Antioxidant Activity of Noni Juice in Vitro and in Human Volunteers

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Abstract

*Morinda citrifolia* (noni) fruit juice has been found to provide a wide range of potential health benefits. Among these are the reduction of free radicals and protection against lipid peroxide DNA damage in heavy cigarette smokers and athletes who had exercised to the point of exhaustion. These benefits have been observed after drinking a beverage containing a blend of noni juice from French Polynesia, Tahitian Noni® Juice (TNJ). To determine if TNJ exerts more immediate antioxidant effects, and under less extreme conditions, in vitro tests and a trial involving healthy young adult men were conducted. In the human study, volunteers drank 200 mL TNJ, orange juice or water following an overnight fast. Blood samples were collected before and 1 hour following ingestion of the beverages. Plasma and red cell lysate (erythrocyte) samples were measured for antioxidant activity potentiometrically. TNJ exhibited high in vitro antioxidant activity in the 2,2-diphenylpicrylhydrazyl (DPPH) radical scavenging, reducing power and lipid hydroperoxide scavenging assays. TNJ also significantly increased mean antioxidant activity in plasma and erythrocytes of healthy volunteers. The effect of TNJ in erythrocytes was approximately 4.6 times greater than that of orange juice. There were no increases observed in the water group. The results of this study reveal that TNJ can provide antioxidant benefits shortly after ingestion and under more everyday conditions, not only in extreme circumstances or when consumed repeatedly.

Keywords: *Morinda citrifolia*, noni juice, antioxidant, clinical trial

1. Introduction

Oxygen is indispensable to life and is involved in many biological processes of the body. Oxygen radicals are produced during many of the biochemical reactions of these processes. These are either utilized in other important chemical reactions that are necessary for homeostasis or are controlled by the antioxidant systems of our cells. However, several situations may result in the production of more reactive oxygen species and other free radicals than can be adequately handled by endogenous antioxidant defenses (Sharifi-Rad et al., 2020). When this occurs, a state of oxidative stress results, thus leading to damage of cellular components and tissues. This damage, when great enough, may lead to impairment of health in a variety of forms (Pham-Hy et al., 2008).

Fruits and vegetables are major sources of dietary antioxidants which may be helpful in preventing or reducing oxidative stress (Wynder et al., 1992). Epidemiological studies have also revealed that diets rich in fruits and vegetables may reduce free-radical-induced oxidative damage and lipid peroxidation in cigarette smokers (Steinmetz and Potter, 1991). *Morinda citrifolia*, commonly known as noni, is a small to medium sized tree that grows in many tropical regions of the world (Morton, 1992). The fruit of this tree has a history of use as food and for the improvement of health among Pacific Islanders and in Southeast Asia (West et al., 2006). Among the reported health benefits of drinking noni fruit juice is antioxidant protection against plasma free radical elevation and lipid peroxide-induced DNA damage from cigarette smoke exposure (Wang et al., 2009; Wang et al., 2012). These benefits were observed in heavy smokers after they had consumed Tahitian Noni® Juice (TNJ) in a 30-day, placebo-controlled, double-blinded, and randomized clinical trial. Additionally, antioxidant activity was observed in a clinical trial involving athletes who had exercised to the point of exhaustion, with improved endurance and decreased lipid oxidation occurring after drinking TNJ for three weeks (Palu et al., 2009).

In these previous human studies, the antioxidant potential of noni juice was evaluated only after multiple 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 ability of noni juice to have a more immediate effect within
the body and if this effect is measurable under more common situations involving nonsmokers who are not involved in exercise to the point of physical exhaustion.

2. Method

2.1 Test Material

Tahitian Noni® Juice (TNJ), previously provided by Morinda, Inc. (now supplied by Partner.co, Midvale, Utah, USA), was evaluated in this study. TNJ is made from noni fruit puree from French Polynesia that is blended with grape and blueberry juice and then pasteurized. All harvesting and processing procedures were done in accordance with good agricultural practices and good manufacturing practices. Pasteurized orange juice (Parmalat Santal brand, Collecchio, Italy) was purchased from a retail food market.

2.2 In Vitro Antioxidant Tests

In the 2,2-diphenylpicrylhydrazyl (DPPH) radical scavenging assay, one mL of sample was filtered and diluted to 10 mL with deionized water. The diluted sample, and a water blank, were combined 1:1 (v/v) with 0.4 mM DPPH in ethanol. The absorbance of each sample and blank was read at 515 nm with a Synergy™ HT microplate reader (BioTek Instruments, Inc., Winooski, VT, USA) after incubation at 25 ± 2°C for 35 minutes. The radical scavenging activity (expressed as a percentage) was calculated by dividing the difference in absorbance between the blank and sample by the absorbance of the blank alone.

In the reducing power assay, one mL of sample, vehicle blank (deionized water), or an ascorbic acid standard is combined with 7.5 μL cumene hydroperoxide at varying concentrations to produce a standard curve. The solution was then incubated at 50 °C for 20 minutes. Immediately after incubation, 2.5 mL of 10% trichloroacetic acid was added, and the solution was centrifuged. Afterwards, 2.5 mL of the supernatant was combined with 2.5 mL deionized water and 0.5 mL 1% ferric chloride. The new solution was transferred to a 96-well clear plastic microplate and incubated at room temperature for 30 minutes. The absorbance at 700 nm was then measured with the microplate reader. Sample results were calculated as mmol Fe²⁺/mL, using the ascorbic acid standard curve generated during the assay (Szteo et al., 2002; Lado et al., 2004). In this test, as well as in the DPPH test, samples were evaluated in triplicate.

In the lipid hydroperoxide scavenging assay, cumene hydroperoxide will oxidize leucomethylene blue (LMB) in the presence of hemoglobin. This produces methylene blue, which absorbs light at 660 nm. Antioxidants react with cumene hydroperoxide and, subsequently, inhibit methylene blue formation. As such, the antioxidant activity of a sample may be determined by the reduction in absorbance at 660 nm. To perform this assay, 5 mg of LMB was dissolved in 8 mL N,N-dimethylformamide. Next, 1.4 mL of Triton X-100 and 5.5 mg of hemoglobin was mixed with 90.6 mL of 0.05 M potassium dihydrogen phosphate buffer (pH 5.0) and sonicated for 30 minutes. Three milliliters of the resulting buffered LMB solution were combined with 7.5 μL cumene hydroperoxide at varying concentrations to produce a standard curve. Absorbance of the reactions were measured at 600 nm in a spectrophotometer (Cary 1C, Varian, Inc., Palo Alto, CA, USA). The reactions were repeated with the addition of TNJ and the absorbance recorded again. The lipid hydroperoxide scavenging activity was determined by the percent reduction in absorbance at 600 nm.

2.3 Human Study

Thirty nonsmoking healthy males, ages 19 to 25 years, were enrolled in the study and assigned to three separate groups. 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 TNJ, orange juice or water. Each group then drank an additional 300 mL of water (500 mL total combined volume). Immediately prior to drinking TNJ, 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 one hour later. Preliminary tests with two volunteers had revealed that the one-hour interval is the optimum sampling time for detecting peak antioxidant activity following ingestion of the juices. Sample collection and evaluation of plasma and erythrocyte antioxidant activities were performed according to a previously reported method (Braining et al., 2009). 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 activity 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 (Brainina et al., 2012). Differences between fasting antioxidant activities and those measured 1 hour after drinking TNJ, 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. The data were evaluated for normality with the Shapiro-Wilk test. Next, 1-way analysis of variance (ANOVA) was conducted to detect intergroup differences. This was followed by Student’s T-test to compare the means of the individual groups. Data were also presented graphically in 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 In Vitro Tests

Results of the in vitro antioxidant tests are summarized in Table 1. DPPH is a free radical that is frequently used in evaluating the antioxidant activity of natural products. The DPPH scavenging activity of TNJ is high and is very similar to that of *Cornus officinalis* (Asian cornelian cherry) fruit juice and somewhat greater than that of *Cornus mas* (European cornelian cherry) fruit puree (West et al., 2012). The relative comparability of the reducing power of all three of these is of interest since they all contain iridoids, a group of biological active phytochemicals with known antioxidant activities (Deng et al., 2011; Wang et al., 2013). However, they differ in their compositions relative to other antioxidant phytochemicals. Even so, 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. In vitro antioxidant activities of TNJ

<table>
<thead>
<tr>
<th>Assay</th>
<th>Mean ± Standard Deviation</th>
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<tbody>
<tr>
<td>DPPH radical scavenging (% activity)</td>
<td>81.41 ± 0.32</td>
</tr>
<tr>
<td>Reducing power (mmol Fe²⁺/mL)</td>
<td>13.05 ± 0.25</td>
</tr>
<tr>
<td>Lipid hydroperoxide scavenging (% activity)</td>
<td>91.45 ± 1.82</td>
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</tbody>
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The lipid hydroperoxide scavenging activity of TNJ was very high, greater than 91%. Lipid hydroperoxides are very biologically relevant compounds and are useful for measuring antioxidant activity. Malondialdehyde (MDA) is a major lipid peroxidation product that is a marker for oxidative stress within the human body, with elevated levels being associated with several chronic health conditions (Menzel et al. 2021). 4-Hydroxyxynenal (HNE) is another lipid peroxidation product that may inhibit DNA and protein synthesis and is associated with arteriosclerosis (Gallo et al., 2020). Such direct lipid hydroperoxide scavenging activity should be considered when interpreting the results from a previous clinical trial of TNJ wherein lipid hydroperoxide levels were significantly decreased in heavy smokers (Wang et al., 2009). Induction of superoxide dismutase activity is one antioxidant mechanism of noni juice (Ma et al., 2013). But the results of this lipid hydroperoxide scavenging assay suggests that additional mechanisms involving more direct neutralizing chemical reactions may also be responsible for the outcomes observed in the previous clinical trial.

3.2 Human Study

Pooled initial erythrocyte and plasma antioxidant activities are compared in Figure 1. There is a significant difference in the ranges of these two groups (P < 0.001). The data indicate that red blood cells have a greater antioxidant system than that of the plasma. This reflects the fact that erythrocytes are under threat of considerable oxidative stress due to their roles in delivering oxygen to, and the removal of carbon dioxide from, the tissues of the body. As a matter of protection against oxidative damage and to maintain hemoglobin’s heme group in a reduced ferrous (Fe²⁺) state, erythrocytes contain substantial antioxidant defenses (Franco et al., 2019). It would seem, therefore, that the antioxidant activities of the hemolysate samples are of more interest in evaluating potential health benefits than plasma alone.
Figure 1. Box plots of initial antioxidant activities of red cell hemolysate and plasma samples from healthy volunteers. The antioxidant activities of hemolysate samples 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 TNJ group, all participants (100%) experienced increases in erythrocyte and plasma antioxidant activities (Figure 2 and Figure 3). Only 30% of participants in the water group experience any increase in plasma antioxidant activity, with declines in values occurring in the other 70%. The same percentages apply to antioxidant activities of the erythrocytes in the water group. In the orange juice group, only 70% of the 1-hour plasma samples had greater antioxidant activity than initial samples. But in this same group, erythrocyte antioxidant activity increased in 90% of volunteers.

Figure 2. Antioxidant activities of erythrocytes from healthy volunteers before (initial) and one hour after drinking 200 mL TNJ. Antioxidant activities increased in all volunteers after drinking TNJ.
Figure 3. Antioxidant activities of plasma samples from healthy volunteers before (initial) and one hour after drinking 200 mL TNJ. Antioxidant activities increased in all volunteers after drinking TNJ.

Box plots of changes in erythrocyte antioxidant activity—from initial to one hour post-ingestion—are compared in Figure 4. ANOVA revealed significant intergroup differences in mean change (P < 0.002). The TNJ group experienced the greatest average increase of 2.79 mM eq. The average change in the orange juice group was 0.601 mM eq, only a fraction of that experienced by the TNJ group (P < 0.016). There was a slight decrease in the average antioxidant activity in the water group, although there was no statistical difference between initial and 1-hour levels. The difference between the TNJ group and the water group was, of course, significant (P < 0.001).

Figure 4. Box plots of changes in erythrocyte antioxidant activity in healthy young adult males 1 hour after drinking TNJ, orange juice or water. The mean antioxidant activity of the TNJ group was significantly greater than that of the orange juice (P < 0.016) 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 TNJ group tended to have larger increases than the orange juice and water groups (P < 0.006, ANOVA). The TNJ group’s average change was roughly double that of the orange juice group. Again, there was essentially no change in the water group. However, the increase seen in the TNJ group was not as great as occurred in erythrocyte hemolysate samples. Nevertheless, the results from both the plasma and erythrocyte samples demonstrate significantly increased antioxidant activity when drinking TNJ.
In this study, TNJ was more effective than pasteurized orange juice at increasing the antioxidant activity of erythrocytes. 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), an inflammatory cytokine, as well as MDA in health people and those at-risk for chronic diseases (Cara et al., 2022). It has already been demonstrated that TNJ provides improved antioxidant protection when compared against fruit juice placebos, including grape, blueberry, and blackberry juices (Wang et al., 2012; Palu et al., 2009). The improved antioxidant response to TNJ over orange juice in this trial further substantiates the exceptional benefits, due to the unique combination of phytochemicals in noni fruit, as compared to other common fruit juices.

As discussed briefly above, erythrocytes require substantial antioxidant defenses since they are constantly exposed to potential oxidative stress. There are several systems which help protect these cells. These include enzymatic conversion of glucose to the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) by glucose-6-phosphate dehydrogenase. The NADPH is then used to regenerate glutathione which is a major intracellular antioxidant molecule used to detoxify oxidants. The use and recycling of glutathione is also carried out by glutathione peroxidase and glutathione reductase (Pizzorno, 2014). While there is some limited evidence that noni fruit extracts increase the activities of these enzymes, this has not yet been investigated in a human study (Muralidharan et al., 2010). But the reduction of serum superoxide anion concentration by TNJ has been demonstrated in human volunteers, and the induction of superoxide dismutase (SOD) activity by the major iridoid in noni juice has been demonstrated in vivo (Wang et al., 2009; Ma et al., 2013). SOD is another antioxidant enzyme that is active in protecting erythrocytes. It may be likely that increased SOD activity is responsible for changes observed in the current study. However, the previously observed decreases in superoxide anion and the increases in SOD activity occurred after several days with no attempt made to measure effects after ingestion of a single dose. Therefore, additional research would be required to determine the exact mechanisms of action responsible for the immediate increase in erythrocyte antioxidant activity caused by TNJ. The conversion of fructose, in fruit juice, to urate has been shown to increase plasma antioxidant capacity (Lotito & Frei, 2004). While this may account for some of what has been observed in this current study, it can’t be responsible for the difference between TNJ and orange juice. One reason for this is that the major effect was seen in erythrocytes not plasma. Further, TNJ contains fewer calories and sugar than orange juice. Therefore, other components in TNJ are contributing to the significant increase in erythrocyte antioxidant activity.

The antioxidant impact of TNJ, as measured by the conversion of $Fe^{3+}$ to $Fe^{2+}$ in this study, may help us to understand the improvements in physical endurance and aerobic capacity seen in clinical trials with athletes. Oxygen
is used to convert glucose into adenosine triphosphate (ATP) in muscle cells. ATP provides the energy for muscle contraction, among other things. For erythrocytes to be able to deliver oxygen to the cells, the iron in hemoglobin must be in the reduced state, or Fe²⁺ (Hoffman et al., 2015). Under conditions of increasing oxidative stress, there is an increasing probability of Fe²⁺ being converted to the ineffective Fe³⁺. The strong antioxidant action of TNJ may inhibit this conversion, thereby allowing more oxygen to be delivered to the muscle cells than may otherwise occur as physical exertion and the mechanisms of fatigue progress. Further, erythrocytes need to be able to deform and squeeze through the capillaries to deliver oxygen. Oxidative stress damages the erythrocyte membrane and impairs deformability, thereby reducing oxygen delivery (Mohanty et al., 2014). Strong antioxidant activity within these cells, such as that provided by TNJ, can help prevent a decline in red blood cell functionality.

4. Conclusion
The results of the in vitro test and human study reveal that TNJ not only has the potential for immediate antioxidant protection but also improves the antioxidant activity of components of the blood within 1 hour of ingestion, especially within the red blood cells. Further, this antioxidant activity is discernable and significant even in nonsmokers. Thus, we have learned that no special health conditions need be present for noni to influence oxidative status within the body. Considering the previous antioxidant effects demonstrated in smokers and athletes, the antioxidant properties of TNJ are robust and repeatable under a variety of experimental and real-life conditions. It is possible that induction of superoxide dismutase may be responsible for this. But additional research is required to elucidate additional mechanisms of action that seem to be involved given that notable antioxidant effects occur within a very short time of ingesting noni juice.

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