Clinical Interventions in Aging

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ORIGINAL RESEARCH

A preliminary study on the effects of star fruit consumption on antioxidant and lipid status in elderly Thai individuals

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Objective: The aims of this preliminary study were to evaluate the antioxidant and lipid status before and after star fruit juice consumption in healthy elderly subjects, and the vitamins in star fruit extracts.

Methods: A preliminary designated protocol was performed in 27 elderly individuals with a mean (±SD) age of 69.5±5.3 years, by planning a 2-week control period before 4 weeks of consumption of star fruit twice daily. Oxidative stress parameters such as total antioxidant capacity, glutathione, malondialdehyde, protein hydroperoxide, multivitamins such as L-ascorbic acid (Vit C), retinoic acid (Vit A), and tocopherol (Vit E), and the lipid profile parameters such as cholesterol, triglyceride, high-density lipoprotein-cholesterol (HDL-C) and low-density lipoprotein-cholesterol (LDL-C) were analyzed. Moreover, Vit C, Vit A, and Vit E levels were evaluated in the star fruit extracts during the 4-week period.

Results: In the 2-week control period, all parameters showed no statistically significant difference; after 4 weeks of consumption, significant improvement in the antioxidant status was observed with increased total antioxidant capacity and reduced malondialdehyde and protein hydroperoxide levels, as well as significantly increased levels of Vit C and Vit A, when compared to the two-time evaluation during the baseline periods. However, glutathione and Vit E showed no statistical difference. In addition, the HDL-C level was higher and the LDL-C level was significantly lower when compared to both baseline periods. But the levels of triglyceride and cholesterol showed no difference. Vit C and Vit A were identified in small quantities in the star fruit extract.

Conclusion: This preliminary study suggested that consumption of star fruit juice twice daily for 1 month improved the elderly people's antioxidant status and vitamins, as well as improved the lipoproteins related to Vit C and Vit A in the star fruit extract.

Keywords: star fruit, elderly, antioxidants, vitamins, lipid, oxidative stress

Introduction

It is well accepted that the aging population is growing rapidly in developed and developing countries worldwide, and is predicted to grow in Thailand from 9.0 million in 2015 to 15.9 million in 2035.[1] In addition, there is a great deal of evidence that demonstrates the growing prevalence of chronic diseases (eg, hypertension, stroke, cardiac disease, type 2 diabetes, and cancer).[2] Furthermore, the aging process is strongly believed to be related to, if not caused by, oxidative stress,[3] which has been linked to many of these chronic diseases, specifically in the elderly.[4] Prior work carried out on the elderly population has shown that low concentrations of antioxidants such as glutathione (GSH) or increased oxidation to both lipid and protein structures increases the

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levels of oxidative stress markers such as malondialdehyde (MDA) and protein hydroperoxide (PrOOH).[3,5] Increased oxidant production together with low antioxidant buffering capacity is a main factor in sarcopenia. Thus, a chronic state of oxidative stress may result in decreased muscle strength, endurance, and function capacity.[6,7] Oxidative stress is proposed in the pathophysiology of hypertension, and a previously reviewed report showed a significant relationship between hypertension and abnormal lipoprotein metabolism, including low levels of high-density lipoprotein-cholesterol (HDL-C) and low-density lipoprotein-cholesterol (LDL-C).[8] Moreover, aging alters the composition of HDL, with a high level of complement C3 and the acute-phase serum amyloid protein, which are involved in endopeptidase/protease inhibition of HDL in elderly subjects.[9] HDL has been suggested as having the potency to promote cholesterol effusion and inhibit the oxidation of LDL molecule by free radicals.[9] Interestingly, previous suggestions indicate that inhibition of oxidation of LDL is related to a reduction in the release of proinflammatory mediators from macrophages, which reduces adhesion molecule expression on the endothelial cells, as well as stimulates nitric oxide (NO) formation that promotes vasodilation in vivo.[10] Therefore, oxidative stress seems to be related with the inflammatory process, lipoprotein metabolism, and possibly physical function in the aging process.

Presently, functional foods for the elderly, such as grape peel, milk, red and green peppers, garlic, onion, vegetables, and fruit juice, have been claimed to reduce the oxidative stress and possibly delay the progression of chronic disease.[11] The bioactive compounds within these foods, which include anthocyanins, calcium, lycopene, curcuminoids, α-tocopherol or vitamin E (Vit E), ubiquinol, and ascorbic acid or vitamin C (Vit C), have all been associated with reducing the oxidative stress that involves the lipoprotein metabolism.[12]

Star fruit (*Averrhoa carambola* L.) is one of the many native fruits grown throughout Thailand and many other Asian countries. When cut in cross section, the five-pointed star is a classic characteristic of this fruit. Star fruit skin is green to yellowish in color and the taste of its flesh ranges from fresh sour to slightly sweet.[13] Reviewed data show its various traditional applications in humans, such as its effect as an appetite stimulant, with antipyretic, laxative, diuretic, and digestive effects, as well as in treating throat inflammation, mouth ulcer, toothache, cough, asthma, or eye-related problems. Moreover, updated reviews show interesting evidence of chemical constituents and nutritional values, including the traditional uses.[14,15] Star fruit has been shown to have high concentrations of Vit C, epicatechin, and gallic acid,[16] as well as flavonoid C-glycoside, which has strong antioxidant activity.[17] A previous report shows that Vit C can decrease glycation and reduce glycohemoglobin in all proteins during the aging process,[18] as well as decrease the osmotic fragility of erythrocytes caused due to free radicals.[19] Therefore, Vit C has strong antioxidant status and star fruit contains a rich, locally grown source of this potent antioxidant. However, star fruit is not popular and not consumed in as much quantity as other fruits such as water melon, banana, guava, and orange. Moreover, Leelarungrayub et al updated their preliminary study on supplementation of star fruit juice twice daily for 1 month to elderly subjects and showed significant inhibition of release of proinflammatory cytokines such as tumor necrosis factor-α and interleukin-23, as well as an increase in walking distance.[20] However, prior work did not investigate the active compounds in the star fruit, nor did the work determine the impact of the star fruit on the lipid profile in elderly subjects. Therefore, this preliminary study was designed to identify the antioxidant compound in star fruit, and also to evaluate the antioxidant status and lipid profile changes in elderly subjects after consumption of the fruit.

Methods

Experimental design

The study design was divided into two parts. In the first part, the effectiveness of star fruit consumption twice daily for 1 month in elderly subjects was determined. Healthy elderly people were included in the protocol designed that had a 2-week baseline period, in which the participants continued their food intake and daily activity as usual without consuming star fruit juice; this was followed by a further 4-week period with star fruit juice added to their daily diet. Star fruit of the same size was purchased from a local farmer in Chiang Mai province, and made available 2 weeks after harvesting for each week of consumption. Therefore, four harvests of star fruit were prepared for elderly subjects during the 4-week experiment. Fresh juice was prepared from each 100 g of fresh, clean star fruit by using a blender for fine homogenization, and was used for consumption immediately after breakfast and dinner daily during the 4-week study period. Basic complete blood count (CBC), and liver (aspartate transaminase [AST], alanine transaminase [ALT]) and kidney (blood urine nitrogen [BUN], creatinine) function, including the antioxidant status (total antioxidant capacity [TAC], GSH, PrOOH, and MDA), as well as Vit A, Vit E, and Vit C levels were evaluated twice in the 2-week control period and after 4 weeks of star fruit juice consumption. In the second part, during the 4-week consumption period, multivitamins (Vit C, Vit A, and Vit E) of ten samplings selected randomly

from fresh star fruits on Monday and Friday of each week were analyzed in the laboratory.

Recruitment of participants

This research protocol was approved by the Human Ethics Committee at the Faculty of Associated Medical Sciences, Chiang Mai University, Thailand, and performed in accordance with the Helsinki Declaration (2001) (ethical approval number 027E/52). The sample size was calculated by a G*Power (3.1.9.2) program with an effect size =0.92, alpha error =0.05, and power =0.95 from the previous study.[20] A minimum of 24 elderly subjects were required for this study. For higher power, 40 healthy elderly subjects (20 males and 20 females) aged between 54 and 87 years from the Piyaman Elderly Health Care Center in Chiang Mai province, of whom 25 were nonsmokers and 15 were ex-smokers (for >5 years), were included in this protocol. Even though this study initially comprised 40 healthy elderly participants, during the course of the 6-week study, 13 individuals elected to drop out because of diarrhea (n=3), loss to follow-up (n=3), and discontinuous supplementation or a supplement loss of more than two times per week (n=7) without any health problems. Therefore, the remaining 27 individuals completed the study. They understood the research protocol and provided written consent before enrolling in the study. All the participants were screened for the exclusion criteria by using previous hospital records, for example, esophageal reflux, hemoptysis, rib fracture, coagulopathy, cardiac arrhythmias, any pulmonary or neurological disorders, and so on, and confirmed by a physician at the AMS Clinical Service Center, Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand. During the 6 weeks of the experiment, all of them strictly controlled their basic daily activities, behavioral aspects, and diet intake. They were instructed to refrain from taking supplementary multivitamins or extra food.

Blood sampling preparation

Of the total 20 mL of blood taken from the anterior cubital vein, 15 mL was transferred to sterile ethylenediamine tetraacetic acid (EDTA) and non-EDTA tubes for evaluating CBC, lipid profiles, and liver and kidney function by a fully automated Olympus AU400 Analyzer (Olympus Diagnostics GmbH, Umkirch, Germany) at the AMS Clinical Service Center. The remaining 5 mL was used for the evaluation of all oxidative stress markers and plasma multivitamins.

Oxidative stress and vitamin evaluation

Four hundred microliters of whole blood was taken for evaluating GSH. The residual plasma was separated by centrifugation at 6,000 rpm for 10 minutes and immediately assayed for TAC. Residual plasma was separated to determine MDA, PrOOH, Vit C, Vit A, and Vit E. GSH was determined by following the previous spectrophotometry protocol of Leelarungrayub et al.[21] The GSH concentration was calculated by comparing with the absorbance of standard GSH (Sigma-Aldrich Co., St Louis, MO, USA), and finally presented as milligrams in 1 g of hemoglobin (mg/g Hb) from CBC analysis.

TAC of fresh plasma was assayed following the 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid decolorization method,[22] by spectrophotometry. TAC was represented as millimoles of standard Trolox per liter (mmol Trolox/L).

Plasma MDA from lipid oxidation was evaluated by following a previous method with the protocol of high performance liquid chromatography (HPLC)[23] under the thiobarbituric acid reactive substances test. The plasma MDA (μmol/L) was calculated by comparing with the standard tetramethoxypropane (Sigma-Aldrich Co.).

Plasma PrOOH was evaluated by following the previous ferrous oxidation xylenol-orange protocol.[24] The PrOOH (μmol/L) was determined by spectrophotometry at 560 nm, and its yield was compared to the standard *tert*-butylhydroperoxide (Sigma-Aldrich Co.).

Antioxidant vitamin evaluation in plasma

Vit A, Vit E, and Vit C were evaluated following the previous HPLC protocol of Talwar et al[25] and Furusawa.[26]

In that protocol, Vit C in plasma was evaluated after precipitation with 60% ethanol containing 1 mM of EDTA, whereas Vit E and Vit A were evaluated in plasma after extraction with hexane. Then, external standard Vit C (100 μg/mL) and Vit E or Vit A (50 mg/L) were added to the samples. All vitamins were analyzed in HPLC composed of a C-18 reverse-phase column (250×4.6×5.0 mm; Phenomenex, Aschaffenburg, Germany) with different mobile phases: 2% (v/v) acetic acid solution in water (pH 2.5) for Vit C, and a mixture of methanol–acetonitrite and tetrahydrofuran (75:20:5, v:v:v) for Vit E and Vit A with a flow rate of 1.0 and 1.2 mL/min, respectively. The peak values of Vit C, Vit E, and Vit A were separately identified at 245, 325, and 294 nm, respectively, by comparing to those of standard ascorbic acid, tocopherol acetate, and retinoic acid (Sigma-Aldrich Co.).

Vitamins in star fruit extract analysis

On Monday and Friday of each week, ten juice samplings (100 g per sampling prepared by using a blender and fine homogenization) were randomly selected from the prepared star fruit materials provided to the elderly subjects. The samples were analyzed as dry powder extracts by using

the dry freezing or lyophilization technique (Vit C, Vit A, and Vit E) with an HPLC protocol.[25,26] Vit C in the extract was analyzed after resolving with distilled water, whereas Vit E or Vit A was extracted with hexane (1:1/w:v) and chloroform (1:1/v:v). Once the samples were processed, the HPLC protocols used for analyzing the start fruit extracts were the same as those used to analyze the human plasma.

Statistical analysis

All data in this study are presented as mean and standard deviation after normal distribution was evaluated by the Kolmogorov–Smirnov test. The parameters of CBC, liver and kidney function, oxidative stress, and vitamins evaluated three times were analyzed with the repeated measures analysis of variance (ANOVA) and the Bonferroni post hoc tests using the Statistical Package for Social Sciences, version 10.0 (SPSS Inc., Chicago, IL, USA). Significance was set at P=0.05. Moreover, the G*Power (3.1.9.2) was used to compute the effect size of outcomes from oxidative stress, vitamins, and lipid profile parameters in this study.

Results

From the overall results, normal distribution was represented after being statistically analyzed with the Kolmogorov-Smirnov test, thus the mean and standard deviation was presented. Although a low sample size (N=27) of elderly subjects was included, the repeated measures ANOVA is still a viable statistical test.

The study results are those of 27 elderly participants, consisting of 19 males and eight females, with a mean age of 69.5±5.3 years. The results of CBC and liver and kidney function tests during the control period and after the consumption period showed no statistical difference, with the repeated measures ANOVA showing a value of $P>0.05$ (Table 1).

Oxidative stress status

Table 2 and Figures 1–4 show the oxidative stress status of the 27 participants, with no significant difference found in TAC, GSH, MDA, and PrOOH during the control period at week 0 and week 2 ($P>0.05$). After 4 weeks of star fruit juice consumption, the results showed significant improvement in some oxidative stress parameters, when compared to week 0 and week 2 ($P<0.05$ for TAC, MDA, and PrOOH), except GSH ($P>0.05$).

Antioxidant vitamins in plasma

There were no significant differences in the plasma concentrations of Vit C, Vit A, or Vit E during the 2-week baseline period. The results presented in Table 2 and Figures 5–7 show a nonsignificant statistical difference in the levels of Vit C, Vit A, and Vit E ($P>0.05$) in all 27 elderly subjects during the control period. After 4 weeks of star fruit juice consumption, the levels of Vit C and Vit A increased significantly ($P<0.05$), but not Vit E ($P>0.05$), when compared to the levels in week 0 and week 2 of the control period.

Table 1 Characteristics, CBC, and LFT in 27 elderly subjects

Characteristics					
Age (years)		69.5±5.3 (56–85)			
Weight (kg)		50.2±7.1 (31.5–68.0)			
Height (m)		1.5±0.9 (1.36–1.65)			
	Reference range	**Control period**		**After 4 weeks of consumption**	***P*-value**
		Week 0	**Week 2**		
CBC					
WBC	4.5–11.5 (10^3/μL)	6.2±1.1 (4.5–9.6)	6.3±1.4 (3.4–9.2)	6.3±1.7 (3.2–9.3)	0.52
RBC	3.8–5.3 (10^6/μL)	5.1±0.2 (3.4–4.4)	4.25±0.5 (3.4–5.7)	4.4±0.2 (4.0–5.7)	0.62
Hb	10–16 (g/dL)	12.1±1.2 (11.3–15.2)	12.4±1.6 (10.5–15.8)	12.2±0.7 (10.2–14.9)	0.71
Hct	36–50 (%)	40.2±1.1 (34.9–43.4)	39.1±3.2 (34.2–50.2)	38.7±2.1 (33.4–40.2)	0.54
PLT	140–440 (10^3/μL)	238.0±36.0 (146–380)	224.0±50.0 (187–411)	230.0±42.0 (140–329)	0.87
Liver function					
AST	10–42 U/L	23.7±3.1 (17–32)	22.2±2.6 (18–31)	22.22±2.8 (18–30)	0.15
ALT	10–40 U/L	22.8±4.3 (17–32)	21.8±4.1 (16–31)	21.8±4.3 (15–32)	0.62
Kidney function					
BUN	7–18 mg/dL	11.2±2.2 (8–16)	10.8±1.8 (9–15)	11.7±1.8 (8–15)	0.29
Creatinine	0.6–1.3 mg/dL	0.9±0.2 (0.6–1.2)	0.9±0.1 (0.6–1.2)	0.9±0.1 (0.7–1.3)	0.16

Notes: Values are presented as mean ± SD; range for each variable is indicated in parentheses. Repeated measures ANOVA was used for statistical comparison.
Abbreviations: ALT, alanine transaminase; ANOVA, analysis of variance; AST, aspartate transaminase; BUN, blood urea nitrogen; CBC, complete blood count; Hb, hemoglobin; Hct, hematocrit; LFT, liver function test; PLT, platelet; RBC, red blood cells; SD, standard deviation; WBC, white blood cells.

Table 2 Oxidative stress, vitamins, and lipid profiles of all 27 volunteers

Variable	Control period		After 4 weeks of consumption	Effect size (*d*)
	Week 0	Week 2		
Oxidative stress status				
TAC (mmol Trolox/L)	0.91±0.2 (0.7–1.2)	0.9±0.1 (0.68–1.22)	1.4±0.3* (0.9–2.1)	2.28
GSH (mg/g Hb)	13.1±1.7 (10.5–13.3)	14.7±1.6 (9.6–15.7)	13.6±2.1 (10.4–16.0)	0.58
MDA (μmol/L)	3.3±0.6 (2.5–4.5)	3.5±0.7 (2.4–4.5)	2.8±0.6* (1.5–3.5)	1.07
PrOOH (μmol/L)	2.8±0.7 (2.0–4.5)	3.1±0.5 (2.5–4.0)	2.0±0.2* (1.25–3.5)	2.88
Plasma vitamins				
Vit C (mg/dL)	70.2±8.8 (52.5–85.0)	68.2±7.1 (45.5–75.0)	82.6±4.1* (56.5–95.0)	2.48
Vit A (μg/mL)	0.3±0.1 (0.2–1.5)	0.3±0.2 (0.1–1.0)	0.4±0.1* (0.3–2.5)	0.68
Vit E (μg/mL)	1.2±0.3 (0.5–2.5)	1.3±0.2 (0.6–2.6)	1.3±0.4 (0.6–2.7)	0.63
Lipid profile				
Triglyceride (mg/dL)	114.3±11.6 (66–179)	112.5±12.3 (56–169)	116.3±13.9 (64–173)	0.28
Cholesterol (mg/dL)	178.0±19.7 (138–315)	198.0±23.7 (142–320)	208.2±39.7 (141–293)	0.31
HDL-C (mg/dL)	53.6±6.5 (32–69)	56.4±9.3 (35–67)	69.1±11.3* (47–88)	1.22
LDL-C (mg/dL)	172.3±25.2 (72.5–262.1)	165.5±32.5 (68.2–256.6)	127.8±39.1* (54.6–221.5)	1.05

Notes: Values are presented as mean ± SD; range for each parameter is indicated in parentheses. *$P<0.05$ compared to both week 0 and week 2 in the control period by Bonferroni test after significance was determined by a repeated measures ANOVA. Effect size (*d*) was analyzed with G*Power 3.1.9.2.
Abbreviations: ANOVA, analysis of variance; GSH, glutathione; Hb, hemoglobin; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein cholesterol; MDA, malondialdehyde; PrOOH, protein hydroperoxide; SD, standard deviation; TAC, total antioxidant capacity; Vit, vitamin.

Lipid profiles in plasma

From the lipid profile results presented in Table 2, it can be found that all parameters, including triglyceride, cholesterol, HDL-cholesterol (HDL-C), and LDL-C, at week 0 were not statistically different from those in week 2 (P=0.68, 0.79, 0.83, and 0.55) of the control period. After consumption of star fruit juice for 4 weeks, a significant increase in HDL-C (P=0.03 and 0.04) and decrease in LDL-C (P=0.02 and 0.03) levels were observed, whereas no statistically significant changes in triglyceride (P=0.87 and 0.65) or cholesterol (P=0.52 and 0.71) levels could be seen when compared to those in week 0 and week 2.

Vitamins in star fruit extracts

In this study, Vit A and Vit C levels could be evaluated following the previous protocols,[25,26] whereas Vit E level was not detectable. Results of vitamin evaluation with HPLC in star fruit extracts during the 4-week consumption period showed nonsignificant concentrations (Table 3) of Vit C and Vit A ($P>0.05$), with the mean average for Vit C and Vit A being ~17 mg and 0.19 μg, respectively.

Discussion

The previous study of star fruit juice consumption showed positive benefits with regard to proinflammatory and functional

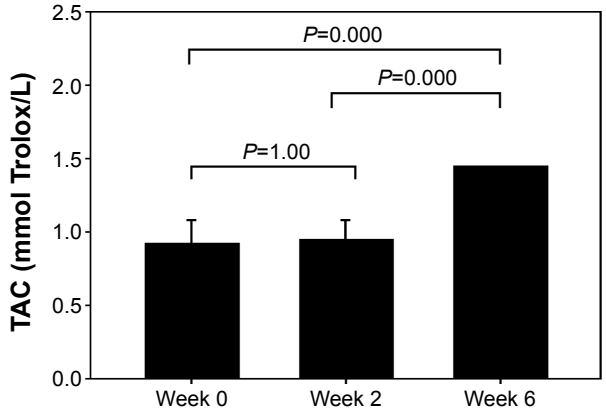

Figure 1 TAC (mmol Trolox/L) of all 27 elderly subjects during the control period (at week 0 and week 2) and after star fruit juice consumption for 4 weeks (at week 6).
Notes: Each bar represents the mean and standard deviation. Repeated measures ANOVA and Bonferroni post hoc tests were used for statistical analysis.
Abbreviations: ANOVA, analysis of variance; TAC, total antioxidant capacity.

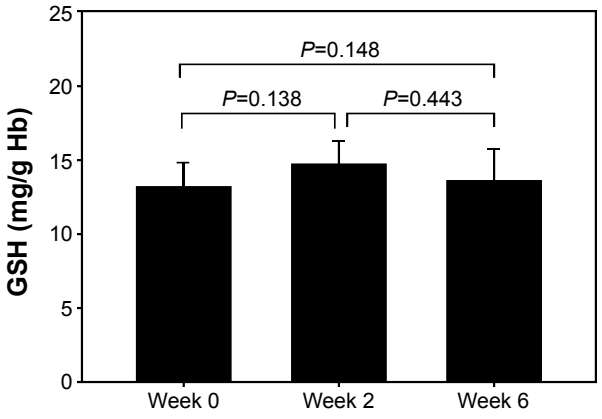

Figure 2 GSH (mg/g Hb) of all 27 elderly subjects during the control period (at week 0 and week 2) and after star fruit juice consumption for 4 weeks (at week 6).
Notes: Each bar represents the mean and standard deviation. Repeated measures ANOVA and Bonferroni post hoc tests were used for statistical analysis.
Abbreviations: ANOVA, analysis of variance; GSH, glutathione; Hb, hemoglobin.

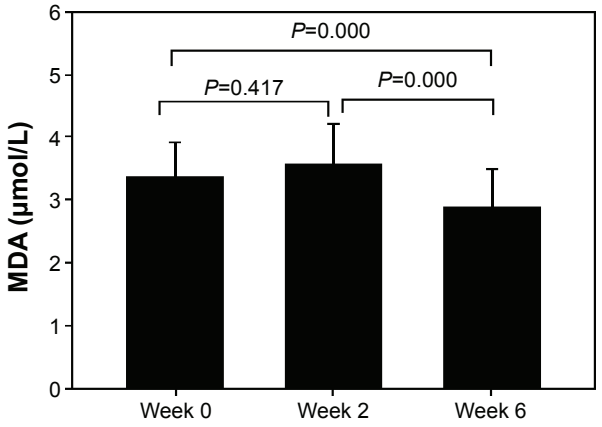

Figure 3 MDA (µmol/L) of all 27 elderly subjects during the control period (at week 0 and week 2) and after star fruit juice consumption for 4 weeks (at week 6).
Notes: Each bar represents the mean and standard deviation. Repeated measures ANOVA and Bonferroni post hoc tests were used for statistical analysis.
Abbreviations: ANOVA, analysis of variance; MDA, malondialdehyde.

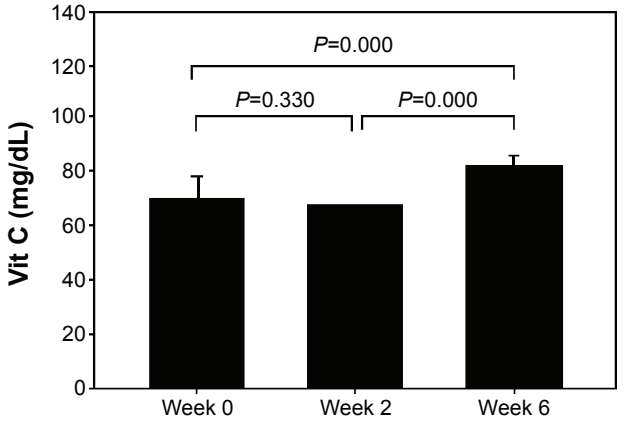

Figure 5 Vit C (mg/dL) of all 27 elderly subjects during the control period (at week 0 and week 2) and after star fruit juice consumption for 4 weeks (at week 6).
Notes: Each bar represents the mean and standard deviation. Repeated measures ANOVA and Bonferroni post hoc tests were used for statistical analysis.
Abbreviations: ANOVA, analysis of variance; Vit, vitamin.

capacity in elderly individuals.[20] Moreover, the benefits of star fruit juice consumption with regard to anti-inflammatory, analgesic, hypoglycemic, anthelmintic, antiulcer, hypotensive, antimicrobial, and antioxidant pharmacological activities have been reported.[14] However, there is little scientific evidence regarding the benefits of star fruit juice intake by elderly subjects. Therefore, the results of this preliminary study possibly support the results of previous one,[20] especially with regard to oxidative stress and lipid profiles. Although the protocol design of this study did not include a formal control group, our data are appropriate for such a preliminary investigation.

The baseline measurements of MDA in this study (3.35±0.56 and 3.55±0.67 µmol/L) (Figure 3) were in line with those reported previously, and they demonstrated a slightly elevated oxidative state in these participants (mean age of 69.5±5.3 years) during the control period. A similar result was reported by Mutlu-Turkoglu et al,[27] who showed higher levels of MDA (3.96±1.12 µmol/L) in 30 healthy elderly people (mean age of 72.7±5.8 years), when compared to the levels (2.55±0.75 µmol/L) in 25 younger participants (mean age of 30.0±4.6 years). This is consistent with a previous report by Mezzetti et al, who suggested that plasma peroxide or MDA in elderly individuals was higher than that in a younger cohort.[5]

In addition, the results of this study show the baseline levels for Vit A (0.3±0.1 and 0.3±0.2 µg/mL), Vit C (1.2±0.3 and 1.3±0.2 µg/mL), and Vit E (1.2±0.3 µg/mL) in plasma, which were similar to those reported in a previous study

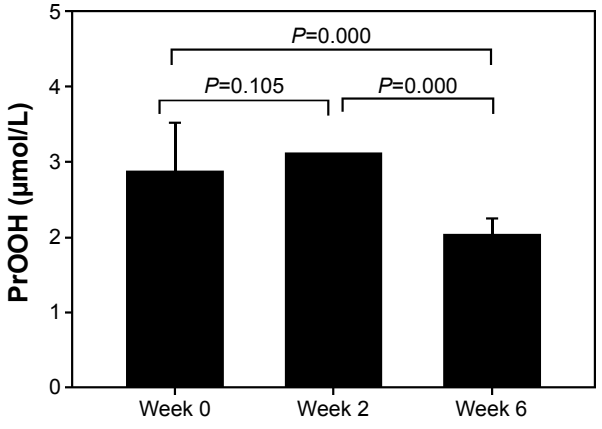

Figure 4 PrOOH (µmol/L) of all 27 elderly subjects during the control period (at week 0 and week 2) and after star fruit juice consumption for 4 weeks (at week 6).
Notes: Each bar represents the mean and standard deviation. Repeated measures ANOVA and Bonferroni post hoc tests were used for statistical analysis.
Abbreviations: ANOVA, analysis of variance; PrOOH, protein hydroperoxide.

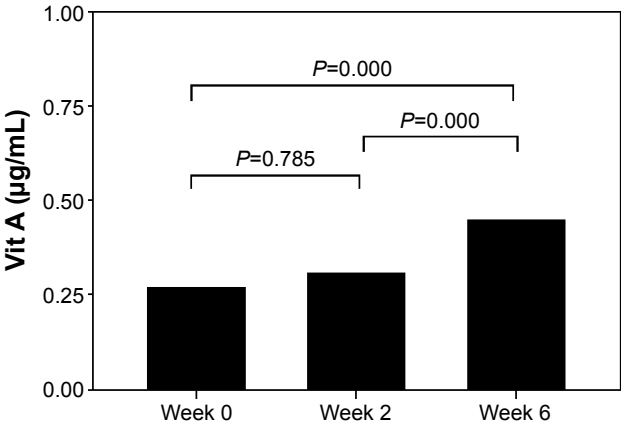

Figure 6 Vit A (µg/mL) of all 27 elderly subjects during the control period (at week 0 and week 2) and after star fruit juice consumption for 4 weeks (at week 6).
Notes: Each bar represents the mean and standard deviation. Repeated measures ANOVA and Bonferroni post hoc tests were used for statistical analysis.
Abbreviations: ANOVA, analysis of variance; Vit, vitamin.

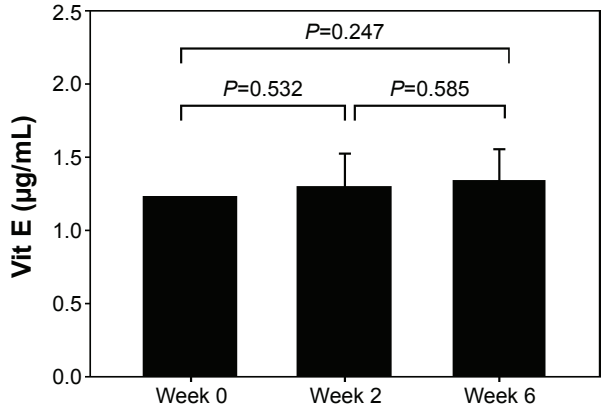

Figure 7 Vit E (µg/mL) levels of all 27 elderly subjects during the control period (at week 0 and week 2) and after star fruit juice consumption for 4 weeks (at week 6).
Notes: Each bar represents the mean and standard deviation. Repeated measures ANOVA and Bonferroni post hoc tests were used for statistical analysis.
Abbreviations: ANOVA, analysis of variance; Vit, vitamin.

of low-income females (1.4±1.3 µmol/L of Vit A and 2.1±1.1 µg/mL of Vit E) and males (1.4±1.8 µmol/L of Vit A and 2.0±1.1 µg/mL of Vit E).[28]

Star fruit is one of the many fruits that have claimed antioxidant properties, and contains many bioactive compounds such as total phenol, and Vit C and Vit A.[29] In addition, updated profiles of star fruit also show benefits for human health, such as anti-inflammatory and antioxidant activities.[14,15,17,30] Significant investigations on the antioxidant activities of vitamin-containing plants or functional food have shown that both Vit A and Vit C can scavenge the free radicals and hydrogen peroxide (H_2O_2).[31] This study evaluated the Vit A, Vit C, and Vit E levels in the star fruit extracts selected on Monday and Friday of each week of the consumption period (Table 3). The HPLC results showed significant changes in Vit C and Vit A levels in the plasma of elderly individuals after consumption (Table 2), which confirmed the previous study result.[29] Lim's work also showed high TAC (131±54 mg/100 g), ascorbic acid (5.2±1.9 mg/100 g), and ascorbic acid equivalent to antioxidant capacity (98±55 mg/100 g), when compared to orange (75±10, 67±9, and 31±10 mg/100 g) and mangosteen (54±7, 5.8±0.8, and 32.3±10.3 mg/100 g).

The results obtained from elderly subjects (Table 2) demonstrate the beneficial effects of consuming star fruit juice by a significant increase in TAC (Figure 1) as well as reduction of MDA (Figure 3) and PrOOH (Figure 4) levels. The antioxidants in the fruit may alter the level of oxidative stress (Figures 5 and 6). But the GSH and Vit E levels did not change either during the control period or after consumption, which is possibly due to the low concentration of Vit E in star fruit. GSH, Vit C, Vit A, and Vit E are antioxidant compounds that protect from oxidative stress in the biological system,[32] which can be evaluated by the marker TAC. Thus, a significantly increased TAC level may be a result of increase in Vit A and Vit E levels.

The interesting results of the lipid profiles presented in Table 2 show a significant increase and decrease of HDL-C and LDL-C, respectively, whereas cholesterol and triglyceride do not show a significant change. However, in a previous study in hamsters, Chau et al reported that the water-insoluble, fiber-rich fraction isolated from the pomace of star fruit has hypocholesterolemic and hypolipidemic activities.[33] The changes in HDL-C and LDL-C in this preliminary study relate to overall health, considering a previous report proposing that oxidative stress induces hypertension and abnormal lipoprotein metabolism.[8] Moreover, some reports suggested that HDL can inhibit the inflammatory process by several mechanisms, including promotion of cholesterol efflux, inhibition of LDL oxidation, and reduction of adhesion molecule expression.[34,35] Increased HDL in the elderly after they consumed star fruit juice possibly helps to explain why the previous preliminary study found significantly reduced proinflammatory cytokines such as tumor necrosis factor-α and interleukin-23.[20] Furthermore, the results of antioxidant status in the blood of elderly subjects can be used to explain why a significant reduction in NO level was observed in this study and the previous

Table 3 Vitamin C and vitamin A in star fruit extracts (100 g) of each week

Variable	First week		Second week		Third week		Fourth week		*P*-value
	Monday (n=10)	Friday (n=10)	Monday (n=10)	Friday (n=10)	Monday (n=10)	Friday (n=10)	Monday (n=10)	Friday (n=10)	
Vitamin C (mg)	17.1±0.5 (16.1–18.2)	16.2±0.28 (15.1–17.3)	16.9±0.6 (16.1–18.2)	17.0±0.2 (65.1–17.9)	17.1±0.6 (16.2–18.1)	16.2±0.7 (14.1–17.1)	17.5±0.5 (16.6–18.2)	16.9±0.4 (14.3–19.0)	0.78
Vitamin A (µg)	0.2±0.0 (0.2–0.3)	0.2±0.0 (0.2–0.3)	0.2±0.0 (0.2–0.3)	0.2±0.0 (0.1–0.2)	0.2±0.0 (0.1–0.2)	0.2±0.1 (0.1–0.2)	0.2±0.03 (0.1–0.2)	0.1±0.0 (0.1–0.2)	0.65

Notes: Data are presented as mean ± SD (min–max). Ten sampling extracts of star fruit at 100 g were randomly selected on Monday and Friday of each week. Repeated measures ANOVA was used to statistically compare between eight periods of 4 weeks during the experiment.
Abbreviations: ANOVA, analysis of variance; max, maximum; min, minimum; SD, standard deviation.

study. Contrasting evidence has been reported in a previous preliminary study of elderly subjects consuming star fruit juice, wherein a significant increase in walking distance with a significantly depressed NO level has been reported.[20] The results of this study can be explained possibly by the same mechanism that confirmed the study of Neville et al in 39 elderly subjects, who were found to have a positive correlation between L-ascorbic acid and grip strength status after fruit or vegetable consumption,[36] but some evidence still controversially confirmed the dose and time of vitamin C supplementation and muscle strength.[37–39]

The overall results of this preliminary study present the possible benefits of star fruit consumption by elderly individuals at 100 g twice daily for 1 month, with its antioxidant activity and also control of lipoproteins such as HDL-C and LDL-C. However, caution should be exercised due to the possible side effects from a prolonged intake, such as diarrhea as presented by three subjects in this study and adverse events from oxalate toxicity.[40] Therefore, for health safety, the CBC, liver function assays such as AST and ALT, and kidney function tests such as BUN and creatinine levels were evaluated during the experiment in this study following a Clinical Trial Application guideline.[41] The oxalate in star fruit may influence red blood cells, hemoglobin, and hematocrit as hemolytic anemia, and possible depress on the white blood cells and platelet production that effects on immune system. If the liver, which is the main organ for detoxification of all chemical compounds, is injured, hepatocellular enzymes such as AST and ALT are released and are found in high concentrations in the blood. Finally, BUN and creatinine are the clinical markers of renal dysfunction, for example, renal obstruction or damage.[42] If these values were elevated at the start of the protocol, subjects would have been excluded. Likewise, if the values were found to be elevated after 4 weeks of star fruit intake, this suggests some potential adverse effect of the supplementation.

Previous evidence suggested that the adverse effects on kidney function may be of concern, especially in patients with uremic condition[43] or chronic kidney disease.[44] However, results from the 27 elderly people in this study showed that BUN and creatinine levels in three experiments were within the reference range with no statistically significant difference (Table 1). Therefore, the consumption protocol in this study suggests safety for the healthy elderly subjects. However, the oxalate level was not evaluated and this should be considered a limitation of the present study. Thus, caution and critical awareness are needed in the future. At the end of this study, it can be stated that application of star fruit as a functional fruit should be developed and studied further for safe dosage and the available methods of administration in humans; also, other antioxidant compounds with strong antioxidant potential such as tannin, saponin, alkaloids,[30] epicatechin, gallic acid, and flavonoid C-glycoside[16,17] should be evaluated in elderly people.

Conclusion and limitations

It was found in this preliminary study that consumption of 100 g of star fruit juice, containing L-ascorbic acid (Vit C) and retinoic acid (Vit A), reduced the oxidative stress by decreasing lipid oxidation and improving the antioxidant status, as well as by increasing the HDL-C level and decreasing the LDL-C level in the blood of 27 healthy elderly people with a mean age of 69.5±5.3 years at the Elderly Health Care Center, Chiang Mai province. Although the low sample size in this study, the effect size of each result was also confirmed by G*Power analysis and was represented in Table 2. Because the previous report strongly suggested that a *P*-value may be not standardized in case of small sample size, calculation of Cohen (*d*) or effect size of each result is required. Cohen classified the effect sizes as small (d=0.2), moderate (d=0.5), and large ($d \geq 0.8$).[45] Thus, a significant result with a large effect size is viewed as more robust. Notably, significant values of each parameter shown in Table 2 correlated with a large or high effect size, for example, TAC (2.28), MDA (1.07), PrOOH (2.88), Vit C (2.48), HDL-C (1.22), and LDL-C (1.05), except Vit A (0.68). Therefore, the Vit A result still needs to be confirmed by a future study with a larger sample size. Future studies should aim to include a larger sample and focus on outcomes in both sexes, as well as elderly individuals living in different parts of the world. This may provide evidence for the more widespread use of star fruit in the future.

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Author contributions

JL was responsible for obtaining funding, designing the study, establishing all protocols, performing laboratory testing, and performing data analysis. JJL, RJB, and RP provided critical

comments, rechecked grammar, and assisted with the initial manuscript editing. Along with AY and DP, they also assisted in rechecking the original and final manuscript versions. All authors read and approved the final version. All authors contributed toward data analysis, drafting and revising the paper and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Institute for Population and Social Research. *Mahidol University Population projections for Thailand 2005–2025*. 1st ed. Bangkok: Mahidol University, Andison Press Product, Ltd; 2006 (March 2006 Revision).
2. Fontana L. Modulating human aging and age-associated diseases. *Biochim Biophys Acta*. 2009;1790:1133–1138.
3. Junqueria VBC, Barros SBM, Chan SS, et al. Aging and oxidative stress. *Mol Aspects Med*. 2004;25:5–16.
4. Cutler RG, Rodriguez H. *Critical Reviews of Oxidative Stress and Aging: Advances in Basic Science, Diagnostics and Intervention*. Singapore: World Scientific Publishing; 2003:1523.
5. Mezzetti A, Lafenna D, Romano F, et al. Systemic oxidative stress and its relationship with age and illness. Associazione Medica "Sabin". *J Am Geriatr Soc*. 1996;44:823–827.
6. Gianni P, Jan KJ, Douglas MJ, Stuart PM, Tarnopolsky MA. Oxidative stress and the mitochondrial theory of aging in human skeletal muscle. *Exp Gerontol*. 2004;39:1391–1400.
7. Rynan MJ, Dudash HJ, Docherty M, et al. Aging-dependent regulation of antioxidant enzymes and redox status in chronically loaded rat dorsiflexor muscles. *J Gerontol*. 2008;63:1015–1026.
8. Kumar A. Correlation between anthropometric measurement, lipid profile, dietary vitamins, serum antioxidants, lipoprotein (a) and lipid peroxides in known case of 345 elderly hypertensive South Asian aged 56–64 y – A hospital based study. *Asian Pac J Trop Biomed*. 2014;4:S189–S197.
9. Holzer M, Trieb M, Konya V, Wadsack C, Heinemann A, Marsche G. Aging affects high-density lipoprotein composition and function. *Biochim Biophys Acta*. 2014;1831:1442–1448.
10. Bursill CA, Lastro ML, Beattie DT, et al. High-density lipoproteins suppress chemokines and chemokine receptors in vitro and vivo. *Arterioscler Thromb Vasc Biol*. 2010;30:1773–1778.
11. Ferrari CKB. Functional foods and physical activities in health promotion of aging people. *Maturitas*. 2007;58:327–339.
12. Ferrari CKB. Functional foods, herbs, and nutraceuticals: towards biochemical mechanisms of healthy aging. *Biogerontology*. 2004;5:275–289.
13. O'Hare TJ. Postharvest physiology and storage of carambola (star fruit): a review. *Postharvest Biol Technol*. 1993;2:257–267.
14. Dasgupta P, Chakraborty P, Bala NN. Averrhoa carambola: an updated review. *Int J Phar Res Rev*. 2013;2:54–63.
15. Manda H, Vyas K, Pandya A, Singhal G. A completed review on: Averrhoa carambola. *World J Pharm Pharmaceut Sci*. 2012;1:17–33.
16. Shui G, Leng LP. Analysis of polyphenolic antioxidants in star fruit using liquid chromatography and mass spectrometry. *J Chromatogr A*. 2004;1022:67–75.
17. Yang D, Xie H, Jia X, Wei X. Flavonoid C-glycosides from star fruit and their antioxidant activity. *J Funct Foods*. 2015;16:204–210.
18. Krone CA, Ely JT. Ascorbic acid, glycation, glycahemoglobin and aging. *Med Hypotheses*. 2004;62:275–279.
19. Arora A, Maurya PK, Shaarma A. Protective role of L-ascorbic acid on erythrocytes subjects to oxidative stress during human aging. *New Biotechnol*. 2004;25:S2.
20. Leelarungrayub J, Laskin JJ, Bloomer RJ, Pinkaew D. Consumption of star fruit juice on pro-inflammatory markers and walking distance in the community dwelling elderly. *Arch Gerontol Geriatr*. 2016;64:6–12.
21. Leelarungrayub D, Ketsuwan N, Pothongsunun P, Klaphajone J, Bloomer RJ. Effects of N-acetylcysteine on oxidative stress, interleukin-2, and running time in sedentary men. *Gazz Med Ital*. 2011;170: 239–250.
22. Re R, Pellegrini N, Proteggente A, Pannala A, Yand M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med*. 1999;26:1231–1237.
23. Chirico S. High-performance liquid chromatograph-based thiobarbituric acid tests. *Methods Enzymol*. 1994;233:314–318.
24. Leelarungrayub D, Saidee K, Pothongsunun P, Pratanaphon S, Yankai A, Bloomer RJ. Six weeks of aerobic dance exercise improves blood oxidative stress status and increases interleukin-2 in previously sedentary women. *J Bodyw Mov Ther*. 2011;15:355–362.
25. Talwar D, Ha TK, Cooney J, Brownlee C, O'Reilly DS. A routine method for the simultaneous measurement of retinol, alpha-tocopherol and five carotenoids in human plasma by reverse phase HPLC. *Clin Chim Acta*. 1998;270:85–100.
26. Furusawa N. Rapid high-performance liquid chromatographic identification/quantification of total vitamin C in fruit drinks. *Food Control*. 2001;12:27–29.
27. Mutlu-Turkoglu U, IIhan E, Oztecan S, Kuru A, Ayka-Toker G, Uysal M. Age-related increases in plasma malondialdehyde and protein carbonyl levels and lymphocyte DNA damage in elderly subjects. *Clin Biochem*. 2003;36:397–400.
28. Olderwage-Theron WH, Samuel FO, Djoulde RD. Serum concentration and dietary intake of vitamin A and E in low-income South African elderly. *Clin Nutr*. 2010;29:119–123.
29. Lim YY, Lim TT, Tee JJ. Antioxidant properties of several tropical fruits: a comparative study. *Food Chem*. 2007;103:1003–1008.
30. Thomas S, Patil DA, Patil AG, Chandra N. Pharmacognostic evaluation and physiochemical analysis of *Averrhoa carambola* L. fruit. *J Herb Med Toxicol*. 2008;2:51–54.
31. Asensi-Fabada M, Munne-Bosch S. Vitamins in plants: occurrence, biosynthesis and antioxidant function. *Trends Plant Sci*. 2010;15:582–592.
32. Lopez-Alarcon C, Denicola A. Evaluating the antioxidant capacity of natural products: a review on chemical and cellular-based assays. *Anal Chim Acta*. 2013;763:1–10.
33. Chau CF, Chen CH, Lee MH. Characterization and physiochemical properties of some potential fibers derived from Averrhoa carambola. *Nahrung*. 2004;48(1):43–46.
34. Barter P. The inflammation: lipoprotein cycle. *Atherosclerosis*. 2005;6:15–20.
35. Holzer M, Gauster M, Pfeifer T, et al. Protein carmamylation renders high-density lipoprotein dysfunction dysfunctional. *Antioxid Redox Signal*. 2011;14:2337–2346.
36. Neville CE, Young IS, Gilchrist SE, et al. Effect of increased fruit and vegetable consumption on physical function and muscle strength in older adults. *Age*. 2013;35:2409–2422.
37. Cesari M, Pahor M, Bartali B, et al. Antioxidants and physical performance in elderly persons: the invecciare in Chianti (InCHIANTI) study. *Am J Clin Nutr*. 2004;79:289–294.
38. Halliwell B. Vitamin C and genomic stability. *Mutat Res*. 2001;475:29–35.
39. McGoinley C, Shafat A, Donnelly AE. Dose antioxidant vitamin supplementation protect against muscle damage? *Sports Med*. 2009;39:1011–1132.
40. Neto MM, Robl F, Netto JC. Intoxication by star fruit (*Averrhoa carambola*) in six dialysis patients (preliminary report). *Nephrol Dial Transplant*. 1998;13:570–572.
41. Lam K, Chan C, Done SJ, Levine MN, Reilly RM. Preclinical pharmacokinetics, biodistribution, radiation dosimetry and acute toxicity studies required for regulatory approval of a Clinical Trial Application for a Phase I/II clinical trial of ^{111}In-BzDTPA-pertuzumab. *Nucl Med Biol*. 2015;42:78–84.

42. Bauer JH, Brooks CS, Burch RN. Renal function studies in man with advanced renal insufficiency. *Am J Kidney Dis*. 1982;11:30–35.
43. Neto MM, Cardeal da Costa JA, Garcia-Cairasco N, Netto JC, Nakagawa B, Dantas M. Intoxication by star fruit (*Averrho carambola*) in 32 uremic patients: treatment and outcome. *Nephrol Dial Transplant*. 2003;18:120–125.
44. Abeysekera RA, Wijetunge S, Nanayakkara N, et al. Intoxication by star fruit toxicity: a cause of both acute kidney injury and chronic kidney disease: a report of two cases. *BMC Res Notes*. 2015;8:796.
45. Sullivan GM, Feinn R. Using effect size-or why the p value is not enough. *J Grad Med Educ*. 2012;4:279–282.