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Antioxidant activities of an iridoid-rich beverage containing noni juice, cornelian cherries, and olive extract *in vitro* and in human volunteers

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Abstract

Plant-based foods are dietary sources of antioxidant compounds that are helpful in preventing or alleviating oxidative stress. Iridoids are a class of secondary plant metabolites with antioxidant activities. An iridoid-rich beverage, Tahitian Noni® Max (Max), that contains *Morinda citrifolia* (noni) juice, *Cornus mas* (cornelian cherry) fruit purée, and *Olea europaea* (olive) extract was evaluated for antioxidant activity *in vitro* and in healthy nonsmoking young adult volunteers. Iridoid analyses were also performed via high performance liquid chromatography (HPLC) with samples of Max to confirm the presence of deacetylasperulosidic acid, asperulosidic acid, scandoside, loganic acid, and oleuropein. The oxygen radical absorbance capacity (ORAC) assay results reveal that Max possesses notable antioxidant potential, with four times more *in vitro* antioxidant potential than pasteurized blood orange juice. In the human trial, all participants in the Max group experienced increases in erythrocyte and plasma antioxidant activities within 40 minutes of ingestion. Further, the average erythrocyte antioxidant activity of this group increased approximately 28 times more, and just over 49 times more for average plasma antioxidant activity, than those in a blood orange juice control group ($P < 0.001$). The findings of the current study indicates that Max is a significant dietary source of iridoids and improves the antioxidant activity of components of the blood within 40 minutes of ingestion.

Keywords: *Morinda citrifolia*; *Cornus mas*; Antioxidant; Iridoids

1. Introduction

Maintenance of the life and health of the human body involves many biochemical reactions that involve oxygen. One result of many of these life-sustaining reactions is the generation of oxygen radicals which may be utilized in other important chemical reactions or controlled by the antioxidant systems of our cells. But some situations may overwhelm endogenous antioxidant defenses with excess reactive oxygen species and other free radicals, creating a state of oxidative stress and subsequent cellular and tissue damage [1]. When such stress is great enough or prolonged, health may be impaired [2].

The foods we ingest may aid or impair antioxidant defenses. Plant-based foods provide dietary antioxidants that help prevent or alleviate oxidative stress [3]. Epidemiological studies have also revealed that diets rich in fruits and vegetables may reduce free-radical-induced oxidative damage and lipid peroxidation [4]. Clinical trials have also demonstrated the antioxidant properties of specific plant-based foods. For example, juice from *Morinda citrifolia* fruit (noni) reduced tobacco smoke-induced oxidative damage of lymphocyte DNA in adult smokers when ingested daily for one month [5]. Daily ingestion of *Cornus mas* fruit (cornelian cherry) extract improved vasomotor symptoms in postmenopausal women, an effect attributed to antioxidant properties [6]. One month of daily oral supplementation with an olive (*Olea europaea*) extract reduced systemic oxidative stress, as measured by urinary levels of 8-isoprostane, in healthy adults [7].

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The specific examples cited immediately above involved *in vitro* tests or antioxidant evaluations after several weeks of daily ingestion. Further, each study involved very specific conditions under which free radicals are produced. The tests in the current study were conducted to evaluate the *in vitro* activity of a blend of these food items as well as measure its ability to exert immediate antioxidant effects within the body.

2. Materials and methods

2.1. Test material

Tahitian Noni® Max (Max, currently supplied by Partner.co, Midvale, Utah, USA), was evaluated in this study. This is a pasteurized beverage containing *Morinda citrifolia* (noni) fruit nectar from pure noni purée and from juice concentrate from French Polynesia, *Cornus mas* (cornelian cherry) fruit purée, grape juice concentrate, blueberry juice from concentrate, red sour cherry juice concentrate, olive extract, and cranberry juice concentrate. Pasteurized blood orange juice (Parmalat Santal brand, Collecchio, Italy) was purchased from a retail food market.

2.2. Iridoid analysis

Samples from separate batches were analyzed. Analysis for iridoids was performed by high performance liquid chromatography (HPLC), according to a previously reported method [8, 9]. Prior to analysis, one g of sample was dissolved in 10 mL 1:1 (v/v) water:methanol and then filtered through a 0.45 μm PTFE filter. Iridoid standards were dissolved in methanol (MeOH) to a concentration of 1 mg/mL and further diluted to produce standard curves for iridoids identified in the samples. Chromatographic separations were performed on a Waters 2690 separations module coupled with 996 PDA detectors (Waters Corporation, Milford, MA, USA), equipped with a C_{18} column. Elution was accomplished with two mobile phases, acetonitrile (MeCN) and 0.1% formic acid in H_2O (v/v), with a flow rate of 0.8 mL/min. A linear gradient of 100% aqueous formic acid (0.1%) for 0-5 min, followed by 70% aqueous formic acid and 30% MeCN for 40 min, was used. The PDA detector was monitored in the range of 210-400 nm. The injection volume was 10 μL for each of the sample solutions. The column temperature was maintained at 25 °C. Iridoids were identified in the samples by comparison of retention times and UV absorbance of compounds in the samples and the standards. Total iridoids per 100 mL, as mean ± standard deviation (SD), were determined by the addition of individual iridoid concentrations.

2.3. Oxygen radical absorbance capacity assay

The oxygen radical absorbance capacity (ORAC) assay was used to evaluate the antioxidant potential of the fruit-based beverages evaluated in this study. This assay was performed according to a previously reported method [10].

A 0.08 μM fluorescein solution was prepared with 75mM potassium phosphate buffer (pH 7.4) and stored in a refrigerator (~ 4 °C) prior to use in the assays. Filtered samples were also diluted with buffer. A solution of 153 mM 2,2'-azobis-2-methyl-propanimidamide, dihydrochloride (AAHP) in phosphate buffer was prepared fresh on the days of assay. Standards of a water-soluble vitamin E analogue, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), were also prepared in buffer at concentrations of 100, 50, 25, 12.5, 6.25 μM on the day of the assay. To separate wells of a black plastic 96-well microplate was added 25 μL of buffer (blank), Trolox standards, or duplicate samples. Next, 150 μL of 0.08 μM fluorescein were loaded into each well, followed by incubation of the plate at 37 °C for 5 minutes. Twenty-five μL 153 mM AAPH was then loaded into each well. The fluorescence of each well was then measured in a Synergy™ HT microplate reader, at 1 minute read intervals for 60 minutes, with 485 ± 20 nm excitation, 528 ± 20 nm emission, and incubation at 37 °C. Areas under fluorescence intensity curves were calculated, with the areas for the blanks being subtracted. The areas under the curve for the various Trolox concentrations were used to prepare a standard curve that was used to determine μmole Trolox equivalents (TE)/mL.

2.4. Human study

Twenty nonsmoking healthy adults (10 male, 10 female), ages 18 to 29 years, were enrolled in the study and assigned to three separate groups, with subsequent crossover to another treatment group after initial testing was completed. Informed consent was obtained from each participant. All groups participated in an overnight fast and then, on an empty stomach, drank 200 mL of either Max, blood orange juice or water. Each group then drank an additional 300 mL of water (500 mL total combined volume). Immediately prior to drinking Max, blood orange juice or water, a blood sample was collected from each participant. They did not drink or eat anything else until after blood samples were collected a second time, which was accomplished 40 minutes later. Sample collection and evaluation of plasma and erythrocyte antioxidant activities were performed according to a previously reported method [11]. Briefly, blood was drawn from the ulnar vein into tubes, treated with an anticoagulant (heparin) and centrifuged. The supernatant was used for plasma antioxidant

activity determination. The red cell mass was frozen at -18°C, and the resulting hemolysate was subjected to antioxidant activity analysis. Antioxidant activity testing was carried out in a measurement cell containing a potassium ferrocyanide and potassium ferricyanide redox mediator solution in a phosphate buffer (pH 7.2). An aliquot of the sample was added to the redox mediator solution, and the electrical potential measured with a platinum screen-printed electrode. The potential of the mediator solution was measured prior to the addition of the sample. Antioxidant activities of the samples were calculated using electrical potential according to a formula described in the previously reported method. Chemical interactions of the antioxidants in the samples will cause a redox potential shift in the mediator solution that can be detected by the electrode. The antioxidant activity (AOA) is the effective equivalent concentration of antioxidant, in reference to a Trolox standard, that can reduce potassium ferricyanide and is expressed as mmol equivalents per liter, or mM eq [12]. Differences between fasting antioxidant activities and those measured 40 minutes after drinking Max, blood orange juice or water were calculated for everyone. This was done for both plasma and red cell (erythrocyte) mass.

Group means and standard deviations were determined. A nonparametric test, the Mann–Whitney U test, was used to determine the significance of observed differences in group means. Data were also presented graphically, including standard box plots with 25th percentile, median and 75th percentile values, as well as whisker boundaries representing values within 1.5 times the interquartile range.

3. Results and Discussion

3.1. Iridoid content

The average, ± standard deviation, concentration of total iridoids in samples of Max was 207.75 ± 7.80 mg/100 mL. The iridoids present in the samples were confirmed as being deacetylasperulosidic acid, asperulosidic acid, scandoside, loganic acid, and oleuropein (a secoiridoid).

3.2. ORAC assay

Results of the ORAC assay are summarized in Table 1. The average value for Max is greater than the ORAC value reported previously for noni fruit [13] but less than that reported for cornelian cherry puree and olive fruit extracts [10, 14]. Max fits within the range of several other antioxidant-rich fruits, while also having higher values than those reported for acai, pomegranate, and goji [15]. Our *in vitro* test results also suggest that Max has four times more potential than blood orange juice. But the ORAC value for the blood orange juice sample is on par with previously published values for pasteurized orange juice, indicating the dampening effect of thermal processing on ORAC values [16]. Further, iridoids are a group of biologically active phytochemicals with known antioxidant activities mediated through increased expression of antioxidant enzymes [5, 8, 17]. This is especially relevant for several of the major iridoids in Max where digestion, absorption and metabolism occur before they exert their influence on the antioxidant systems of the body. As such, *in vitro* testing provides only an indication or estimate of potential antioxidant activity in humans. True antioxidant activity must be determined in adult volunteers.

Table 1 Mean, ± standard deviation, oxygen radical absorbance capacities (ORAC) of beverages evaluated in the trial

	uM TE/mL
Tahitian Noni® Max	22.82 ± 0.99
Blood orange juice	5.51 ± 0.25

3.3. Human study

The initial erythrocyte and plasma antioxidant activities of all study participants are compared in Figure 1. As is demonstrated by the significant difference in ranges of these two measurements ($P < 0.001$), red blood cells have a greater antioxidant system than that of plasma. This is likely an adaptation to the high potential for oxidative stress that accompanies the delivery of oxygen to, and the removal of carbon dioxide from, the tissues of the body. To mitigate oxidative damage and maintain hemoglobin's heme group in a reduced ferrous (Fe^{2+}) state, erythrocytes contain substantial antioxidant defenses [18]. Accordingly, the antioxidant activities of the hemolysate samples may be more pertinent for evaluating potential health benefits than plasma alone.

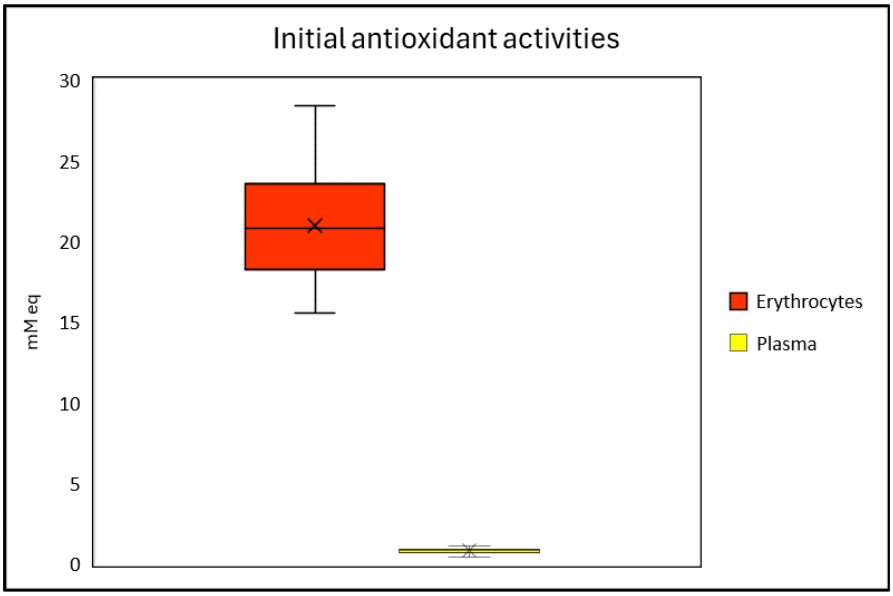

Figure 1 Box plots of initial antioxidant activities of erythrocyte mass and plasma samples from healthy volunteers. The antioxidant activities of hemolysate samples (from erythrocytes) were significantly greater than those of plasma samples ($P < 0.001$). Average values, denoted by an X, are included within the box plots

In the Max group, all participants (100%) experienced increases in erythrocyte and plasma antioxidant activities (Figure 2 and Figure 3). Only 40% of participants in the water group had any increase in erythrocyte antioxidant activity, with declines in values occurring in the other 60%. Only 30% of this group saw increases in plasma antioxidant activity. In the blood orange juice group, 60% of the volunteers had increases in plasma antioxidant activity, with 60% also experiencing the same with their red blood cells.

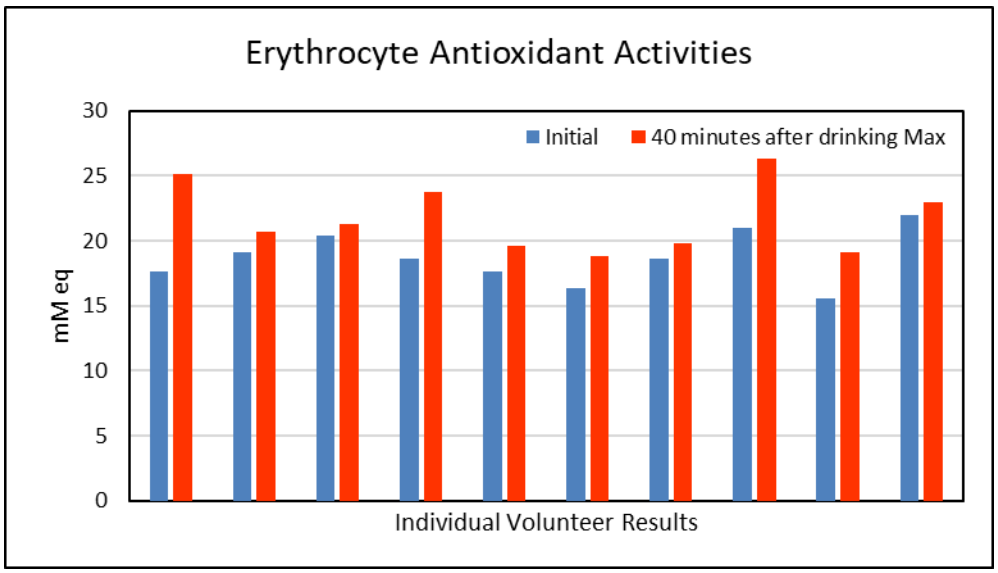

Figure 2 Antioxidant activities of erythrocytes from healthy volunteers before (initial) and 40 minutes after drinking 200 mL Max. Antioxidant activities increased in all volunteers after drinking Max.

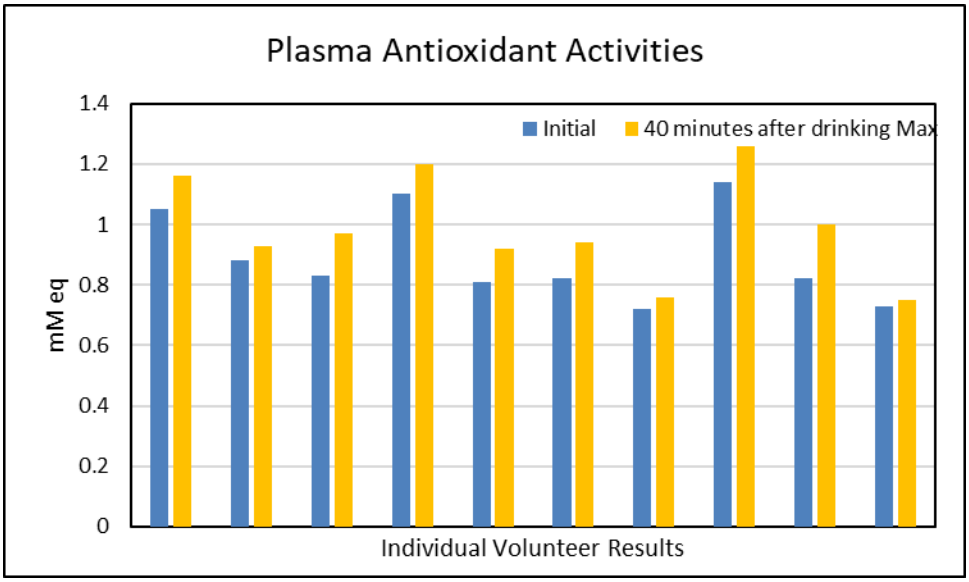

Figure 3 Antioxidant activities of plasma samples from healthy volunteers before (initial) and 40 minutes after drinking 200 mL Max. Antioxidant activities increased in all volunteers after drinking Max

Box plots of changes in erythrocyte antioxidant activity—from initial to 40 minutes post ingestion—are compared in Figure 4. Significant intergroup differences in mean change between Max and the other two treatments were evident ($P < 0.001$). The Max group experienced the largest average increase of 3.06 mM eq, approximately 28 times more than the average change (0.11 mM eq) in the blood orange juice group ($P < 0.001$). There was a slight decrease in the average antioxidant activity in the water group, although there was no statistical difference between initial and 40-minute levels. The difference between the Max group and the water group was, of course, significant ($P < 0.001$).

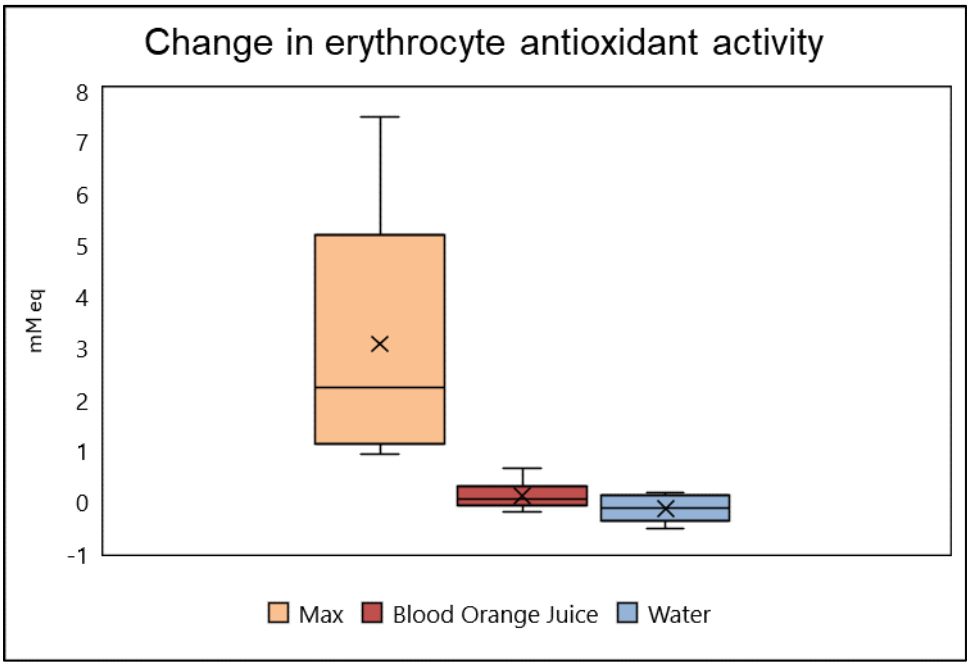

Figure 4 Box plots of changes in average erythrocyte antioxidant activity 40 minutes after drinking Max, blood orange juice or water. The mean antioxidant activity of the Max group was significantly greater than that of the blood orange juice ($P < 0.001$) and water group ($P < 0.001$). Average values, denoted by an X, are included within the box plots

The changes in plasma antioxidant activities are compared in Figure 5. As can be seen, the Max group had a greater average increase (0.10 mM eq) than the blood orange juice (0.002 mM eq) and water (-0.02 mM eq) groups ($P < 0.001$). The Max group's average change was just over 49 times more than that of the blood orange juice group. Again, there was essentially no change in the water group. The findings from both the plasma and erythrocyte samples demonstrate significantly increased antioxidant activity within 40 minutes of drinking Max.

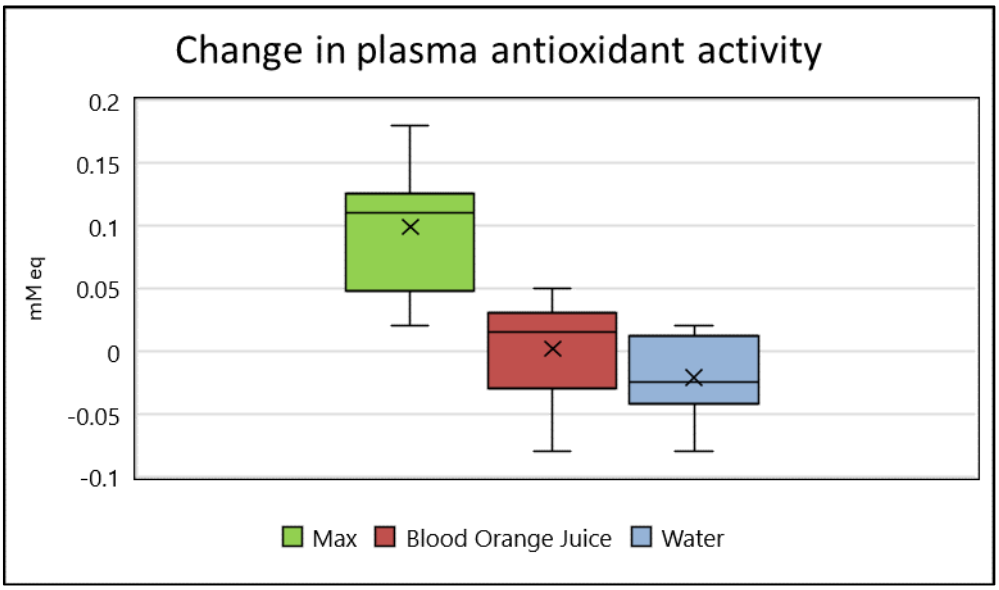

Figure 5 Box plots of changes in average plasma antioxidant activity 40 minutes after drinking Max, blood orange juice or water. The average increase in plasma antioxidant was approximately 49 times greater in the Max group than in the blood orange juice group ($P = 0.001$). Average values, denoted by an X, are included within the box plots

In this study, Max was more effective than pasteurized blood orange juice at increasing the antioxidant activity of erythrocytes and plasma. This is notable since the antioxidant action of orange juice is well established. A scoping review, systematic review, and meta-analysis of 21 human intervention studies of orange juice indicates that consumption of orange juice lowers interleukin-6 (IL-6), and inflammatory cytokine, as well as MDA in health people and those at-risk for chronic diseases [19]. The improved antioxidant response to Max over blood orange juice in this trial suggests exceptional benefits when compared to other common fruit juices, due to the unique combination of phytochemicals in noni fruit, cornelian cherries and olive extract.

4. Conclusion

The results of the *in vitro* test and human study reveal that Max improves the antioxidant activity of components of the blood within 40 minutes of ingestion, especially within the red blood cells. As such, daily ingestion may maintain or enhance the overall health of the human body. This is especially relevant under environmental, metabolic or lifestyle conditions that lead to increased oxidative stress.

Compliance with ethical standards

Acknowledgments

The potentiometric antioxidant activity test with human volunteers was conducted by Mark Khodos and Khiena Brainina, Ural State University.

Disclosure of conflict of interest

Both authors are employed by Partner.co which produces the investigational product of this study.

Statement of ethical approval

The human portion of this study was conducted with reference to the ethical principles outlined in the World Medical Association's Declaration of Helsinki.

Statement of Informed consent

Informed consent was obtained from all study volunteers.

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