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Research Article

Biological Activities of Solid Dispersion Kollicoat® IR (PVA-co-PEG) - *Kaempferia parviflora* Dichloromethane Extract (KPD) After Six-week Consumption by Middle-aged Male Rats

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Abstract

Background: We previously demonstrated that consumption of *Kaempferia parviflora* (KP) rhizome dichloromethane extract (KPD) caused some beneficial changes in cardiovascular parameters, decreased body fat and up-regulated NO in middle-aged rats. KPD is poorly soluble and was developed to improve its solubility in solid dispersion form with Kollicoat® IR (PVA-co-PEG). Thus the present study aimed to investigate whether Kollicoat (K), an excipient, affected any biological activities of the KPD.

Methods: Solid dispersion of the KPD was prepared by dissolving KPD and K separately and then mixing together after drying to yield a solid dispersion of KPD (K-KPD). Middle-aged male rats were gavaged 200 mg/kg K-KPD, K (100 mg/kg) or distilled water (DW), twice a day, for 6 weeks.

Results: In comparison to control group (DW), K did not affect any of the study parameters. K-KPD caused decreases in body fat and liver cell lipid accumulation, plasma level of glucose and triglycerides, plasma level of alkaline phosphatase, and blood platelets count. K-KPD did not affect basal blood pressure or heart rate in anesthetized rats. K-KPD caused decreased maximal contractile response of thoracic aortic rings to phenylephrine, and this effect disappeared in the presence of DL-propargylglycine (PAG) or removal of the vascular endothelium, but not by nG-nitro-L-arginine (L-NA). K-KPD potentiated vasodilatation of the aortic ring precontracted with phenylephrine to acetylcholine and glyceryl trinitrate, and these effects were abolished by PAG. Western blot analysis showed an increase in blood vessel CSE, but not eNOS, protein expression.

Conclusion: Taken together, Kollicoat did not affect the beneficial cardiovascular health parameters of the KPD, except for the mechanism of the vascular function which was found to cause increased blood vessel H_2S instead of the NO. K-KPD did not have any adverse effects on internal organ gross toxicity, liver and kidney functions, or on blood cells. Thus, the K-KPD would be a novel health product to prolong cardiovascular health functions in human. Further development of the K-KPD in a dosage form as tablets or capsules for convenient human consumption would be worthwhile.

Keywords: *Body Fat; Liver Lipid; Blood Vessel; CSE; Nitric Oxide; DL-propargylglycine*

Abbreviations

Ach: Acetylcholine; CSE: Cystathionine-γ-lyase; DW: Distilled Water; eNOS: Endothelial Nitric Oxide Synthase; GTN: Glyceryl Trinitrate; H_2S: Hydrogen Sulfide; K: Kollicoat® IR (PVA-co-PEG); KPD: *Kaempferia parviflora* Rhizomes Dichloromethane Extract; K-KPD: Solid Dis-

persion Kollicoat® IR (PVA-co-PEG)-*Kaempferia parviflora* Dichloromethane Extract; L-NA: N^G-nitro-L-arginine; NO: Nitric Oxide; PAG: DL-propargylglycine

Introduction

Cardiovascular disease is still the leading cause of death. Its etiology is multifactorial but age and nutrition are two of the most important risk factors. Population aging is accelerating in nearly all countries of the world [1, 2]. Unlike age, nutrition is modifiable, and thus consumption of supplementary cardiovascular health nutrients may prolong a healthy life and facilitate aging with less healthcare burden.

Advancing age is associated with increased intra-abdominal visceral fat accumulation [3-7]. This leads to endothelial dysfunction [8-10] due to decreased vascular eNOS expression, and thereby diminished nitric oxide (NO) production [11], which represents the early stage of pathophysiological changes in the development of cardiovascular disease [12-14]

Kaempferia parviflora (KP) Wall.Ex Baker or black ginger belongs to the family Zingiberaceae, found in the northern part of Thailand. Its rhizome has been used in folk medicine for health promotion [15]. To date, a number of investigations have claimed therapeutic benefit such as: an aphrodisiac [16-19], anti-inflammatory [20,21], anti-hypertensive [22], cardioprotective [23,24], and antiobesity [25] effects. Our group Yorsin., *et al.* [26] also found that consumption of dichloromethane extract of the plant rhizomes (KPD) by middle-aged male rats caused increased blood vessel eNOS protein expression resulting in an increased NO production, as well as decreased body and visceral fat and liver cell lipid accumulation, with no changes in liver and kidney functions. Thus KPD would be a good choice to develop as a health product to prevent and/or prolong the development of obesity and/or cardiovascular diseases in human.

KPD is poorly water soluble. To enhance its solubility the KPD was prepared as in a solid dispersion form with Kollicoat® IR (PVA-co-PEG). This solid dispersion-based KPD/Kollicoat® IR (PVA-co-PEG) (K-KPD) was found to be effective both as regards solubility and the *in vivo* pharmacokinetics [27]. Therefore, in the present study we aimed to further investigate whether K-KPD still has the same beneficial cardiovascular health activity as previously reported by Yorsin., *et al.* [26]. The studies were performed in middle-aged male rats and followed the same methodology as previously described by Yorsin., *et al.* [26].

Materials and Methods

Kaempferia parviflora extract preparation

The same method as previously described by Yorsin., *et al.* [26] was used. Briefly, fresh rhizomes of *Kaempferia parviflora* were blended and extracted with 95% ethanol, twice, and followed by extracting three times with 100% dichloromethane. The dichloromethane soluble part was collected and evaporated to yield a yellowish gummy dichloromethane *Kaempferia parviflora* extract (KPD).

Preparation of KPD/PVA-co-PEG solid dispersion

Preparation of the solid dispersion of KPD followed the previous method described by Weerapol., *et al.* [27]. Briefly, KPD (1g) and K (1g) were separately dissolved in 5 mL dichloromethane, then mixed together and dryness at 50°C for 24 h or more in a water bath to obtained a yellowish solid dispersion product: K-KPD. The product was tested for dichloromethane residue by Gas Chromatograph-micro-Electron Capture Detector (GC-uECD) using the service operated by the Equipment Center, Prince of Songkla University. The product without residual solvent was used for animal consumption by dissolving in distilled water.

The K-KPD was analyzed by high performance liquid chromatography (HPLC) for its major chemical profile as previously reported [26]. Briefly, analytical HPLC was carried out on a HP1100 system equipped with a photodiode array detector (Agilent Technologies). The

K-KPD was analyzed on a Symmetry C_{18} column (5 µm, 3.9 x 150 mm i.d: Waters), with a gradient of CH_3OH: H_2O+ 0.05% of trifluoroacetic acid (10: 90 to 100: 0) with a flow rate of 1 mL/min. The UV traces of the eluants were measured at 210 and 254 nm and the UV spectra (DAD) were recorded between 200 and 500 nm.

Pharmacological studies

Middle-aged (13-14 month old) male Wistar rats were bought from the National Laboratory Animal Center, Mahidol University. The animals were housed in controlled environmental conditions at 25 ˚C on a 12 h dark and 12 h light cycle and allowed access to standard food (Perfect Companion Group Co. Ltd, Thailand), and tap water *ad libitum*. The animal methods employed in this study were approved by the Prince of Songkla University Animal Ethics Committee (Ethics Number: Ref. 10/2018). The investigation conformed to the Guide for the Care and Use of Laboratory Animals (CIOMS Guidelines). The rats were randomly divided into three groups, with six animals in each type of experiment except for the Western blot analysis in which 4 rats were used in each group. The experimental group was treated by oral administration of K-KPD (200 mg/kg which is equal to 100 mg/kg KPD), Kollicoat (100 mg/kg) and the control group animals received distilled water (DW) twice a day for six weeks. The body weight and 24 h food intake (one day before receiving oral gavage of K-KPD, K or DW) were recorded at day 0, and again every consecutive 7th day over a 6-week period.

Effects of the K-KPD, K or DW treatment on the basal blood pressure and on the haematology and clinical biochemical analysis

The same methods as previously described by Yorsin., *et al.* [26] were used. At the end of the 6-week treatment, blood pressure and heart rate were recorded in anesthetized rats. Following this, blood samples were collected for the analysis of glucose and lipid levels and for the analysis of total blood cell count. Then the rat was killed by decapitation with a guillotine.

Effects of K-KPD, K or DW treatment on internal organs and lipid accumulation

The decapitated rats were dissected as previously described [26]. The heart, lung, liver, adrenal gland, kidney and testes, and the visceral fats from the epididymis, testis, and retroperitoneal and subcutaneous fats were removed and weighed.

Two pieces of liver were cut, embedded into a cryostat gel, and the sections (20 µm thick) stained with oil red O, and mounted with glycerine jelly for observation by light microscopy. The oil red O of each slide of the liver tissue was extracted with 1 mL of 100% dimethyl sulfoxide (DMSO), and its absorbance was measured at 520 nm. The area of a thin whole-liver section was measured using the Auto CAD 2005 program. The amount of accumulated liver lipid was expressed in terms of µg/mL/cm^2 of the thin liver-tissue section area.

Preparation of the thoracic aortic rings

The thoracic aortic rings were prepared as previously described [26]. Six adjacent rings of 4-5 mm in length were cut. Each aortic ring was mounted in a 20 mL organ bath containing Krebs-Henseleit solution, maintained at 37 ˚C and bubbled with carbogen. The tissues were equilibrated for 60 min under a resting tension of 1 g and the bath solution was replaced with pre-warmed oxygenated Krebs-Henseleit solution every 15 min.

At the end of the equilibration period, each aortic ring was tested for viability of the endothelium by precontraction with phenylephrine (3 µM) until the response reached a plateau (5-8 min), followed by the addition of acetylcholine (30 µM). Endothelial viability was judged by a > 65% vasorelaxation back to the tension generated by the ring before the addition of the phenylephrine. After 45 min equilibration, the basal tension of the thoracic aortic rings adjusted to the optimal tension of 2 g before the experimental protocol began.

Effects of the K-KPD, K or DW treatment on the pharmacological vascular functions

Effects on contraction to phenylephrine and role of NO and H_2S

After equilibration, the contractile response to the cumulative concentration-response (*C-R*) curve of phenylephrine was obtained. This was followed by several washings, and the aortic rings were allowed to fully relax for 50 min. They were then pre-incubated with L-NA for 40 min, and the second *C-R* curve to phenylephrine was then obtained. After repeated washings and re-equilibrations for 40 min, the third *C-R* curve to phenylephrine was obtained in the presence of both L-NA and DL-propargylglycine (PAG).

Effects on relaxation to acetylcholine and glyceryl trinitrate and role of NO and H_2S

Another set of aortic rings was precontracted with phenylephrine (3 μM) for 10-15 min (plateau phase) following which the cumulative dilator *C-R* curves to acetylcholine were determined. After repeated washing to remove the agonists and re-equilibration for 40 min, the second *C-R* curve to acetylcholine was obtained in the presence of DL-propargylglycine (PAG).

Using the above protocol and separate sets of aortic rings, the cumulative dilator *C-R* curves to glyceryl trinitrate (GTN) were obtained in the presence of L-NA alone and then together with PAG.

eNOS and CSE Western blot analysis

The thoracic aortas of the K-KPD, K- or DW- treated groups (n = 4) were obtained in order to measure the expression level of the enzymes, eNOS and CSE. After removal of the adhering connective tissue, each blood vessel was cut into small rings and kept at -70 ˚C until used. Protein extraction from the tissues and Western blot analysis were carried out as previously described [26]. Briefly, the total proteins were extracted in RIPA buffer, and quantitated by Bradford assay. Protein at 50 μg was separated by 12% SDS-PAGE and transferred onto nitrocellulose membranes. The membranes were blocked with 5% low fat dry milk in TBS-T, followed by primary antibody incubation against eNOS at 1:250 (Cell Signalling, USA), CSE at 1:1,000 (Abnova, USA) and β-actin at 1:1,000 (Cell Signalling, USA). The membranes were incubated with HRP-conjugated IgG (1:5,000) and detected by chemiluminescence detection kit (Pierce, Rockford, USA).

Drugs

The following chemicals were used: PVA-co-PEG (Kollicoat® IR) was obtained from BASF (Thai) Co. Ltd. (Bangkok, Thailand). Acetylcholine chloride, N^G-nitro-L-arginine (L-NA), phenylephrine hydrochloride, DL-propagylglycine (PAG), pentobarbital, and oil red O from Sigma, USA. GTN was obtained from Mycomed, Denmark. The acetylcholine chloride and phenylephrine were dissolved in a solution containing NaCl 9 g/L, NaH_2P0_4 0.19 g/L and ascorbic acid 0.03 g/L.

Statistical analysis

The results were expressed as the mean ± standard error of the mean (SEM) (n = 6 for vascular function study and n = 4 for western blot analysis). Statistical differences were determined by unpaired *t*-test or by one-way analysis of variance (ANOVA), followed by Tukey's range test, using GraphPad Prism 5.00. A P value < 0.05 was considered to indicate a significant difference between values.

Results

Preparation of KPD/PVA-co-PEG solid dispersion (K-KPD)

The solid dispersion of KPD: K-KPD dissolved well in distilled water as previously reported by Weerapol., *et al.* [27]. The HPLC chromatograms of the K-KPD and its three major substances: 3, 5, 7, 3′, 4′-pentamethoxyflavone, 5, 7-dimethoxyflavone (DMF) and 5, 7, 4′-trimethoxyflavone (TMF) together with their retention times and corresponding UV spectra are shown in Figure 1.

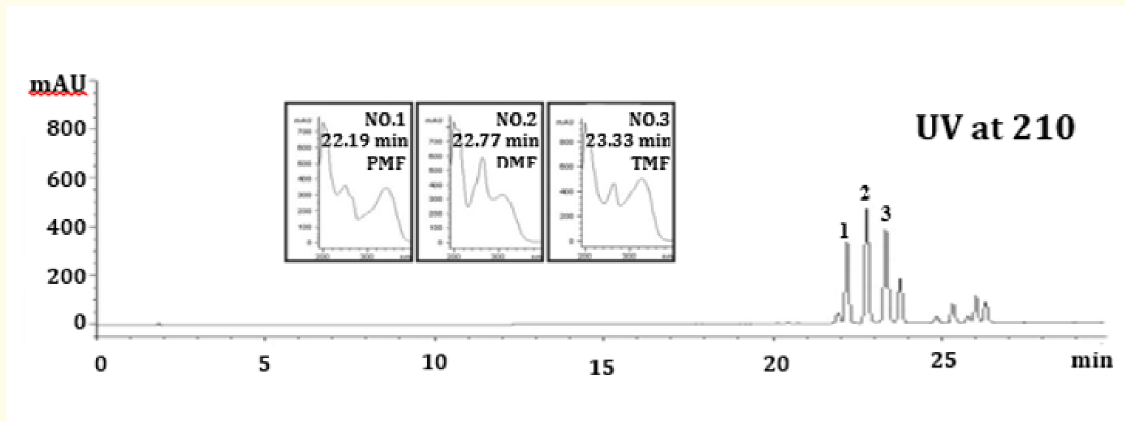

***Figure 1:** HPLC chromatogram of the K-KPD, and their major methoxyflavones: 3, 5, 7, 3′, 4′-pentamethoxyflavone (PMF), 5, 7-dimethoxyflavone (DMF) and 5, 7, 4′-trimethoxyflavone (TMF). The column eluent from each extract was scanned at the wavelength 210 nm.*

Effects on body weight, food intake, blood biochemistry, internal organ weight and body fat accumulation

In comparison to DW control groups, K, the excipient of the KPD, did not affect any parameters studied. K-KPD caused decreased animal body weight with no change in food intake (Figure 2). Fat accumulation at the epididymis, mesentery, retroperitoneum and subcutaneous site (Table 1), as well as the lipid accumulation in the liver cells, were found to be decreased (Figure 3). It also caused a decrease in fasting plasma levels of glucose, triglycerides and alkaline phosphatase, and platelet count (Table 2-4). None of the internal organ weights was found to be affected after treatment with K-KPD or K (Table 5).

Treatments	Body weight (g)		n	Absolute adipose tissue weight (g)				
	Initial	Final		Epididymis	Prostate	Mesentery	Retroperitoneum	Subcutaneous
DW	621.5 ± 14.2	667.1 ± 10.7	6	17.40 ± 1.39	1.06 ± 0.20	17.75 ± 0.78	26.06 ± 2.64	53.87 ± 5.21
K	621.4 ± 15.8	659.5 ± 14.0	7	18.06 ± 1.33	1.10 ± 0.10	18.50 ±1.71	24.07 ± 3.53	53.39 ± 6.73
K -KPD	619.9 ± 10.7	600.5 ± 13.2*	6	13.79 ± 1.34*	1.03 ± 0.14	14.35 ± 1.11*	18.54 ± 2.18*	30.40 ± 5.01*

***Table 1:** Effect of K-KPD, K or DW consumption by middle-aged male rats on adipose tissue accumulation at the internal organs and at the subcutaneous site. * Significantly lower than the K and DW control group, P < 0.05.*

NLAC- MU normal range	n	Glucose (mg %) 122.1 – 180.8	Triglyceride (mg %) 61.0 – 164.0	Cholesterol (mg %) 46.0 – 98.0	HDL-C -	LDL-C -	LDL/HDL ratio
DW	6	135.0 ± 5.7	78.2 ± 7.9	110.8 ± 4.8	105.3 ± 7.9	35.1 ± 7.6	0.33 ± 0.03
K	7	127.7 ± 6.3	68.3 ± 13.8	126.2 ± 5.7	115.0 ± 15.3	33.0 ± 2.7	0.30 ± 0.03
K -KPD	6	106.3 ± 3.2*	51.1 ± 8.4*	99.7 ± 7.6	115.5 ± 12.0	37.1 ± 6.3	0.31 ± 0.03

***Table 2:** Effect of K-KPD, K or DW consumption by middle-aged male rats on fasting plasma glucose and lipid profile. *Significantly lower than the K and DW control group, P < 0.05.*

NLAC- MU normal range	n	ALP (U/L) 46.0 - 92.0	SGOT (U/L) 111.0 - 225.0	SGPT (U/L) 25.0 - 64.0	BUN (mg %) 10.3 - 23.6	CREAT (mg %) 0.5 - 0.7
DW	6	57.9 ± 8.5	123.9 ± 13.3	91.9 ± 14.2	23.1 ± 2.2	0.6 ± 0.09
K	7	56.9 ± 3.2	109.3 ± 17.2	89.3 ± 11.4	22.6 ± 3.0	0.6 ± 0.04
K -KPD	6	32.4 ± 3.4*	118.0 ± 15.7	92.0 ± 13.6	21.4 ± 3.9	0.6 ± 0.04

Table 3: *Effect of K-KPD, K or DW consumption by the middle-aged male rats on the plasma levels of alkaline phosphatase (ALP), Blood urea nitrogen (BUN) and Creatinine (CREAT). * Significantly lower than the K and DW control group, P < 0.05.*

NLAC- MU Normal range	n	HCT (%) 33.2 - 46.0	HGB (g/dl) 13.5 - 17.6	MCV (fl) 47.5 - 54.7	MCH (pg) 17.4 - 26.5	MCHC (%) 34.7 - 51.8	WBC ($x10^3$/µl) 3.0 - 7.2	Neutrophil (%) -	LYMPH (%) 59.0 - 91.0	Plt ($x10^5$/µl) 4.9 - 11.3
DW	6	45.6 ± 2.4	16.1 ± 0.5	52.6 ± 0.7	17.6 ± 0.2	33.6 ± 0.5	5.0 ± 0.6	69.1 ± 4.3	32.7 ± 4.0	8.5 ± 0.3
K	7	46.9 ± 2.7	16.1 ± 0.7	55.1 ± 1.1	19.0 ± 0.5	34.5 ± 1.0	4.2 ± 0.4	69.9 ± 2.6	28.9 ± 2.5	7.5 ± 0.5
K -KPD	6	47.0 ± 2.9	16.6 ± 0.9	55.0 ± 0.9	19.3 ± 0.3	34.5 ± 0.6	4.4 ± 0.3	62.5 ± 3.5	34.5 ± 2.9	6.9 ± 0.3*

Table 4: *Effect of K-KPD, K or DW consumption by the middle-aged male rats on complete blood count * Significantly lower than the DW control group, P < 0.05.*

Treatments	Absolute organs weight (g)								
	Heart	Lung	Liver	Kidney	Adrenal gland (mg)	Spleen	Testis	Epididymis	Prostate gl.
DW	1.70 ± 0.04	1.77 ± 0.04	16.84 ± 0.67	3.10 ± 0.24	91.22 ± 3.46	1.18 ± 0.08	4.06 ± 0.10	1.20 ± 0.05	1.66 ± 0.03
K	1.60 ± 0.09	1.72 ± 0.10	15.53 ± 0.78	3.22 ± 0.11	81.71 ± 5.85	1.17 ± 0.08	4.00 ± 0.15	1.33 ± 0.09	1.51 ± 0.09
K -KPD	1.60 ± 0.05	1.76 ± 0.04	16.21 ± 0.74	3.19 ± 0.22	92.33 ± 3.81	1.16 ± 0.05	3.95 ± 0.16	1.23 ± 0.16	1.55 ± 0.10

Table 5: *Effect of K-KPD, K or DW consumption by the middle-aged male rats on absolute internal organ weight.*

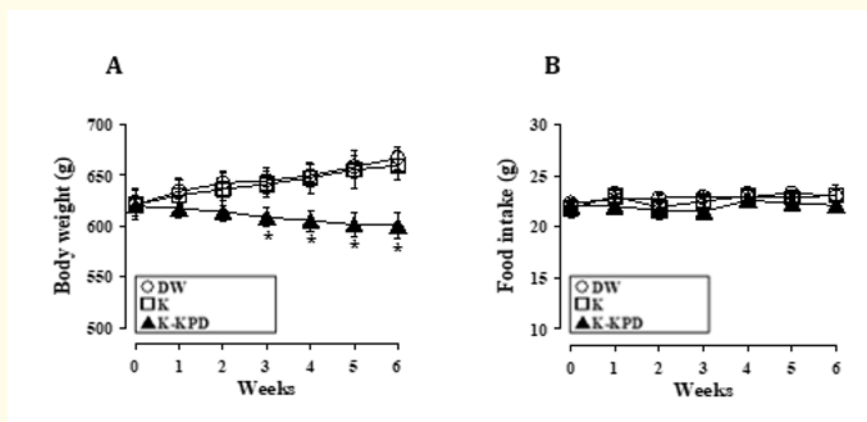

Figure 2: *Effects of K-KPD, K or DW consumption by middle-aged male rats on body weight (a) and food intake (b). Each point represents mean ± SEM of 6 rats. * Significantly lower than the K and DW control group, P < 0.05.*

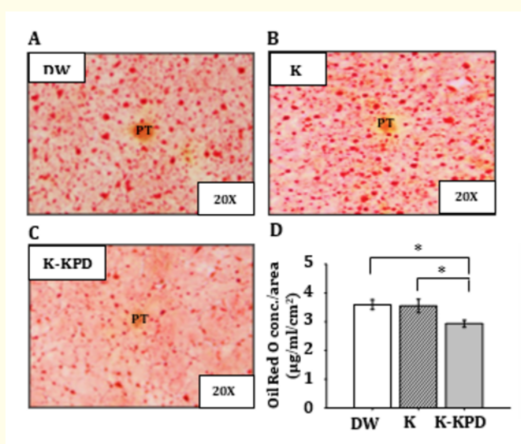

Figure 3: *Effect of K-KPD, K or DW consumption by middle-aged male rats on liver cell lipid accumulation. (A) Distilled water (DW), (B) Kollicoat (K), (C) K-KPD and (D) oil red O concentration. Values represent mean ± SEM of 6 experiments. * Significantly lower than that of the K and DW control group, P < 0.05. (PT = Portal triad; oil red O staining of liver tissue frozen section, 20 mm thick, 20X magnification).*

Effects of on blood pressure

K-KPD or K treatment did not affect the basal arterial blood pressure or heart rate of the anesthetized middle-aged rats when compared to that of the DW control group (Table 6).

Treatments	n	Basal systolic BP (mmHg)	Basal diastolic BP (mmHg)	Mean Arterial Pressure (mmHg)	Basal heart rate (bpm)
DW	6	152.6 ±7.3	117.3 ± 6.0	127.2 ± 2.1	433.3 ±8.9
K	7	142.0 ± 6.5	115.7 ± 4.4	124.1 ± 5.0	427.9 ± 5.1
K -KPD	6	141.2 ± 6.6	116.7 ± 5.7	124.8 ± 6.0	429.0 ± 7.6

Table 6: *Effect of K-KPD, K or DW consumption by middle-aged male rats on blood pressure and heart rate in anesthetized middle-aged male rats.*

Effects of the K-KPD, K or DW treatment on vascular functions

Effect on contraction and relaxation of the thoracic aorta

In comparison to the DW control group, K did not modify the aortic ring function, whereas K-KPD lowered maximal contractile response of the endothelium intact thoracic aortic ring to phenylephrine, and this effect persisted in the presence of N^G-nitro-L-arginine (L-NA), but disappeared when DL-propargylglycine (PAG) was also added (Figure 4 A-C). These effects were not found when the vascular endothelium had been removed (Figure 4 D-F). K-KPD also potentiated vasorelaxation to acetylcholine of the thoracic aortic ring precontracted with phenylephrine, and this effect was abolished in the presence of PAG (Figure 5). Similarly, K-KPD potentiated vasorelaxation

to glyceryl trinitrate of the thoracic aortic ring in the presence of L-NA which had precontracted with phenylephrine; and this effect was abolished when PAG was also added (Figure 6).

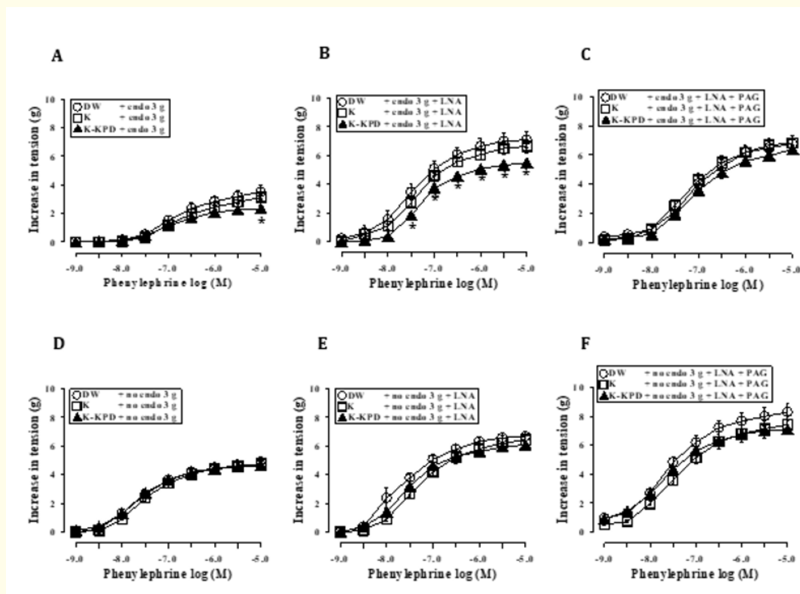

Figure 4: *Effect of K-KPD, K or DW consumption by middle-aged male rats on contractile response to phenylephrine of endothelium-intact (upper panel, endo, A) and endothelium-denuded (lower panel, no endo, D), with L-NA (B, E), and in the presence of L-NA and PAG thoracic aorta (C, F). Values represent mean ± SEM; n = 6. * Significantly lower than the K and DW control group, P < 0.05.*

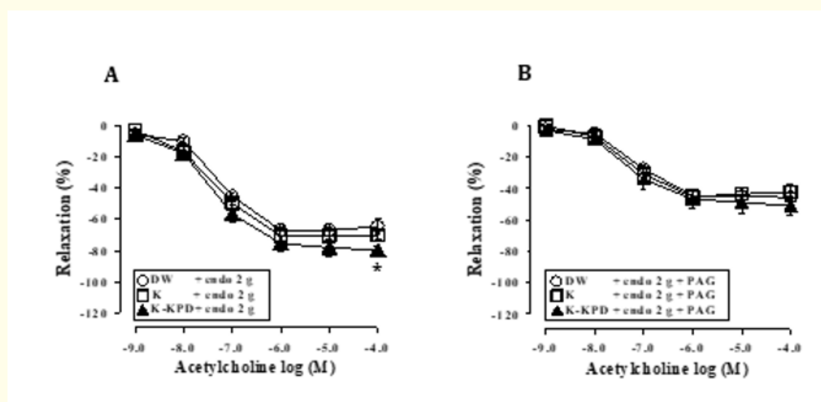

Figure 5: *Effects of K-KPD, K or DW consumption by middle-aged male rats on relaxation of the endothelium-intact thoracic aortic ring precontracted with phenylephrine to acetylcholine (A) and in the presence of PAG (B). Values represent mean ± SEM; n = 6. * Significantly lower than the DW control group, P < 0.05.*

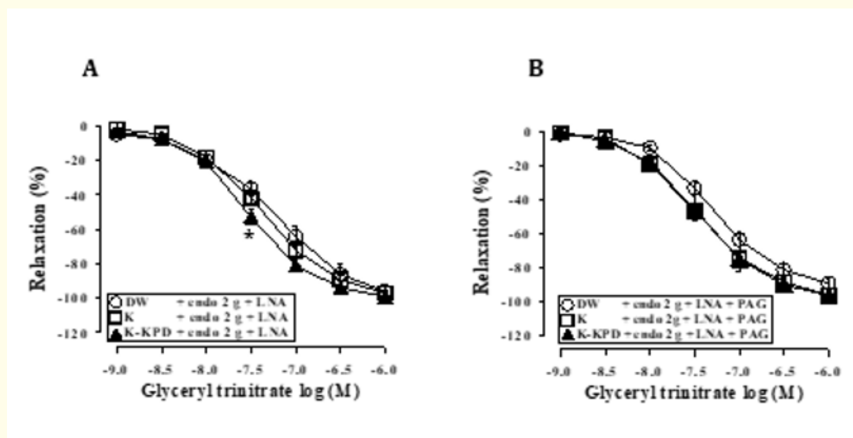

Figure 6: *Effects of K-KPD, K or DW consumption by middle-aged male rats on relaxation of the endothelium-intact thoracic aortic ring precontracted with phenylephrine to glyceryl trinitrate in the presence of L-NA (A) and in the presence of L-NA and PAG (B). Values represent mean ± SEM; n = 6. * Significantly lower than the DW control group, P < 0.05.*

eNOS and CSE western blot analysis

Western blot results were compared among DW, K- and K-KPD-treated groups. For each group, the intensity of the protein concerned was divided by the intensity of actin for all of four independent tissue samples. The results showed significantly increased CSE, but not e-NOS protein expression between the K-KPD-treated and DW or K-treated groups (Figure 7).

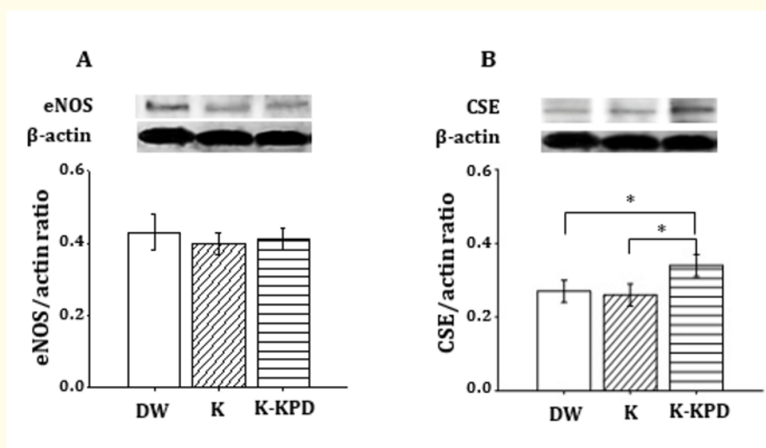

Figure 7: *Effects of K-KPD, K or DW consumption by middle-aged male rats on eNOS protein expression (A) or CSE protein expression (B) of the thoracic aorta. For each blot, β-actin expression is shown as a loading control. Values represent mean ± SEM; n = 4. * Significantly higher than the K and DW control group, P < 0.05.*

Discussion

Kollicoat IR® is a poly(vinyl alcohol)-poly(ethylene glycol). Kollicoat IR® (PVA-co-PEG), is a pharmaceutical excipient developed as a coating polymer for instant release dosage form [28-30]. Weerapol., *et al.* [27] reported that Kollicoat IR® used as a recipient for making solid dispersion of KPD, a very poor water soluble extract, was successful regarding both its solubility and the *in vivo* pharmacokinetis in adult male rats. However, K itself might affect the biological activities of the KPD. Therefore the present study aimed to investigate these, as well as its toxicity in the middle-aged male rats after 6 weeks of oral gavage of the K-KPD in comparison to K and in DW control. In the present study dosage of the K-KPD was used at 200 mg/kg which would be equal to 100 mg/kg of the KPD as used in our previous reported by Yorsin., *et al.* [26]. The reason for this is that the K-KPD was prepared by mixing the KPD and the K in the ratio of 1: 1.

The present study demonstrates that K-KDP consumption caused decreased body weight, body and visceral fat and liver cell lipid accumulation, as well as decreased plasma glucose and triglyceride levels, which are similar to our previous report on KPD consumption by the middle-aged male rats [26], whereas K alone showed no changes in any of these parameters. These results indicated that K, an excipient of the solid dispersion of KPD, did not disturb the beneficial activities of the KPD. In addition, the basal blood pressure and heart rate of the middle-aged rats treated with K-KPD or K alone were not different from those of the DW control group. It was also found that K-KPD, not K, caused decreased contractile response of the thoracic aortic with increased relaxation to acetylcholine and glyceryl trinitrate. These effects are also similar to those of KPD reported by Yorsin., *et al.* [26]. However, the mechanism responsible for the effect was different. In the present study, the lowering of the aortic ring contraction to phenylephrine was abolished by PAG; a cystathionine-γ- lyase inhibitor; but not by an L-NA, a nitric oxide synthase inhibitor; or by removal of the vascular endothelium. This suggests that H_2S, but not NO was responsible for the effect. Analogously, the higher dilatation of the aortic rings to acetylcholine or to glyceryl trinitrate of the K-KPD treated rats was also abolished by PAG, suggesting that these effects might be modified by up-regulation of the H_2S. To this end, results from the Western blot analysis could be confirmed by the finding that blood vessel CSE protein expression obtained from the K-KPD-, but not from the K treated rats, was significantly higher than in the DW control rats.

The major components of the KPD are PMF, DMF and TMF [26]. All of these substances have been reported to have vasodilatation effects on the isolated thoracic aortic rings in the *in vitro* experiment in organ baths by partly stimulated released of NO [24, 31, 32]. In case of PMF, Yorsin., *et al.* [31] found that PMF also stimulated release of H_2S in addition to NO to modulate the blood vessel functions. The effect persisted in the *in vivo* experiments after consumption of the PMF for 6 weeks [33]. Therefore, the active component that was responsible for the K-KPD above mentioned effect would be the three major substances of the KPD. However, from the pharmacokinetic studies of the KPD and K-KPD using PMF, DMF and TMF as the markers found some differences. Yorsin., *et al.* [26] studied the PKD dissolved in a mixture of tween 80: carboxymethylcellulose: distilled water = 0.2 g: 0.2 g: 10 mL, and found all three substances reached their peaks at 60 min with the same ranges in the plasma levels. In contrast, Weerapol., *et al.* [27] studied K-KPD which was dissolved in distilled water, and found that the three substances reached their peaks at 90 min with the plasma levels about two-fold higher for the DMF and TMF, but not the PMF, than that of the KPD. Thus, it is possible DMF and/or TMF might be the active components of the K-KPD that are responsible for the up-regulation of the blood vessel CSE protein expression and resulting increased H_2S production to modify the blood vessel functions. However, further study by isolation of the DMF and TMF and investigating of their activities both *in vitro* and *in vivo* are needed to confirm the aforementioned possibilities.

Conclusion

Taken together, K-KPD consumption caused decreased body fat and liver cell lipid accumulation, lowered plasma levels of glucose and triglycerides and up-regulation of blood vessel CSE protein expression resulting increased H_2S production to modulate vascular functions in middle-aged male rats. It also decreased numbers of blood platelets with no changes in internal organ weight, blood cells count or the

marker enzymes of liver and kidney functions. Thus, the K-KPD, a solid dispersion KPD product, is a novel cardiovascular and/or metabolic health product, and worth further development in the form of a tablet or capsule for toxicity test and human clinical trial.

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Conflict of Interest

All authors declare no any conflict of interest exists.

Bibliography

1. National Institute on Aging. NIA. Global Health and Aging (2019).
2. United Nation. UN. World Population Ageing (2020).
3. Huffman D M and N Barzilai. "Role of Visceral Adipose Tissue in Aging". *Biochimica et Biophysica Acta* 1790.10 (2009): 1117-1123.
4. Kotani K., *et al*. "Sexual Dimorphism of Age-Related Changes in Whole-Body Fat Distribution in the Obese". *International Association for the Study of Obesity* 18.4 (1994): 207-212.
5. Ponti F., *et al*. "Aging and Imaging Assessment of Body Composition: From Fat to Facts". *Frontiers in Endocrinology* (*Lausanne)* 10 (2019): 861.
6. Tchkonia T., *et al*. "Fat Tissue, Aging, and Cellular Senescence". *Aging Cell* 9.5 (2010): 667-684.
7. Zamboni M., *et al*. "Adipose Tissue, Diet and Aging". *Mechanisms of Ageing and Development* 136-137 (2014): 129-137.
8. Arcaro G., *et al*. "Body Fat Distribution Predicts the Degree of Endothelial Dysfunction in Uncomplicated Obesity". *International Association for the Study of Obesity* 23.9 (1999): 936-942.
9. Chait A and LJ den Hartigh. "Adipose Tissue Distribution, Inflammation and Its Metabolic Consequences, Including Diabetes and Cardiovascular Disease". *Frontiers in Cardiovascular Medicine* 7 (2020): 22.
10. Pazos F. "Range of Adiposity and Cardiorenal Syndrome". *World Journal of Diabetes* 11.8 (2020): 322-350.
11. Novella S., *et al*. "Aging-Related Endothelial Dysfunction in the Aorta from Female Senescence-Accelerated Mice Is Associated with Decreased Nitric Oxide Synthase Expression". *Experimental Gerontology* 48.11 (2013): 1329-1337.
12. Bhayadia R., *et al*. "Senescence-Induced Oxidative Stress Causes Endothelial Dysfunction". *The journals of gerontology Series A Biological Sciences and Medical Sciences* 71.2 (2016): 161-169.
13. Marchesi S., *et al*. "Short-Term Atorvastatin Treatment Improves Endothelial Function in Hypercholesterolemic Women". *Journal of Cardiovascular Pharmacology* 36.5 (2000): 617-621.

14. Rudolph TK., *et al*. "Acute effects of various fast-food meals on vascular function and cardiovascular disease risk markers: the Hamburg Burger Trial". *The American Journal of Clinical Nutrition* 86 (2007): 334-340.

15. Mekjaruskul CM Jay and B Sripanidkulchai. "Pharmacokinetics, Bioavailability, Tissue Distribution, Excretion, and Metabolite Identification of Methoxyflavones in *Kaempferia Parviflora* Extract in Rats". *Drug Metabolism and Disposition* 40.12 (2012): 2342-2353.

16. Chaturapanich G., *et al*. "Enhancement of Aphrodisiac Activity in Male Rats by Ethanol Extract of *Kaempferia Parviflora* and Exercise Training". *Andrologia* 44.1 (2012): 323-328.

17. Jansakul C., *et al*. "Relaxant Mechanisms of 3, 5, 7, 3', 4'-Pentamethoxyflavone on Isolated Human Cavernosum". *European Journal of Pharmacology* 691.1-3 (2012): 235-244.

18. Sudwan P., *et al*. "Effect of *Kaempferia Parviflora* Wall. Ex. Baker on Sexual Activity of Male Rats and Its Toxicity". *The Southeast Asian Journal of Tropical Medicine and Public Health* 37.3 (2006): 210-215.

19. Wattanathorn J., *et al*. "Aphrodisiac Activity of *Kaempferia parviflora*". *American Journal of Agricultural and Biological Sciences* 7.2 (2012): 114-120.

20. Jin S and Mi-Young L. "*Kaempferia parviflora* Extract as a Potential Anti-Acne Agent with Anti-Inflammator, Sebostatic and Anti-Propionibacterium acnes Activity". *International Journal of Molecular Sciences* 19.11 (2018): 3457.

21. Tewtrakul S., *et al*. "Anti-inflammatory effects of compounds from *Kaempferia parviflora* and *Boesenbergia pandurata*". *Food Chemistry* 115.2 (2009): 534-538.

22. Saokaew S., *et al*. "Clinical Effects of Krachaidum (*Kaempferia Parviflora*): A Systematic Review". *Evidence-Based Complementary and Alternative Medicine* 22.3 (2017): 413-428.

23. Malakul W., *et al*. "The Ethanolic Extract of *Kaempferia Parviflora* Reduces Ischaemic Injury in Rat Isolated Hearts". *Journal of Ethnopharmacology* 137.1 (2011): 184-191.

24. Tep-Areenan PP Sawasdee and M Randall. "Possible Mechanisms of Vasorelaxation for 5,7-Dimethoxyflavone from *Kaempferia Parviflora* in the Rat Aorta". *Phytotherapy Research* 24.10 (2010): 1520-1525.

25. Akase T., *et al*. "Antiobesity Effects of *Kaempferia Parviflora* in Spontaneously Obese Type Ii Diabetic Mice". *Journal of Natural Medicines* 65.1 (2011): 73-80.

26. Yorsin S., *et al*. "Effects of *Kaempferia Parviflora* Rhizomes Dichloromethane Extract on Vascular Functions in Middle-Aged Male Rat". *Journal of Ethnopharmacology* 156 (2014): 162-174.

27. Weerapol Y., *et al*. "Retracted article: Enhanced dissolution and oral bioavailability of poorly water-soluble herb (*Kaempferia parviflora*) extract using solid dispersions: effect of surfactants and concentrations". *International Journal of Pharmaceutical Investigation* 243 (2020): 51.

28. Fouad E. A., *et al*. "Technology Evaluation: Kollicoat IR". *Expert Opinion on Drug Delivery* 8.5 (2011): 693-703.

29. Fussnegger B., *et al*. "Kollicoat® IR: Minimizing the Risks for Oxidative Degradation of Drugs". *Journal of Analytical and Pharmaceutical Research* 2.3 (2016): 20.

30. Janssens S., *et al*. "The Use of a New Hydrophilic Polymer, Kollicoat Ir, in the Formulation of Solid Dispersions of Itraconazole". *European Journal of Pharmaceutical Sciences* 30.3-4 (2007): 288-294.

31. Yorsin S., *et al*. "Vasorelaxant effects of 3,5,7,3',4'-pentamethoxyflavone isolated from *Kaempferia parviflora*: partly stimulating the release of NO and H_2S by rat thoracic aorta". *Journal of Physiological and Biomedical Sciences* 28.1 (2015): 5-14.

32. Tep-Areenan P and P Sawasdee. "Vasorelaxant Effects of 5,7,4'-Trimethoxyflavone from *Kaepmferia parviflora* in the Rat Aorta". *International Journal of Pharmacology* 6.4 (2010): 419-424.

33. Yorsin S., *et al*. "Increased Vascular Enos and Cystathionine-Gamma-Lyase Protein after 6 Weeks Oral Administration of 3, 5, 7, 3', 4'-Pentamethoxyflavone to Middle-Aged Male Rats". *Naunyn-Schmiedeberg's Archives of Pharmacology* 389.11 (2016): 1183-1194.

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