Bioactivities and Iridoid Determination of a Beverage Containing Noni, Cornelian Cherries and Olive Leaf Extract

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Abstract: The aim of the current study was to evaluate the iridoid content, as well as the in vitro and in vivo bioactivities, of a beverage containing noni fruit, Cornelian cherries, and olive leaf extract (Thrive Adaptogenics Max). The average total iridoid content of the beverage was 2.09 mg/mL. The major iridoids present were identified as asperulosidic acid, deacetylasperulosidic acid, oleuropein, morroniside, loganic acid, and loganin. In the 2, 2-Diphenylpicrylhydrazyl (DPPH) radical scavenging assay, remarkably high in vitro antioxidant activity was observed, with an IC_{50} of 3.8 \mu M/mL. In vivo bioactivities were evaluated in type 2 diabetic Sprague Dawley rats. In a dose-dependent manner, Thrive Adaptogenics Max reduced abnormal weight gain, blood glucose levels, and serum Advanced Glycation End products (AGEs), as well as improved immunity via increased T cell counts and CD4+/CD8+ ratios. These results suggest that this blend of ingredients is beneficial for improving and maintaining health in the general population, as well as among those with metabolic imbalance.

Key words: Advanced glycation end products, antioxidant, cornelian cherries, iridoids, noni, olive leaf extract

INTRODUCTION

Noni (Morinda citrifolia), Asian Cornelian cherry (Cornus officinalis), European Cornelian cherry (Cornus mas), and olive (Olea europaea) have been used for centuries as foods and to improve health (Morton, 1992; Seeram et al., 2002; Rop et al., 2010; Erbay and Icier, 2010). Significant antioxidant activity has been found in products derived from each of these plants (Tural and Koca, 2008; Lee et al., 2012; Wang et al., 2009; Kontogianni and Gerothanassis, 2012). However, other notable bioactivities have also been discovered. Reduction of Advanced Glycation End products (AGEs) in vitro and in vivo is a reported activity of noni and the Cornelian cherries, as well as olive leaf extract (Kusirisin et al., 2009; Yamabe et al., 2007; Sasaki et al., 2007). Such benefits indicate that consumption of these foods is not only useful for maintaining health among the general population but also for improving health in those with impaired or imbalanced metabolism.

In addition to a number of other biologically active phytochemicals, each of these plants produces iridoids (Japon-Lujan et al., 2006; Deng et al., 2011; Gu et al., 1996). Iridoids found in noni fruits exhibit DNA protective activities, as well as anti-inflammatory and antioxidant activities (Choi et al., 2005; Kim et al., 2005; West et al., 2011). Oleuropein, a secoiridoid found in olive leaves, possesses antioxidant activity (Visioli and Galli, 1994). But it has also been shown to improve blood pressure and lipid levels (Perrinjaquet-Moccetti et al., 2008). The Cornelian cherries produce iridoid glycosides and secoiridoids which reduce AGEs, improve immune function, lower blood glucose levels and have neuroprotective and renal protection properties (Xu et al., 2006; Wang et al., 2010; Yokozawa et al., 2008). As different iridoids vary in their specific activities, combinations of these from safe food sources may provide synergistic health benefits. The aim of the current study was to evaluate the iridoid content, as well as the in vitro and in vivo bioactivities, of a pasteurized beverage containing noni fruit, Cornelian cherries and olive leaf extract, thereby gaining a better understanding of the utility of such a blend for the promotion of health.

MATERIALS AND METHODS

This study was conducted by the research and development department of Morinda, Inc. (American Fork, Utah, USA) and the pharmacology department of Tianjin Medical University (Tianjin, China) from June 2011 to January 2012.

Preparation of test product: Noni fruits were harvested in French Polynesia and allowed to fully ripen. The fruit was then processed into a puree by mechanical removal of the seeds and skin at a good manufacturing certified fruit processing facility in Mataiea, Tahiti. Olive leaf extract was prepared by extraction of dried O. europaea leaves...
with ethanol, followed by removal of the solvent and residual water by evaporation. The resulting extract was reduced to a powder with a particle size <0.420 mm and total moisture <6%. Ripe C. mas and C. officinalis fruits were harvested during the autumn season. C. mas fruit was harvested in the Anatolia region of Turkey, whereas C. officinalis fruit was harvested in the Shaanxi and Hunan provinces of China. The seed pits were removed from fruit with automatic fruit pitters. Batches of C. mas fruit were processed into puree in an auger press micro-mesh (4 mm) screen fruit pulper. Depitted C. officinalis fruits were dried at low heat and then shipped to the U.S. where the reconstituted juice was extracted by pumping/filtration through a stainless steel mesh screen. All ingredients were blended and pasteurized at a good manufacturing certified fruit processing facility in American Fork, Utah to produce the finished product used in this study, Thrive Adaptogenics Max (Morinda, Inc. Utah, USA). The total solids content (mean±standard deviation) of the final test product was 20.24±0.48%.

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 (Deng et al., 2011). 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 C18 column. Elution was accomplished with two mobile phases, MeCN and 0.1% formic acid in H2O (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.

2. 2-Diphenylpicrylhydrazyl (DPPH) radical scavenging assay: In the DPPH test, the beverage was diluted serially with deionized water. The diluted samples, and a water blank, were combined 1:1 (v/v) with 0.4 mM DPPH in ethanol. The absorbance of each sample, in triplicate, 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 60 min. Percent radical scavenging activity was calculated by dividing the difference in absorbance of the blank and the sample by the absorbance of the blank alone. The concentration of Thrive Adaptogenics Max which scavenged 50% of the DPPH radicals (IC50) was calculated with the linear equation that best fit the plot of concentration versus scavenging activity (R²>0.99).

Efficacy in type 2 diabetic Sprague Dawley (SD) rats: Fifty healthy male SD rats, 200-220 g, were acquired from the Tianjin Drug Research Center (Tianjin, China). These were acclimated for one week while receiving standard rodent feed, composed of 23% protein, 53% carbohydrates, and 5% fat, respectively. Following acclimation, the rats were randomly divided into five groups of 10 animals each. One group, the normal control, continued to be fed the standard diet. The four other groups were fed a high fat and sugar diet composed of 20% protein, 48% carbohydrates, and 22% fat. Water and feed were provided ad libitum. The normal control group and one of the groups receiving the high fat diet, the diabetic controls, were fed 2 mL saline twice per day by gavage. The remaining three groups received, twice daily by gavage, 2 mL of solutions containing 25% (low dose), 50% (mid dose), or 100% (high dose) Thrive Adaptogenics Max. All animals were fed saline or test product solutions at the same time every morning and evening. After four weeks of the various treatments and feeding of the assigned diets, the animals were fasted for 12 h and administered a single abdominal injection, at 30 mg/kg body weight (b.w.), of 0.5% streptozotocin in citrate buffer (pH 4.2). The normal control animals did not receive the streptozotocin solution, but were administered citrate buffer only. All groups were provided water and the standard diet for another 7 days then fasted over night.

On the final day of the study (day 35), all animals received an abdominal injection of glucose solution at 2 g/kg b.w. Glucose levels in blood from the tail vein of each animal were measured with a glucometer (Changsha Sannuo Biologic and Sensing Technology, Inc., Changsha, Hunan, China) at 0 and 2 h after glucose injection. Following the glucose test, the rats were anesthetized with ether, and 1.5 mL blood was removed from the orbital sinus. For the detection of T-cell subsets, 1 mL blood was mixed with heparin. For detection of AGEs, 0.5 mL blood was centrifuged at 3000 rpm for 10 min, followed by collection of the serum.

Heparinized blood samples were prepared for flow cytometry analysis with a T cell subset detection kit (MultiSciences Biotech Co., Ltd., Hangzhou, Zhejiang, China). This involved labeling of specific lymphocytes with fluorescently-labeled antibodies for CD3+, CD4+ and CD8+ proteins, according to the kit manufacturer’s instructions. Labeled cells were detected with a flow cytometer (Beckman Coulter, Brea, CA, USA) and the percentages of CD3+, CD4+ and CD8+ T-cell subsets were determined. The CD4+/CD8+ ratios were also calculated.
Centrifuged blood samples were prepared for AGE detection with an enzyme linked immunosorbent assay (ELISA) kit (Shanghai YanHui Biotechnical, Inc., Shanghai, China), according to the kit manufacturer’s instructions. Fluorescent detection of labeled AGE’s was performed with a Bio-Rad 550 microplate ELISA reader (Bio-Rad, Hercules, CA, USA). Animal weights were also recorded throughout the study.

The means and standard deviations of each measurement were calculated for each group. Statistical differences between control and test groups were evaluated with Student’s t-test, using SPSS® software version 16.0 (IBM Corporation, Armonk, NY, USA).

RESULTS AND DISCUSSION

Iridoid analysis: The phytochemical analysis revealed that Thrive Adaptogenics Max is an iridoid rich beverage, with total iridoid content (mean±standard deviation) of 2.09±0.08 mg/mL. The major iridoids present were identified as asperulosidic acid deacetylasperulosidic acid, oleuropein, morroniside, loganic acid and loganin. Noni fruit puree is the source of asperulosidic acid and deacetylasperulosidic acid (West et al., 2011). Oleuropein is a well known and well researched iridoid in olive leaf extract (Japon-Lujan et al., 2006). Morroniside and loganin are contributed primarily by C. officinalis fruit (Gu et al., 1996; Du et al., 2008). Our analysis resulted in the first discovery of loganic acid in C. mas fruit. The concentrations found in the beverage indicate that processing conditions are suitable for the preservation of the iridoids.

DPPH radical scavenging assay: In the DPPH radical scavenging assay, remarkably high in vitro antioxidant activity was observed, with an IC50 of 3.8 μL/mL. The antioxidant activity of this beverage is much greater than those reported for several fruits and fruit juices. The IC50’s reported for mangosteen, orange, pomelo, grapes and papaya ranged from 11.18-32.80 mg/mL, while those of orange juice, grape, rose apple, and jackfruit were from 50.62-110.46 mg/mL (Surinut et al., 2005). Different varieties of pomegranate juice are also reported to have a higher IC50’s, ranging from 15.98-23.98 μL/mL (Elfallah et al., 2009). The DPPH radical scavenging activity of Thrive Adaptogenics Max is at least three times greater than that reported for any of these other fruit products.

Efficacy in type 2 diabetic rats: Average weights throughout the study are summarized in Table 1. There were no differences in the initial weights of the groups. However, significant differences from normal controls were evident by week 2 in the diabetic control and low dose groups. At the same time point, mid and high dose group weights were no different than those of normal controls. At week 4, high dose group weights were not significantly different from those of normal controls. During the entire study, weight gain was greatest in the diabetic control group, which received no test product. Significant reductions in weight gain of the high and mid dose groups, as compared to the diabetic control group, were also apparent throughout the entire study. A trend of lower weight was also present in the low dose group, reaching statistical significance at week 4. Overall, weight differences were dose-dependent and demonstrated that supplementation with the test product reduced weight gain in rats fed a high fat and high sugar diet.

Blood glucose levels at 0 and 2 h after glucose injection, or challenge, are presented in Table 2. Glucose levels in all groups fed the high fat and sugar diet were significantly greater than the control group. But significant dose-dependent reductions in blood glucose, as compared to the diabetic controls, were seen in the low, mid and high dose groups. Animals with 0 and 2 h glucose levels >7.0 and >11.0 mmol/L, respectively, were categorized as diabetic. The percentage of animals categorized as diabetic in each group is also presented in Table 2. All animals in the diabetic control group were above these limits, demonstrating the successful induction of diabetes. As with mean blood glucose, a dose-dependent decrease in the percentage of diabetic rats occurred in the test product groups. It is remarkable that no animals met the diabetic threshold in the high dose group.

T cell subsets in each group are summarized and compared in Table 3. The percentage of T cells among total lymphocytes is expressed by CD3+ values. CD4+ cells are T helper cells that play a central role in regulating the immune system, whereas CD8+ cells (cytotoxic or suppressor cells T cells) down regulate immune function (Hung et al., 1998; Jiang and Chess, 2006; WHO, 2007). The CD4+/CD8+ ratio, also referred to as the T-lymphocyte helper/suppressor profile, is an indicator of immune system function. In a properly functioning immune system, this ratio is greater than one, as CD4+ cell count should be greater than CD8+. Reductions in CD4+/CD8+ ratios have been associated with immune deficiency (Reinherz and Schlossman, 1980; Kiecolt-Glaser et al., 1986). Human studies have demonstrated an inverse association between T helper cell...
Table 2: Mean blood glucose, ±standard deviation, and percentage of animals categorized as diabetic (%DM) by glucose level, at 0 and 2 h after glucose challenge

<table>
<thead>
<tr>
<th>Group</th>
<th>Glucose (mmol/L)</th>
<th>%DM</th>
<th>Glucose (mmol/L)</th>
<th>%DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>4.32±0.39</td>
<td>0</td>
<td>6.28±0.53</td>
<td>0</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>7.93±0.57</td>
<td>100</td>
<td>12.1±0.58</td>
<td>100</td>
</tr>
<tr>
<td>Low dose</td>
<td>6.90±0.57</td>
<td>50</td>
<td>11.3±0.91</td>
<td>60</td>
</tr>
<tr>
<td>Mid dose</td>
<td>6.48±0.46</td>
<td>20</td>
<td>10.7±0.68</td>
<td>30</td>
</tr>
<tr>
<td>High dose</td>
<td>6.16±0.47</td>
<td>0</td>
<td>10.2±0.55</td>
<td>0</td>
</tr>
</tbody>
</table>

*: p<0.05 (compared to normal controls); #: p<0.05; ##: p<0.01 (compared to diabetic controls);

Table 4: Mean AGE level, ±standard deviation, by treatment group

<table>
<thead>
<tr>
<th>Group</th>
<th>AGE (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>26.4±3.74</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>36.9±4.67</td>
</tr>
<tr>
<td>Low dose</td>
<td>32.7±4.26</td>
</tr>
<tr>
<td>Mid dose</td>
<td>31.4±4.32</td>
</tr>
<tr>
<td>High dose</td>
<td>30.1±5.19</td>
</tr>
</tbody>
</table>

*: p<0.05; **: p<0.01 (compared to normal controls); *: p<0.05; **: p<0.01 (compared to diabetic controls)
Union, is ever increasing (Ford et al., 2004). In 2006, 34% of the US adult population met the criteria for metabolic syndrome (Ervin, 2009). Surprisingly, similar percentages were reported for adult males in the Okayama Prefecture of Japan (Miyatake et al., 2006). Metabolic syndrome is also an increasing public health problem in emerging economies, such as Malaysia and Russia (Tan et al., 2011; Metelskaya et al., 2011) and effective interventions may have a major impact on these populations.

Previous studies of the ingredients in the test product have revealed a wide range of possible positive health effects. Therefore, the benefits of Thrive Adaptogenics Max are not likely to be limited to what has been demonstrated in the current study. Rather, our data may represent just a fraction of a larger set of biological activities.

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REFERENCES


