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ARTICLE

Bioavailability and Antioxidant Effects of a Xanthone-Rich Mangosteen (*Garcinia mangostana*) Product in Humans

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Oxidative damage is involved in many chronic diseases including those cited as the major causes of death in Western societies such as cardiovascular disorders and cancer. Antioxidants may prevent these degenerative processes by various mechanisms including the scavenging of free radicals. Intake of antioxidant supplements is associated with preventing oxidative damages. This study investigated the absorption and antioxidant effects of a xanthone-rich mangosteen liquid in healthy human volunteers after the acute consumption of 59 mL of the supplement. The liquid contained mangosteen, aloe vera, green tea, and multivitamins. Results indicated that α-mangostin and vitamins B_2 and B_5 were bioavailable, with observed C_{max} at t_{max} of around 1 h. The antioxidant capacity measured with the oxygen radical absorbance capacity (ORAC) assay was increased with a maximum effect of 18% after 2 h, and the increased antioxidant level lasted at least 4 h. Overall, this study demonstrated the bioavailability of antioxidants from a xanthone-rich mangosteen product and its in vivo antioxidant effects.

KEYWORDS: Aloe vera; antioxidant; bioavailability; green tea; mangosteen; α-mangostin; multivitamin; ORAC

INTRODUCTION

Oxidative damage is involved in many chronic diseases including some of the prominent causes of death in Western societies such as cardiovascular disorders and cancer (*1*−*5*). Antioxidants may prevent these degenerative processes by various mechanisms including scavenging of free radicals. There are numerous reports about the reduction of the incidence of degenerative diseases due to the consumption of fruits and vegetables (*6*−*9*). These positive bioactivities are considered mainly to be due to the presence of various antioxidants in fruits and vegetables. Most of the antioxidant activity in foods is thought to be due to vitamins C and E, polyphenols, and carotenoids (*10*, *11*).

Mangosteen (*Garcinia mangostana*) is a tropical evergreen tree originated in Southeast Asia and used for centuries as a folk medicine. The rind has been used for internal and external infections, and poultices can be used to treat skin conditions; an extract of mangosteen pulp has even been used to control fever (*12*). Recently, the consumption of mangosteen products has increased as a dietary supplement in the United States, because of their potent antioxidant properties (*13*, *14*). Xanthones, a particular class of plant phytochemicals from mangosteen, are highly biologically active, possess anti-inflammatory properties such as COX inhibition, and have cardiovascular protective effects (*15*−*18*).

Mangosteen Plus with Essential Minerals, a commercial product, contains a wide variety of natural antioxidant sources including mangosteen, green tea (*Camellia sinensis*), aloe vera, and multivitamins. Some ingredients in this product are believed to provide preventive effects against diseases associated with aging. However, there have been no human bioavailability studies using commercial mangosteen juice to our knowledge. Due to the health benefits now attributed to mangosteen consumption and the rising popularity of mangosteen botanical supplements, human intervention studies using mangosteen extract supplements are crucial for determining the efficacy of mangosteen extracts in the prevention of chronic diseases and establishing science-based dosing recommendations. In this study, we determined the bioavailability of the free form of α-mangostin and vitamins B_2 and B_5 found in a xanthone-rich mangosteen product and its efficacy on plasma antioxidant status in the human body.

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MATERIALS AND METHODS

Subjects and Study Protocol. A randomized, double-blind, placebo-controlled clinical trial was conducted with generally healthy male and female subjects between 20 and 23 years of age. All subjects were screened by evaluation of a medical history and assessment of diet history and supplement history using a self-developed semiquantitative questionnaire. It was required that subjects had not supplemented their diet with vitamins, minerals, or antioxidants for at least 6 months prior to the study. Written informed consent was obtained from each volunteer participating in this study. All procedures of the protocol were approved by the Institutional Review Board for the Protection of Human Subjects of the Capital Medical University (Beijing, China). Ten men and 10 women participated in this study. Participants were randomly divided into two groups, study or placebo group, with the same number of male and female participants in each group. The average age of both study and placebo groups was 22 years old. The trial duration was 24 h. On the morning before the study started, a blood sample was drawn after a fast of at least 8 h. Each participant was then given a single dose (59 mL) of Mangosteen Plus with Essential Minerals, the Vemma formula, or a matching 59 mL

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Table 1. Energy, Nutrient, Mineral, and Specific Component Intake with the Mangosteen Product (59 mL) and the Placebo Juice (59 mL)[a]

content per dose	mangosteen product	placebo
energy, kcal	35	35
total carbohydrate, g	8	8
vitamin A (100% as β-carotene), IU	5000	BLD[b]
vitamin C (as ascorbic acid), mg	300	BLD
vitamin D_3 (as cholecalciferol), IU	1000	BLD
vitamin E (as *d*-α-tocopheryl acetate), IU	60	BLD
thiamin (as thiamin hydrochloride), mg	1.5	BLD
riboflavin (as riboflavin USP), mg	1.7	BLD
niacin (as niacinamide), mg	20	BLD
vitamin B_6 (as pyridoxine hydrochloride), mg	5	BLD
folate (as folic acid), μg	800	BLD
vitamin B_{12} (as cyanocobalamin), μg	15	BLD
biotin (as *d*-biotin), μg	300	BLD
pantothenic acid (as calcium *d*-pantothenate), mg	10	BLD
selenium (as amino acid chelate), μg	140	BLD
mangosteen, whole leaf aloe vera and green tea blend, g	25.2	BLD
xanthones		
mangostins, mg	94.2	BLD
catechins	119	BLD
epigallocatechin gallate, mg	3.6	BLD
plant-sourced mineral blend,[c] mg	956	BLD

[a] Energy and nutrient composition are data provided by the manufacturers. [b] Below the limit of detection. [c] Includes a full spectrum of minerals.

dose of fructose liquid before breakfast on the trial morning. **Table 1** lists the ingredients of each liquid. Blood samples were then collected from each subject at 1, 2, 4, and 6 h after administration of the Vemma formula or the placebo liquid. Plasma was obtained by centrifugation. One milliliter of each plasma sample was freeze-dried and stored at −80 °C until analysis.

Material. α-Mangostin was purchased from Chromadex (Irvine, CA), and vitamin B_2 was from Acros (Geel, Belgium). Vitamin B_5 was obtained from Sigma-Aldrich (St. Louis, MO). 2,2′-Azobis(2-amidinopropane) dihydrochloride was purchased from Wako Chemicals USA (Richmond, VA). Mangosteen Plus with Essential Minerals and placebo (fructose liquid) were provided by Vemma Nutrition Co. (Scottsdale, AZ).

LC-MS/MS Analysis of Plasma Samples. Freeze-dried samples were reconstituted to 1 mL with deionized water. LC-MS/MS analyses were performed using a system consisting of a model SIL-HTC Shimadzu autosampler (Shimadzu Scientific Instruments, Inc., Columbia, MD) and an API-4000 Qtrap mass spectrometer with a turbo-ion spray source (Applied Biosystem, Foster City, CA). Sample preparations and conditions for LC-MS analyses were as give below. The chemical structures of tested analytes are illustrated in **Figure 1**.

Standard Solutions and Calibration Curves. Each standard weighed accurately was dissolved into the appropriate volume of methanol to give 1 mg/mL of stock solution. Working standard solutions were diluted to a series of concentrations with methanol. Standards, at the concentration ranges shown in **Table 2** with seven to eight concentration levels, were injected to obtain calibration curves. Peak areas from the LC chromatogram were plotted against the known corrected concentrations of standard solutions to establish calibration equations. A weighed linear regression analysis (weighing factor $1/x2$) was used to calculate the concentration of analytes. For recovery studies, five sets of spiked plasma samples were prepared to give the concentrations listed in **Table 3**. Presented values were adjusted by subtracting the base concentration of 3.31 ng/mL vitamin B_2 and 13.8 ng/mL vitamin B_5 in blank plasma.

α-Mangostin. One hundred microliters of reconstituted human plasma was extracted using 300 μL of methanol. The supernatant was collected after centrifugation at 14000 rpm for 10 min (4 °C). Ten microliters was injected onto the LC-MS/MS for mangostin analysis. Chromatography was carried out on a 2.1 × 50 mm, 3 μm, MacMod HyroBond PS-C18 column (MAC-MOD Analytical, Inc., Chadds Ford, PA), and the solvent system consisted of a gradient system with water (0.4% formic acid, v/v) (A) and acetonitrile (B). Gradient elution was performed at 0.6 mL/min

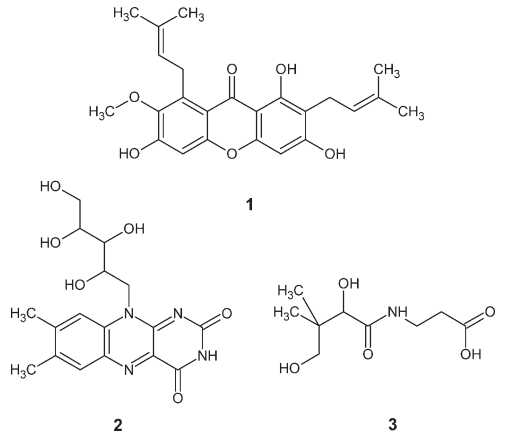

Figure 1. Structures of tested analytes: **1**, α-mangostin; **2**, vitamin B_2; **3**, vitamin B_5.

Table 2. Concentration Range and Linearity of Calibration Curve

		regression parameters		
analyte	concentration (ng/mL)	slope	intercept (ng/mL)	*r*
α-mangostin	0.4−100	0.000273	0.00	0.996
vitamin B_2	1−200	179	−33.8	0.997
vitamin B_5	8−200	186	−3.38	0.998

with the following conditions: 1 min hold at 100% A, 0.5 min linear gradient from 100 to 60% A, 4.5 min linear gradient from 60 to 2% A, 1.1 min hold at 2% A, 0.1 min linear gradient from 2 to 100% A, and 2.9 min hold at 100% A. The mass detector was equipped with a turbo-ion spray (electrospray ionization, ESI) source and operated in multiple reaction monitoring mode (MRM) under negative ion mode. The heated capillary and voltage were maintained at 550 °C and −4500 V, respectively. The optimized instrument setting is listed in **Table 4**. Representative chromatograms of MRM scans are shown in **Figure 2**. The lower limit of quantification (LLOQ) for α-mangostin was 0.4 ng/mL.

Vitamins B_2 and B_5. Two hundred microliters of reconstituted human plasma was mixed with 280 μL of methanol and 20 μL of trifluoroacetic acid. The supernatant was collected after vortexing and centrifugation at 14000 rpm for 10 min (4 °C). Five microliters was injected onto the LC-MS/MS for vitamin B analysis. Chromatography was carried out on a 50 × 4.6 mm, 3 μm, Ascentis RP-AMIDE column (Sigma-Aldrich), and the solvent system consisted of a gradient system with water (0.4% formic acid, v/v) (A) and acetonitrile (B). Gradient elution was performed at 0.6 mL/min with the following conditions: 0.9 min hold at 100% A, 0.1 min linear gradient from 100 to 90% A, 3.0 min linear gradient from 90 to 40% A, 1 min linear gradient from 40 to 2% A, 2.0 min hold at 2% A, 0.1 min linear gradient from 2 to 100% A, and 3.0 min hold at 100% A. The mass detector was equipped with a turbo-ion spray (ESI) source and operated in MRM under positive ion mode. The heated capillary and voltage were maintained at 550 °C and 5500 V, respectively. The optimized instrument settings for vitamins B_2 and B_5 analysis are listed in **Table 4**. Representative chromatograms of MRM scans are shown in **Figure 2**. The LLOQ for α-mangostin was 0.4 ng/mL. The LLOQs for vitamins B_2 and B_5 were 1 and 8 ng/mL, respectively.

Antioxidant Capacity (ORAC Assay). Plasma antioxidant capacity was determined by ORAC assay as previously described (*19*, *20*). Peroxyl radicals were generated by AAPH, and Trolox was used as a control standard. Fluorescence was monitored at 485 nm excitation and 528 nm emission on a Bio-Tek Synergy KC4 fluorescence plate reader (Bio-Tek Instruments, Winooski, VT).

Statistical Analysis. Means ± SEMs were used to summarize the characteristics of the subjects. The measured values were used for the maximum plasma concentration, C_{max}, and the time to reach the maximum plasma concentration, t_{max}. A paired *t* test was performed to determine the significance of the test using Microsoft Excel software (Office 2003, Microsoft Corp., Redmond, WA).

Table 3. Recovery of Tested Analytes in Human Plasma

analyte	spiked concentration (ng/mL)	mean measured, ng/mL (n = 5)	CV (%)	recovery (%)
α-mangostin	0.4	0.37	11.9	92.2
	2	2.05	8.67	102
	10	9.78	3.55	97.8
	50	44.1	3.58	88.2
	100	106	2.49	106
vitamin B_2	1	0.89	9.22	88.8
	8	7.13	4.94	89.2
	100	85.3	2.91	85.3
	200	172	2.68	85.9
vitamin B_5	8	7.66	4.70	95.8
	100	82.8	4.43	82.8
	200	177	2.49	88.7

Table 4. Molecular Weights (MW) and Optimized Instrument Settings

		transitions (m/z)				
analyte	MW	parention	production	DP[a] (V)	CE[b] (V)	CXP[c] (V)
α-mangostin	410.5	409.0	351.1	−90	−35	−10
vitamin B_2	376.4	377.3	243.2	85	35	5
vitamin B_5	219.2	220.4	90.2	50	20	12

[a] Declustering potential. [b] Collision energy. [c] Collision cell exit potential.

RESULTS AND DISCUSSION

LC-MS/MS Analysis of Plasma Samples. One xanthone and two vitamins in human plasmas were measured by LC-MS/MS methods. α-Mangostin, one of the most abundant xanthones in mangosteen, is known as an antioxidant. The plasma level of α-mangostin was analyzed in this study. The bioavailabilities of vitamins B_2 and B_5 were also investigated. Although they are not antioxidants, they are essential nutrients and important for cell metabolism. Therefore, they are interesting as a part of one's health promotion. A complete data set was not available for several subjects due to concentrations being below LLOQ. For that reason the missing values were replaced by 0 to obtain the mean for all subjects. **Figure 3** illustrates the mean plasma concentration−time curves of tested analytes.

A significant increase of α-mangostin was detected in the study group, whereas no α-mangostin was found in the placebo group within the detection range. The α-mangostin plasma concentration reached its maximum (C_{max}), 3.12 ± 1.47 ng/mL, at t_{max} of 1 h after the Vemma formula consumption. After that time point, the concentration of α-mangostin decreased to one-third of C_{max} by the fourth hour, and the level remained through the sixth hour. After mangosteen product consumption, at 2 h, C_{max} values of vitamins B_2 and B_5 were 7.52 ± 2.72 and 48.9 ± 11.7 ng/mL, respectively. Significant increases of vitamins B_2 and B_5 were observed in the treatment group (P = 0.022 and 0.041, respectively), whereas no significant changes were seen in the placebo group (data not shown). As seen in **Figure 3**, the concentrations of vitamins B_2 and B_5 roughly doubled within 1 h and gradually decreased to the level at time 0.

Antioxidant Capacity (ORAC Assay). The human plasma antioxidant capacity increased by >16% at 1 h following consumption of the Vemma formula (**Figure 4**). The ORAC value increased as much as 18% after 2 h and stayed elevated through 6 h, the last blood draw in the trial. In the placebo group, no change was observed (data not shown).

The bioavailability of vitamins B_2 and B_5 is low after the ingestion, and they are not antioxidants themselves. For that reason, they are not expected to contribute to ORAC value. α-Mangostin has antioxidant activity both in vitro (*13*) and in vivo, but the increase in ORAC after the consumption of the xanthone-rich product could not be explained solely by the increase in plasma α-mangostin. The possible reasons are due to the t_{max} and plasma level of α-mangostin. The t_{max} of α-mangostin and that of plasma ORAC value do not match: 1 and 2 h, respectively. Plasma α-mangostin concentration was increased by 3.12 ng/mL, which accounts for only a small percentage of the significant increases in plasma ORAC. Therefore, we can conclude that α-mangostin is not the main contributor to the increase in plasma ORAC.

Even though the increase of antioxidant capacity may be related to α-mangostin, other antioxidants in the Vemma formula are likely to contribute to the increase. Vitamins C and E are well-known antioxidants contained in this xanthone-rich product. However, significant increases of vitamins C and E were not observed (data not shown). The increase of vitamins C and E in plasma would not be sufficient to give such a large impact on plasma ORAC (*21*), and there is no potentiation observed on the antioxidant effects by vitamins C and E (*22*) due to the high base concentrations. Although one serving (59 mL) of the Vemma formula contains 300 mg of vitamin C and 60 IU of vitamin E, more than double the recommended daily values, they would not be main contributors to the increase in ORAC value in this study.

This xanthone-rich product also contains green tea, which is full of catechins. Green tea catechins belong to the flavonoid family and are known to have fairly strong antioxidant activity with ORAC values of 81,000−220,000 μmol of Trolox equiv/g (*23*). As possible contributors to ORAC, the bioavailability of catechins was tested. The assay was carried out as described by Henning et al. (*24*). Because the majority of catechins are present as glucuronides and sulfates, plasma samples were hydrolyzed using β-D-glucuronidase type X-A from *Escherichia coli* and sulgatase type VIII from abalone entrails. Analyzed catechins are, namely, catechin, epicatechin catechin gallate, epicatechin gallate, gallocatechin gallate, and epigallocatechin gallate. However, we did not detect any green tea catechins in the plasma samples (data not shown). This may be due to the low concentration of catechins in one serving. Data presented by Henning et al. showed that the bioavailability of four catechins including epicatechin, epicatechin gallate, epigallocatechin, and epigallocatechin gallate. The intake of the four catechins was approximately 680 mg, and plasma level at t_{max} was 395 ng/mL in total. Whereas the total catechin concentration in Vemma formula was 119 mg, we would expect the lower concentration in plasma. As we expected, none of eight catechins were detected by HPLC-ECD system. Therefore, we can conclude that green tea catechins do not make a major contribution to the observed ORAC increase.

There are other possible contributors to the ORAC increase in this product including flavonoids, some other phenolics, and plant-sourced minerals. Particularly, some flavonoids are known to have several times higher antioxidant activities than those of vitamins C and E (*19*, *25*, *26*), which may contribute greatly to the increasing ORAC. However, this study did not focus on the bioavailability and structural elucidation of flavonoids except green tea catechins. Moreover, this study did not reveal if the ORAC increase was due to some strong antioxidants or synergistic effects of many components. Therefore, a detailed study is required to confirm all possible contributors and the mechanism of the antioxidant capacity increase.

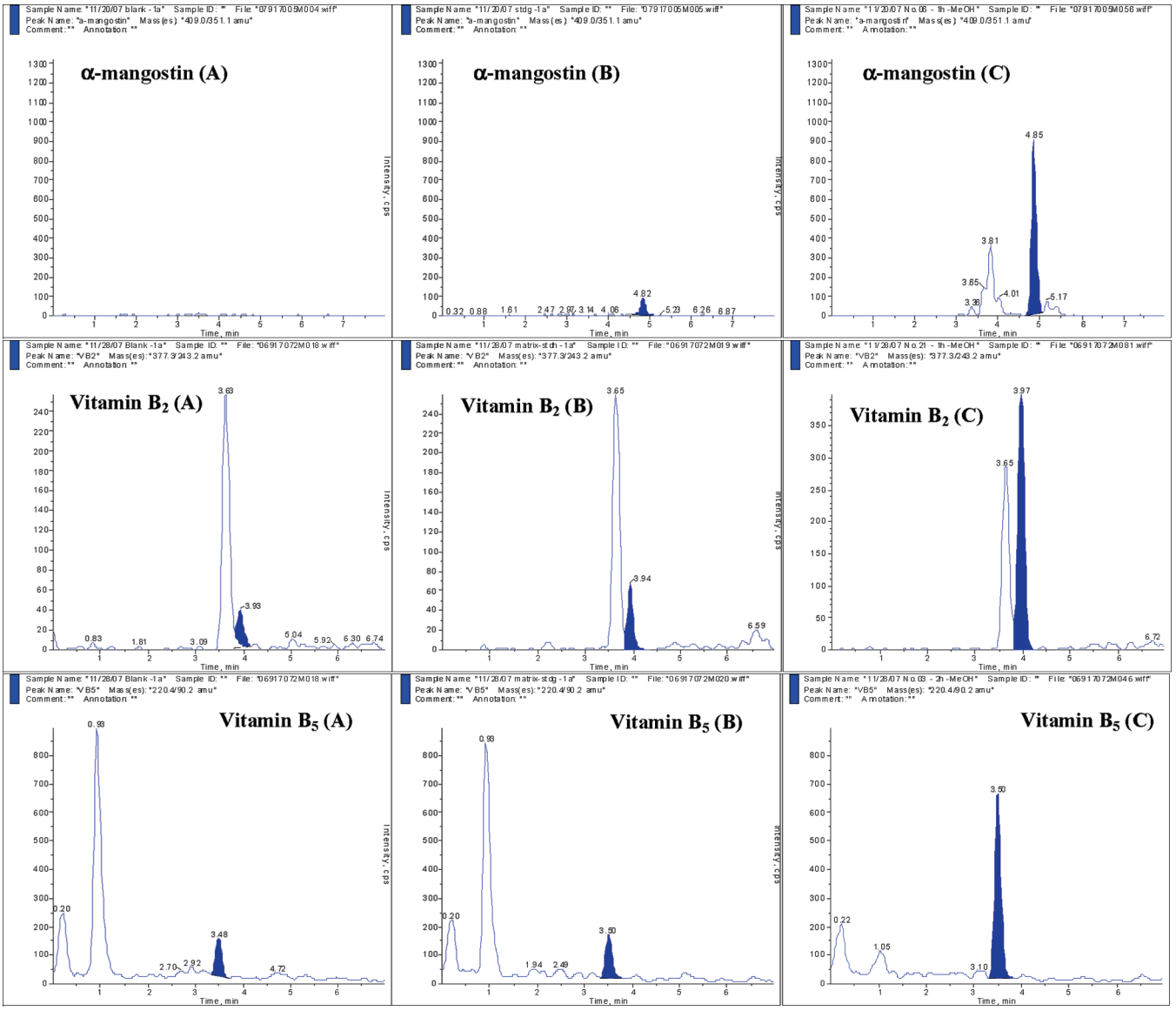

Figure 2. LC-MS/MS MRM chromatograms of blank human plasma (**A**), spiked human plasma at limit of quantitation (**B**), and representative sample (**C**) for each standard compound.

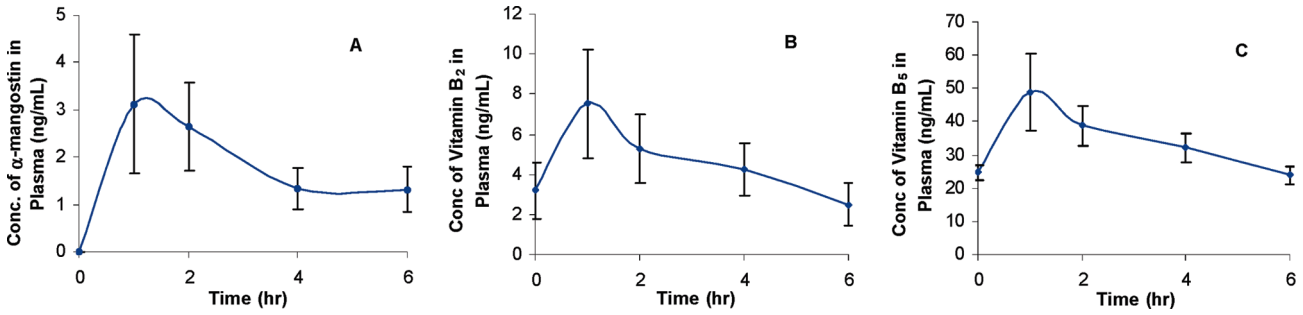

Figure 3. Concentration of each analyte in human plasma versus time curve after a single oral administration of a xanthone-rich mangosteen product (59 mL): (**A**) α-mangostin; (**B**) vitamin B_2; (**C**) vitamin B_5. Values were expressed as means ± SEM.

In summary, we determined the physiological availability of xanthones and vitamins found in a xanthone-rich product and their effects on the degree of antioxidant potency in the human body. Noteworthy bioavailability was seen in α-mangostin and vitamins B_2 and B_5 along with the increase of antioxidant capacity. In the experiment group within 2 h after consumption of the xanthone-rich product, recognizable increases of α-mangostin, vitamins B_2 and B_5, and ORAC (antioxidant levels) were observed in these subjects. In the placebo group, no significant change was observed in vitamins B_2 and B_5, and no α-mangostin was detected. Our results suggest that Mangosteen Plus with Essential Minerals is an excellent source to increase the blood

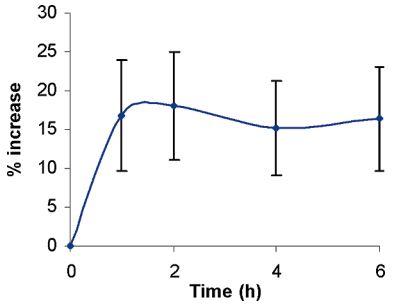

Figure 4. Increase of human plasma antioxidant capacity after a single oral administration of a xanthone-rich mangosteen product (59 mL).

level of antioxidants, thus possibly offering protection against chronic diseases caused by the aging process. However, further studies need to be done to characterize the antioxidant properties of the xanthone-rich product as a dietary supplement.

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