RESEARCH ARTICLES

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

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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

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ผลในการคลายความกังวลของน้ำลูกยอ (โนนิ)ในหนูขาว

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บทคัดย่อ

น้ำลูกยอ หรือน้ำโนนิ (*Morinda Citrifolia* L.) เป็นเครื่องดื่มที่มีสรรพคุณเป็นยาพื้นบ้านที่ใช้กันแพร่หลายในประเทศแถบร้อนชื้นและหมู่เกาะแปซิฟิค ในปัจจุบันเป็นที่ทราบกันดีว่าน้ำลูกยอมีฤทธิ์ในการด้านอนุมูลอิสระ และสามารถป้องกันการก่อมะเร็งได้ทั้งในสัตว์ทดลองและในหลอดทดลอง อย่างไรก็ดีในกลุ่มผู้บริโภคได้มีการกล่าวถึงผลของน้ำลูกยอในการลดความเครียดและทำให้หลับสบาย ซึ่งการทดลองที่มีการรายงานถึงผลของสารสกัดที่มีต่อระบบประสาทนั้น มีเพียงการทดลองที่ใช้สารสกัดจากรากต้นยอในการบรรเทาความเจ็บปวด และทำให้เกิดอาการซึมในหนูทดลอง การศึกษาครั้งนี้ผู้วิจัยสนใจศึกษาถึงการให้น้ำลูกยอในหนูขาวและวัดผลในการลดความกังวลโดยใช้ elevated-plus maze ซึ่งเป็นวิธีทดสอบมาตรฐานในการวัดความกังวลในหนูขาว พบว่าในหนูที่ได้รับน้ำลูกยอหรือน้ำโนนิให้ผลในการลดความกังวลไม่ต่างจากหนูที่ได้รับ diazepam ซึ่งเป็นยาที่ใช้ในการลดความกังวลทางการแพทย์ ผลที่พบคือน้ำลูกยอ และยา diazepam ทำให้หนูใช้เวลาอยู่ในส่วนเปิดของ elevated-plus maze มากกว่าหนูในกลุ่มควบคุม ซึ่งเป็นดัชนีหนึ่งที่ใช้ในการชี้วัดความกังวล โดยผลของน้ำลูกยอและยา diazepam ไม่มีผลต่ออัตราการเคลื่อนที่ของหนูในอุปกรณ์ทดสอบ นอกจากนั้นในการศึกษาครั้งนี้พบว่าการได้รับน้ำลูกยอติดต่อกันเป็นเวลา 30 วัน ไม่มีผลต่อการทำงานของตับหรือไต หรือผลต่ออัตราการเจริญเติบโต หรือปริมาณอาหารที่กินต่อวันในหนูขาว

คำสำคัญ: ความกังวล การคลายความกังวล โนนิ น้ำลูกยอ 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[1].

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, ursolic acid, rutin, a putative proxeronine, glycosides, and a tri-saccharide fatty acid ester[1-5]. It had been proved recently that noni juice extract contained antioxidant, anti-cancer[5] and anti-inflammatory[6] property both *in vivo* and *in vitro*[7] experiments. The *in vitro* experiment revealed that glycosides in noni fruit extract were responsible for the anti-cancer activity[2].

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[8]. Similarly, in Thailand, the use of herbal products is also arising. Despite of becoming a popular herbal product, in 2000, there was a case report of unfavorable effect of noni juice on chronic renal failure patient[9]. It is thus likely that the consumers are sometime unaware of a serious health consequence that might occur.

In this study, we were interested in the anxiolytic action of noni juice beverage, as it is claimed to produce happiness and reduce stress. To date, the only reported central effect of noni is that the noni root extract contained sedative property along with the central analgesic activity in mice[10]. Unfortunately, the active substance has not been yet identified, it is therefore could not make an assumption that the fruit would have similar effect as the root; since some constituents found in the root like anthraquinone could not be detected in the juice[8]. In order to test for anxiolytic effect, the elevated plus-maze (EPM) is selected; this test is widely used and specific for anxiety. It bases on unconditioned responses to a potentially dangerous environment, the combination of height, luminosity and open space is assumed to induce fear or anxiety in the rodents. The degree of anxiety is assessed by measuring the time spent on the opened- and closed-arms, and the number of entries made into each arm[11-13]. 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[11-13]. 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[14]. 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.

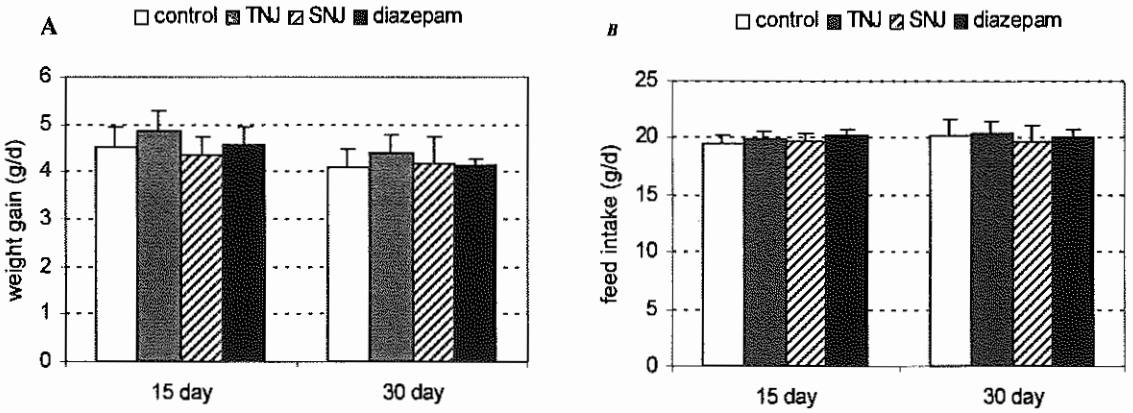

Figure 1 Effects of noni feeding on daily weight gain (A) and daily feed intake (B) There was no different between treatments when noni juices were fed for 15- or 30- days. Data presented as mean ± SE.

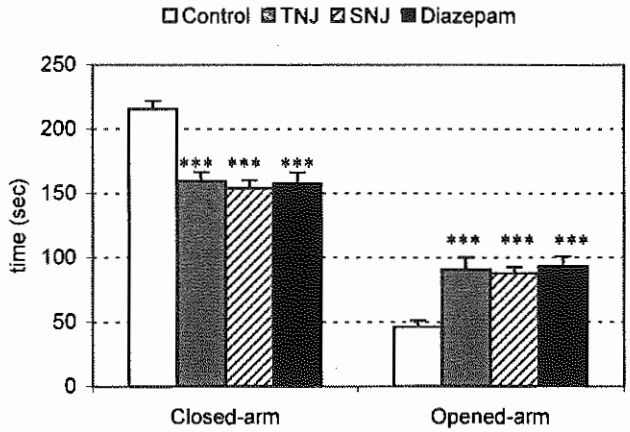

Figure 2 Effect of 15-day noni feeding on opened- and closed- arm time. Noni juice and diazepam were able to induce an anxiolytic like-behavior upon exposure to the EPM as they spent more time on the opened-arm and less time on the closed-arm compared to the controls. *** Significantly different from control at $p < 0.001$, one-way ANOVA followed by Student-Newman-Keuls test. Data presented as mean ± SE.

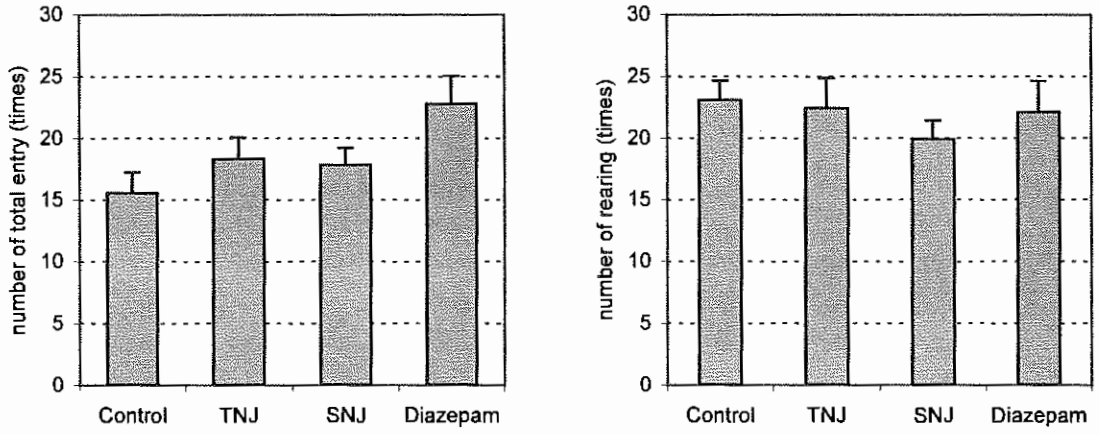

Figure 3 Effect of 15-day noni feeding on motor activity; total arm entry (A) and rearing (B). The total arm entry (A) and the number of rearing (B), the indicators of motor activity in rats tested with EPM were not different between treatments. Data presented as mean ± SE.

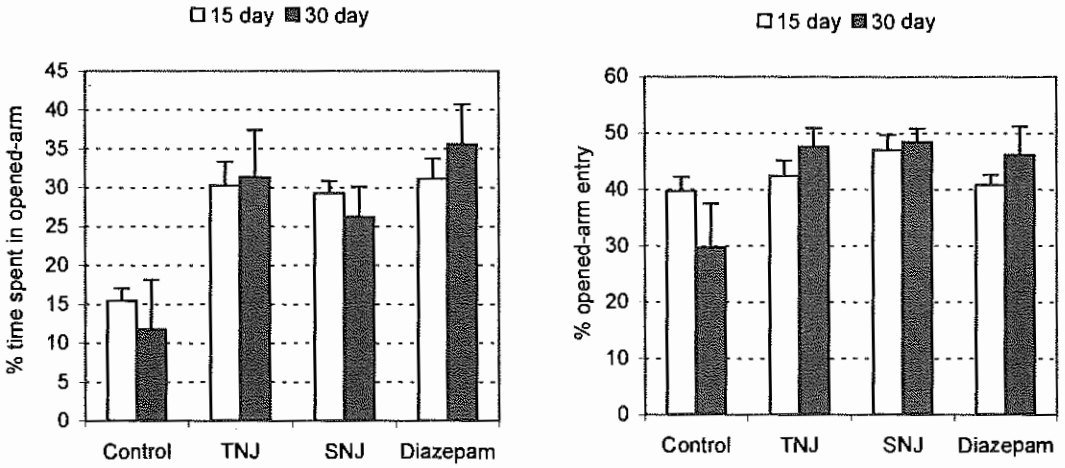

Figure 4 Effect of length of noni feeding on percentage of time spent on opened-arm (A) and percentage of opened-arm entry (B). There was no different in the percentage of time (A) or number of entry (B) into opened-arm when noni juices were fed for 15 or 30 days. Data presented as mean ± SE.

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.

	Control	**TNJ**	**SNJ**	**Diazepam**
AP (U/L)	633.00±92.47	421.25±29.08	478.75±36.32	504.75±83.25
GPT (U/L)	22.15±1.27	27.45±1.15	22.50±1.24	27.25±2.13
GOT (U/L)	68.68±6.78	81.10±8.58	85.88±14.15	77.65±5.37
BUN (mg%)	47.20±3.83	39.94±2.71	49.69±4.25	45.43±1.20
Na^+ (mEq/L)	137.75±4.39	137.25±2.87	139.50±2.18	138.25±4.39
K^+ (mEq/L)	4.33±0.15	3.90±0.07	3.95±0.21	4.25±0.29
Cl^- (mmol/L)	98.00±1.68	99.75±0.75	102.00±1.08	99.25±0.75
Glucose (mg/dL)	116.75±2.50	135.50±4.33	120.75±4.31	138.25±5.25

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 the 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[11]. 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[11,15]. 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[9] 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.

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