Anxiolytic-like effects of Morinda citrifolia L. (noni) in rats

Sarinee Kalandakanond, Jantarima Pandaranandaga, Siripen Komolvanich and Sutthasinee Poonyachoti

1Department of Physiology, Faculty of Veterinary Science; 2Graduate student, Interdepartment of Physiology, Graduate School; Chulalongkorn University, Bangkok Thailand.

Abstract

Noni juice (Morinda Citrifolia L.) had long been known as a medicinal plant in folklore medicine in tropical countries and Pacific islands. To date, the only available scientific knowledge was focused on antioxidant and anti-cancer effects, while it was claimed to have beneficial effects on stress relieved and happiness feeling by the noni juice-consumers. The central effect of noni was demonstrated from the noni root extract to contain sedative and analgesic properties. In this study, we utilized an elevated-plus maze (EPM), a standard test for anxiety in rats to determine whether noni beverages contained an anxiolytic property. We found that noni juices from two commercially available sources had marked effect on the anxiety-related behavioral parameter on EPM in rat. This effect was comparable to that of diazepam, a clinically effective anxiolytic drug. Noni juices and diazepam can increase time spent in the opened-arm of the EPM, the indicator of anxiolytic-like behaviors without effect on locomotor activity. Additionally, we did not find any detrimental effect on liver and kidney functions when the noni juice was fed for 30 days; the daily weight gain and feed intake were not affected as well.

Key words: anxiety, anxiolytic, elevated-plus maze, Morinda citrifolia, noni

Address correspondence and reprint requests to: Sarinee Kalandakanond. Department of Physiology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330 Thailand. Sarinee.Kai@chula.ac.th
ผลในการคลายความกังวลของน้ำสูกถึง (โมนี) ในหนู

สาเนี กลิ่นทานเหล็ก จันทบุรี ปีพรรณนท ศิริพิมัย ไก่ล้านนิสิต และ สุทธาสินิ์ ปุญญาזכิริย์

1 ภาควิชาวิทยาการค้นคว้าพยาบาลศาสตร์, 2 นิสิตปริญญาเอก สาขาวิชาวิทยาการ กีฬาพยาบาลภัย
จุฬาลงกรณ์มหาวิทยาลัย กรุงเทพ

บทคัดย่อ

น้ำสูกถึง หรือน้ำโมนี (Morinda Citrifolia L.) เป็นเครื่องดื่มที่มีสรรพคุณเป็นยาพื้น
บ้านที่ใช้กันแพร่หลายในประเทศไทย แต่ยังไม่มีการปริญญา ว่า
น้ำสูกถึงมีที่มาในการด้านอนุสระ และสามารถป้องกันการก่อเริ่มต้นในสุขภาพดีและ
ในสุขภาพดี อย่างไรก็ตาม นุ่มสูกถึงไม่มีการกล่าวถึงผลของการดื่มน้ำสูกถึงในการดื่ม
ความเครียดและทำการให้สุขภาพ ซึ่งการทดลองที่มีการร่างนิสิตของสารกัดที่มีต่อระบบ
ประสิทธิ์ มีความสูสัมพันธ์ที่ใช้สารกัดและการดื่มก็ในการบรรเทาความเจ็บปวด และทำให้
เกิดอาการภูมิคุ้มกันในสุขภาพดี สามารถจัดเป็นน้ำสูกถึงที่สามารถใช้ในการดื่มน้ำ
ดื่มน้ำโมนี พบว่าในหนูที่ได้รับน้ำสูกถึงหรือน้ำโมนีให้ผลในการลดความกังวลไม่ต่างจากหนู
ที่ได้รับ diazepam ซึ่งเป็นยาที่ใช้ในการลดความกังวลทางการแพทย์ ผลที่พบคือน้ำสูกถึง และยา
diazepam ทำให้หนูได้รับภูมิคุ้มกันในส่วนต่างของ elevated-plus maze มากกว่าหนูในกลุ่มน้ำโมนี
ซึ่งเป็นตัวหนึ่งที่ใช้ในการวัดความกังวล โดยทดลองน้ำสูกถึงและยา diazepam ไม่มีผลต่อ
อัตราการเคลื่อนไหวของหนูในสุขภาพดี หนูที่ได้รับน้ำสูกถึงอยู่ต่อเนื่องเป็นเวลา 30 วัน ในผลสัมประสิทธิ์การดื่มน้ำสูกถึงหรือในส่วนต่างของตัวเครื่องภาวะร่วม
ใด หรือผลต่ออัตราการเจริญเติบ
โต หรือปริมาณอาหารที่กินต่อวันในหนู

คำสำคัญ: ความกังวล การคลายความกังวล โมนี น้ำสูกถึง elevated-plus maze, Morinda citrifolia
Introduction

Noni (Morinda citrifolia Linn. Rubiaceae) is a small evergreen plant that can be found from India through Southeast Asia to Eastern Polynesia. Its common names are noni, nonu, Indian mulberry, duppy soursop, cheese fruit, Ba Ji Tian, mergadu, yor and nhau. The noni has been used in various aspects; different parts of a plant (e.g. fruit, leaf, bark, root, flower and seed) have long been employed in folklore medicine to treat a broad range of diseases including diabetes, hypertension, infections, colds, and cancer

There are more than 160 identified chemicals in noni, the major components are scopoletin, octanoic acid, terpene compounds, alkaloids, anthraquinones, β-sitosterol, carotene, vitamin A, vitamin C, potassium, flavone glycosides, linoleic acid, amino acids, acubin, asperuloside, acubin, caproic acid, caprylic acid, ursofolic acid, rutin, a putative proxeronine, glycosides, and a tri-saccharide fatty acid ester. It had been proved recently that noni juice extract contained antioxidant, anti-cancer and anti-inflammatory property both in vivo and in vitro experiments. The in vitro experiment revealed that glycosides in noni fruit extract were responsible for the anti-cancer activity.

These scientific evidences are not only supported the knowledge of noni as a medicinal plant in folklore medicine, but also supported some health benefits of the juice claimed by the consumer. Nowadays, noni juice is commercially market as a health-promoting beverage and according to data obtained in the USA for 2001, an average number of 46,603 people purchased Tahitian Noni® Juice per month. Additionally, the effect of noni juice on growth and the clinical chemistry specific for liver and kidney profiles were determined as well.

Materials and Methods

Noni Juice

Noni juice used in the experiments were Tahitian Noni® Juice (TNJ; Morinda International Inc, Thailand) and Siam Noni® (SNJ; Suprederm International, Thailand). TNJ was a fruit juice mixture of 89% Tahitian noni juice and 11% common grape and blueberry juice concentrates. SNJ was produced from Thai noni and composed of 99% noni juice.

Animals

Male Wistar rats weighing 200-250 gm at the beginning of the experiments were obtained from National Laboratory Animal Center, Mahidol University (NLAC-MU), Thailand. All animals were housed in shoebox cage under 12h light/dark cycle (lights on at 0700 h) at
room temperature (25±2°C). Standard rat chow and water were supplied ad libitum. Body weight and amount of food consumed were measured daily. After 7-day adaptation period, rats were assigned randomly into 4 groups: control, diazepam, TNJ and SNJ (n = 9 per group; except n = 12 for SNJ group). The rats were fed with 1 ml of water or noni juices for 15 or 30 days. For the diazepam treated groups, the rats were daily gavaged with 1 ml water, and diazepam dissolved in distilled water was given orally at a single dose of 15 mg/kg on the day of behavioral test. All procedures were done under the approval of Animal Used Committee, Faculty of Veterinary Science, Chulalongkorn University.

Behavioral assessment

The behavioral experiments were performed using an EPM, the standard test to assess anxiety-like behaviors in rats. The EPM was made of wood, elevated 50 cm above the floor, and consisted of four arms of equal dimension (10 x 50 cm) in which two arms enclosed by high wall (30 cm) and two arms opened. The last day of experiment, rats were placed in the center of the EPM facing a corner of the platform 15 min after receiving water or noni juice. Rats treated with diazepam, a standard anxiolytic agent (Azepam 5, Macrophar Co. Ltd, Thailand) was tested on EPM within 5 min after received drug. The behavioral test was conducted during the light phase of the cycle, between 0900-1100 h in a low natural light room. Each rat was allowed to explore freely on the EPM for 5 min and recorded on video cassette recorder for later analysis. The parameters measured were time spent in opened-arm, closed-arm and center platform, including number of entries into each arm. Number of times of rearing and grooming were also recorded. An arm entry was defined as the placement of at least both forefeet into one arm.

Clinical chemistry

Blood was collected from the heart at the end of the experiment and tested for liver and kidney profiles using Reflotron® test strips for alkaline phosphatase (AP), glutamate pyruvate transaminase (GPT), glutamate oxaloacetate transaminase (GOT) and creatinine (Roche Diagnostics, Thailand). For blood urea nitrogen (BUN), the plasma concentration was determined by colorimetrically measuring the product formed in the direct reaction of urea and diacetyl mono-oxime. Blood glucose was measured by using Accu-Chek® advantage II blood glucose test strips (Roche Diagnostics, Thailand). The plasma was analyzed for sodium and potassium concentration by flame photometry (model 410C clinical flame photometer, Corning, Halstead, Essex, UK) and for chloride concentration by chloride titration (model 925 Chloride analyzer, Corning).

Data analysis

For each rat, the total number of entries (opened + closed arm), the percentage of opened-arm entries (100 x opened-arm entry/ total entries), and the percentage of time spent on the opened-arm (100 x opened-arm time/300) were calculated. Then, the effects of noni juice on all parameters were analyzed using one-way analysis of variance (ANOVA) followed by Student-Newman-Keuls test to compare between groups. The unpaired t-test was used to compare the different effects of noni fed for 15 days and 30 days. The data were expressed as mean ± SE. A difference of the means of p < 0.05 was considered as statistically significant.

Results

Body weight and feed intake

There was no different in body weight, daily weight gain or daily feed intake between treatments after noni juices were given orally for 15 days. Similarly, when the treatment was extended to 30 days, there was no different in all measured parameters as shown in figure 1.
Behavioral assessment

The rats treated with diazepam (15 mg/kg, PO), the standard anxiolytic agent, spent more time in an aversive opened area compared to control (p < 0.001) as shown in figure 2. Similar to diazepam treated-rats, 15-day noni treated-rats spent longer time on opened-arm than control (p < 0.001; figure 2). Moreover, the number of total entry into both arms and the number of rearing, the indicator of motor activity in rats fed with noni juices or diazepam were not different from control (figure 3). Interestingly, when noni juice was fed for 30 days, the percentage of time spent in opened-arm and the percentage of opened-arm entry were not significantly different from those of 15 days as shown in figure 4.

![Figure 1](image1)

**Figure 1** Effects of noni feeding on daily weight gain (A) and daily feed intake (B). There was no different between treatments when noni juices were fed for 15- or 30- days. Data presented as mean ± SE.

![Figure 2](image2)

**Figure 2** Effect of 15-day noni feeding on opened- and closed- arm time. Noni juice and diazepam were able to induce an anxiolytic like-behavior upon exposure to the EPM as they spent more time on the opened-arm and less time on the closed-arm compared to the controls. *** Significantly different from control at p < 0.001, one-way ANOVA followed by Student-Newman-Keuls test. Data presented as mean ± SE.
Figure 3 Effect of 15-day noni feeding on motor activity; total arm entry (A) and rearing (B). The total arm entry (A) and the number of rearing (B), the indicators of motor activity in rats tested with EPM were not different between treatments. Data presented as mean ± SE.

Figure 4 Effect of length of noni feeding on percentage of time spent on opened-arm (A) and percentage of opened-arm entry (B). There was no different in the percentage of time (A) or number of entry (B) into opened-arm when noni juices were fed for 15 or 30 days. Data presented as mean ± SE.

Table 1 Clinical chemistry of rats fed with noni juice for 30 days. There was no different in clinical chemistries between treatments. Data presented as mean ± SE.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>TNJ</th>
<th>SNJ</th>
<th>Diazepam</th>
</tr>
</thead>
<tbody>
<tr>
<td>AP (U/L)</td>
<td>633.00±92.47</td>
<td>421.25±29.08</td>
<td>478.75±36.32</td>
<td>564.75±83.25</td>
</tr>
<tr>
<td>GPT (U/L)</td>
<td>22.15±1.27</td>
<td>27.45±1.15</td>
<td>22.50±1.24</td>
<td>27.25±2.13</td>
</tr>
<tr>
<td>GOT (U/L)</td>
<td>68.68±6.78</td>
<td>81.10±6.58</td>
<td>85.88±14.15</td>
<td>77.65±5.37</td>
</tr>
<tr>
<td>BUN (mg%)</td>
<td>47.20±3.83</td>
<td>39.94±2.71</td>
<td>49.69±4.25</td>
<td>45.43±1.20</td>
</tr>
<tr>
<td>Na⁺ (mEq/L)</td>
<td>137.75±4.39</td>
<td>137.25±2.87</td>
<td>139.50±2.18</td>
<td>138.25±4.39</td>
</tr>
<tr>
<td>K⁺ (mEq/L)</td>
<td>4.33±0.15</td>
<td>3.90±0.07</td>
<td>3.95±0.21</td>
<td>4.25±0.29</td>
</tr>
<tr>
<td>Cl⁻ (mEq/L)</td>
<td>98.00±1.68</td>
<td>99.75±0.75</td>
<td>102.00±1.08</td>
<td>99.25±0.75</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>116.75±2.50</td>
<td>135.50±4.33</td>
<td>120.75±4.31</td>
<td>138.25±5.25</td>
</tr>
</tbody>
</table>
The grooming activity recorded from the EPM was not different between treatments. However, it should be noted that the number of grooming was reduced in rat treated with noni juice and diazepam (data not shown). Moreover, when feeding was prolonged to 30 days, the noni-fed rat had no grooming activity in all parts of the maze.

**Clinical chemistry**

At the end of experiments, blood was collected for measuring AP, GPT, GOT, BUN, creatinine, Na⁺, K⁺, Cl⁻, glucose. There was no different in all parameters compared to control group after feeding for 15 days (data not shown). Similarly, in a 30-day treated group, all blood chemistries were not significantly different from control (table 1).

**Discussion**

In this study, we examined the effects of noni juice on anxiety related behavior, along with the effects on body weight gain, feed intake and blood chemistry related to liver and kidney functions. We found that noni juices in a form of supplemented beverages (TNJ® and SNJ®) contained an anxiolytic activity in rats when tested with elevated plus maze (EPM). The EPM is a valid behavioral test for the selective anxiolytic and anxiogenic agent in the rat. The correlation of behavior, physiologically and pharmacologically have been stated. Since the opened elevated area could evoke an unconditioned fear, rats usually made fewer entries into the opened arm than into the closed arm, and spent less time in opened-arm. The agents that can increase the percentage of time spent on the opened-arm and the number of entries into the opened-arm is therefore implied as an anxiolytic agent. Diazepam, a clinically effective anxiolytic had been shown previously to increase both time and number of entry into opened-arm. In this study, the rats treated with single oral dose of diazepam spent more time in the opened-arm when compared to control, and this finding was in agreement with others. Similarly, we found that noni juice when given orally for 15 days could produce an effect resembling to diazepam in that rat fed with noni juices spent more time in the aversive opened area, while control rats avoid it and stayed in a protected closed-arm. Since there were some concerns that the increase in opened-arm time could be confounded by an increase in motor activity of the rats, the total number of entries into both arms and the rearing behavior were usually recorded. We did not find any different in total entries or number of rearing, suggesting that noni juices and diazepam contained an anxiolytic effect without any effect on motor activity in this test.

Additionally, the prolongation of noni juice feeding to 30 days had no greater effects than those of 15 days demonstrated by the percentage of opened-arm time and opened-arm entry were not different between 15- and 30-day treatment. Although it is likely that prolonged taking of noni juice had no more additive effect but it should be noted that the grooming activity, another indicator of anxiety, was reduced in rats treated with noni juices for 30 days.

From this study, we can conclude that the noni juices (TNJ® and SNJ®) could reduce anxiety-related behavior in rats and SNJ® yielded similar effect to that of TNJ®. In addition, body weight, daily weight gain, daily food consumption, the liver and kidney profiles were not affected as well even when the feeding was lengthened to 30 days. However, Mueller and coworkers has reported previously that noni juice caused an unfavorable effect on chronic renal patient due to high concentration of potassium in noni juice. It is possible that in healthy animal, the body can excrete unwanted materials from the body compared to sick animal. Nevertheless, it should take into account that potassium concentration in the noni juice was quite high, and this could cause an undesirable effect to patient or animal with health problem.

In conclusion, we found that noni juices in a form of supplemented beverages could reduce anxiety with no
detrimental effect on liver and kidney functions in rats. The exact mechanism or the active substance(s) responsible for this anxiolytic effect will be required further investigations.

Acknowledgements

This work was supported by Veterinary Research Fund of the Faculty of Veterinary Science, Chulalongkorn University, Thailand. We would like to thank Dr. Damri Darawiroj for his helps on blood chemical analysis.

References