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Glucose tolerance test and pharmacokinetic study of *Kaempferia parviflora* extract in healthy subjects

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Abstract

Kaempferia parviflora Wall. ex Baker (KP), Krachaidam in Thai or Thai ginseng, is an herbal medicine that has many potential pharmacological effects. This study focused on the oral glucose tolerance test and pharmacokinetic study in healthy volunteers administered with KP extract (90 and 180 mg/day, placebo). The oral glucose tolerance tests were performed at baselines and 28-days of administration. The pharmacokinetics were determined after a single dose administration of the tested products using 3,5,7,3′,4′-pentamethoxyflavone (PMF) and 5,7,4′-trimethoxylfavone (TMF) as markers. The results showed that glucose metabolism via oral glucose tolerance test was not affected by KP extract. The results of pharmacokinetics study revealed that only TMF and PMF, but not DMF levels could be detected in human blood. The given doses of KP extract at 90 and 180 mg/day showed a linear dose-relationship of blood PMF concentration whereas blood TMF was detected only at high given dose (180 mg/day). The half-lives of PMF and TMF were 2–3 h. The C_{max}, AUC and T_{max} values of PMF and TMF estimated for the 180 mg/day dose were 85.37±11.31, 73.23±29.93 mg/ml; 291.89±48.23, 412.20±203.69 mg.h/ml; and 3.89±0.37, 4.50±0.96 h, respectively. PMF was quickly eliminated with higher Ke and Cl than TMF at the dose of 180 mg/day of KP extract. In conclusion, the results demonstrated that KP extract had no effect on glucose tolerance test. In addition, this is the first demonstration of the pharmacokinetic parameters of methoxyflavones of KP extract in healthy volunteers in a phase I study in drug development. The data suggest the safety of the KP extract and will be of benefit for further clinical trials using KP extract as food and sport supplements as well as a drug in health product development.

Keywords. Glucose tolerance, pharmacokinetic, *Kaempferia parviflora*, methoxyflavone

1. Introduction

Kaempferia parviflora Wall. ex Baker (KP) (Thai ginseng, Black ginger or Krachaidam in Thai) belongs to the family of Zingiberaceae. Several flavonoids are constituents of KP with three major compounds having been used as the markers for quantitative analysis, namely 3,5,7,3′,4′-pentamethoxyflavone (PMF, PubChem CID: 97332), 5,7,4′-trimethoxylfavone (TMF, PubChem CID: 79730), and 5,7-dimethoxyflavone (DMF, PubChem CID: 88881) [1]. Ethnopharmacologically, the plant rhizomes have been traditionally used in folk medicine for centuries for longevity promotion, anti-fatigue, appetite induction, male sexual stimulation, anti-stomachache, and laxative [2-3]. KP extract and its major constituents have been reported to confer several health beneficial effects through *in vitro* and animal studies, including aphrodisiac activity [4-7], anti-inflammation [8], anti-cancer [9-10] , cardioprotection [11-12], anti-peptic ulcer [13], antimicrobial [14], anti-allergy [15], anti-mutagenicity [16], anti-depression [17], anti-cholinesterase activity [17-18], prevention of the brain from valproic acid-induced the impairments of spatial memory [19], inhibition of intrinsic aging process [20], anti-osteoporosis [21], reduction of pain threshold and severity of osteoarthritis [22], anti-obesity and preventing obesity-induced dermatopathy [23-24], inhibition of fat accumulation and muscle atrophy [25], increased whole-body energy expenditure [26] and promotion of the differentiation of brown adipose tissue [27-28]. In terms of antidiabetic activity, the ethanol extract of *K. parviflora* was reported to decrease blood glucose in streptozocin-induced diabetic rats [29]. Later on 5,7,3′,4′-tetramethoxyflavone, TMF and PMF constituents from *K. parviflora* showed *in vitro* inhibitory effects on α-glucosidase activity [30]. Moreover, the ethyl acetate extract of *K. parviflora* also suppressed the glucose and lipid metabolism in Tsumura, Suzuki, Obese Diabetes (TSOD) mice [23, 30]. Several clinical trials showed the potential activities of KP extract including increased physical fitness in elderly volunteers and in soccer players [31-

32], decreased abdominal fat in overweight and pre obese subjects [33], improved self-assessed sex health in men [34] and reduction of stress and anxiety in adult subjects [35]. Moreover, the safety of KP has been reported. A study of acute toxicity in mice found that the LD_{50} value of KP is more than 13.3 g/kg. Oral administration of KP extract at a single dose of 2 g/kg was safe. For chronic toxicity study, daily giving KP extract up to 2,000 mg/kg to rats for six months did not show any abnormality in histopathological examination of organs, behaviors, physical examination and body weight [8, 36]. Accordingly, these efficacies and its safety indicate that KP has a potential to be the new product from Thai herbal plants for this century. Although pharmacokinetic data of KP extract in rats have been reported [37], to our knowledge, there are no reports of pharmacokinetics in human. It is necessary to have a better understanding of the pharmacokinetics of this plant in humans. Moreover, there are no reports regarding an oral glucose tolerance test which explain glucose metabolism in humans after administration of KP extract. Therefore, the objectives of this study were to evaluate the effect of a single dose administration of 90 and 180 mg/day of KP extract on oral glucose tolerance test and its pharmacokinetic parameters in healthy volunteers.

2. Materials and Methods

2.1 Chemicals and test product description

Glucose powder was purchased from Utopian Co. Ltd., Thailand. 17-α-hydroxyprogesterone was purchased from Sigma, China. Acetonitrile was analytical grade from RCI Labscan, Thailand. Methanol was HPLC grade from RCI Labscan, Thailand.

Dried powder of KP Romkaou stain rhizomes obtained from Phurue, Loei province of Thailand was authenticated and kept as voucher specimen (No. KP-BS-2010) at the Center for Research and Development of Herbal Health Products, Khon Kaen University, Thailand. KP extract was prepared by maceration in 95% ethanol (following the pretty patent of Thailand No. 4048) and the crude extract was obtained at 5.71 % yield. The KP extract was analyzed for the content of methoxyflavones using HPLC (Figure 1). The tested KP product was prepared in the dosage form of capsule to contain 90 mg of KP extract and other excipients (Table 1). Placebo capsule containing no KP extract, composed of the same excipients and capsule color. The entire study was conducted using a single batch of KP extract to optimize product consistency.

2.2 Study design

Randomized, double blinded, and placebo control trials were conducted in 45 healthy volunteers (Figure 2). The clinical study was approved by the Khon Kaen University Ethics Committee in human research, Thailand (No. 4.2.11:10/2552) following the Declaration of Helsinki and ICH Good Clinical Practice. All volunteers had signed the informed consent form before participating in the study. Inclusion criteria included healthy individuals, 20 to 50 years old, fasting blood glucose between 70 and 100 mg/dL, and normal range of AST, ALT and serum creatinine levels. Participants who had impaired glucose tolerance test, were allergic to KP, preganant, lactating, consuming other medicines or herbal medicines, and participating in other trials were excluded from this study. All of the recruited participants

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were screened for healthy status by a physician. Their blood biochemical parameters and complete blood count (CBC) were determined as the baseline data and after 28-day study period to monitor the safety of the treatment.

After overnight fasting of 8–12 h, the volunteers were randomly divided to three groups of 15 people, each subject was daily and orally taking KP extract 90 mg/day (one capsule of KP extract and one capsule of placebo), KP extract 180 mg/day (two capsules of KP extract), and placebo (two capsules of placebo) for groups 1, 2, and 3, respectively for 28 days consecutively. At baseline and day 28 after taking the capsules, all subjects were given 20% glucose solution at a dose of 1.75 g/kg BW. Then blood samples (3 mL) were drawn from the median cubital vein or cehali vein, or basilica vein to sodium fluoride tubes at 0, 30, 60, and 120 min after glucose loading to determine plasma glucose levels.

At day 1 after taking the KP capsules, another 3 ml of blood were drawn to EDTA-tube at 0, 15, 30, 45 min, 1, 2, 4, 6, 8, 10, and 12 h for pharmacokinetic study. The blood samples were frozen at -20^{o}C until analysis. The methoxyflavones concentration in blood samples were further analyzed by using HPLC (Figure 1).

2.3 Analysis of blood biochemical parameters

The blood samples were analyzed for complete blood count, electrolytes, glucose, lipid profile, liver and kidney functions by the Clinical Chemistry Unit at the Faculty of Associated Medicine, Khon Kaen University, using an automated clinical analyzer (Hitachi 912, Japan)

2.4 Quantization of methoxyflavones by HPLC method

2.4.1 Sample preparation

The blood sample (2.5 mL) was transferred to a 15-mL tube, and then spiked with 10 µL 17-α-hydroxyprogesterone (55 µg/mL) as internal standard. Six mL of acetonitrile was added as an extraction solvent, mixed for 1 min and sonicated for 10 min, then centrifuged at

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1,000 rpm for 10 min. The organic solvent layer was collected with three time repeats in crucible. The solvents were pooled and evaporated to dryness at room temperature. Then, the residue was reconstituted with 1 mL of methanol and further injected into the HPLC system.

Methoxyflavone concentration in the KP capsule was also determined by dissolving the content of the capsule in methanol and filtration through a 0.45 µm-syringe filter, then the filtrate was injected to HPLC.

2.4.2 HPLC system

Methoxyflavones were analyzed by Agilent® 1200 series HPLC (Germany) with UV detector using Agilent® Hypersil ODS column (5 µm, 4.6 x 250 mm) as the stationary phase, set at 55ºC. A mixture of 0.2% orthophosphoric acid in water (mobile phase A) and methanol (mobile phase B) was used [38]. Briefly, the mobile phase was used as gradient elution with a linear gradient from 40% of mobile phase A and 60% of mobile phase B at flow rate of 1.2 mL/min at 0 min, 47% of mobile phase A and 53% of mobile phase B at flow rate of 1 mL/min at 5 min, 60% of mobile phase A and 40% of mobile phase B at flow rate of 0.7 mL/min at 40 min, and 44% of mobile phase A and 56% of mobile phase B at flow rate of 1 mL/min at 80 min. The injection volume was 20 µL. The wavelength of UV detection was set at 254 nm.

2.5 Data analysis

Area under the blood concentration and time profiles (AUC, ng.h/mL) was calculated by using Phoenix WinNonlin program (Pharsight, Mountain View, CA) by noncompartment methods. The maximum concentration (C_{max}, ng/mL) and time to C_{max} (T_{max}, h) were obtained directly from the blood concentration and time profiles. Elimination rate constant (Ke, h^{-1}) was obtained from slope of log-linear regression of elimination range of blood concentration-time profile. Half-life ($T_{1/2}$, h) was calculated following the equation 0.693/Ke.

Statistical analyses were performed using SPSS version 17.0. *P*-values less than 0.05 were considered statistically significant.

3. Results

3.1 Subject characteristics

From 45 volunteers that fulfilled the inclusion/exclusion criteria, one subject was lost at day 28 (Figure 2). The volunteers were generally healthy, there was no clinically significant finding of hematology and clinical chemistry, and an absense of chronic disease. The placebo group included 7 male and 8 female subjects. Six male and 9 female subjects were in KP extract 90 mg/day group. The volunteers who received 180 mg/day of KP extract were 8 males and 6 females. The general characteristics of the volunteers were as followed: the age ranged from 25 to 28 years old and their weights were from 54 to 58 kg with BMI levels around 20-22 kg/m^2 (Table 2). Blood biochemical parameters of all subjects were in normal ranges. No significant differences of all parameters among three groups were detected. Compliance of all volunteers ranged from 96–100% within 28 days of treatment. The incidences of adverse events throughout 28 days of treatment were as follows: one volunteer from the placebo group experienced constipation at 1st week and recovered in the 2nd week; one volunteer from KP 90 mg/day group experienced a hunger throughout 28 days of administration. Nausea, vomiting, and anorexia were found in one volunteer who received 180 mg of KP extract at 2nd and 3rd week although it recovered at 4th week. No severe adverse events were reported in this study. In addition, the parameters of blood hematology, biochemistry, liver function, and kidney function of the all volunteers enrolled this study for 28 days were within normal limits and there were no clinically important (Table 3).

3.2 Oral glucose tolerance test

The mean levels of plasma glucose following administration of KP extract, were in the range 129–135 mg/dL in all groups within 30 min (Table 4). At 120 min, the plasma glucose levels of all volunteers declined to 83.67, 82.47, and 82.79 mg/dL for placebo, KP 90 mg/day, and KP 180 mg/day groups, respectively. The plasma glucose levels at every time

point of both dose of KP groups were not significantly different with those of the placebo group.

Oral glucose tolerance test results of all subjects at 28 days of KP treatment are presented in Table 5. The plasma glucose levels at 30 min after glucose loading reached 126.87, 120.60, and 142.50 mg/dL in placebo, KP 90 mg/day, and KP 180 mg/day groups, respectively. After that, the plasma glucose levels continuously decreased to normal ranges of 79.53, 91.27, and 94.21 mg/dL respectively and without statistical significant differences among groups.

3.3 Pharmacokinetics

Chromatograms of standard methoxyflavones, KP capsule and the blood methoxyflavones are shown in Figure 1. The validation method of the HPLC system for determination of methoxyflavones was successful with a correlation coefficient of PMF and TMF at R^2 0.98. Limit of detection (LOD) and limit of quantitation (LOQ) of TMF were 0.002 and 0.005 µg/mL, respectively. LOD and LOQ of PMF were 0.003 and 0.007 µg/mL, respectively. Intra-day and inter-day precision of PMF and TMF ranged 0.1 to 1.6 % RSD. Recovery of PMF and TMF ranged from 95.6 to 98.3% and 96.5 to 100.7% , respectively. Amounts of PMF and TMF in KP capsule in this study were 38.37 and 29.75 mg/g of powder in capsule, respectively. Therefore, KP capsule contained PMF and TMF at 13.43 and 10.41 mg/capsule, respectively.

After treatment with 90 and 180 mg/day of KP extract, the blood samples of each subject were drawn at various times for 12 h and analyzed for DMF, TMF and PMF by the HPLC method. The extraction efficacy of the spiked methoxyflavones in blood was more than 75% recovery. PMF and TMF concentrations can be detected in a certain number of subjects. Only 9 subjects of 14 subjects receiving 180 mg/day of KP extract gave the complete data for blood PMF profile with time point interval, whereas blood TMF of four

subjects can be determined. In the case of subjects receiving 90 mg/day of KP extract, blood PMF could be determined in only nine subjects. As illustrated in Figure 3, the human blood concentrations of PMF reached a peak of 25.95±4.68 ng/mL at 4.02±0.54 h and 71.20±11.31 ng/mL at 4.02±0.37 h after taking KP extract of 90 mg and 180 mg, respectively. PMF levels in the blood of both initial dosage levels reached zero concentration at 10 h. TMF levels in blood samples can be detected only at dose of 180 mg/day of KP extract, which displayed C_{max} (63.00±18.02 ng/mL) at 6.03±0.96 h. At the final point of blood drawing (12 h), TMF was still detected in blood at concentration of 20–25 ng/mL. TMF concentrations in blood samples at dose of 90 mg/day of KP extract were lower than the limit of quantitation. Therefore, the pharmacokinetics of TMF of 90 mg/day dose of KP extract were not able to be reported. All pharmacokinetic parameters of both doses of KP extract are shown in Table 6. Ke of PMF and TMF ranged from 0.32±0.13–0.75±0.26 h^{-1}. Half-life levels of both of PMF and TMF were ranging between 1.83–3.30 h. Vd values of PMF in volunteers who received 90 and 180 mg/day of KP extract were 218.11±99.26 and 199.02±48.59 L, respectively. At a dose of 180 mg/day of KD extract, Vd value of TMF (88.28±24.27 L) was lower than that of PMF (199.02±48.59 L). Cl values of PMF at doses of 90 and 180 mg/day and for TMF at the dose of 180 mg/day of KP extract were 57.81±20.80, 54.68±11.15, and 12.97±6.59 L/h, respectively.

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4. Discussion

To our knowledge, this is the first report on oral glucose tolerance test and the pharmacokinetic study of KP extract in healthy volunteers. KP has centuries of traditional uses for longevity promotion, anti-fatigue and providing energy expenditure. It is safe and efficient and there are currently several KP products on the market as food and sport supplements. Our initial clinical trials on KP extract have demonstrated that at 180 mg/day dose, it significantly enhanced physical fitness, increased muscle strength and improved aerobic capacity in soccer players [32] and KP extract at a dose of 90 mg/day can enhance the physical fitness in healthy elderly volunteers [32]. Sport supplement formulation containing the combination of KP extract with two other plants also increased physical endurance in healthy volunteers [39]. Considering the clinical effects on physical fitness, promotion of energy expenditure, anti-obesity and the hyperglycemic effect in an animal model [32, 6, 33, 26], the effect of KP extract on glucose tolerance test at doses of 90 and 180 mg/day for one month consecutive consumption was conducted in healthy volunteers in this study. It is interesting to find that KP extract at doses of 90 and 180 mg/day had no effect on the capacity of the body to manage glycemic condition after the passage of glucose into the human gut and kept the normal limit of blood glucose within 2 h as similarly observed in the placebo group. The results suggest that KP extract does not interfere in the process of blood glucose homeostasis in the normal subjects, which is in contrast to a previous report on the hypoglycemic effect of KP extract in streptozocin-induced diabetic animals [29]. It may imply that KP has a differential effect in glucose metabolism in the diabetic status. The mechanism involved may be via glucose uptake or insulin related- glucose metabolic pathways that need to be further elucidated. However, our finding on the glucose tolerance test in this study is in agreement with the report in normal rats [23]. Moreover, KP extract also decreased blood glucose after glucose loading in obese diabetic mice, which supports the

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selective effect of KP extract on glucose metabolism in various types of diabetes. To understand the energy enhancement effect of KP, further studies on the other mechanisms of action including glucose uptake, glycolysis/gluconeogenesis, lipolysis/lipogenesis need to be conducted. There are some supportive studies on this matter that reported that KP improved lipid metabolism. KP extract decreased body weight gain and visceral fat accumulation in obese diabetic mice [23]. The reduction of triglyceride level, increment of oxygen consumption and promotion of energy metabolism were via the expression of uncoupling protein 1 (UCP 1) in brown adipose tissue (BAT) causing the enhancement of thermogenesis in mice [27]. The expression of UCP 1 in BAT causing a decrease of body weight gain, reduction of fat accumulation and decrease of blood lipid levels were also reported in obese diabetic mice [28]. Clinical trials on the effect of KP in human subjects have also confirmed these findings as observed that a single dose of KP extract capsule effected the enhancement of whole-body energy expenditure and decreased the body fat without effect on food uptake [26]. Moreover, KP extract decreased body weight gain and abdominal fat accumulation in overweight and pre obese subjects [33]. Taken together, our finding that giving the KP extract at both 90 and 180 mg/day doses for 28 days did not affect the blood glucose under glucose tolerance test in healthy volunteers, suggests the safety effect of KP extract on normal blood glucose level and that KP may facilitate the available sources of energy expenditure in terms of the enhancement of lipid metabolism.

The pharmacokinetic data in healthy volunteers are important and necessary for drug development, especially for the dosage design of other clinical studies. To the best of our knowledge, clinical pharmacokinetic study of KP extract in healthy volunteers has never been investigated. Although three major compounds in KP extract that possess pharmacological effects were used, but only TMF and PMF can be detected in some human blood samples. Therefore, TMF and PMF were used as chemical markers in this pharmacokinetic study. A

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single dose pharmacokinetic trial was performed in 30 healthy subjects as designed for the glucose tolerance test (n = 15 each for both 90 and 180 mg/day doses of KP extract). PMF and TMF levels were detected by HPLC in only a certain number of blood samples. Unfortunately, DMF cannot be detected in all blood samples according to the limit sensitivity of the system. PMF levels were detectable in 33% and 64% of blood samples of subjects receiving 90 and 180 mg/day of KP extract (n=9) whereas TMF levels can be determined only in four blood samples (29%) of subjects receiving 180 mg/day of KP extract. These may reflect the wide variation of methoxyflavone absorption in the gastrointestinal tract. However, our results can be taken as preliminary evidence to support the notion that there is a certain bioavailability of methoxflavones of KP extract in human blood enabling them to exert their pharmacological effects in the clinical trials. Even though with the above mentioned limitations of this study, the obtained pharmacokinetic parameters can provide an insight for the dosage regimen in further clinical studies. AUC and C max values of PMF in this study were in the linear relationship of the doses. The noticeable variation in the values of T_{max} between PMF and TMF implied that methoxyflavones may be differentially absorbed and metabolized within the gastrointestinal tract as well. Considering the amount of these two methoxyflavones in the 180 mg/day dose, they are equivalent to 26.86 and 20.83 mg of PMF and TMF, respectively. Even though at this dose, the amount of PMF was higher than that of TMF, but AUC of PMF was lower than that of TMF. Whereas their C_{max} values were in a linear relationship with the dose of these methoxyflavones, i,e, the C_{max} value of PMF was higher than that of TMF. The lower value of AUC but higher Cmax values of PMF may result from the higher Ke and Cl of PMF than that of TMF. Accordingly, TMF which possessed the lower Ke and Cl, had higher AUC than that of PMF. In addition, gender factor was also considered in the pharmacokinetics of KP in humans. The detectable amount of PMF and TMF are similar in blood samples of both male and female volunteers. In 90

mg/day of KP extract-treated group, PMF can be determined from 9 subjects (4 males, 5 females). In 180 mg/day of KP extract-treated group, both TMF and PMF levels were detected from 4 subjects (each two males and females) and 9 subjects (4 males and 5 females). Although too small number of subjects for statistical analysis, the results suggested the similar pharmacokinetic data from male and female subjects, suggesting that there are no gender differences of pharmacokinetic parameters of KP extract. In comparison to the animal study in rats [36], AUC values of TMF were also higher than those of PMF. In rats, TMF and PMF reached the maximum blood concentration at 0.85 and 1.71 h, respectively, which were faster than those in humans. The half-life of TMF was longer than those of PMF in rats (5.91 and 3.12 h), which were also longer than those in humans (3.30 and 1.83 h), respectively. The elimination of PMF was faster than TMF in both rats and humans. These phenomena imply that there is a some degree of similarity in the pharmacokinetics of KP extract in rats and humans. The obtained data from the pharmacokinetic parameters can be used to guide and to design the appropriate dose regimen for future efficacy studies of KP extract. Furthermore, this study also confirmed the safety of an administration of KP extract up to 180 mg/day for 28 days consecutively. Blood biochemical and CBC parameters of all KP extract-treated subjects were in the normal limits. The majority of volunteers (93%) showed good compliance to the capsules of KP extract used in the study, only 3 subjects (7%) including one in the placebo group showed mild complains on nausea, vomiting and anorexia. Moreover, the capsules KP extract were previously used in the studies on the physical fitness in both soccer players and healthy elderly subjects [31-32].

5. Conclusions

In conclusion, this study has demonstrated for the first time the oral glucose tolerance test and pharmacokinetics of healthy male and female volunteers who had taken KP extract at doses of 90 and 180 mg/day. KP extract does not affect the blood glucose homeostasis and

the results suggest that methoxyflavones in KP extract may modulate multi-pathways in both carbohydrate and lipid metabolism to promote their energy expenditure effect. In addition, TMF and PMF can be detected and used as markers in human blood and their pharmacokinetic parameters in human were firstly revealed.

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Author contributions

Bungorn Sripanidkulchai had formulated the idea, performed the experiments and wrote the manuscript. Catheleeya Mekjaruskul and Rosawan Areemit performed the experiments and wrote the manuscript. Areewan Cheawchanwattana and Jiraporn Sithithaworn performed the experiments

Conflict of interest

There was no conflict of interest in this study.

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Figure legends

Figure 1 HPLC chromatograms of methoxyflavones (A = standard compounds; B = blood sample from a healthy subject following oral KP extract 180 mg/day and C= KP capsule)

Figure 2 Flowchart illustrating the phases of the study.

Figure 3 Blood concentration and time profile of PMF and TMF in normal subjects after receiving of 90 and 180 mg/day of KP extract (●: PMF concentration in KP extract 90 mg/day group (n=5), ▲: PMF concentration in KP extract 180 mg/day group (n=9), ✕: TMF concentration in KP extract 180 mg/day group (n=4))

Table 1 Composition of test products

Composition (mg)	serving size: 300 mg/capsule	
	Placebo	**KD extract**
KP extract	0	90
Microcrystalline cellulose	282	192
Silica dioxide	15	15
Magnesium stearate	3	3

Table 2 General characteristics of participants.

Characteristics	Placebo (n=15)	KP extract 90 mg/day (n=15)	KP extract 180 mg/day (n=14)	Total (n=44)	*P*-value
Age (year)	26.9±7.2	25.3±5.1	28.3±9.7	26.8±7.4	0.932
Gender (male/female)	7/8	6/9	8/6	21/23	0.655
Body weight (kg)	54.3±7.4	55.1±7.8	57.0±7.4	55.5±7.4	0.452
Height (cm)	163.0±7.5	162.8±9.2	164.2±6.0	163.2±7.6	0.706
BMI (kg/m^2)	20.5±2.3	20.8±2.2	21.3±2.2	20.8±2.2	0.478
Systolic Blood pressure (mmHg)	110.3±15.1	113.0±15.7	110.4±13.5	111.3±14.5	0.992
Diastolic Blood pressure (mmHg)	69.2±22.4	70.6±8.5	66.9±6.2	68.9±13.9	0.423
Pulse rate (beats/min)	75.1±8.6	76.5±11.3	70.3±12.8	74.0±11.0	0.203
Body temperature (°C)	35.5±2.9	36.2±0.3	36.2±0.4	36.0±1.7	0.975

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Table 3 Blood biochemical parameters of subjects at base line and at 28 days after KP treatment in glucose tolerance test.

Parameters (normal limits)	**Placebo (n=15)**		**KP 90 mg/day (n=15)**		**KP 180 mg/day (n=14)**	
	day 0	**day 28**	**day 0**	**day 28**	**day 0**	**day 28**
RBC (3.9-5.6 *10^6µL)	5.18 (4.82, 5.54)	5.15 (4.77, 5.52)	5.40 (4.99, 5.82)	5.26 (4.81, 5.71)	5.42 (5.11, 5.74)	5.25 (4.93, 5.58)
WBC (3.8-10.8 x10^3/µL)	5.94 (5.34, 6.53)	6.18 (5.37, 7.02)	6.62 (5.97, 7.27)	6.98 (6.22, 7.75)	7.00 (5.28, 8.71)	7.30 (5.74, 8.86)
Hb (12-18g/dL)	13.00 (11.91, 14.09)	13.14 (12.06, 14.22)	12.69 (11.44, 13.98)	13.03 (12.16, 13.91)	14.09 (13.27, 14.92)	13.73 (12.95, 14.51)
Hct (37-42%)	40.29 (37.09, 43.49)	41.00 (37.77 44.23)	40.21 (37.49, 42.92)	39.88 (37.09, 42.67)	43.03 (40.47, 45.60)	41.64 (39.42, 43.87)
Plt.count (140-400x10^3)	249.86 (215.64, 284.07)	246.07 (214.78, 277.35)	276.73 (238.78, 314.69)	281.80 (249.27, 314.69)	253.47 (231.49, 275.44)	226.29 (205.70, 246.87)
MCV (80-95fL)	78.93 (72.50,85.36)	80.15 (74.05, 86.25)	75.90 (68.78, 83.02)	77.51 (70.06, 84.97)	76.09 (68.18, 83.99)	80.44 (75.62, 85.25)
MCH (27-32)	25.54 (23.60, 27.47)	25.76 (23.92, 27.60)	25.18 (23.04, 27.32)	25.20 (23.03, 27.37)	26.30 (24.87, 27.73)	26.12 (24.71, 27.54)
MCHC (23-36)	32.49 (31.61, 33.36)	32.35 (31.49, 33.20)	33.36 (32.58, 34.14)	32.71 (31.89, 33.54)	33.21 (32.57, 33.86)	32.54 (32.08, 33.01)
RDW (10.9-15.7)	14.77 (13.41, 16.13)	14.78 (13.55, 16.01)	14.53 (13.34, 15.72)	15.05 (13.70, 16.40)	14.13 (13.45, 14.80)	14.22 (13.63, 14.81)

Neutrophil (44.3-70.9%)	49.50 (44.87, 54.13)	50.53 (46.01, 55.06)	53.27 (48.88, 57.65)	55.13 (50.97, 59.30)	50.13 (47.02, 53.24)	52.71 (47.23, 58.20)
Lymphocyte (20.1-44.5%)	38.14 (34.02, 42.27)	37.87 (32.95, 42.78)	36.07 (31.9, 40.21)	34.47 (30.04, 38.89)	37.13 (33.77, 40.50)	35.14 (30.21, 40.07)
Monocyte (3.4-9.8%)	6.14 (4.99, 7.29)	5.93 (5.29, 6.58)	6.73 (5.79, 7.68)	6.07 (5.22, 6.92)	6.60 (5.94, 7.26)	5.93 (4.93, 6.93)
Eosinophil (0.7-9.2%)	6.14 (3.06, 9.23)	5.53 (2.63, 8.43)	3.73 (2.13, 5.33)	3.87 (2.46, 5.27)	5.73 (2.43, 9.03)	4.86 (1.97, 7.75)
Basophil (0-2.6%)	0.21 (0.00, 0.43)	0.20 (0.00, 0.43)	0.29 (0.06, 0.52)	0.47 (0.18, 0.75)	0.49 (0.23, 0.74)	0.57 (0.27, 0.87)
Na (135–146 mEq/L)	140.4 (139.1, 141.7)	143.8 (142.0, 145.6)	139.8 (138.7, 140.9)	144.2 (142.6, 145.8)	138.7 (137.8, 139.6)	143.9 (142.6, 145.2)
K (3.5-5.5 mEq/L)	4.3 (4.2, 4.4)	4.0 (3.8, 4.2)	4.1 (4.0, 4.3)	3.9 (3.8, 4.1)	4.2 (4.0, 4.4)	4.0 (3.7, 4.2)
Cl (95–112 mEq/L)	104.2 (102.7, 105.7)	103.9 (102.7, 105.0)	103.7 (102.3, 105.0)	104.2 (103.0, 105.4)	102.3 (101.3, 103.3)	103.6 (102.7, 104.6)
CO_2 (22–32 mEq/L)	22.9 (22.0, 23.8)	19.0 (18.0, 20.0)	22.1 (21.0, 23.1)	18.7 (17.8, 19.6)	23.8 (22.8, 24.9)	19.8 (18.5, 21.0)
Mg (1.8-2.6 mg/dL)	2.2 (2.1, 2.3)	2.2 (2.1, 2.3)	2.2 (2.1, 2.3)	2.2 (2.1, 2.3)	2.3 (2.2, 2.3)	2.2 (2.1, 2.3)
Phosphate	3.5	4.0	3.9	4.1	3.8	4.0

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(2.5-4.5 mg/dL)	(3.3, 3.8)	(3.6, 4.3)	(3.7, 4.1)	(3.8, 4.4)	(3.6, 4.1)	(3.7, 4.2)
BUN (6-20 mg/dL)	12.6 ± 3.5 (7.0, 19.0)	12.67 (11.47, 13.86)	13.1± 4.0 (6.0, 19.0)	12.40 (10.26, 14.54)	13.5 ± 4.7 (9.0, 26.0)	12.79 (10.62, 14.95)
Cr (0-1.5 mg/dL)	0.9 ± 0.3 (0.5, 1.4)	0.93 (0.80 , 1.07)	1.0 ± 0.3 (0.5, 1.4)	0.93 (0.83, 1.04)	1.0 ± 0.3 (0.4, 1.5)	0.94 (0.87, 1.01)
FPG (70-110 mg/dL)	87.1± 4.8 (81.0, 96.0)	86.73 (82.78, 90.69)	87.7 ± 7.9 (69.0, 100.0)	87.47 (83.99, 90.94)	87.0 ± 5.6 (75.0, 93.0)	89.21 (83.12, 95.31)
AST (12-32 U/L)	19.4 ± 4.2 (16.0, 29.0)	19.60 (17.22, 21.98)	18.3 ± 4.6 (12.0, 27.0)	19.73 (13.48, 25.99)	21.9 ± 11.0 (12.0, 57.0)	22.86 (11.92, 33.80)
ALT (4-36 U/L)	16.4 ± 5.7 (10.0, 27.0)	17.80 (14.87, 20.73)	15.9 ± 5.9 (9.0, 29.0)	17.87 (13.99, 21.74)	18.4 ± 8.7 (7.0, 32.0)	19.14 (13.75, 24.53)
ALP (42-121U/L)	65.1 ± 14.6 (43.0, 99.0)	58.60 (50.48, 66.72)	61.5 ± 13.1 (36.0, 83.0)	55.40 (49.29, 61.51)	59.2 ± 14.3 (35.0, 88.0)	54.64 (45.65, 63.64)
Direct bilirubin (0-0.3 mg/dl)	0.10 ± 0.10 (0.0, 0.2)	0.14 (0.10, 0.18)	0.10 ± 0.10 (0.00, 0.30)	0.13 (0.08, 0.18)	0.10 ± 0.10 (0.00, 0.30)	0.11 (0.08, 0.13)
Indirect bilirubin (0-1.2 mg/dl)	0.50 ± 0.20 (0.20, 0.80)	0.39 (0.25, 0.52)	0.40 ± 0.30 (0.10, 1.30)	0.41 (0.23, 0.60)	0.50 ± 0.20 (0.30, 1.00)	0.34 (0.25,0.43)
Total cholesterol (< 200 mg/dL)	189.10 ± 37.60 (113.00, 239.00)	195.7 (176.00, 215.40)	194.30 ± 31.60 (154.00, 280.00)	189.90 (177.60, 202.30)	198.20 ± 34.00 (138.00, 253.00)	185.80 (164.50, 207.00)
Triglyceride (< 160 mg/dL)	78.10 ± 38.80 (46.00, 171.00)	84.90 (48.00, 121.80)	70.10 ± 21.10 (41.00, 107.00)	80.30 (63.30, 97.30)	78.30 ± 36.90 (30.00, 162.00)	79.60 (61.40, 97.80)

LDL (< 100 mg/dL)	128.50 ± 27.60 (70.00, 165.00)	133.3 (117.30, 149.20)	134.60 ± 37.10 (67.00, 220.00)	123.90 (110.70, 137.00)	136.90 ± 30.20 (97.00, 186.00)	119.60 (104.30, 135.00)
HDL (40 - 60 mg/dL)	49.40 ± 14.00 (33.00, 77.00)	45.50 (39.60, 51.40)	44.30 ± 11.20 (27.00, 64.00)	50.10 (44.00, 56.20)	47.60 ± 9.80 (32.00,62.00)	50.10 (44.60, 55.70)

Values are expressed as mean (95% CI)

Abbreviations: RBC, red blood cell count; WBC, white blood cell count; Hb, hemoglobin; Hct, hematocrit; Plt, platelet count; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red blood cell distribution width; Na, sodium; K, potassium; Cl, chloride; CO2, carbon dioxide; Mg, magnesium; BUN, blood urea nitrogen; Cr, creatinine; FPG, fasting plasma glucose; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; TC, total cholesterol; TG, triglyceride; LDL, low density lipoprotein; HDL, high density lipoprotein.

Table 4 Blood glucose levels of volunteers after glucose loading at baseline

Group	FPG (mg/dL, mean (95% CI))			
	0 min	**30 min**	**60 min**	**120 min**
Placebo	82.60	134.67	120.93	83.67
(n=15)	(78.94, 86.26)	(122.35, 146.98)	(101.11, 140.76)	(73.84, 93.49)
P-value*	-	<0.001	0.002	0.847
KP 90 mg	81.93	128.53	113.93	82.47
(n=15)	(78.33, 85.53)	(113.64, 143.43)	(93.34, 134.53)	(74.79, 90.14)
P-value*	-	<0.001	0.01	0.902
P-value**	0.940	0.491	0.432	0.893
KP 180 mg	83.79	130.79	125.50	82.79
(n=14)	(79.86, 87.71)	(120.31, 141.26)	(108.94, 142.06)	(73.06, 92.51)
P-value*	-	<0.001	0.001	0.711
P-value**	0.816	0.73	0.764	0.94
P-value***	0.759	0.73	0.278	0.952

*Paired t-test (compared with FPG at 0 min)

**P-value compared with placebo group using One way ANOVA with LSD multiple comparison

***P-value compared with KP 90 mg group using One way ANOVA with LSD multiple comparison

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Table 5 Blood glucose levels of volunteers after glucose loading at 28 days of the treatment.

Group	FPG (mg/dL, mean (95% CI))			
	0 min	**30 min**	**60 min**	**120 min**
Placebo	86.73	126.87	122.00	79.53
(N=15)	(83.12, 90.35)	(113.58, 140.15)	(100.38, 143.62)	(71.57, 87.50)
P-value*	-	<0.001	0.004	0.088
KP 90 mg	87.47	120.60	115.07	91.27
(N=15)	(84.29, 90.64)	(113.44, 127.76)	(98.47, 131.67)	(80.95, 101.58)
P-value*	-	<0.001	0.005	0.502
P-value**	0.949	0.588	0.549	0.312
KP 180 mg	89.21	142.50	129.36	94.21
(N=14)	(83.69, 94.74)	(118.63, 166.37)	(95.77, 162.98)	(74.35, 114.08)
P-value*	-	<0.001	0.018	0.542
P-value**	0.833	0.186	0.533	0.214
P-value***	0.882	0.064	0.226	0.802

*Paired t-test (compared with FPG at 0 min)

**P-value compared with placebo group using One way ANOVA with LSD multiple comparison

***P-value compared with KP 90 mg group using One way ANOVA with LSD multiple comparison

Table 6 Pharmacokinetic parameters

	PMF		TMF
Parameters*	**KP extract 90 mg/day (n=9)**	**KP extract 180 mg/day (n=9)**	**KP extract 180 mg/day (n=4)**
AUC (ng.h/mL)	86.65 ± 18.75	291.89 ± 48.23	412.20 ± 203.69
Cmax(ng/mL)	25.95 ± 4.68	71.20 ± 11.31	63.00 ± 18.02
Tmax(h)	4.02 ± 0.54	4.02 ± 0.37	6.03 ± 0.96
Ke(h^{-1})	0.75 ± 0.26	0.66 ± 0.13	0.32 ± 0.13
$T_{1/2}$ (h)	2.51 ± 0.88	1.83 ± 0.36	3.30 ± 0.96
Vd(L)	218.11 ± 99.29	199.02 ± 48.59	88.28 ± 24.27
Cl (L/h)	57.81 ± 20.80	54.68 ± 11.15	12.97 ± 6.59

*Values are extract as mean ± SE

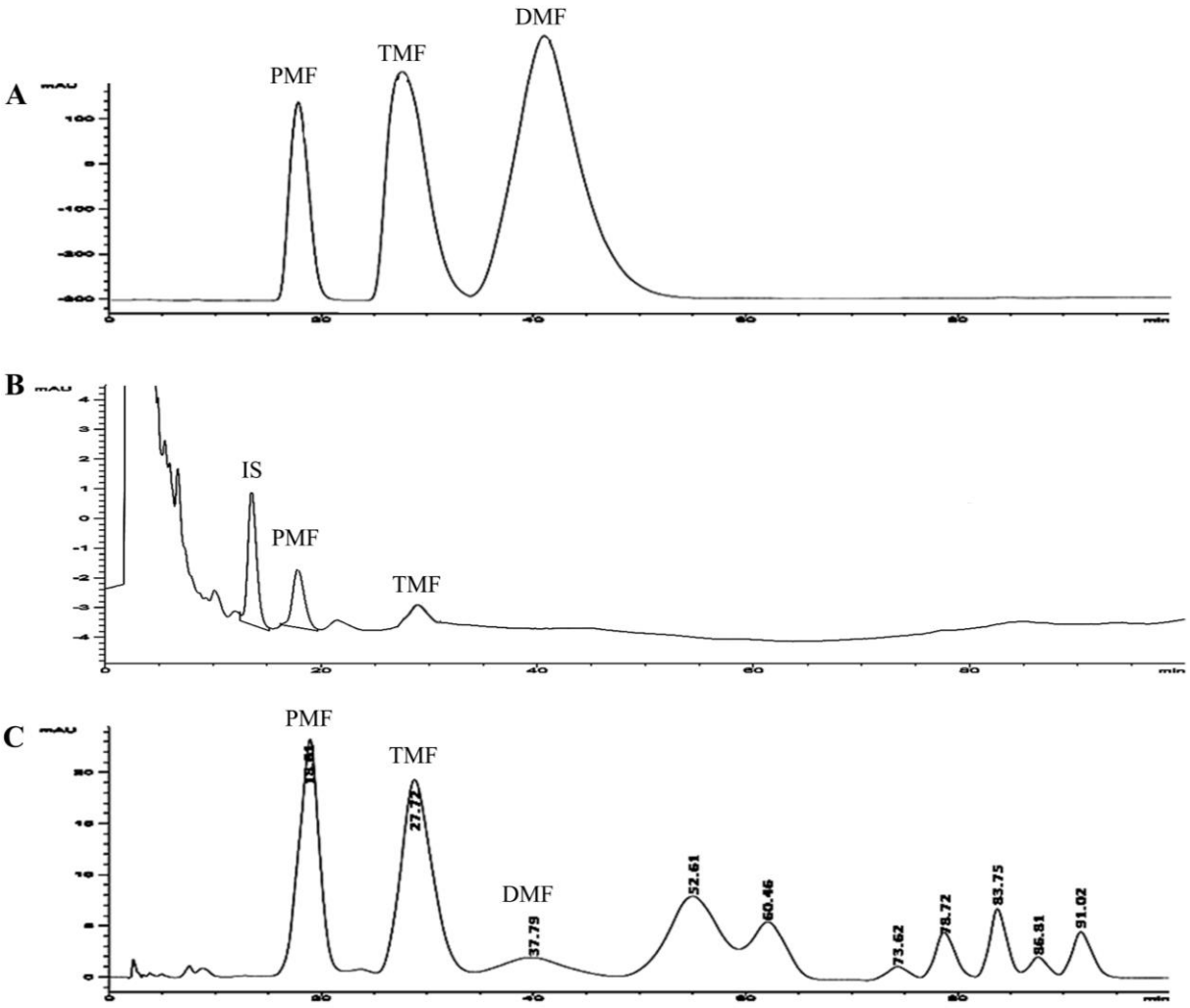

Figure 1

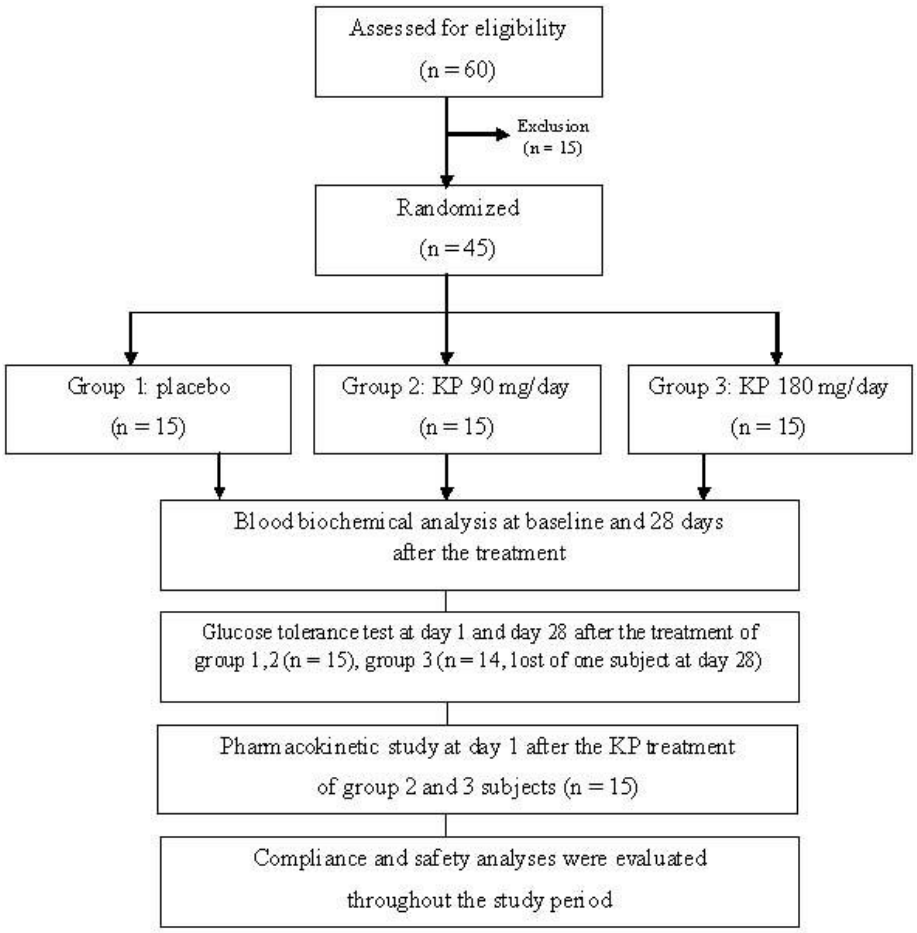

Figure 2

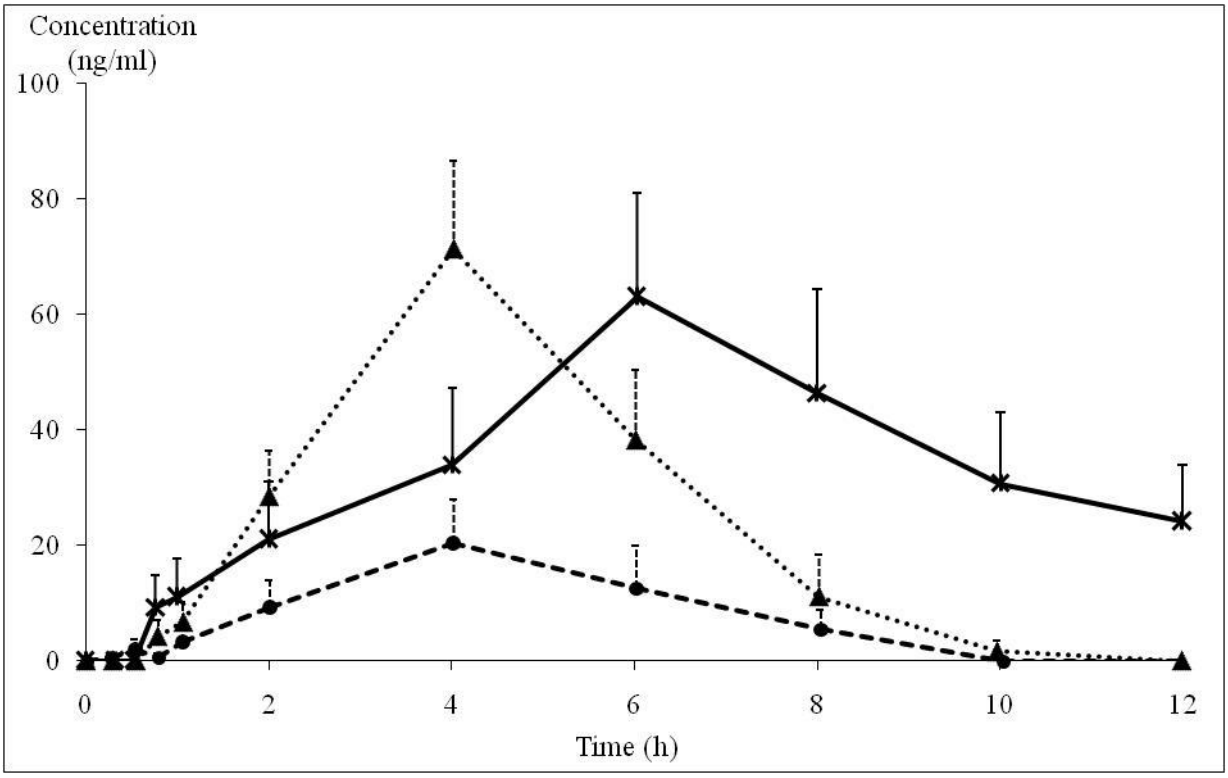

Figure 3