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Original article

Effect of mangosteen pericarp extract on skin moisture and arterial stiffness: Placebo-controlled double-blinded randomized clinical trial

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Abstract

***Purpose*:** Glycative stress further enhances defects of skin and blood vessels caused by aging. This research investigated how the intake of water extract of mangosteen pericarp (WEM) affected skin moisture and arterial stiffness, as anti-glycative effect of WEM had already been identified.

***Method*:** Subjects that participated in this research were forty healthy females from 25 to 59 years of age (20 subjects in WEM group and 20 subjects in the placebo group). After they took 200 mg of WEM or placebo per day for 12 weeks, a blood test, a skin moisture test and a vascular function test were conducted before intake as well as 4, 8 and 12 weeks after intake. To evaluate the efficacy of WEM, a placebo-controlled double-blinded randomized clinical trial was performed.

***Results*:** In WEM group (19 accomplished the trial), levels of blood sugar and HbAlc did not decrease after the intake of WEM, compared with the levels examined before intake. Concerning serum concentrations of pentosidine, no significant difference between WEM and the placebo groups were identified. However, compared with the level obtained before intake, the value of WEM group decreased significantly 8 weeks after intake, while no significant change was confirmed in the placebo group. Regarding moisture content in cheek skin, WEM group significantly increased by taking WEM, compared with the placebo group (19 accomplished the trial). In addition, owing to WEM intake, in terms of API (Arterial Pressure volume Index), an indicator of arterial stiffness, WEM group showed a lower value than the placebo group 8 weeks after intake.

***Conclusion*:** The results of this study suggested that the ingestion of WEM decreased healthy women's glycative stress and improved their skin moisture content as well as arterial stiffness.

***KEY WORDS*:** mangosteen (*Garcinia mangostana*), glycative stress, advanced glycation endproducts (AGEs), skin moisture, arterial stiffness

Introduction

Protein non-enzymatically reacts to saccharides, produces Amadori compound in the intermediate process and forms advanced glycation endproducts (AGEs) [1]. Stress on a human body, which is caused by excessive sugar reduction associated with metabolic disorders and the production of carbonyl compound, is called "glycative stress" [2,3] and is considered to be one of the aggravating factors in aging. It is also known that the accumulation of AGEs in the human body proceeds in an age-dependent manner [4,5] and enhanced by the onset of diabetic complications [6-10] and arteriosclerosis [11-15]. Pentosidine, is a cross-linking compound of AGEs, accumulating with aging in long-lived tissue proteins, including collagen, a primary structural protein, which forms skin, blood vessels and bone [16]. Since pentosidine forms cross-links between molecules in collagen in a non-specific fashion, it is considered to impair the flexibility of collagen and greatly influence functions of skin and blood vessels.

In order to prevent the accumulation of AGEs, it is considered to be effective not only to improve exercising and eating habits on a daily basis but also to take food ingredients that prevent the generation of AGEs. It is also known that anti-oxidant compounds, such as flavonoid, catechin and procyanidin, which are included in vegetables and fruits,

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prevent Maillard reaction and inhibit formation of AGEs *in vitro* [18-20]. Prengkuan *et al*. evaluated antioxidant activity in edible fruit extracts to inhibit Maillard reactions and reported that mangosteen pericarp extracts possessed high inhibitory activity [21]. Mangosteen is a fruit tree cultivated in Southeast Asia, such as Thailand, and is called the Queen of Fruits [22]. Mangosteen pericarp includes α-mangostin, one representative xanthone and also a variety of polyphenol, such as anthocyanin, epicatechin and procyanidin B2 [22-24]. In the U.S., juice produced by squeezing the whole fruit, including mangosteen pericarp and alcoholic extracts of mangosteen pericarp, are used as health foods [25, 26]. In particular, it has been reported that a water soluble component of mangosteen pericarp has a strong anti-oxidative effect [27]. In Japan, therefore, mangosteen pericarp extract has been more widely used as a raw material for health food.

Until recently, it was confirmed that after healthy women in their 30s and 40s took hot water extract of mangosteen pericarp (WEM), a skin fluorescence value, an index of AGE accumulation, became lower, and also serum pentosidine concentrations decreased, compared to the values before intake [28]. In this study, therefore, it was verified that WEM intake reduced glycative stress and influenced functions of skin and blood vessels. The subjects in this research were healthy females at 25 through 59 years of age. After they took 200 mg of WEM or placebo per day for 12 weeks, blood, skin function and vascular function tests were carried out before intake and 4, 8 and 12 weeks after intake. To evaluate the efficacy of WEM, a placebo-controlled double-blinded randomized clinical trial was performed.

Method

Subjects

The subjects in this research were healthy Japanese women in their 20s to 50s who had subjective symptoms of sagging skin or dull skin. Interview-based inquiry was conducted on the telephone for clinical test volunteer registrants, when they were recruited. In order to select the subjects, the following exclusion criteria were set as follows: 1) subjects who are currently taking medicine or supplements, 2) subjects who have a past medical history of critical illnesses related to liver, kidney, heart, lung and blood, or have a history of present illness, 3) subjects with skin diseases, including atopic dermatitis, 4) subjects who are currently participating in other human clinical studies or participated in those kinds of studies within three months, 5) subjects who are likely to change their life style (due to a night shift or long-term travel) during the test and 6) subjects who are considered to be inappropriate for this clinical test by the doctor in charge.

During the clinical test, the subjects were required to comply with the following precautions: 1) not keeping irregular hours, including excessive drinking and eating or lack of sleep, 2) fasting from 4 hours before blood sampling until the end of the test, 3) not changing their life style, 4) being prohibited from exposing themselves to direct sunlight outdoors and to expose their measurement site to ultraviolet rays in everyday life, 5) not taking new health food and 6) not changing the kinds of skincare products they use or how to use them.

Clinical test food

Mangostin aqua (Nippon Shinyaku CO., LTD., Kyoto, Japan) was used as WEM. After mangosteen pericarp was scalded for 2 minutes, it was dried at 70°C for 8 hours. The dried mangosteen pericarp was extracted with hot water, filtrated and concentrated. After 33% of solid weight dextrin was added to the concentrated mangosteen, it was powdered using a spray drying method. As a result, WEM containing 0.078% of rhodanthenone B was obtained. The clinical test foods were gelatin capsule types of food containing 100 mg of WEM per capsule and placebo capsules containing 100mg of crystalline cellulose per capsule instead of WEM, which apparently looked like the WEM ones (***Table 1***). The amount of intake per day was two capsules. ***Table 2*** shows the nutrient composition.

Table 1. Test food composition
(Compound amount: mg/2 capsules)

	WEM	Placebo
Water extract of Mangosteen	200	0
Saflower oil	340	340
Bees wax	30	30
Glycerine fatty acid ester	30	30
Crystalline cellulose	0	200

WEM, water extract of mangosteen.

Table 2. Test food nutritional constitutes
(Administration amount per day)

	WEM	Placebo
Energy (Kcal)	5.5	5.5
Protein (g)	0.21	0.21
Lipid (g)	0.40	0.40
Carbohydrate (g)	0.27	0.28
Sodium (mg)	0.87	0.66

WEM, water extract of mangosteen.

Clinical study design

In order to design the human study, a placebo-controlled double-blinded randomized clinical trial was implemented. An assignment supervisor randomly divided 40 subjects, who had been selected through a screening process, into two groups, and determined one group as WEM group and the other one as the placebo group. The allocation assignment list was sealed up tightly and kept until the list was unsealed. Two capsules of the test food per day (200mg as WEM) were taken for 12 weeks. Before intake (0 week), 4, 8 and 12 weeks after intake, measurement of evaluation items as well as physician interviews were conducted. The subjects took the capsules from February through April in 2016.

Evaluation items

Skin function index

As skin function indices, transepidermal water loss and horny cell layer water moisture were measured. After the measurement site of skin was cleaned and acclimated in a temperature and humidity controlled room (21°C ± 1°C, 50% ± 10%) for 20 minutes, the measurement was carried out. Tewameter (TM-300; Courage-Khazaka, Köln, Germany) was used to measure transepidermal water loss, whereas Corneometer (CM825; Courage-Khazaka) was used to measure horny layer water moisture. In both cases, a section of skin in the apex of the cheekbones on the left side of their face was measured.

Vascular function index

As vascular function indices, API (arterial pressure index) as well as AVI (arterial velocity pulse index) were measured [29]. PASESA AVE-1500 (Shisei Datum, Tokyo, Japan) was employed to measure a site of their right upper arm.

Serum pentosidine quantitative

After blood serum (0.2 mL) was hydrolyzed with hydrochloric acid (6N HCl, 100°C, 24 hours), it was evaporated and solidified under reduced pressure. Then it was dissolved in 1 mL of distilled water. This solution was added to an equilibrated cation exchange column (Strata-X-Cv Polymeric Strong Cation, 30 mg/mL; Phenomenex, Torrance, CA, USA) and cleaned with 3mL of 0.1 M HCl. After being eluted with 3mL of 7% ammonia, it was evaporated and solidified under reduced pressure. This substance was redissolved in a heptafluorobutyric acid (HFBA) solution and filtrated with 0.20 μm. Then it was quantified using high performance liquid chromatography (HPLC) [28].

Safety index

As safety indicators, blood pressure/pulse, body weight/body fat percentage/body mass index (BMI), a hematologic test, a blood biochemical test and a urine test were conducted. Blood pressure/pulse were measured using PASESA AVE-1500, while body weight/body fat percentage/body mass index (BMI) were measured using DC-320 (Tanita, Tokyo, Japan).

Hematologic examination items are shown as follows: leucocyte count, erythrocyte count, hemoglobin, hematocrit, erythrocyte indices (MCV, MCH, MCHC) and blood platelet count.

Biochemical examination of blood items are shown as follows: total protein (TP), albumin (Alb), urea nitrogen (UN), creatinine (CRE), uric acid (UA), aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (γ-GT), creatine kinase (CK), CRP quantitative (CRP), total cholesterol (T-C), triglyceride (TG), LDL cholesterol (LDL-C), HDL cholesterol (HDL-C), sodium (Na), potassium (K), chloride (Cl), iron (Fe), fasting plasma glucose and HbA1c.

Statistical analysis

The results of the research were indicated with mean ± standard deviation. As examination methods, a multiple test (Sidak test) was used to measure the comparison between before and after intake, whereas an unpaired t-test (two-sided test) was performed to measure a comparison between the two groups. The significance level was below 5%.

Ethical principles

This study was conducted in accordance with Declaration of Helsinki (the declaration was corrected at WMA Fortaleza General Assembly in 2013) as well as ethical principles concerning medical researches (notification of Ministry of Education, Culture, Sports, Science and Technology as well as Ministry of Health, Labor and Welfare). In order to secure the subjects' human rights and security, and ensure the reliability of examination data, this research was carried out after being deliberated and approved by Oriental Ueno Medical Examination Center Ethics Review Committee (2016-01-06), Nippon Shinyaku Co., Ltd. Research Ethics Committee (No.A151005) and "Ethics Committee for Human Subject Research" at Tokai University. Furthermore, this study was registered by University hospital Medical Information Network-Clinical Trials Registry (UMIN-CTR) and implemented (Registration No: UMIN000032310).

Results

After researchers obtained approval from 40 volunteers from 25 through 59 years of age, the study started with 20 subjects in the WEM group and 20 subjects in the placebo group. Because two of them voluntarily quit, 19 subjects in the WEM intake group and 19 subjects in the placebo group actually participated in the research. The backgrounds of these subjects are shown in ***Table 3***.

Table 3. Clinical characteristics

	WEM (n=19)	Placebo (n=19)
Age	43.1 ± 10.3	42.9 ± 10.2
Weight (kg)	51.8 ± 6.8	51.6 ± 6.1
Height (cm)	158.5 ± 5.0	156.0 ± 6.8

Values: means ± standard deviation. WEM, water extract of mangosteen.

Serum pentosidine concentration in the WEM group was significantly greater than that of the placebo group before intake (94.0 fmol /μL vs. 79.3 fmol /μL, p = 0.036). In the WEM group, serum pentosidine concentration significantly lowered 8 weeks after intake, compared with the level before intake, whereas no significant change was determined in the placebo group throughout the trial period, compared with the level before intake (***Table 6***). Meanwhile, no significant difference in blood glucose level was identified between before and after intakes as well as in the two groups throughout the trial period. Although HbA1c of the WEM group was significantly lower than that of placebo group 8 weeks after intake, no obvious change was observed between the levels before and after intakes (***Table 6***).

Table 6. Hematologic testing

Parameter	Unit	Reference range	Group	0W	4W	8W	12W
Pentosidine	fmol/μL	–	WEM	94.0 ± 20.8*	94.0 ± 23.9	80.7 ± 21.8##	97.2 ± 23.0
			Placebo	79.3 ± 20.9	83.3 ± 26.8	70.8 ± 19.8	83.5 ± 19.7
Glucose	mg/dL	70 – 109	WEM	89 ± 6	88 ± 8	90 ± 7	90 ± 6
			Placebo	89 ± 6	90 ± 6	89 ± 6	87 ± 6
HbA1c	%	4.6 – 6.2	WEM	5.2 ± 0.2	5.2 ± 0.2##	5.3 ± 0.2#*	5.3 ± 0.2#
			Placebo	5.3 ± 0.2	5.3 ± 0.3	5.5 ± 0.2#	5.4 ± 0.3
Total protein	g/dL	6.7 – 8.3	WEM	7.7 ± 0.4	7.4 ± 0.5#	7.5 ± 0.4	7.6 ± 0.4
			Placebo	7.7 ± 0.4	7.4 ± 0.3#	7.4 ± 0.5#	7.5 ± 0.4
Albumin	g/dL	3.8 – 5.3	WEM	4.7 ± 0.3	4.5 ± 0.2	4.6 ± 0.2	4.5 ± 0.2
			Placebo	4.6 ± 0.3	4.4 ± 0.3	4.5 ± 0.3	4.5 ± 0.2
Urea nitrogen	mg/dL	8 – 22	WEM	12 ± 3	13 ± 4	12 ± 4	13 ± 3
			Placebo	11 ± 3	12 ± 3	11 ± 3	12 ± 3
Creatinine	mg/dL	0.47 – 0.79	WEM	0.59 ± 0.07	0.61 ± 0.08	0.62 ± 0.09	0.64 ± 0.08##
			Placebo	0.55 ± 0.09	0.57 ± 0.09	0.58 ± 0.09	0.61 ± 0.09##
Uric acid	mg/dL	2.5 – 7.0	WEM	4.3 ± 0.9	4.5 ± 1.1	4.3 ± 0.8	4.4 ± 1.0
			Placebo	4.0 ± 0.7	4.0 ± 0.6	4.0 ± 0.5	4.1 ± 0.6
AST (GOT)	U/L	10 – 40	WEM	21 ± 6	21 ± 6	20 ± 6	20 ± 6
			Placebo	20 ± 5	20 ± 4	19 ± 3	21 ± 5
ALT (GPT)	U/L	5 – 45	WEM	17 ± 4	16 ± 6	16 ± 5	15 ± 4
			Placebo	17 ± 6	15 ± 5	16 ± 4	16 ± 4
γ-GT	U/L	< 45	WEM	21 ± 11	22 ± 11	22 ± 12	22 ± 12
			Placebo	24 ± 11	21 ± 10	20 ± 7#	18 ± 6##
CK	U/L	45 – 210	WEM	101 ± 63	81 ± 20	85 ± 26	86 ± 32*
			Placebo	105 ± 48	104 ± 57	100 ± 42	126 ± 73
CRP	mg/dL	< 0.30	WEM	0.04 ± 0.03	0.05 ± 0.03	0.06 ± 0.10	0.05 ± 0.04
			Placebo	0.04 ± 0.02	0.13 ± 0.28	0.08 ± 0.10	0.04 ± 0.02
T-C	mg/dL	130 – 219	WEM	217 ± 39	205 ± 38##	210 ± 40	211 ± 33
			Placebo	232 ± 34	222 ± 29	225 ± 26	226 ± 28
TG	mg/dL	35 – 149	WEM	70 ± 31	68 ± 21	61 ± 20	71 ± 21
			Placebo	76 ± 33	74 ± 37	78 ± 33	73 ± 33
LDL-C	mg/dL	70 – 139	WEM	118 ± 32	111 ± 29#	111 ± 31	110 ± 27#
			Placebo	136 ± 36	127 ± 31	126 ± 33	127 ± 32
HDL-C	mg/dL	40 – 96	WEM	89 ± 19	81 ± 19#	88 ± 20	84 ± 17
			Placebo	85 ± 17	81 ± 19	85 ± 17	84 ± 17
Sodium (Na)	mEq/L	135 – 147	WEM	139 ± 1	141 ± 2##	141 ± 1##	141 ± 2#
			Placebo	140 ± 1	141 ± 1##	141 ± 1##	141 ± 1#
Potassium (K)	mEq/L	3.6 – 5.0	WEM	3.9 ± 0.2	4.2 ± 0.2#	4.0 ± 0.2	4.0 ± 0.2
			Placebo	4.0 ± 0.4	4.1 ± 0.4	4.1 ± 0.4	4.0 ± 0.4
Chloride (Cl)	mEq/L	98 – 108	WEM	101 ± 2*	104 ± 2##	105 ± 2##	104 ± 2##
			Placebo	103 ± 2	104 ± 2#	105 ± 2##	105 ± 2##
Serum Iron (Fe)	μg/dL	40 – 170	WEM	98 ± 41*	94 ± 32	96 ± 35	91 ± 38
			Placebo	70 ± 40	80 ± 42	79 ± 38	82 ± 44

Values: means ± standard deviation. * $p < 0.05$, ** $p < 0.01$: Comparison with the Placebo group by t-test. # $p < 0.05$, ## $p < 0.01$: Comparison with before administration by Sidak. WEM, water extract of mangosteen.

Transepidermal water losses in the cheek skin before intake were 18.0 in WEM group and 16.9 in the placebo group, which indicated no significant difference between the two groups ($p = 0.468$). Compared with the value obtained before intake, the WEM group revealed −3.0%, 4.5% and −5.3%, respectively, 4, 8 and 12 weeks after intake, which were lower than those of the placebo group, because the placebo group showed 1.0%, 9.9% and 1.7%, respectively. However, there was no significant difference between the two groups before and after intakes (***Fig. 1-A***). On the other hand, skin moisture content of the WEM and the placebo groups before intake were 56.8 and 56.4, respectively, which revealed no significant difference between the two groups ($p = 0.865$). Compared with the value before intake, skin moisture content of the WEM and placebo groups demonstrated 3.6%, −2.7% and 7.4%, respectively, whereas those of the placebo group were −3.0%, −8.4% and −3.9% respectively, 4, 8 and 12 weeks after intake. This result, therefore, clarified that skin moisture content of the WEM group was significantly greater than that of placebo group 8 and 12 weeks after intakes (***Fig. 1-B***).

API before intake were 23.0 and 22.2 in the WEM group and the placebo group, respectively, which showed no significant difference between the two groups ($p = 0.674$). Compared with API before intake, API values of the WEM group were −7.4%, −13.4% and −8.7%, respectively, while those of the placebo group were 5.2%, 7.5% and 1.9%, respectively. The result, therefore, clarified that API of the WEM group was significantly lower than that of the placebo group 8 weeks after intake and also lower, compared with the level before intake (***Fig. 2-A***). Meanwhile, the AVI of the WEM and the placebo groups before intakes were 18.7 and 17.6, respectively, which indicated no significant difference between the two groups ($p = 0.540$). Compared with the value before intakes, the AVI of the WEM group were −14.9%, −9.8% and −0.7%, while those of the placebo group were −0.7%, 5.2% and 9.9%, respectively, 4, 8 and 12 weeks after intake. This result showed that the AVI of the WEM group was significantly lower 4 weeks after intake than AVI before intake ($p = 0.010$).

Concerning the subjects' body weight, body fat percentage and BMI, no significant difference associated with WEM intake was determined between the two groups. Moreover, there was no change between before and after intakes, except that the body fat percentage of WEM group was significantly lower 4 weeks after intake than before intake (***Table 4***). Regarding systolic blood pressure, diastolic blood pressure and pulse rate, no significant change was identified in both the WEM and the placebo groups throughout the test period (***Table 4***). Meanwhile, the decrease of erythrocyte count, 4 and 8 weeks after intakes as well as the decrease of hemoglobin count 8 weeks after intake were determined in the WEM group, compared with the levels before intake. In terms of hemoglobin and hematocrit, MCV, MCH, MCHC and platelet count, a significant difference between the two groups was identified from the levels before intakes (***Table 5***). Furthermore, values of TP, CRE, γ-GT, T-C, LDL-C, HDL-C, Na, K and Cl in WEM group changed after intake, compared with those before intake. However, they did not deviate from reference values. CK of the WEM group was lower than that of the placebo group 12 weeks after intake, but the value still fell within the reference range (***Table 6***). In addition, the result of urine tests fell within the reference range. No adverse event that had a causal relationship with WEM intake was observed.

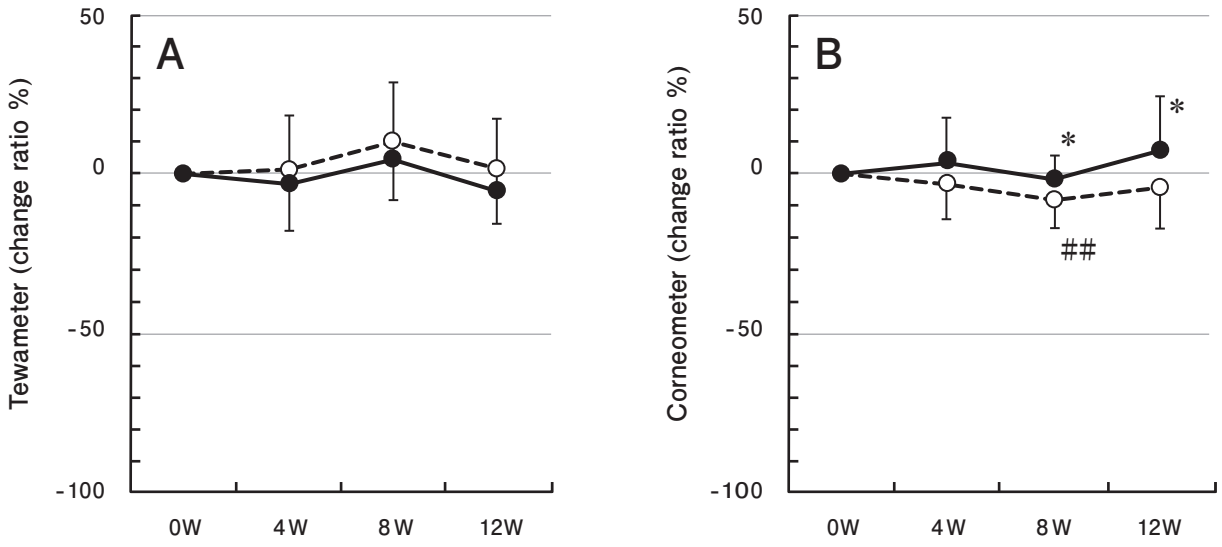

Fig. 1. **Values of percutaneous water transpiration quantity measured with Tewameter (A) and skin moisture measured with Corneometer (B).**

Results of change ratio (%) of WEM group (●) and Placebo group (○) are expressed as mean ±standard deviation.
* $p<0.05$: Comparison with the Placebo group by t-test. ## $p<0.01$: Comparison with before administration by Sidak.

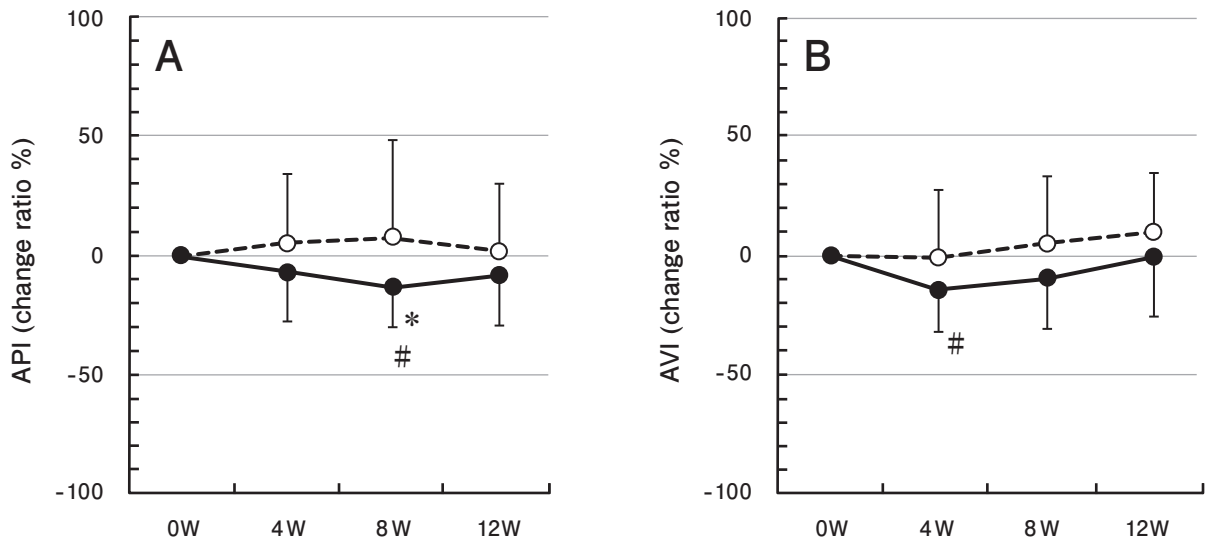

Fig. 2. **Values of API (A) and AVI (B) measured with the PASESA.**

Results of change ratio (%) of WEM group (●) and Placebo group (○) are expressed as mean ±standard deviation.

* $p<0.05$: Comparison with the Placebo group by t-test. # $p<0.05$: Comparison with before administration by Sidak. API, Arterial Pressure Index; AVI, Arterial Velocity pulse Index.

Table 4. Physical testing

	Parameter	Unit	Group	0W	4W	8W	12W
Body composition	Weight	kg	WEM	51.8 ± 6.7	51.9 ± 6.8	52.1 ± 6.7	52.1 ± 6.9
			Placebo	51.6 ± 6.1	51.9 ± 6.4	52.1 ± 6.4	51.8 ± 6.4
	Body fat	%	WEM	29.2 ± 5.4	28.7 ± 5.5 #	28.9 ± 5.5	28.9 ± 5.1
			Placebo	29.7 ± 5.2	30.4 ± 4.6	30.3 ± 4.7	30.1 ± 4.4
	MBI	kg/m^2	WEM	20.6 ± 2.7	20.7 ± 2.7	20.8 ± 2.7	20.7 ± 2.7
			Placebo	21.2 ± 2.4	21.4 ± 2.5	21.4 ± 2.4	21.3 ± 2.4
Blood pressure	Systolic	mmHg	WEM	110 ± 12	105 ± 13	107 ± 12	108 ± 13
			Placebo	108 ± 18	106 ± 17	113 ± 21	108 ± 17
	Diastolic	mmHg	WEM	67 ± 10	63 ± 10	68 ± 9	67 ± 10
			Placebo	63 ± 11	63 ± 11	69 ± 12	67 ± 10
Pulse rate		bpm	WEM	63 ± 9	63 ± 9	68 ± 9	65 ± 10
			Placebo	66 ± 9	69 ± 9	71 ± 13	68 ± 8

Values: means ± standard deviation. # $p < 0.05$: Comparison with before administration by Sidak. WEM, water extract of mangosteen; BMI, body mass index.

Table 5. Hematologic testing

Parameter	Unit	Reference range	Group	0W	4W	8W	12W
Leukocyte count	/μL	3500 – 9100	WEM	5958 ± 1245	5568 ± 1818	5826 ± 1505	5658 ± 1060
			Placebo	6132 ± 1812	6053 ± 2001	6026 ± 1707	5984 ± 1605
Erythrocyte count	× 10^4/μL	376 – 500	WEM	452 ± 28	447 ± 28 #	441 ± 26 #	440 ± 29
			Placebo	453 ± 22	443 ± 18	443 ± 23	443 ± 21
Hemoglobin	g/dL	12.3 – 15.2	WEM	13.6 ± 0.7*	13.5 ± 0.8**	13.3 ± 0.6 #**	13.3 ± 0.9*
			Placebo	12.7 ± 1.5	12.4 ± 1.3	12.3 ± 1.3	12.4 ± 1.4
Hematocrit	%	33.4 – 44.9	WEM	42.1 ± 1.9*	41.3 ± 2.3*	40.7 ± 1.9 ##*	41.2 ± 2.4
			Placebo	40.1 ± 3.5	39.1 ± 3.1	38.9 ± 3.1 #	39.4 ± 3.4 #
MCV	fL	79 – 100	WEM	93 ± 5*	93 ± 4*	93 ± 4*	94 ± 4*
			Placebo	89 ± 7	88 ± 7	88 ± 7	89 ± 7
MCH	pg	26.3 – 34.3	WEM	30.1 ± 1.4**	30.2 ± 1.4**	30.1 ± 1.4**	30.3 ± 1.5**
			Placebo	28.1 ± 2.9	28.0 ± 2.9	27.9 ± 2.9	28.0 ± 2.8
MCHC	%	30.7 – 36.6	WEM	32.3 ± 0.6*	32.7 ± 0.5**	32.6 ± 0.5**	32.3 ± 0.7*
			Placebo	31.6 ± 1.2	31.7 ± 1.1	31.7 ± 1.0	31.5 ± 1.2
Platelet count	× 10^4/μL	13.0 – 36.9	WEM	23.6 ± 3.7**	23.2 ± 3.8**	23.7 ± 3.6**	24.0 ± 4.3*
			Placebo	30.2 ± 6.4	29.2 ± 6.9	28.7 ± 6.9	29.1 ± 7.2

Values: means ± standard deviation. * $p < 0.05$, ** $p < 0.01$: Comparison with the Placebo group by t-test. # $p < 0.05$, ## $p < 0.01$: Comparison with before administration by Sidak. WEM, water extract of mangosteen.

Discussion

Serum pentosidine concentrations of the WEM group significantly decreased 8 weeks after intake, compared with the value obtained before intake. This result corresponded with the previous report [28]. However, serum pentosidine concentrations of both WEM and placebo groups increased 12 weeks after intake. Since there is not enough information for healthy people's variation factors for pentosidine concentration in blood, the cause for the change in pentosidine concentration 12 weeks after intake is unknown.

Because moisture content of skin of WEM group was significantly higher than that of placebo group 8 and 12 weeks after intakes, it was considered that WEM intake improved the water retention function of skin. Pentosidine, one type of AGE, forms cross-links between collagen molecules in the dermis in a nonspecific manner [17]. It is, therefore, considered that improvement of glycative stress reduces production of cross-links between collagen molecules, enhances turnover of skin and leads to the improvement of three-dimensional structures of skin collagen. Moreover, it has been reported that N^{ε}-(carboxymethyl) lysine (CML), a type of AGE, induces apoptosis of fibroblasts, which secretes hyaluronan contributing to water retention capacity of the skin [30, 31]. It was, therefore, considered that the reduction of glycative stress could improve functions of dermal fibroblasts.

API reflects arterial stiffness of brachial artery in the measurement site [29]. Since due to the intake of WEM, API value of the WEM group was lower than that of the placebo group 8 weeks after intakes (***Fig.2-A***), and it was considered that the ingestion of WEM enhanced arterial softness. According to Reddy GK's report, glycation of rabbit tendons increased their stiffness [32]. This was because pentosidine was formed in collagen [32]. However, considering that WEM intakes decreased serum pentosidine concentration, there is also a possibility that stiffness of arterial collagen could be improved by taking WEM. The result indicated that AVI value reflected the function of vascular endothelium [33, 34]. Moreover, it was reported that AGEs elicited inflammation through RAGE (Receptor of AGEs) and impaired blood vessels by suppressing NO (nitric oxide) [35, 36]. An epidemiological study reported a relationship between API value as well as AVI value and the risk of arteriosclerosis that could happen in the future [37]. According to the result shown in ***Fig. 2-B***, the function of vascular endothelium was improved in the WEM group. It is, therefore, expected that reduction of AGE accumulation by taking WEM will alleviate vascular injury through RAGE and lead to reduction of the risk of arteriosclerosis that might occur in the future.

Saito *et al.* clarified that pentosidine concentration in bone collagen was related to stiffness of bone [38]. On the other hand, Arai *et al.* reported a correlation between serum concentrations of pentosidine and schizophrenia [39]. Thus, by monitoring levels of AGEs on a daily basis, there is a possibility that we can recognize the changes in our health conditions that we cannot notice through regular health check-ups. It is, therefore, expected that inhibition of AGE production associated with our lifestyle will greatly contribute to mental health as well as physical health in our everyday life and reduce the risk of becoming ill in the future.

Conclusion

It was suggested that ingestion of WEM may reduce glycative stress, improve moisture content of skin and contribute to retention of skin moisture. Furthermore, it was suggested that the ingestion of WEM not only improves flexibility of blood vessels but also may reduce the risk of arteriosclerosis that could occur in the future. It is, therefore, considered that since the intake of WEM decreases glycative stress, as a secure health food, WEM will play a key role in reducing risks of the future illness.

Declaration of Conflict of Interest

The clinical test of this research was sponsored by Nippon Shinyaku Co., Ltd., and was conducted in TES Holdings. Meanwhile, Nippon Shinyaku Co., Ltd., invested on research funding of the quantitative analysis of serum pentosidine and it was carried out at Tokai University.

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