# Cashew Apple Juice Improves Physical Fitness and Oxidative Stress Status in the Middle-Aged and Elderly Volunteers


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***Objective:*** *Based on the crucial role of oxidative stress on physical performance and muscular strength, we aimed to determine the effect of a 12 week-consumption period of cashew apple juice, a substance possessing antioxidant effect, on the physical fitness and oxidative stress status of the middle aged and the elderly.*

***Material and Method:*** *A randomized double-blind placebo-controlled design was performed. Forty-five healthy middle-age and elderly volunteers received 1,000 ml of either cashew apple juice (120 and 240 mg/day) or placebo once daily for 12 weeks. The changes of malondialdehyde (MDA) level and the activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) enzymes in the plasma together with the health related-physical fitness was assessed every four weeks throughout the 12-week consumption period and four weeks after the cessation of the consumption.*

***Results:*** *The subjects who consumed the cashew apple juice at dose of 240 mg/day showed improved oxidative stress status and enhanced performance in 30-second chair stand test and 6-minute walk test. These data suggested that a 12-week consumption period of cashew apple juice consumption enhance muscle strength of lower extremities and the cardiopulmonary endurance.*

***Conclusion:*** *The possible underlying mechanism might occur partly via the improved oxidative stress status. Therefore, cashew apple fruit juice may be a potential candidate to enhance the health-related physical fitness for the middle aged and elderly volunteers. However, further research is required to provide better understanding about the precise underlying mechanism.*



***Keywords:*** *Cashew apple juice, Physical fitness, Oxidative stress*

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The number of geriatric population is on the rise, worldwide. The numbers are expected to more than double from 841 million in 2013 to two billion by 2050, particularly among less developing countries in Asia(1). Though ageing is a natural process, it often leads to biological and physiological changes that are primarily associated with a decrease in muscle mass ranging from 1% to 2% per year after the age of 50, including strength, endurance, and difficulty to maintain balance(2). In addition, the prevalence of slow movements, imbalance, immobility, falls, and disability increases(3,4). Additionally, aging also decreases the daily physical activity level(5). Since physical activity is an essential factor to delay the age-associated decline in body function and maintain the quality of life of older adults(6), a strategy to improve physical fitness of the older adults is required. Although both exercise and diet can successfully modify the physical fitness of the older adults, the time limitation is the big burden for physical exercise. Recent findings also showed that antioxidant supplement could provide beneficial effects to age-related disorders and slow down aging process(7) (Bonnefoy et al, 2003). Moreover, the polyphenol rich compound such as quercetin could extend lifespan and increase stress resistance(8,9) (Peng et al, 2011; Saul et al, 2008). Based on the crucial role of free radicals and anti-aging property of substance possessing


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antioxidant, the health benefit of polyphenol-rich fruit has attained much concentration. Therefore, the modulations of physical fitness by the functional foods and functional drinks have gained much attention.

Recent evidence shows that aging is associated with a general loss in oxidative stress tolerance[10]. As the age advances, the generation of free radicals and oxidative stress appears to be overwhelm the natural cellular antioxidant scavenging response resulting in the impaired homeostasis. Several studies have demonstrated that antioxidant is required to maintain healthy muscularactivity[11,12]. In addition, the physical performance and muscular strength are improved[13,14]. On the basis of the findings that antioxidant plays the crucial role on the physical performance and muscular strength, the beneficial effect of substance possessing antioxidant on the physical fitness of the middle aged and the elderly has been focused.

Cashew or *Anacardium occidentale* L., a plant in a family of Anacardiaceae, is native to South America. To date, it is widely distributed in tropical countries such as Thailand, Vietnam, and India. In Thailand, Cashew is widely cultivated in many regions of Thailand, including South, Northeast, North, and the Eastern part of Thailand. It has been recognized as one of the new economic plants. Cashew apple (pseudofruit) has been reported to possess many medicinal properties such as antitumor, antimicrobial[15], and antioxidant activity[16]. A preclinical study showed that cashew apple juice improved muscle performance in rats (unpublished data). Based on the effect of cashew apple juice on muscle performance and its antioxidant activity, it was hypothesized that consumption of cashew apple fruit juice for 12 weeks might improve physical fitness of the middle aged and the elderly. Since no scientific evidence is available, this study was carried out to examine the effects of a 12-week-consumption period of cashew apple on physical fitness of the middle aged and the elderly. In addition, the alterations of serum oxidative stress markers were also investigated to explore for the possible underlying mechanism.

## Material and Method

### *Preparation of samples*

Ripen cashew apples (var. Kaopayam) were obtained from Srisuphaluck Company, Phuket province. They were harvested between January and April and authenticated by Taxonomist of the Department of Agriculture, Ministry of Agriculture and Cooperatives, Thailand. After being cleaned, all fruits were washed and kept in the refrigerator until used. Cashew apple juice was extracted from cashew apple fruit by the juice blender, filtered, and heated to inactivate the natural enzymes presented in the juice. Cashew apple fruit juice was blended with water and sugar to provide the final concentrations of cashew apple fruit beverage at 23% and 33%, respectively (These concentrations were the doses that enhanced memory in our preclinical study). Then, they were pasteurized at 90°C for 10 minutes and hot filled with sterile bottles. Placebo was also prepared with the same procedures without cashew apple fruit juice. Each bottle contained 500 ml of fruit juice extract and provided approximate 160 k calories/bottle (serving). The appearances of both placebo and cashew apple fruit juice were the same.

### *Analysis of sample*

The content of total phenolic compounds in the plant extract was determined according to the well-established colorimetric assay[17]. Gallic acid was used as a standard and the results were expressed as milligram Gallic acid equivalent/ml.

Sample analysis was performed via gradient high performance liquid chromatography (HPLC) system consisting of 515 HPLC pump and 2998 Photodiode array detector of Waters company, USA. Chromatographic separation was carried out using Purospher®STAR, C-18 encapped (5 µm), LiChroCART® 250-4.6 and HPLC-Cartridge, Sorbet Lot No. HX255346 (Merk, Germany). Two mobile phases consisting of methanol and 2.5% acetic acid in deionized (DI) water were used to induce gradient elution. The injection volume was 20 µL and the flow rate was 1.0 mL/min. During HPLC analysis the solvent gradient was programmed as shown in Table 1 and data analysis was performed using Empower TM 3.

**Table 1.** Gradient program of HPLC analysis

| Times (minutes) | Solvents (%) | |
|---|---|---|
| | A (Methanol) | B (2.5% acetic acid) |
| 0 | 10 | 90 |
| 17 | 70 | 30 |
| 18 | 100 | - |
| 20 | 100 | - |
| 20.5 | 10 | 90 |
| 25 | 10 | 90 |

### *Subjects*

This study was approved by the Khon Kaen University Ethical Committee on Human Research (Project NO. HE531319) and Clinical Trial registration number: NCT02560077, and written informed consents were then obtained from subjects before conducting the study. Forty-five volunteers were recruited to the study. The inclusion criteria were described as (1) healthy and aged between 55 and 70 years, (2) body mass indices (BMI) ranged between 18.5 and 24.9, (3) no history of the following diseases, cardiovascular diseases, respiratory diseases, neuropsychological diseases, diabetes, liver diseases, cancer, autoimmune disease, or hematological disorders, (4) no consumption of the nutraceutical compounds or herbs or drugs that influence the nervous system functions at least one month before participating in this present study, (5) no history of head injury, and (6) smoking no more than 10 cigarettes/day. On the day of the study, all volunteers felt well and refrained from drinking tea or coffee. To ensure that all subjects were healthy, they were subjected to a physical examination by a physician. The subjects who were not healthy according to the physical examination or showed serious allergies and side effects or could not follow the whole study protocol or failed to complete the study either via side effects or unwilling to participate in the study were excluded.

Each volunteer was requested to complete a medical health questionnaire before participating in the study. All subjects underwent extensive medical evaluation in order to ascertain subject suitability for entering the phase of clinical trial. They were randomly allocated to either the treatments or placebo groups. In addition to their normal diet, the subjects had to consume 1,000 mL/day (2 x 500 ml) of cashew apple fruit juice or placebo (natural mineral water with sucrose and color in order to mimic the apple fruit juice which contains the same calorie) for 12 weeks. No significant differences in mean age, education, and body mass index among groups were observed. No participants withdrew from the study and no adverse effects were reported throughout the study. Volunteers were also asked to complete a simple diet questionnaire throughout the study to determine whether their diet and fluid intake changed over the course of the study. No significant changes were recorded (data were not shown).

### *Study design and intervention*

A randomized, double-blind, placebo-controlled was carried out to determine the effect of a 12-week-consumption period of cashew apple on physical fitness of the middle aged and the elderly. The subjects in experimental groups were instructed to consume total 1,000 ml of functional drink which contained cashew apple juice at dose of 120 or 240 mg/day for 12 weeks. All subjects must consume the assigned drink both after breakfast and after dinner. On the arrival at Integrative Complementary Alternative Research and Development Center, subjects were allowed to rest for 30 minutes in a quiet, temperature-controlled room. Subsequently, anthropometric measurements and vital signs were recorded. Then, blood was collected in the fasted state for the determination of oxidative stress markers, including malondialdehyde (MDA) level and the activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) enzymes in the plasma followed by food. After meal, they subjected to a battery test of health related physical fitness comprising of four main domains, including strength and endurance of skeletal muscles, joint flexibility, body composition, and cardio respiratory endurance[18]. All parameters mentioned earlier were determined two weeks prior to the intervention as baseline data. In addition, these parameters were also assessed every four weeks throughout the 12 week-study period and four weeks after the cessation of cashew apple based-drink consumption (Fig. 1).

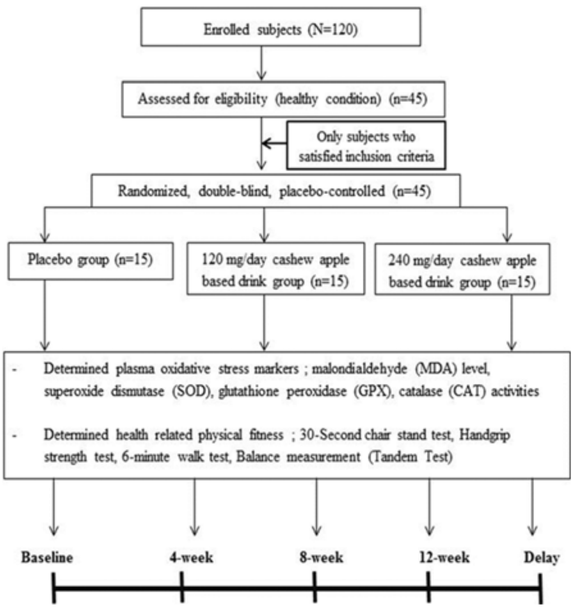


**Fig. 1** Schematic diagram showing study protocol.

***Outcome measures***

***Health-related physical fitness assessment***

To assess the health related physical fitness in the volunteers, the battery tests for physical assessment were carried out[18,19]. The battery tests were composed of various tests as described below.

*30-second chair stand test:*

The strength of lower extremities muscles was assessed using ability to sit down and stand up from the chair within the limited time. According to this test, the ability to rise fully from a seated position with back straight and feet flat on the floor, without pushing off with the arms within 30 seconds of each individual was recorded and used as an index.

*Handgrip strength test:*

According to this test, the subjects had to squeeze the dynamometer with maximum isometric effort, which was maintained for about five seconds. Each subject must perform five trials per each hand with a one-minute inter trial period. The best value of two trials for each hand was chosen and the average value of both hands was recorded. This value was used as an index to indicate the upper body muscular strength.

*6-minute walk test:*

The cardio-pulmonary endurance was assessed by measuring the maximum distance (meters) that an individual can walk within six minutes along a 45.7-meter-rectangular course.

*Tandem stance test:*

This test was performed with both eyes opened and with eye closed while one foot place in front of the other foot when both feet touching each other. Standing duration without swaying was recorded.

***Determination of oxidative stress markers***

Blood was drawn after overnight fasting with a dry disposable syringe and needle, under all aseptic conditions by venipuncture in the antecubital vein in sterile dry acid washed vials[20] and was collected in the ethylene diaminotetra acetic acid (EDTA). The tubes were kept on ice, and then centrifuged at 4°C (3,000 rpm for 15 minutes) for the separation of plasma. Then, this plasma was used for the estimation of malondialdehyde level (MDA) and the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) enzymes.

***Determination of malondialdehyde level***

Malondialdehyde (MDA), an end product of unsaturated fatty acid peroxidation, is measured as an index of lipid peroxidation. Plasma MDA level was measured via thiobarbituric acid reactive products (TBAR)[21]. In brief, 150 µl of plasma was reacted with 125 µl of 10% triochloroacetic acid, 125 µl of 5 mM EDTA, 125 µl of 8% SDS, and 10 µl of 0.5 µg/ml of butylatedhydroxytoluine (BHT). The mixture was left for 10 minutes, then 0.6% thiobarbituric acid (TBA) was added in an equal volume and mixture was heated for 30 minutes in a boiling water bath. After cooling at room temperature, the mixture was centrifuged at 25°C, 10,000 g for 10 minutes. The absorbance of the supernatant was determined at 532 nm.

***2.8 determination of scavenging enzymes activities***

The activity of SOD was performed via cytochrome c-based spectrophotometric assay. According to this process, xanthine/xanthine oxidase system and native cytochrome c were served as the $O^{2-}$ source and indicating scavenger respectively. SOD was determined by measuring the capacity to lower the rate of $O^{2}$-mediated reduction of ferricytochrome c at 550 nm[22]. SOD activity was presented as units per milligram of protein (U/mg protein). One unit of enzyme activity was defined as the quantity of SOD required to inhibit the reduction rate of cytochrome C by 50%.

CAT activity was measured by recording the reduction rate of $H_2O_2$ absorbance at 240 nm[23] and expressed as µmol $H_2O_2$/min/mg protein. In this study, GSH-Px was measured based on the oxidation of glutathione (GSH) to oxidized glutathione (GSSG) catalyzed by GSH-Px, which was coupled to the recycling of GSSG back to GSH utilizing glutathione reductase (GR) and NADPH (b-Nicotinamide Adenine Dinucleotide Phosphate, reduced). The decrease in NADPH absorbance measured at 340 nm during the oxidation of NADPH to NADP+ reflected GSH-Px activity because it was the rate limiting factor of the coupled reactions[24]. GSH-Px activity was expressed as U/mg protein. One unit of the enzyme was defined as micromole (µmol) of reduced nicotinamideadenine dinucleotide phosphate (NADPH) oxidized per minute.

***Statistical analysis***

Data of malondialdehyde level and scavenging enzymes activities were expressed as means $\pm$ SEM whereas data of physical fitness were expressed as mean $\pm$ SD. Statistical analysis was performed by using one-way ANOVA. The results were considered statistically significant at *p*-value <0.05.

## Results

### *Sample analysis*

The data obtained from this present study showed that placebo contained total phenolic compounds at concentration of 0.06±0.02 mg GAE/mL whereas the 120 mg/day and 240 mg/day cashew apple juice contained total phenolic compounds at concentrations of 0.54±0.02 and 0.63±0.13 mg GAE/mL, respectively.

The HPLC fingerprint of cashew apple juice based drink (Fig. 2). Gallic acid and quercetin were identified according to their retention times and spectral characteristics of their peaks compared with standard. The ultraviolet spectrum of chromatographic bands presented in the fingerprinting of the samples indicated the presence of gallic acid in the low and high doses of cashew apple juice drink at concentrations of 0.12 and 0.28 mg GA/500 ml sample whereas the presence of quercetin in the low and high concentrations of cashew apple fruit juice drink were 6.46 and 9.34 mg QE/500 ml sample.

### *The general characteristic of volunteers*

Forty-five healthy volunteers were enrolled in this present study. All volunteers were participated in the study throughout the study period. No withdrawal case was observed. The general characteristic data of all groups were summarized in Table 2. There were no significant differences between the groups.

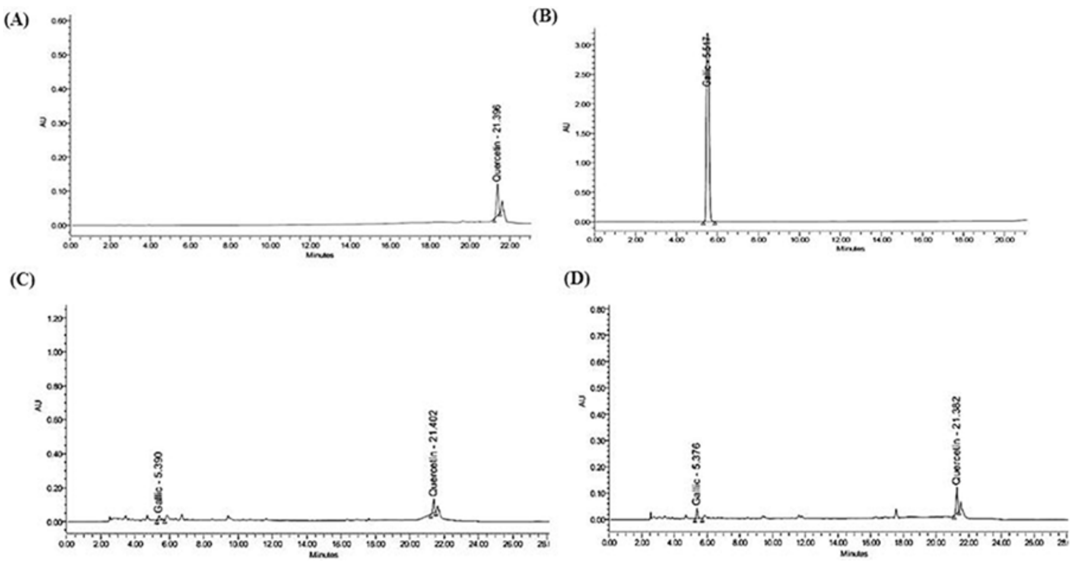


**Fig. 2** High performance liquid chromatography (HPLC) chromatogram of A) standard of gallic B) standard of quercetin C) cashew apple juice at dose of 120 mg/day D) cashew at dose of 240 mg/day.

**Table 2.** Demographics speech assistants (SAs) (n = 6)

| Characteristics | Placebo (mean ± SD) | 120 mg/day (mean ± SD) | 240 mg/day (mean ± SD) | *p*-value |
|---|---|---|---|---|
| Age (years) | 55.13±2.50 | 56.27±3.63 | 55.20±2.48 | 0.9422 |
| Weight (kilogram) | 58.07±8.68 | 58.29±5.44 | 58.88±9.78 | 0.8129 |
| Body mass index | 23.57±3.59 | 23.50±2.49 | 23.48±3.33 | 0.9430 |
| Heart rate (bpm) | 72.27±6.22 | 72.27±13.30 | 70.93±5.86 | 0.5504 |
| Respiratory rate (times/minute) | 21.27±1.28 | 21.47±1.51 | 20.53±2.58 | 0.6571 |
| Systolic blood pressure (mmHg) | 131.40±12.41 | 131.67±12.26 | 127.87±9.43 | 0.3874 |
| Diastolic blood pressure (mmHg) | 78.80±9.44 | 80.27±7.25 | 79.13±7.55 | 0.9157 |
| Metabolic rate (kcal) | 1,351.33±197.96 | 1,420.60±237.28 | 1,319.87±193.07 | 0.6628 |

### *Effects of cashew apple juice on physical fitness*

The effects of various doses of cashew apple juice on various parameters indicating physical fitness are shown in Table 3. It was found that at 12-week-consumption period, the subjects who consumed cashew apple juice at dose of 240$\pm$mg/day had significant increase by 30-second chair stand test and 6-minute walk test (*p*-value <0.01; compared to placebo group). However, no other significant effects were observed. In addition, these changes were not observed at four weeks after the cessation of cashew apple juice consumption.

### *Effects of cashew apple juice on malondialdehyde level*

Based on the crucial role of oxidative stress on memory impairment as previously mentioned, this part of study was focused on the effects of cashew apple juice on MDA level, an index reflecting lipid peroxidation induced by oxidative stress. It was demonstrated that the subjects who consumed cashew apple juice at dose 240 mg/day for 12-week had significant decrease in the MDA level (Fig. 3) (*p*-value <0.05; compared to placebo group).

### *Effects of cashew apple juice on scavenging enzymes activities*

The effects of cashew apple juice on the activities of main scavenger enzymes, including superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) in plasma were also determined (Fig. 4). It was shown that consumption of cashew apple juice of 240 mg/day for 12 weeks had significant increase in plasma GSH-Px activity in the middle aged and elderly volunteers (*p*-value <001; compared to placebo group) whereas no changes of superoxide dismutase (SOD) and catalase (CAT) activities were observed. At four weeks after the cessation of cashew apple juice consumption, no change of GSH-Px activity was presented.

## Discussion

This present study is the first study to demonstrate that a 12-week-consumption period of cashew apple juice based beverage could improve the performance of 30-second chair stand test and 6-minute walk test, which reflected the enhanced strength of muscle of lower extremities and the enhanced cardiopulmonary endurance together with the improved oxidative stress status and scavenging enzyme activity of the middle aged and elderly volunteers.

It has been reported that as the age advances, the physical fitness declines. The loss of muscle strength begins around the age of 30. This loss results from a muscle mass reduction that is associated with alterations in the percentage of intramuscular contractile tissue and a reduced capacity for muscular activation[25]. The age-related muscle mass reduction is reported to involve the excess free radicals due to the decreased glutathione peroxidase enzyme activity in skeletal muscle[26]. In addition to the decreased muscle mass, the decreased oxygen uptake and the decreased mitochondria production of energy (ATP) are also observed[27]. Moreover, recent study also demonstrated that the muscle strength was closely correlated with increased muscle blood flow[28]. This present study showed that the subjects who consumed the high dose of the functional drink that contained cashew apple extract at dose of 240 mg/kg BW showed the enhanced strength of muscle of lower extremities via 30 second-chair stand test together with the elevation of GSH-Px but decreased plasma MDA level. Since muscle is the largest component of body and largest source of oxidative stress production, the plasma oxidative stress markers should indicate oxidative stress status in the muscle. It has been reported that the elevation of oxidative stress can promote muscle weakness via two mechanisms. It can either induce muscle loss or modify the function of contractile protein, which in turn induces contractile dysfunction[29]. Due to these pieces of information, it has been suggested that the improved muscle performance of lower extremities via 30 second-chair stand test observed in the subjects who consumed high dose of cashew apple juice might occur partly via the enhanced GSH-Px activity, which in turn decreased oxidative stress and finally gave rise to the improved muscle performance.

Beside the improved muscle performance of the lower extremities, the improved cardiopulmonary endurance via 6-minute walk test was also observed in the subjects who consumed the high dose of cashew apple juice. Cardio-pulmonary fitness, a health-related component of physical fitness, is defined as the ability of the circulatory, respiratory, and muscular systems to supply oxygen during sustained physical activity. It is found that the increased cardiopulmonary fitness is associated with the enhancement of baroreceptor reflex sensitivity (BRS)[30]. Recently, it has been shown that increased plasma lipid peroxidation is associated with reduction in baroreflex sensitivity[31,32]. Therefore, the enhanced GSH-Px activity induced by cashew apple juice might decrease oxidative stress which in turn

**Table 3.** Effects of various doses of cashew apple juice based drink on health related physical fitness (compared to placebo)

| Index | Group | Baseline (mean ± SD) | 4-week (mean ± SD) | 8-week (mean ± SD) | 12-week (mean ± SD) | Delay (mean ± SD) |
|---|---|---|---|---|---|---|
| 30 second Chair-stand test | Placebo | 17.07±2.15 | 18.00±2.39 | 18.27±2.19 | 18.60±2.44 | 18.47±2.67 |
| | 120 mg | 17.80±2.04<br>F (1, 28) = 0.916, $p$ = 0.347 | 18.60±2.20<br>F (1, 28) = 0.512, $p$ = 0.480 | 18.07±2.37<br>F (1, 28) = 0.058, $p$ = 0.812 | 18.40±2.38<br>F (1, 28) = 0.051, $p$ = 0.822 | 18.80±2.76<br>F (1, 28) = 0.113, $p$ = 0.739 |
| | 240 mg | 16.93±3.03<br>F (1, 28) = 0.019, $p$ = 0.891 | 18.07±3.10<br>F (1, 28) = 0.004, $p$ = 0.948 | 19.07±2.60<br>F (1, 28) = 0.830, $p$ = 0.370 | 20.60±1.24*<br>F (1, 28) = 7.985, $p$ = 0.009 | 18.33±3.58<br>F (1, 28) = 0.013, $p$ = 0.909 |
| Grip strength Right side (kg) | Placebo | 21.73±2.28 | 21.53±1.73 | 21.60±2.20 | 21.33±2.32 | 21.53±2.29 |
| | 120 mg | 21.27±1.79<br>F (1, 28) = 0.388, $p$ = 0.538 | 21.47±1.68<br>F (1, 28) = 0.011, $p$ = 0.916 | 21.53±1.64<br>F (1, 28) = 0.009, $p$ = 0.926 | 21.20±1.74<br>F (1, 28) = 0.032, $p$ = 0.860 | 21.20±1.66<br>F (1, 28) = 0.208, $p$ = 0.652 |
| | 240 mg | 21.53±2.00<br>F (1, 28) = 0.065, $p$ = 0.800 | 21.87±1.92<br>F (1, 28) = 0.250, $p$ = 0.621 | 21.53±2.00<br>F (1, 28) = 0.008, $p$ = 0.931 | 21.53±1.60<br>F (1, 28) = 0.076, $p$ = 0.785 | 21.47±1.73<br>F (1, 28) = 0.008, $p$ = 0.929 |
| Grip strength Left side (kg) | Placebo | 19.27±1.87 | 19.20±1.78 | 19.27±1.87 | 19.40±1.76 | 19.20±1.93 |
| | 120 mg | 20.87±3.40<br>F (1, 28) = 2.552, $p$ = 0.221 | 20.80±2.40<br>F (1, 28) = 3.651, $p$ = 0.366 | 20.87±3.18<br>F (1, 28) = 2.820, $p$ = 0.104 | 21.07±3.24<br>F (1, 28) = 3.062, $p$ = 0.091 | 21.07±3.31<br>F (1, 28) = 3.564, $p$ = 0.069 |
| | 240 mg | 19.53±2.67<br>F (1, 28) = 0.100, $p$ = 0.754 | 19.53±2.20<br>F (1, 28) = 0.208, $p$ = 0.652 | 19.60±2.56<br>F (1, 28) = 0.166, $p$ = 0.687 | 19.73±2.40<br>F (1, 28) = 0.187, $p$ = 0.668 | 19.53±2.67<br>F (1, 28) = 0.153, $p$ = 0.698 |
| 6 minute walk test (meters) | Placebo | 539.27±22.31 | 539.80±21.98 | 541.53±19.06 | 543.60±29.94 | 541.20±25.12 |
| | 120 mg | 541.60±29.60<br>F (1, 28) = 1.497, $p$ = 0.231 | 539.47±17.37<br>F (1, 28) = 0.002, $p$ = 0.964 | 547.27±23.19<br>F (1, 28) = 0.547, $p$ = 0.466 | 543.93±28.16<br>F (1, 28) = 0.001, $p$ = 0.975 | 535.80±23.94<br>F (1, 28) = 0.363, $p$ = 0.552 |
| | 240 mg | 539.67±31.24<br>F (1, 28) = 1.193, $p$ = 0.284 | 542.47±19.61<br>F (1, 28) = 0.123, $p$ = 0.728 | 565.27±37.22<br>F (1, 28) = 3.054, $p$ = 0.091 | 580.00±37.16**<br>F (1, 28) = 8.728, $p$ = 0.006 | 558.93±40.68<br>F (1, 28) = 2.062, $p$ = 0.162 |
| Tandem (R) foot in front, (EO) | Placebo | 121.33±8.27 | 120.13±8.90 | 120.27±13.66 | 121.87±13.38 | 120.60±9.64 |
| | 120 mg | 124.00±9.67<br>F (1, 28) = 0.659, $p$ = 0.204 | 122.80±12.01<br>F (1, 28) = 0.477, $p$ = 0.495 | 118.53±10.27<br>F (1, 28) = 0.154, $p$ = 0.697 | 121.40±9.77<br>F (1, 28) = 0.012, $p$ = 0.914 | 120.60±6.90<br>F (1, 28) = 0.108, $p$ = 0.745 |
| | 240 mg | 123.20±11.60<br>F (1, 28) = 0.257, $p$ = 0.616 | 123.93±10.41<br>F (1, 28) = 1.155, $p$ = 0.292 | 121.40±14.40<br>F (1, 28) = 0.049, $p$ = 0.827 | 128.33±5.70<br>F (1, 28) = 2.966, $p$ = 0.096 | 124.47±9.95<br>F (1, 28) = 0.649, $p$ = 0.427 |

* $p$-value <0.05, ** $p$-value <0.01 compared to placebo

**Table 3.** cont.

| Index | Group | Baseline (mean ± SD) | 4-week (mean ± SD) | 8-week (mean ± SD) | 12-week (mean ± SD) | Delay (mean ± SD) |
|---|---|---|---|---|---|---|
| Tandem (L) foot in front,(EO) | Placebo | 77.80±13.59 | 78.07±13.23 | 80.13±15.34 | 75.30±14.51 | 78.07±19.15 |
| | 120 mg | 79.93±20.71 | 81.33±21.78 | 79.60±19.15 | 83.27±18.17 | 80.80±18.60 |
| | | F (1, 28) = 0.111, *p* = 0.741 | F (1, 28) = 0.247, *p* = 0.623 | F (1, 28) = 0.007, *p* = 0.934 | F (1, 28) = 1.717, *p* = 0.201 | F (1, 28) = 0.157, *p* = 0.695 |
| | 240 mg | 81.80±18.44 | 81.87±19.17 | 80.73±14.57 | 85.93±20.61 | 81.53±18.83 |
| | | F (1, 28) = 0.457, *p* = 0.504 | F (1, 28) = 0.399, *p* = 0.533 | F (1, 28) = 0.012, *p* = 0.913 | F (1, 28) = 2.620, *p* = 0.117 | F (1, 28) = 0.250, *p* = 0.621 |
| Tandem (R) foot in front,(EC) | Placebo | 32.20±12.03 | 28.67±8.95 | 31.60±10.29 | 30.67±11.54 | 30.80±9.52 |
| | 120 mg | 28.87±10.51 | 29.40±11.02 | 26.67±8.65 | 30.07±8.61 | 28.47±10.75 |
| | | F (1, 28) = 0.653, *p* = 0.426 | F (1, 28) = 0.040, *p* = 0.843 | F (1, 28) = 2.362, *p* = 0.136 | F (1, 28) = 0.026, *p* = 0.873 | F (1, 28) = 0.396, *p* = 0.534 |
| | 240 mg | 31.40±9.63 | 28.27±8.01 | 30.73±9.68 | 29.93±8.98 | 30.73±10.22 |
| | | F (1, 28) = 0.040, *p* = 0.842 | F (1, 28) = 0.017, *p* = 0.898 | F (1, 28) = 0.056, *p* = 0.814 | F (1, 28) = 0.038, *p* = 0.847 | F (1, 28) = 0.000, *p* = 0.895 |
| Tandem (L) foot in front,(EC) | Placebo | 17.53±4.66 | 19.13±5.79 | 18.27±5.97 | 19.07±5.35 | 18.80±5.54 |
| | 120 mg | 20.87±5.00 | 20.40±4.26 | 21.67±4.73 | 20.47±5.88 | 22.00±6.04 |
| | | F (1, 28) = 3.571, *p* = 0.069 | F (1, 28) = 0.466, *p* = 0.501 | F (1, 28) = 2.989, *p* = 0.095 | F (1, 28) = 0.465, *p* = 0.501 | F (1, 28) = 2.287, *p* = 0.142 |
| | 240 mg | 19.87±6.10 | 18.13±5.89 | 20.80±5.91 | 21.67±6.14 | 21.27±6.10 |
| | | F (1, 28) = 1.385, *p* = 0.249 | F (1, 28) = 0.009, *p* = 0.926 | F (1, 28) = 1.365, *p* = 0.253 | F (1, 28) = 1.529, *p* = 0.226 | F (1, 28) = 1.343, *p* = 0.256 |

* *p*-value <0.05, ** *p*-value <0.01 compared to placebo

leading to the enhanced BRS resulting in the enhanced cardiopulmonary fitness. However, the determination of baroreceptor reflex change was required to confirm this postulation.

Previous study has revealed that quercetin, a substance possessing antioxidant activity[33], can improve baroreceptor reflex sensitivity[34] and muscle performance[35]. Therefore, quercetin might be the possible active ingredient which was responsible for both the improved muscle strength of the lower extremities and the enhanced baroreceptor reflex sensitivity.

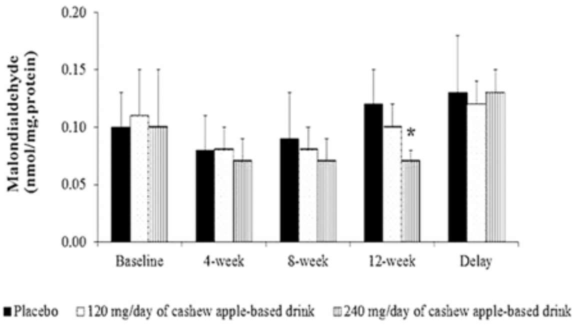


**Fig. 3** Effects of cashew apple-based drink on the level of malondialdehyde (MDA) in plasma. The column and bar represent mean ± SEM (n = 15/group). * The *p*-value <0.05 when compared to placebo group.

## Conclusion

The present study has clearly demonstrated that a 12-week-consumption period of cashew apple juice-based drink can improve the muscle strength of lower extremities and cardiopulmonary fitness of the middle aged and elderly volunteers. The possible underlying mechanism might occur partly via the decreased oxidative stress status in the body. However, further researches are still required to provide the better understanding about the precise underlying mechanism.

## What is already known on this topic?

The previous study the cashew apple juice can improved enhance the muscle power in animal and no seriously the side effect. Little is known about beneficial effect of cashew apple juice on physical fitness in human.

## What this study adds?

The results obtained from this study demonstrated that the cashew apple juice based drink

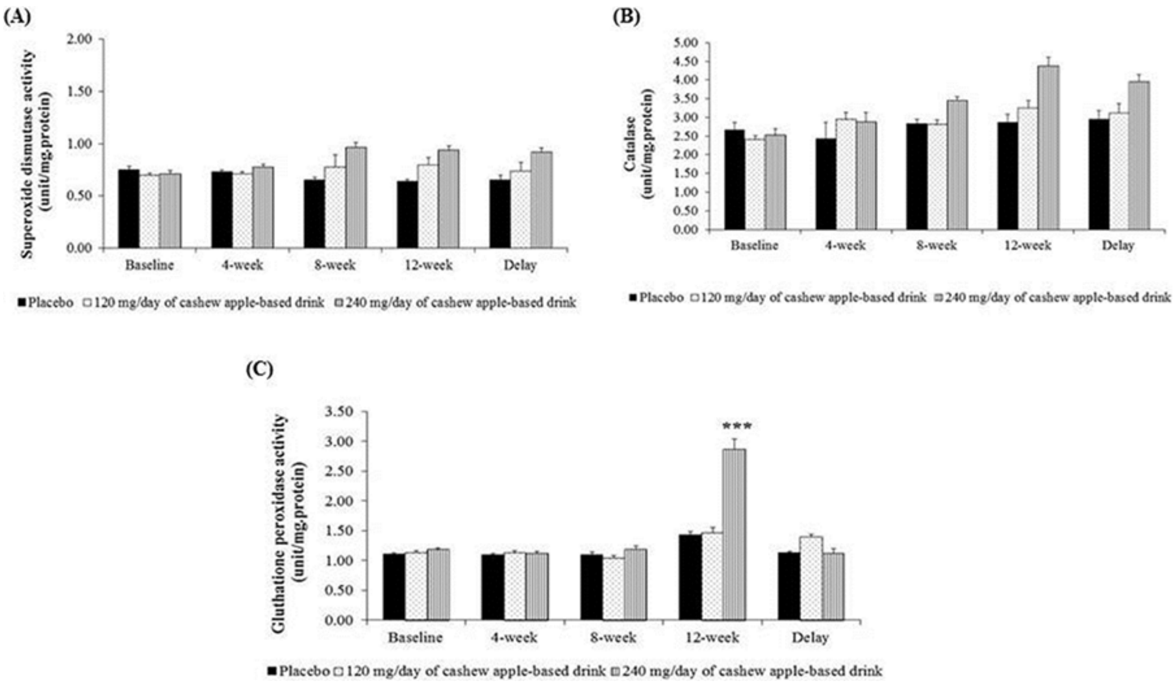


**Fig. 4** Effects of cashew apple-based drink on the activities of scavenger enzymes in plasma, including: A) Superoxide dismutase (SOD) activity, B) Catalase (CAT) activity, and C) Glutathione peroxidase (GSH-Px) activity. The column and bar represent mean ± SEM (n = 15/group). *** The *p*-value <0.001 when compared to placebo group.

improved physical fitness. Therefore, the developed cashew apple juice based drink has the potential to be served as the functional drink in order to enhance physical fitness for the middle-aged and elderly.

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**Potential conflicts of interest**

None.

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***ศักยภาพของน้ำมะม่วงหิมพานต์ต่อการเปลี่ยนแปลงสมรรถภาพทางกายและสารอนุมูลอิสระในอาสาสมัครปกติวัยกลางคนและวัยสูงอายุ***

*ศศลักษณ์ แก้วบุตรา, จินตนาภรณ์ วัฒนธร, วิภาวี ทูคำมี, สุภาพร มัชฌิมะปุระ, เทอดไท ทองอุ่น*

***วัตถุประสงค์:*** *ผลของสารอนุมูลอิสระมีฤทธิ์ต่อสมรรถนะทางกายและความแข็งแรงของกล้ามเนื้อ การศึกษาครั้งนี้จึงมุ่งศึกษาผลของการบริโภคเครื่องดื่มน้ำมะม่วงหิมพานต์ 12 สัปดาห์ที่น่าจะมีศักยภาพการต้านอนุมูลอิสระ เพื่อประเมินสมรรถนะทางกายและการทำงานของเอนไซม์ต้านอนุมูลอิสระของอาสาสมัครวัยกลางคนและวัยสูงอายุ*

***วัสดุและวิธีการ:*** *การศึกษาในครั้งนี้อาสาสมัครที่ได้รับการคัดเลือกเข้าร่วมโครงการจะถูกแบ่งเป็นกลุ่มต่างๆ คือ กลุ่มที่ได้รับยาหลอก และกลุ่มที่ได้รับเครื่องดื่มสุขภาพน้ำมะม่วงหิมพานต์ขนาดวันละ 120 มิลลิกรัมและ 240 มิลลิกรัม อาสาสมัครจะต้องบริโภคสารทดสอบเป็นเวลา 12 สัปดาห์ และจะได้รับการประเมินดังนี้ 1) ประเมินกลไกการทำงานของเครื่องดื่มสุขภาพน้ำมะม่วงหิมพานต์ประเมินดัชนีความเครียดออกซิเดชัน (oxidative stress) โดยใช้ระดับสาร malondialdehyde (MDA) 2) การทำงานของเอนไซม์ต้านอนุมูลอิสระ ได้แก่ superoxide dismutase (SOD), catalase (CAT) และ glutathione peroxidase (GSH-Px) เป็นดัชนี 3) ประเมินสมรรถนะทางกาย โดยจะทำการประเมินดัชนีที่กล่าวข้างต้น ก่อนเริ่มบริโภคเครื่องดื่มสุขภาพน้ำมะม่วงหิมพานต์หลังบริโภคไปทุก 4 สัปดาห์ตลอดระยะเวลาการบริโภค 12 สัปดาห์นอกจากนั้นหลังจากหยุดบริโภคไปแล้ว 4 สัปดาห์ของอาสาสมัครวัยกลางคนและวัยสูงอายุ*

***ผลการศึกษา:*** *อาสาสมัครที่บริโภคเครื่องดื่มสุขภาพน้ำมะม่วงหิมพานต์มีการเปลี่ยนแปลงดังนี้ มีการเพิ่มความแข็งของของกล้ามเนื้อส่วนขาและเพิ่มความทนทานของหัวใจและหลอดเลือด กลไกการเพิ่มความแข็งแรงและความทนทานนี้น่าจะเกิดจากการลดความเครียดออกซิเดชันโดยเพิ่มการทำงานของ GSH-Px และลด MDA ในเลือด*

***สรุป:*** *ผลการศึกษาแสดงให้เห็นว่าเครื่องดื่มสุขภาพน้ำมะม่วงหิมพานต์เป็นอาหารสุขภาพที่มีฤทธิ์ เพิ่มทั้งสมรรถนะทางกายดังนั้นน้ำเครื่องดื่มสุขภาพมะม่วงหิมพานต์จึงเป็นอาหารสุขภาพที่มีศักยภาพสำหรับวัยกลางคนและสูงอายุ*