system.

Study of Pharmacokinetic

Twelve rats were divided into two groups: Group I received a single captopril at a dose of 2.5 mg/kg orally, while Group II was mixture captopril and celery extracts orally (40 mg/kg). Serial blood samples (0.5 mL per sample) were collected before dosing and after 30, 45, 60, 120, 180, 300, and 420 min. The harvested plasma samples were treated in the pre-used plasma preparations mentioned before. The captopril concentration was determined using LC-MS/MS, and the values of elimination constant (K_e), maximum concentration (C_{max}), maximum time (T_{max}), half-time ($T_{1/2}$), and area under the curve (AUC) were calculated based on this blood data.

The data were represented in a plasma level–time curve, which was used to calculate the $AUC_{0\text{-}7h}$ using the trapezoid rule. The C_{max} and T_{max} were obtained directly from the generated data. The K_e and $T_{1/2}$ were determined from the semi-log plot of the data. The mean plasma concentration–time curve for captopril (2.5 mg/kg) alone and captopril + celery extract (40 mg/kg) was determined. The study was conducted for 7 h since the half-life of captopril is 1–2 h (Vancea, 2009). The results were analyzed statistically using the Student's T-test.

RESULTS

Characteristics of Physico-Chemical

The result of physicochemical features of celery extract such as loss on drying 4.87% w/w, water content 8.89% v/w, ash values 6.7% w/w, and essential oil content 3.34% v/w, are same with the previous research (Siska, Munim, Bahtiar & Suyatna, 2018)

Optimization of LC-MS/MS Parameters

We used Water Xevo TQD (Waters) equipped with electrospray ionization (ESI) source in positive ions in multiple reaction monitoring (MRM) mode for mass detection. The following operational parameters of the ion cone and collision energies are presented in Table 2. Captopril was detected at an *m/z* of 271.13 -> 153.07, while propranolol was discovered at an *m/z* of 260 -> 183.17, which was used as an internal standard (Siska, Munim, Bahtiar & Suyatna, 2018).

Calibration Curve and Lower Limit of Quantification (LLOQ)

The curves of calibration were found to be linear, over the concentration range of 3–100 ng/mL and with linearity of 0.9961, and the lower limit of quantification (LLOQ) was 3 ng/mL. The precision value (%CV) of the within-run analysis was 6.91–8.37%, which is less than 20% (Table 1).

Table 1. Accuracy and precision of captopril in presence of apigenin

Concentration (ng/ml)	Mean measured concentration (ng/ml) ± SD	(% CV)	(% diff)
3	3.13 ± 0,25	7.91	4.46
9	8.46 ± 0,71	8.37	-5.82
40	39.36 ± 2,93	7.46	-1.41
80	77.37 ± 5,34	6.91	-3.10

Sumber: Siska, Munim, Bahtiar, & Suyatna, 2018.

Pharmacokinetics of Captopril

The pharmacokinetics of the combined captopril and celery extract were subsequently examined in Sprague-Dawley rats after oral administration. The pharmacokinetic parameters

are listed in Table 2 and Figure 1. The C_{max} and AUC_{total} was decreased in Group II compared with Group I ($p < 0.05$).

Table 2. Pharmacokinetic Parameters of Captopril.

Pharmacokinetic Parameters	Group I ($n = 6$) (single captopril)	Group II ($n = 6$) (captopril + celery extract)
C_{max} (ng/mL)	57.94 ± 19.540	17.13* ± 6.083
T_{max} (hour)	0.5	1
K_e (hour^{-1})	0.217 ± 0.094	0.114 ± 0.060
$T_{1/2}$ (hour)	3.62 ± 1.534	7.42 ± 3.892
AUC_{total}	54.44 ± 1.984	28.94* ± 2.186

Values are mean ± SEM, $p > 0.05$ when compared to captopril alone. *AUC*: area under the curve; C_{max}: maximum concentration; T_{max}: maximum time; K_e: elimination constant; $T_{1/2}$: half-time.

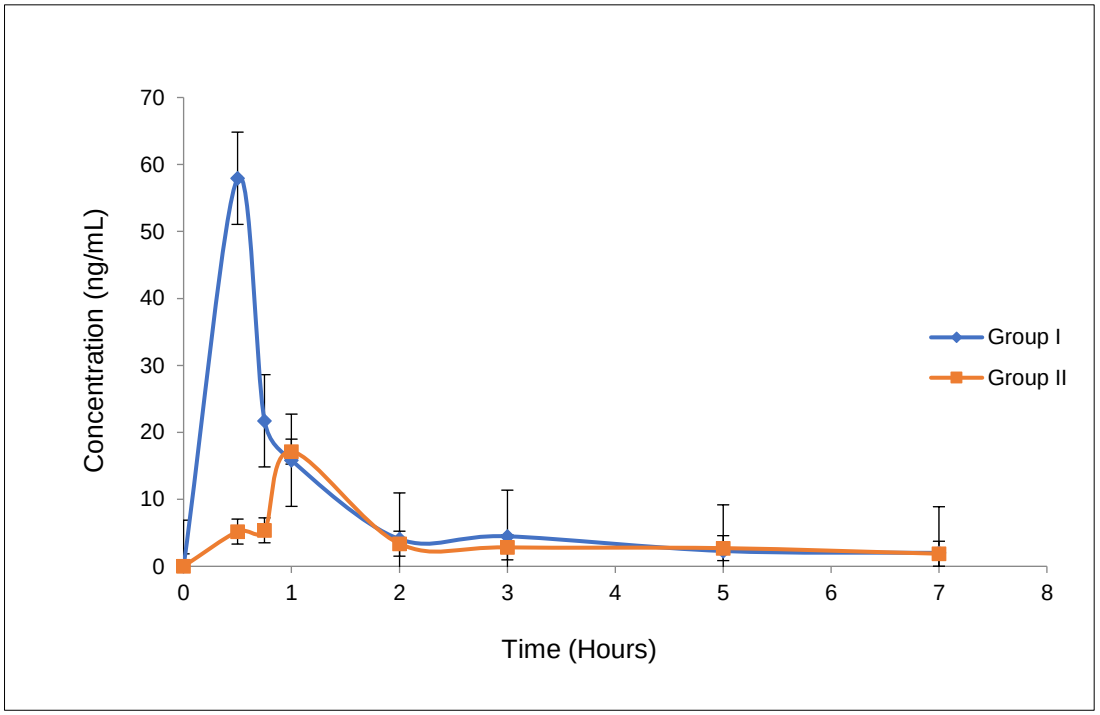

Figure 1. Pharmacokinetics of captopril. Plasma captopril levels were measured with LC-MS/MS. Symbols represent the mean concentration ± standard error of the mean (SEM). Group I ($n = 6$) was given a single dose of captopril (2.5 mg/kg, orally), while Group II ($n = 6$) was given captopril (2.5 mg/kg) with celery extract (40 mg/kg, orally).

DISCUSSION

The physicochemical characteristics of celery extract determine their quality. Water content or moisture is sufficient to facilitate the activation of enzymes and the proliferation of microorganisms. These are certain components of crude medicines and must be eliminated as much as possible. Ash values are useful in determining the authenticity and purity of a drug as well as its critical quantitative standards. The total ash value of the extract provides an indication of earthy matter or mineral composition as well as the impurities present. The quality of herbal medicines can be affected by many factors, such as light exposure, temperature, water availability, the amount of nutrients, the period and time of collection, the methods by which the medicine is collected, dried, packed, stored, and transported, age, and which part of the plant is collected (World Health Organization, 2003, Departemen Kesehatan RI, 2000).

Captopril is an unstable compound that undergoes oxidation. The degradation product is a dimer that also binds to endogenous compounds (cysteine and glutathione) (Vancea, 2009). A derivatizing agent, 2,4-dibromo acetophenone, was added to improve the stability of the compound, which prevents captopril from binding to plasma constituents and is also a chemical stabilizer (Vancea, 2009).

The equation of the captopril calibration curve was obtained from linear regression between captopril (X) and absorbance (Y) levels and was used to calculate captopril levels in blood samples. Based on the results of a linear regression calculations obtained by the standard curve equation $Y = 0.0048\ X + 0.0122$ ($R^2 = 0.9961$).

The precision and accuracy of captopril in the presence of apigenin (a marker from celery extract) were calculated by our within-run variation of QC samples at four different concentrations with five replicates. The precision (%CV) value from the within-run analysis is 6.91–8.37%, while the accuracy (%diff) of captopril is less than 20%. The accuracy and precision values indicate the adequate reliability and reproducibility of the method within an analytical range (EMEA, 2011).

Based on the calculation of pharmacokinetic parameters and statistical tests performed (Table 2), there was a significant decrease in the value of C_{max} (70.43%) and AUC_{total} (46.84%). The K_e (47.47%) also decreased but not substantial. There was prolonged elimination half-life ($T_{1/2}$) of captopril in the presence of celery from 3.62 to 7.42. The time needed to reach the peak

(T_{max}) plasma concentration of captopril increased from 0.5 hours to 1 hour. The upward trend of C_{max} and *AUC* of captopril combined with celery extract in our study suggests that celery extract inhibited the absorption of captopril, thereby increasing the T_{max} to reach maximum level (Jakovljevic, Raskovic, Popovic & Sabo, 2002). In this situation, the celery extract could act as foods, because food may decrease captopril absorption by up to 54% (Weir, Hanes, Klassen & Wasser, 2015).

In our previous research, when celery extract gave one hour before captopril administration, there ware increasing of C_{max} and AUC, it seems that celery extract inhibits the metabolism of captopril (Siska, Munim, Bahtiar & Suyatna, 2018) since it was a potent inhibitor of cytochrome P450, which is responsible for captopril metabolization (Jakovljevic, Raskovic, Popovic & Sabo, 2002). Another research showed that captopril has pharmacodynamical interactions when combined with garlic, which has the synergetic effect of preventing the damage caused by isoproterenol in rats (He, 2014).

The mechanism of action for many herbs has not been determined, and the exact mechanisms of drug-herb interaction are also unknown (Asdaq & Inamdar 2010). To our knowledge, this is the first report showing the possible pharmacokinetics interaction of celery extract when combined with captopril.

CONCLUSIONS

The administration of celery extracts, when given in combination with captopril, can decrease the bioavailability of captopril which is probably caused by inhibition of captopril absorption.

RECOMMENDATION

The result of this study could be used as a reference for herb-drug interaction research. Patient should be careful when using combination of herb and prescription drug and it is better to consult with their doctor or pharmacist.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

REFERANCES

Al-Snafi, A. E. (2014). The pharmacology of Apium graveolens.-A review. *International Journal for Pharmaceutical Research Scholars*, *3*(1-1), 671-677.

Asdaq, S. M. B., & Inamdar, M. N. (2010). Pharmacodynamic interaction of captopril with garlic in isoproterenol-induced myocardial damage in rat. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, *24*(5), 720-725.

Asdaq, S. M. B., & Inamdar, M. N. (2010). Pharmacodynamic interaction of captopril with garlic in isoproterenol-induced myocardial damage in rat. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, *24*(5), 720-725.

Committee for Medicinal Products for Human Use. (2011). Guideline on bioanalytical method validation. *European Medicines Agency*.

Departemen Kesehatan RI (2000). Parameter standar umum ekstrak tumbuhan obat. *Jakarta: Departemen Kesehatan Republik Indonesia*, 9-12.

Donáth-Nagy, G., Vancea, S., & Imre, S. (2011). Comparative study of captopril derivatization reaction by LC-UV, LC-MS and CE-UV methods. *Croatica Chemica Acta*, *84*(3), 423-427.

Gusmira, S. (2012). Evaluasi Penggunaan Antihipertensi Konvensional dan Kombinasi Konvensional Bahan Alam pada Pasien Hipertensi di Puskesmas Wilayah Depok. *Makara Kesehatan*, *16*(2), 77-83.

He, Z. X. (2014). Clinical herb-drug interactions as a safety concern in pharmacotherapy. *J. Pharmacol. Drug. Metab*, *1*, 1-3.

Izzo, A. A. (2005). Herb–drug interactions: an overview of the clinical evidence. *Fundamental & Clinical Pharmacology*, *19*(1), 1-16.

Izzo, A. A., Di Carlo, G., Borrelli, F., & Ernst, E. (2005). Cardiovascular pharmacotherapy and herbal medicines: the risk of drug interaction. *International journal of cardiology*, *98*(1), 1-14.

Jakovljevic, V., Raskovic, A., Popovic, M., & Sabo, J. (2002). The effect of celery and parsley juices on pharmacodynamic activity of drugs involving cytochrome P450 in their metabolism. *European journal of drug metabolism and pharmacokinetics*, *27*(3), 153-156.

Kaufman, D. W., Kelly, J. P., Rosenberg, L., Anderson, T. E., & Mitchell, A. A. (2002). Recent patterns of medication use in the ambulatory adult population of the United States: the Slone survey. *Jama*, *287*(3), 337-344.

Kooti, W., Ali-Akbari, S., Asadi-Samani, M., Ghadery, H., & Ashtary-Larky, D. (2015). A review on medicinal plant of Apium graveolens. *Advanced Herbal Medicine*, *1*(1), 48-59.

Nasri, S., Ramazani, M., & Yasa, N. (2009). Antinociceptive and anti-inflammatory effects of hydro-alcoholic extract of Apium graveolens. *Journal of Shahrekord Uuniversity of Medical Sciences*, *10*.

Setiawati, A. (2007). *Drug Interaction, in Pharmacology and Therapeutics*. Department of Pharmacology and Therapeutic Faculty of Medicine Universitas Indonesia: Gaya Baru, Jakarta, Indonesia, pp. 800–801.

Siska, S., Munim, A., Bahtiar, A., & Suyatna, F. D. (2018). Effect of Apium graveolens Extract Administration on the Pharmacokinetics of Captopril in the Plasma of Rats. *Scientia pharmaceutica*, *86*(1), 6.

Vancea, S., Imre, S., Donáth-Nagy, G., Béla, T., Nyulas, M., Muntean, T., & Borka-Balás, R. (2009). Determination of free captopril in human plasma by liquid chromatography with mass spectrometry detection. *Talanta*, *79*(2), 436-441.

Weir, M.R, Hanes, D.S., Klassen, D.K., Wasser, W.G. (2015). In *Brenner and Rector's The Kidney E-Book*. Elsevier Health Sciences, 1640-701.

World Health Organization. (2003). *WHO guidelines on good agricultural and collection practices [GACP] for medicinal plants*. World Health Organization.