Comparative Hypolipidemic Properties between the Lyophilized Fruit Juice of Morinda citrifolia L. (Rubiaceae) and Lyophilized Commercial Noni Juice in Triton and Atherogenic Diet-Induced Dyslipidemic Rats

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Research Article

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Abstract

This study evaluates the hypolipidemic activities of lyophilized commercial Noni juice (NJ) in animal models of dyslipidemia and determine if this property is exhibited by the lyophilized fruit juice of locally harvested Morinda citrifolia Linn. (FJ). Triton and atherogenic diet-fed dyslipidemic rats were assessed by serum lipid profiles. With FJ treatment, the serum total cholesterol (TC) lowering effect between tritonized and diet-fed rats was comparable; with NJ treatment, however, a much significantly higher inhibition was achieved in tritonized rats than in diet-fed rats. In lowering serum triglycerides (TG), both FJ and NJ gave higher inhibitions in diet-fed rats than in tritonized rats. FJ, but not NJ, significantly increased serum high-density lipoprotein cholesterol (HDL-C) in tritonized rats. FJ, but not NJ, significantly increased serum lipid concentrations was achieved with FJ as it gave a lower atherogenic index of 2.97 as compared to NJ which gave a higher value of 3.59. These results showed that FJ is generally more potent than NJ in exhibiting hypolipidemic activities in the animal models used. More experimental models should be designed to test the efficacy of FJ in this therapeutic area.

Keywords: Morinda citrifolia, Noni, triton, hypolipidemic

Introduction

In a compilation of anecdotal information on Morinda citrifolia, the beneficial effects of the Noni fruit juice in diabetes mellitus has been recorded (McClatchey, 2002). The fruit seed oil given orally significantly reduced total cholesterol and triglyceride levels in mice fed with an atherogenic diet (Pazos et al., 2011). In an in-vitro experiment, this hypolipidemic properties are mediated by inhibition of lipoprotein lipase (Pak-Dek et al., 2011). The hydroalcoholic fruit extract of Morinda citrifolia hypolipidemic properties in both triton and diet induced dyslipidemic rats (Mandukhail et al., 2010). Studies have yet to been undertaken on the serum lipid lowering effects of any aqueous preparations of the fruit using animal models of dyslipidemia. This investigation took on the initiative to compare the hypolipidemic activities between a lyophilized form of a fruit juice obtained from locally harvested Morinda citrifolia fruit (FJ) and a lyophilized form of a commercial preparation of Noni juice (NJ) in animal models of dyslipidemia.

Material and Methodology

Sample Preparation

Fifty kg or mature ripe fruits of Morinda citrifolia, consisting of 3 batches, was collected by the author from a single tree in a farm located at the island municipality of Anda, Pangasinan, Philippines during the months of April and May of 2002. Fruits were stored in a cooler while in transport. The specimen submitted for authentication at the University of Santo Tomas Herbarium, Manila, and was assigned a repository number of 4847 by Prof. Rosie Madulid of the Section of Botany, College of Science. After peeling and removing of the seeds, fruit pulps were homogenized in a blender. Homogenates were filtered in a Buchner funnel with gentle suction until clear and then measured for pH. The fruit juice from each batch of fruit collected were lyophilized at -40°C and 200 mtorr pressure using a Virtis freeze-dryer. The residue (2.63% w/v) was designated as the lyophilized Morinda citrifolia fruit juice (FJ). Five liters of commercial Tahitian Noni...
juice manufactured by Morinda Holdings, Inc. was purchased online. The contents of this product were lyophilized similarly and designated as the lyophilized Noni juice (NJ, 2.52% w/v). In all bioassays, FJ was and NJ were administered to test animals as 50% aqueous stock solutions.

**Determination of Median Lethal Dose**

The median lethal dose (LD$_{50}$) was performed using a modified method (Ekwall et al., 1998). Dose levels started at 1 g/kg which was increasingly spaced at 0.1 log intervals to obtain 7 consecutive values. Eight mice (4 males and 4 females) were assigned per dose level of FJ which was given by oral gavage. Fatalities were noted within 7 days during which mice were given free access to food and water. The LD$_{50}$ was determined by linear regression analysis using the “all or none response” probit method.

**Phytochemical Screening**

Phytochemical screening for secondary metabolites, such as alkaloids, flavonoids, anthraquinones and pectins, were carried out using FJ as sample according to standard qualitative and thin-layer chromatographic methods (Evans, 2006).

**General Considerations in Pharmacological Screenings**

Healthy male Sprague-Dawley rats weighing at least 200 grams were obtained from the Bureau of Animal Industry and bred at the Department of Pharmacology, University of the Philippines, Manila. Experiments were undertaken following the specifications of the Institutional Animal Care Use Committee and the US NIH Guideline No. 85-23 series of 1985. The project was given a protocol number Noni – 2000 – 2003 – 105. Prior to any experiment, animals were acclimatized in individual cages for 1 week with a 12 hour light-dark cycle with free access to water and a standard rodent diet containing 24% protein, 12% fat, 50% carbohydrates, 7% ash, 6% fibers and vitamins. In case of pharmacological screening 28 days of observation, rats were given free access to food and water throughout. During measurement of biochemical profiles, 1mL of blood was withdrawn from rats per test interval by tail-puncture. Blood was centrifuged at 800 rpm to obtain serum. In cases where left lobes of liver and blood obtained by cardiac puncture were required, rats were sacrificed by cervical dislocation and dissected by midline incision of the peritoneum.

**Reagents, Chemicals and Apparatuses**

Enzymatic kit for serum triglyceride assay was obtained from Bioquant. Reagents for serum cholesterol and HDL-cholesterol assays were provided by Teco Diagnostics. Gemfibrozil (positive control drug) was prepared as a 700mg/100mL suspension in 5% aqueous povidone. Triton WR 1339 was prepared as 4g/50mL in normal saline. The atherogenic diet consisted of cholesterol which was prepared as a 4g/250mL suspension in coconut oil.

**Biochemical Assays**

Serum total cholesterol levels (TC) were determined by the cholesterol oxidase colorimetric methods (Bauer, 1982). Serum glucose levels were assayed by the ortho-toluidine method (Dubowski, 1962). Serum high-density lipoprotein cholesterol (HDL-C) by the precipitation method. (Jensen et al., 2002). Serum triglyceride (TG) levels were measured by the enzymatic method (Bucolo and David, 1973).

**Screening in Triton-Induced Dyslipidemic Rats**

Triton WR-1339 was injected IP at 400 mg/kg, followed immediately by FJ and NJ administered at 1.6, 2.6. 4.1, 6.3 and 10 g/kg PO. After an 18-hour induction period during which rats were given free access to water only, sera from blood obtained by cardiac puncture were determined for total cholesterol (TC) levels.

In a 2nd experiment, Triton WR-1339 was given at 400 mg/kg IP. FJ at 4.1 g/kg and NJ at 6.3 g/kg were given PO to groups of 8 rats, with gemfibrozil (100 mg/kg PO) and 3 mL of distilled water (PO) as controls. After 18 hours, sera from blood obtained by cardiac puncture were assayed for TC, TG, HDL-C, LDL-C and glucose levels. Fecal collections were determined for TC contents (LAi et al., 2004).

**Screening in Rats Fed with Atherogenic Diet**

FJ at 4.1 g/kg and NJ at 6.3 g/kg were given PO to groups of 8 rats, with 3 ml of distilled water (PO) as control. An atherogenic diet consisting of 400 mg/kg cholesterol in 5 mL of coconut oil was orally given freely and concomitantly with each treatment group. This scheme was performed daily for 21 days. At the end of the 21st day, rats were fasted overnight for at least 12 hours. On the 22nd day, fecal TC content and sera obtained by cardiac puncture were analyzed for glucose, TC, TG, HDL-C and LDL-C (Pellizon et al., 2008).

**Statistical Analyses**

Replicate data were expressed as mean ± standard error of the mean (S.E.M.) and analyzed by student t-test, 2-way analysis of variance and 90% confidence interval, such that a minimum of p < 0.05 is considered significant. Linear regression was used to analyzed any dose-response correlation.

**Results and Discussion**

**Exploratory Screening for Hypolipidemic Activities**

During the acute toxicity study, an LD$_{50}$ value of 16.4 g/kg PO was computed for FJ, a value considered non-toxic according (Loomis and Hayes, 1996). Twenty-five percent of this value correspond to 4.1 g/kg which was used as the basis for assigning dose levels for FJ and NJ during this exploratory screening, from 1.6 g/kg to 10 g/kg and spaced at 0.2 log interval. Figure 1a compares mean serum...
total cholesterol (TC) levels 18 hours after treatments were given concomitantly with triton at 400 mg/kg IP. Significantly lower serum TC levels compared to water were obtained with FJ starting at 4.1 g/kg and with NJ starting at 6.3 g/kg. Both FJ and NJ were, therefore, given at these dose levels in subsequent screenings for hypolipidemia.

Table 1: Comparison of Lipid Lowering Effects Between the Lyophilized Fruit Juice of Morinda citrifolia (FJ) and Lyophilized Commercial Noni Juice (NJ) in Tritonized and Atherogenic Diet-Fed Rats

<table>
<thead>
<tr>
<th>Serum Biochemical Parameters (mg/dL)</th>
<th>Animal Model</th>
<th>FJ 4.1 g/kg PO</th>
<th>NJ 6.3 g/kg PO</th>
<th>Gemfibrozil – 100 mg/kg PO</th>
<th>Mean % Inhibition (± S.E.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cholesterol (TC)</td>
<td>Tritonized Rats</td>
<td>16.7 ± 1.8</td>
<td>19.0 ± 2.1**</td>
<td>55.2 ± 1.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Diet-fed Rats</td>
<td>14.0 ± 1.2Δ</td>
<td>8.8 ± 2.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglycerides (TG)</td>
<td>Tritonized Rats</td>
<td>5.6 ± 0.2 **</td>
<td>2.6 ± 1**</td>
<td>59.2 ± 6.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Diet-fed Rats</td>
<td>33.0 ± 7.0Δ</td>
<td>10.5 ± 2.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL-Cholesterol (LDL-C)</td>
<td>Tritonized Rats</td>
<td>13.4 ± 2.8*</td>
<td>13.1 ± 3.0</td>
<td>62.5 ± 3.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Diet-fed Rats</td>
<td>22.8 ± 1.7</td>
<td>13.0 ± 1.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>Tritonized Rats</td>
<td>10.2 ± 1.0Δ</td>
<td>29.8 ± 1.9</td>
<td>6.0 ± 1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Diet-fed Rats</td>
<td>4.0 ± 1.1Δ</td>
<td>27.1 ± 2.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* p < 0.01; ** p < 0.001 versus Diet-fed Rats; Δ p < 0.001 versus NJ

Table 2: Comparative Atherogenic Indices

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean Serum Lipoproteins (mg/dL)</th>
<th>AI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VLDL-C</td>
<td>LDL-C</td>
</tr>
<tr>
<td>FJ – 4.1 g/kg</td>
<td>9.66</td>
<td>65.0</td>
</tr>
<tr>
<td>NJ – 6.3 g/kg</td>
<td>12.9</td>
<td>70.4</td>
</tr>
<tr>
<td>Water – 3 mL</td>
<td>13.74</td>
<td>83.2</td>
</tr>
</tbody>
</table>

To compute for median effective dose (ED₅₀), for each dose level, rats with serum TC below 310 mg/dL (i.e., the lower S.E.M. from mean serum TC levels obtained with the water-treated group) 18 hours after triton injection were considered responsive to treatment. Percentage efficacy (when n = 6) were transformed into probit values and plotted against log of corresponding dose levels. The inverse of the log dose extrapolated to a probit of value of 5 (~50%) is the computed ED₅₀. A larger ED₅₀ value was computed for NJ over FJ (3.63 versus 2.29 g/kg) which means that FJ is more potent than NJ in lowering serum TC in tritonized rats. The therapeutic indices were computed as the ratio of the LD₅₀ over ED₅₀. The LD₅₀ of FJ (16.4 g/kg) was adopted for NJ. Therapeutic indices of 7.16 and 4.52 were computed for FJ and NJ, respectively, meaning a larger margin of safety can be allowed with FJ over NJ when used as hypcholesterolemic agent in tritonized rats.

Figure 1a. Comparative Effects on Mean Serum Total Cholesterol between Lyophilized Morinda citrifolia Fruit Juice (FJ) and Lyophilized Commercial Noni Juice (NJ) After 18 Hours of Treatments in Tritonized Rats

Figure 1b. Comparative Hypocholesterolemia (% Inhibition) between Lyophilized Morinda citrifolia Fruit Juice (FJ) and Lyophilized Commercial Noni Juice (NJ) After 18 Hours of Treatments in Tritonized Rats

Comparative Lipid Lowering Effects

Table 1 compares serum lipid lowering between FJ and NJ in both tritonized and diet-fed rats. With FJ treatment, the TC lowering effect between tritonized and diet-fed rats was comparable; with NJ treatment, however, a much significantly higher inhibition was achieved in tritonized rats than in diet-fed rats. This difference could be due to enhancement of lipoprotein lipase (LPL) activity, suppression of hepatic cholesterol biosynthesis or enhanced hepatobiliary excretion of cholesterol.

Comparative Atherogenic Indices
NJ between the 2 animal models was comparable. This is justified with the use of highly saturated coconut oil as part of the atherogenic diet which was manifested by a marked elevation of serum TG, a property not shared by triton. Serum TG lowering is often more advantageous than TC lowering alone, since in most clinical cases, normal serum TC levels does not often equate to normal serum TG levels and, thus, elevated TG levels will require therapy with fibrates such as gemfibrozil.

Figure 2 Graphical Determination of the Hypolipidemic Oral Median Effective Dose (ED50 in g/kg-BW) of FJ

FJ was able to lower the serum glucose levels much more effectively in tritonized rats than in diet-fed rats; with NJ, results were comparable between the 2 animal models. Both treatments were more hypoglycemic in tritonized rats than in diet-fed rats. It is advantageous that a hypolipidemic plant extract hypoglycemia as diabetes mellitus is a co-morbidity of dyslipidemia in the so-called metabolic syndrome.

Figure 3. Comparative Effects on Mean Serum HDL-C Levels between Lyophilized Morinda citrifolia Fruit Juice (FJ) and Lyophilized Commercial Noni Juice (NJ) in Tritonized and Atherogenic Diet-Fed Rats (n = 8; *p < 0.05 versus water)

Comparative Effects on Serum HDL-C Levels
Figure 2 compares the effects of FJ and NJ on serum HDL-C levels between tritonized rats 18 hours after induction and atherogenic diet–induced rats treated with daily with FJ or NJ for 21 days. All 3 treatments showed significant increase in fecal cholesterol excretion compared to water in both set of rats. This process requires activating cholesterol 7-α-hydroxylase which is the rate-limiting step in cholesterol catabolism to bile acids. The observed hypocholesterolemia observed in this experiment support the bile acid sequestration of of cholesterol by cholestyramine, a resin drug that upregulates hepatic LDL receptors (Goto et al., 1997).

Atherogenic Indices
The atherogenic indices (AI) was computed to assess efficacy of hypolipidemia during sub-acute treatments, thus: AI = (VLDL-C – TC)/HDL-C, such that VLDL-C = TG/5 according to the Friedewald equation. Table 2 gives the AIs computed for each treatment which was given concomitantly with an atherogenic diet, daily, for 21 days. Treatment with FJ at 4.1 g/kg gave lower AI value than with NJ at 6.3 g/kg, indicating better efficacy of FJ over NJ in the long-term treatment of dyslipidemia in rats fed with an atherogenic diet. Lower AI values with FJ (34.4%) and NJ (20.8%) compared with water indicates better protection from atherogenesis.

Phytochemical Analysis
Qualitative phytochemical and thin-layer chromatographic analyses of FJ showed the presence polyphenolic substances (i.e., flavonoids, tannins, lignans, iridoids). These large group of atherogenic small dense LDL particles. An showed that an increase in serum HDL-C level prevents the development of atherosclerotic lesions (Kapur et al., 2008). An increase in serum HDL-C levels with FJ will be great significance during long-term treatment as this reverses cholesterol transport and inhibits oxidation of LDL.
The oxidation of LDL would induce inflammatory responses by producing leukocyte and cytokine on endothelial tissues. Oxidation of LDL would generate reactive oxygen species that are toxic. Flavonoids reduce LDL oxidation and prevent inflammation in endothelial tissues. It is interesting to note that these polyphenolics compounds have been isolated from the fruit of *M. citrifolia*, and their antioxidant scavenging activity against free radicals have been demonstrated in vivo and in vitro. In addition, the ethanol-insoluble precipitate of FJ is a mixture of pectin substances. Pectins have long been associated with hypolipidemic properties due to their ability to sequester cholesterol and facilitate their hepatobiliary excretion in the liver (Marounek et al., 2010).

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**References**


