Pharmacokinetic aspect of Carica papaya leaf extract after oral administration

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Pharmacokinetic aspect of Carica papaya leaf extract after oral administration

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Abstract. The pharmacokinetic data were needed to determine the dose and frequent of drug. Carica papaya leaves have potency to develop a new herbal medicine. The research aimed to explore the excretion rate of oral administration of Carica papaya leaf extract. The time series experimental was conducted to six male and health rats. Single dose (900 mg) of Carica papaya leaf extract was given orally. Urines were collected in range 0 to 6 hr, 6 to 12 hr, 12 to 24 hr and 24 to 48 hr after oral administration. Flavonoids urine were measured by HPLC. Flavonoids detected in range 0 to 6 hr were 0.0721mg/mL. The level of flavonoid increased in 6 to 12 hr (0.0722 mg/mL), 12 to 24 hr (0.1306 mg/mL) and 24 to 48 hr (0.1800 mg/mL). The excretion rate in range 0 to 6 hr was 0.02662 mg/hr, 6 to 12 hr was 0.02888 mg/hr, 12 to 24 hr was 0.03428 mg/hr and 24 to 48 hr was 0.03574 mg/hr. Total flavonoid have been excreted in 48 hour was 4.73 %. Carica papaya leaf extract was excreted in 6 hr after oral administration and need more than 48 hr to clear all flavonoid from plasma.

1. Introduction

Carica papaya leaves are widely used as tradisional medicine. Aqueous and chloroform leaf extract of papaya have antimicrobial activity which inhibited the growth of many microorganism such as Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa and Staphylococcus aureus. The observed antimicrobial effects are believed to be due to the presence of alkaloids, tannins and flavonoids which have been shown to posse antimicrobial properties [1]. The high concentrations of steroids in chloroform leaf extract of Carica papaya slows glucose and lipid absorption in the digestive organs which could be responsible for the hypoglycemic and hypolipidemic effect of the chloroform extract in diabetic rats [2]. Quercetin and Rutin stimulate β-cells to release more insulin [3].

Fresh leaves of Carica papaya had total flavonoid 0.275±0.0015 mg quercetin eq/g and total phenolic level 2.35±0.01 mg gallic acid/g [4]. Young leaves of Carica papaya had total flavonoid level 333,14±1,03 mg rutin equivalen/100 gram dry weight [5]. Flavonoids are widely spread plant secondary metabolites called C6–C3–C6 phenolic. Chemically flavonoids are based upon a fifteen-carbon skeleton consisting of two benzene rings linked via a heterocyclic pyrane ring [6].

Flavonoids are absorbed in small intestine. After being absorbed in intestines, flavonoids are bound with blood albumin and transported via hepatic portal vein to liver. Flavonoids are conjugated in liver through glucoronidation, sulfation, and methylation to be a smaller phenolic compound. The liver metabolites are excreted to bile and with bile they return to the small intestine and are reabsorbed, thus forming flavonoids enterohepatic circulation [7]. In addition, the liver metabolites are transported to target cells/tissues and kidney via blood. Flavonoids undergo filtration in glomerulus, reabsorption, and

secretion in renal tubule. Whereas, flavonoid that are not absorbed in the intestine, will reach the colon and be hydrolyzed by colonic microflora. In addition, colonic microflora also degradate flavonoid structure to be phenolic acids [8].

Pharmacokinetic study is required to investigate the safety and effectivity included absorption, distribution, metabolism, and excretion. Excretion study of flavonoid on Carica papaya leaf exctract is useful to determine the effect, side effects, and the toxicity which can provide the suitable dosage and manner of administration Carica papaya leaf extract [9]. The aim of the study to analyse the flavonoid excretion through parameters urine flavonoid level, excretion rate, and percentage flavonoid which excreted in urine within 48 hours after orally administration of Carica papaya leaf extract.

2. Materials and method

2.1. Preparation of Extract

Carica papaya leaf extract was prepared in UNNESs biochemistry laboratory using distilled water extractor [10]. One kilogram of wet Carica papaya leaves produced 32.514 gram extract (powder). The number of 900 mg extract was dissolved into 2 mL distilled water. The total of 900 mg extract contained 33.903 mg of flavonoid.

2.2. Experimental study

The sample of this study were six health male Wistar rats between 2-3 months old and average weight 225 gram. The maintenance and the experiment were done at Animal Physiological laboratory Universitas Negeri Semarang. Each rat was caged in metabolic cage. The acclimatization was done during 5 days. On the sixth day, rats were fasted for 12 hour with free access to water (ad libitum) [11]. The single dose 900 mg/rat of Carica papaya leaf extract were administered orally. The urine were collected for 0-6 hour, 6-12 hour, 12-24 hour, and 24-48 hour. The urine were collected in the tube and stored in freezer at -200C until HPLC analysis time. The analysis of flavonoid content were done using HPLC UV-VIS C-18 column.

2.3. Standard curve of flavonoid

Standard solution used five difference concentration of Rutin that is 200, 100, 50, 25, and 12,5 ppm. Each concentration was injected 20µl into HPLC-UV-VIS C18 column to obtain standard flavonoid curve. The standard flavonoid curve was shown in figure1. From the standard flavonoid curve were obtained equation y = 53243x – 143400.

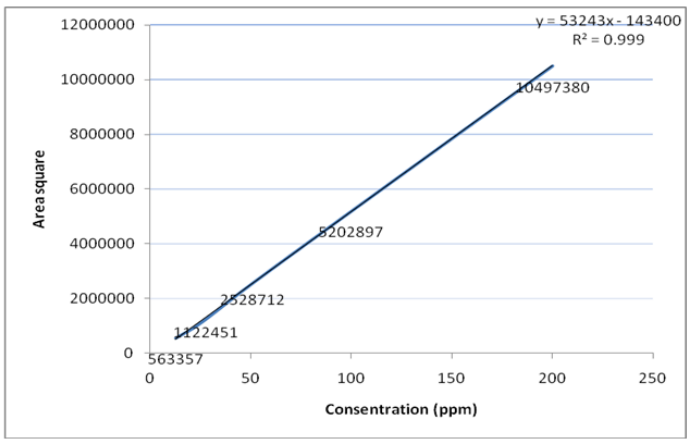

Figure 1. The standard flavonoids curve.

2.4. Flavonoid analysis

Flavonoid were analyzed using HPLC UV-VIS C-18 column (Shimadzu, Japan). The mobile phase consisted of 73 % methanol and water (99.5:0.5) (solvent A) and 27 % acetonitrile (solvent B), pH adjusted to 3.64 with glacial acetic acid. The separation was performed under an isocratic condition with a constant flow rate 1.5 ml/min, column temperature 25oC and the detector wavelength 347 nm [12]. Urine sample of 0.1 ml were added to methanol 1,0 ml. The Mixture was shaken for 2 minute. The mixture was filtrated use Sartorius stedim Minisart RC 15 membrane. Urine sample of 20 µl was injected into HPLC.

2.5. Data analysis

The data of flavonoid in rat urine in range 0-6 hour, 6-12 hour, 12-24 hour, and 24-48 hour were analyzed descriptively. Pharmacokinetic parameters were analyzed in this study included urine flavonoid level, excretion rate (dDu/dt) and the percentage of flavonoid which excreted in 48 hour after orally administration of Carica papaya leaf extract

3. Result and discussion

3.1. Result

Flavonoid level of Carica papaya leaf extract were calculated use equation y = 53243x – 143400. The y value was an area square. Whereas the value of x was flavonoid level, then multiplied by dilution factors. The result flavonoid level of Carica papaya leaf extract in urine, urine volume, flavonoid excreted, and excretion rate were shown in Table 1.

Table 1. Total flavonoid and excretion rate of flavonoid of Carica papaya extract.

Range (dt) (hour)	Urine volume (mL) (n= 6)	Urine flavonoid level (mg/mL)	Flavonoid exreted (Du) (mg)	Excretion Rate (Du/dt) (mg/hr)
0-6 (6)	2.217	0.0721	0.15973	0.02662
6-12 (6)	2.400	0.0722	0.17330	0.02888
12-24 (12)	3.150	0.1306	0.41139	0.03428
24-48 (24)	4.767	0.1800	0.85781	0.03574
Total	12.533	0.45483	1.60224	0.0334

The average of flavonoid rate excretion was determined from the amount of flavonoid excreted (∑Du) divided 48 hour. So, the The average of flavonoid rate excretion of Carica papaya leaf extract in 48 hour was 0.0334. The curve of flavonoid rate excretion were shown in figure 2.

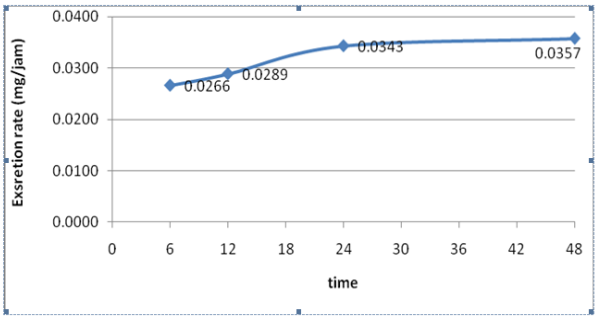

Figure 2. Flavonoid rate excretion of *Carica papaya* leaf.

3.2. Discussion

Flavonoid are generally present as glycosides form. Flavonoid glycosides are hydrolysed from sugar moieties by LPH (Lactase Phloridzin Hydrolase) in the intestinal brush border or by colonic micro-organism. An alternatif mechanism, flavonoid are transported via sodium-dependent glucose transporter (SGLT1) into enterocytes, where the sugar moieties are removed by β-glucosidases. LPH is an important step in the absorption and metabolism of dietary phenolic glycosides including monoglycosides, diglycosides, and triglycosides. On the other hand, some phenolic glucosides such as Rutin and baohuoside I, which may not be the substrates for LPH, could only be hydrolyzed by bacterial glycosidases in the colon or absorbed by enterocytes directly. LPH is not a contributing factor for these glycosides and their disposition could be quite different [13,14].

The flavonoid aglycones undergo conjugated reactions involving glucuronidation and sulfation with or without methylation. The conjugations occur in enterocytes and liver. Almost all flavonoids in plasma and urine are as conjugated forms. Flavonoid conjugates may bind to some cell receptors or cellular components, resulting in their accumulation after prolonged ingestion. Moreover, flavonoid are hydrophobic, they tend to partition into membranes, resulting in their accumulation in the body [13].

Then, the metabolites circulate in blood and be excreted into bile, feces, and urine. When excreted into bile, the conjugated metabolites may be reabsorbed and enter into enterohepatic circulation, appeared second peak of serum profile [15]. The heavy molecular of flavonoid and their extensive binding to protein could favor their biliary excretion [16]. Water soluble flavonoid, commonly light molecular, are slowly biotransformated by the liver and eliminated by renal [17]. A molecular < 20 000 kD cross the glomerular capillaries. Macromolecules with ≥ 69 000 kD such as albumin, do not. So, glomerular filtration is not the main excretion mechanism of compounds bound to this protein.

The flavonoid in form glucuronide and sulfate conjugates in the bloodstream [16]. Rutin are absorbed rapidly and then slowly declined (elimination) [11]. Rutin are hydrolyzed by the intestinal microflora, R-rhamnosidase and α-glucosidase to isoquercitrin (quercetin 3-glucoside and Quercetin). Within 48 hour, Quercetin are absorbed and excreted into the bile and urine as glucuronide and sulfate conjugates. Quercetin is further degraded as phenolic acids such as 3-hydroxyphenylacetic acid and 3,4-dihydroxyphenylacetic acid through B-ring fission by intestinal bacteria [18].

The bioavaibility of flavonoid varies between different kinds of flavonoids, isoflavones have the best absorbed, followed by flavanols, flavanones, flavonols, proanthocyanidins, and anthocyanins. But, excretion rate of anthocyanins were rapid. While flavonols were slowest [13]. So, it may possible that flavonoid metabolites of Carica papaya leaf extract in urine were flavonols and their metabolites in the form of glucoronides/sulfates conjugation. In this study, the metabolites detected on HPLC were unknown, therefor to know the sort of flavonoid metabolites of Carica papaya leaf extract were required a test using LC-MS.

Flavonoids have been detected in range 0-6 hour. It was estimated that flavonoid have been excreted via urine before 6 hour after intake. The lowest level of flavonoid was shown in range 0-6 hour. It might the flavonoid were still distributed or stayed in cells, tissues or blood. Previous study showed a difference maximum concentration (Cmaks) and maximum absorbed time (tmaks) of rutin metabolites in plasm after orally administration of buckwheat tea dan pure Rutin (containig 662 µMol Rutin). The maximum level of Rutin metabolites are 0.6±0.7 µg/ml and 0.3±0.3 µg/ml, respectively. The maximum level was reached 4.3 hour after intake buckwheat tea and 7 hour after intake Rutin [19]. Whereas, the administration of 40 mg pure Rutin were reached its maximum level in plasm in 7.4 hour with 47.6 µg/l [20].

Other study suggested the Rutin metabolites were detected on interval 0-3 hour after consumption of tomato juice which containing 176 µmoles Rutin. Rutin metabolites appeared in plasm in 4 hour after intake. It indicated Rutin absorption from large intestine. While in interval 2-5 hour, small quantities of quercetin-based compounds (Rutin metabolites) were detected [21]. Whereas the orally administration of Rutin tablets of 60 and 30 mg by two healthy volunteers showed urine rutin level reached its maximum on 16.64 and 8.06 µg/ml in 2.5 hour. Then, Rutin level rapidly declined until reached the lowest level on 0.73 and 0.35 µg/ml in 8 hour [22].

Flavonoids of Carica papaya leaf extract were still detected indeed reached its maximum in 48 hours after orally administration. Indicated flavonoid Carica papaya leaf extract required more than 48 hours to clear from the body. Study about the excretion of six flavonoid after 2.5 mL/kg single dose oral administration of Fructus Sophorae extract were shown completely in bile after 36 hour and completely in urine after 96 hour [9].

The percentage urine flavonoid level within 48 hour was 4.73%. The previous study about urinary excretion of 60 mg pure Rutin in human subject, a total Rutin which excreted in 8 hour was 58.5 mg (88.3%). Urine flavonoid level reached its maximum after 3 hour and declined sharply within a few hours [23]. The percentage urine flavonoid level of Carica papaya leaf extract in 48 hour was low. It proved that there was flavonoid accumulation in the cell or tissues. The other possibility was flavonoid of Carica papaya leaf extract undergo excreted via feces and bile (enterohepatic circulation). Therefore, future study needed to investigate the level of flavonoid excreted via bile and feces.

Flavonoid of Carica papaya leaf extract are rapidly absorbed then slowly eliminated via kidney. In a common, factors which influence the metabolism and excretion of flavonoid are gender [20,24,25], age [26], body weight, species [27], Flavonoid structures, molecular weight [28,29], genetic polymorphism [26,30] and liver and kidney physiology.

4. Conclusion

Urine flavonoid excretion parameters were required for development of Carica papaya leaves as herbal medicine. Flavonoid of Carica papaya leaf extract detected in rat urines on period 0-6 hour. Urine flavonoid level were still high in period 24-48 hour. Flavonoid is estimated still in plasma, tissue, or cells in long enough time. Required more than 48 hour to excrete all flavonoid from body. Future study required to investigate flavonoid excretion using closer time series urine collection before sixth hour and after 48 hour. So, the apt period of the beginning excretion and the period of all flavonoid excreted from the body can be known.

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