Anxiolytic-like Effects of Noni juice (Morinda citrifolia L.) on the Respective Changes of Neurotransmitters in Rat Brain in the Elevated Plus-maze Test

Sarinee Kalandakanond-Thongsong1* Jantarima Charoenphandhu2

Abstract

Noni (Morinda citrifolia L.) has long been used to treat various conditions in folklore medicine. Presently, most studies focus on antioxidant and anti-cancer activities while it is also claimed by noni juice-consumers to produce happiness. Mood disorders, e.g. anxiety, are symptoms that reflected the disorder of neurotransmitters, one of the most prominent is the monoaminergic system. Anxiety affects people throughout the world; people have been seeking medicine for treatment while natural products may also alleviate this unfavorable symptom. The present study examined whether noni contained anxiolytic-like effect when tested with elevated plus-maze (EPM), a standard test for anxiety, and whether this effect was related to change in monoamine transmitters in brain. Male Wistar rats were fed either noni juice or water (1 ml/day, PO for 15 days), and the anxiety level was measured with EPM. The brain monoamines were then analyzed with HPLC-electrochemical detector. We found that the noni-treated rats spent more time in the opened-arm than the control rats, indicating that the noni-treated rats were less anxious than the control rats. The neurochemical analysis revealed significant changes in noradrenergic, dopaminergic or serotonergic systems in amygdala, hippocampus and substantia nigra of the noni-treated group. These findings indicated that the noni juice produced anxiolytic-like behaviors in rats partially by modulating neurochemical metabolisms in brain regions related to anxiety.

Keywords: anxiolytic, elevated-plus maze, Morinda citrifolia L., noni, neurotransmitter, rat

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ผลของน้ำลูกยอ (Morinda citrifolia L.) ในการลดภาวะวิตกกังวลในหนูขาวที่ทดสอบด้วย elevated plus-maze ที่สัมพันธ์กับการเปลี่ยนแปลงสารสื่อประสาทในสมอง

บทคัดย่อ

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สุนันต์ กลันทกานนท์ ทองทรง จันทริมา เจริญพันธุ์

น้ำลูกยอ (Morinda citrifolia L.) เป็นพืชที่ถูกนำมาใช้ในการรักษาอย่างแพร่หลายตามความเชื่อแพทย์แผนโบราณในปัจจุบัน การศึกษาส่วนใหญ่แสดงให้เห็นว่าลูกยอฤทธิ์ต้านอนุมูลอิสระและเซลล์มะเร็งแต่ในขณะเดียวกันข้อมูลจากผู้บริโภครายงานว่าการบริโภค้น้ำลูกยอทำให้เกิดความสบายใจ ความผิดปกติทางอารมณ์เป็นอาการทางจิตที่เกิดจากความผิดปกติของสารสื่อประสาทในสมอง โดยเฉพาะกลุ่มโมโนเอมีน ซึ่งผู้ป่วยที่มีความผิดปกติต่างกันมีการใช้ยาเพื่อการรักษาในขณะที่การศึกษาทางคลินิกอาจเป็นทางเลือกหนึ่งที่น่าสนใจ งานวิจัยครั้นนี้มีวัตถุประสงค์เพื่อศึกษาฤทธิ์ในการลดภาวะวิตกกังวลของน้ำลูกยอ โดยการป้อนน้ำหรือน้ำลูกยอปริมาณ 1 ml. ให้หนูเพศผู้แต่ละตัวก่อนที่จะทำการทดสอบ 15 วัน ถัดไปการวัดพฤติกรรมทางจิตใจของหนูศักรายตามที่ใช้วัดความกังวล elevated plus-maze และการวัดสารสื่อประสาทกลุ่มโมโนเอมีนในสมองโดยใช้เทคนิค HPLC ผลการทดลองพบว่าหนูที่ได้รับน้ำลูกยอใช้เวลาอยู่ในส่วนแขนเปิดมากกว่าหนูกลุ่ม对照 ชี้ให้เห็นว่าหนูที่ได้รับน้ำลูกยอต้านวิตกกังวลที่น้อยกว่า และจากการวัดสารสื่อประสาทในสมองพบว่ามีการเปลี่ยนแปลงของสารสื่อประสาทกลุ่มโมโนเอมีนในสมองส่วนอะมิกดาลา ฮิปโปแคมปัสและซับแสตนเชียไนกรา ในหนูกลุ่มที่ได้รับน้ำลูกยอ การศึกษาครั้นนี้ชี้ให้เห็นว่าผลลัพธ์มีการลดภาวะวิตกกังวลในหนูโดยมีการทำงานแนวส่วนที่เกี่ยวกับการปรับเปลี่ยนการทำงานของสารสื่อประสาทในสมองส่วนที่เกี่ยวกับควบคุมพฤติกรรมกังวล

คำสำคัญ: การลดความกังวล Elevated-plus maze Morinda citrifolia L. น้ำลูกยอ สารสื่อประสาท หนูขาว

Introduction

Noni (Morinda citrifolia L.) 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, and recognized as nhau (yor) in Southeast Asia. The different parts of plant (e.g. fruit, leaf, bark, root, flower and seed) have long been employed in folklore medicine to treat broad range of diseases including diabetes, hypertension, infections, colds and cancer (Wang et al., 2002). 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, caprylic acid, caprylic acid, ursolic acid, rutin, a putative proxeronine, glycosides, and a tri-saccharide fatty acid ester (Liu et al., 2001; McKoy et al., 2002; Wang et al., 2002; Hornick et al., 2003). These evidences not only support the knowledge of noni as in folklore medicine, but also support some health benefits of the juice claimed by consumers. Nowadays, noni juice is commercially marketed as a health-promoting beverage; it was accepted in the European Union (Scientific committee on Food, 2002) and was patented and trademark in the USA (Chan-Blanco et al., 2006). Interestingly, other than antioxidant or anticancer properties, one interesting claim from consumers was feeling of happiness and relax while there have been limited numbers of studies on the central effect of noni. Younos et al. (1990) have shown that the root extract of noni contains sedative effect along with analgesic activity. However, Potterat et al. (2007) demonstrated that some ingredients presented in roots and leaves may not be found in juices. Nevertheless, the anxiolytic effect of noni juice has been reported (Kalandakanond et al., 2004).

Anxiety is a form of mood disorders. It has been proposed that managing various neurotransmitters using different medications can alleviate this disorder. The utilization of either cancer, anti-inflammatory and anti-angiogenic properties (Liu et al., 2001; McKoy et al., 2002; Wang et al., 2002; Hornick et al., 2003). These evidences not only support the knowledge of noni as in folklore medicine, but also support some health benefits of the juice claimed by consumers. Nowadays, noni juice is commercially marketed as a health-promoting beverage; it was accepted in the European Union (Scientific committee on Food, 2002) and was patented and trademark in the USA (Chan-Blanco et al., 2006). Interestingly, other than antioxidant or anticancer properties, one interesting claim from consumers was feeling of happiness and relax while there have been limited numbers of studies on the central effect of noni. Younos et al. (1990) have shown that the root extract of noni contains sedative effect along with analgesic activity. However, Potterat et al. (2007) demonstrated that some ingredients presented in roots and leaves may not be found in juices. Nevertheless, the anxiolytic effect of noni juice has been reported (Kalandakanond et al., 2004).

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pharmacologically active substances or genetically modified animals has shown that anxiety can be modified through monoaminergic activities including serotonin (5-HT), norepinephrine (NE) and dopamine (DA). The serotonergic system has been well accepted to play a key modulatory role in central nervous system processes that appear to be dysregulated in psychiatric disorders including anxiety. The usage of serotonin selective reuptake inhibitor (SSRI) or various agonists or antagonists to 5-HT receptors has been proven to induce anxiolytic effect both in animal models and human subjects (for review see Gingrich and Hen, 2001). Not only the serotonergic system, but the noradrenergic system is also suggested to be partially involved in the provocation and attenuation of anxiety. For example, the α2-adrenergic receptor antagonist was found to induce anxiety in rats (Tanaka et al., 2000).

Furthermore, the dopaminergic system also took responsibility in regulating anxiety, the dopamine D3 receptor agonists was found to produce anxiolytic-like effects in behavioral test models (Rogoz et al., 2003). Therefore, it is likely that anxiety is not a simple result of one abnormality, but indeed an integration of different dysfunction/dysregulation of monoaminergic systems.

In this study, the effect of noni juice on reducing anxiety was tested using an elevated plus-maze, a standard model specific for anxiety. This test is based on unconditioned responses to a potentially dangerous environment, the combination of height, luminosity and open space is assumed to induce fear or anxiety in rodents. The degree of anxiety is assessed by measuring the time spent on the open- and closed-arms, and the number of entries made into each arm (Pellow et al., 1985; Cruz et al., 1994; Rodgers and Dalvi, 1997). Furthermore, in order to correlate the anxiolytic effect of noni on neurotransmitter modulation; the changes in monoaminergic activities were also measured in various brain regions related to anxiety (e.g. frontal cortex, amygdala, hippocampus, etc.).

Materials and Methods

Animals: Male Wistar rats weighing 200-250 g 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 12 hrs light/dark cycles (lights on at 0700 hr) at room temperature (25±2°C). Standard rat chow and water were supplied ad libitum. After 7-day adaptation period, the rats were assigned randomly into 2 groups (6 rats per group): control and noni. The rats were fed 1 ml of water or noni juices for 15 days. The juice of noni (30 cm) and two arms opened. On the day of behavioral test, 15 min following being fed water or noni juice, each rat was placed in the center of the EPM facing a corner of the platform. The behavioral test was conducted during the light phase between 0900-1100 hr in a low natural light room. Each rat was allowed to explore freely on the EPM for 5 min and was recorded on VCR for later analysis. The parameters measured were time spent in opened-arm and closed-arm, including number of entries into each arm (opened-arm entry; closed-arm entry). 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. The maze was carefully wiped with a wet towel after each animal’s experiment.

Neurochemical analysis: After the behavioral test, the rats were euthanized with an overdosage of isoflurane, brains were rapidly removed, individually wrapped, frozen in liquid nitrogen and stored at -80°C for further analysis. Various brain regions known to contribute to anxiety were obtained according to the instruction of Heffner et al. (1980). These isolated brains were sonicated in iced-cold 0.1M perchloric acid containing 3, 4-dihydroxy-benzyl-amine hydrobromide (DHBA; Sigma, St. Louis, MO, USA), as an internal standard. The samples were centrifuged and the supernatant was collected for the measurement of monoamine neurotransmitters using high-performance liquid chromatography with electrochemical detector (HPLC-EC). The HPLC-EC system was composed of a glassy carbon-working electrode, an amperometric control (sensitivity 20 nA and oxidative potential +0.70 V; Bioanalytical systems, West Lafayette, IN, USA) and a 15-cm Luna® column (Phenomenex, Torrance, CA, USA). The mobile phase solution was 1 mM Heptane sulfonate, 100 mM Sodium dihydrogen phosphate, 1 mM Na2·EDTA and 5% Methanol, adjusted to pH 4.1 with saturated citric acid. The supernatant (40 μl) was injected into the HPLC-EC system to detect the monoamines including dopamine (DA) and its metabolites 3, 4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA); norepinephrine (NE) and its metabolite 3-methoxy-4-hydroxyphenylglycol (MHPG); and serotonin (5-HT) and its metabolite 5-hydroxyindole acetic acid (5-HIAA). The HPLC peaks were integrated and analyzed with Delta 5.0 software (Digital Solutions, Margate, QLD, Australia). The retention time and the concentration of neurotransmitters and its metabolites were calculated by injecting different concentrations of standard solutions into HPLC system. The concentrations of neurotransmitters and its metabolites were then converted and expressed as pmol/mg protein of brain tissue. Protein concentration of each brain regions was determined by the method of Lowry et al. (1951).

Statistical analysis: All data were presented as means and standard errors of means (SEM). For comparisons between two groups, unpaired Student’s t-tests were used. Differences were considered statistically significant at p<0.05.
Results

The anxiolytic effect of noni juice

After receiving noni juice for 15 days, the noni-treated rat spent more time in the opened-arm and less time in the closed-arms than the control rats (Table 1), resulting in a significantly greater percentage of time on the opened-arms of the plus-maze (Fig 1A; Table 1). In addition, the time per entry into the opened-arms (Time/Entry) was also higher in noni rats than control rats (Fig 1B). These data indicated an anxiolytic property of the noni juice in the EPM. There was no significant difference in the number of entries into the opened-arms (Table 1). The locomotor activity of animals, represented by the number of closed-arm and total arm entries (Table 1), was not different between these two groups. Therefore, a decrease in anxiety, as displayed by an increase in the time spent on the opened-arms, percentage of time on the opened-arms and the time per entry in the opened-arm, is independent of any change in locomotor activity. Furthermore, the number of times rearing and grooming activity was recorded from the EPM was also not different between groups (Table 1). However, it should be noted that the number of grooming in the noni group was less than the control group although the difference was not significant.

Brain neurochemistry and noni juice

The effects of noni juice on monoamines and their metabolite levels in brain areas that contribute to anxiety are shown in Table 2. There was no significant difference in NE, DA, 5-HT or their metabolites in the frontal cortex, caudate putamen, anterior hypothalamus, nucleus accumbens and septum. In the amygdala, the NE level in the noni group was lower than the control group (\( p = 0.0241 \)) with no significant change in its metabolite or ratio. The 5-HT level was tended to be lower in the noni group (\( p = 0.0976 \)) with no change in the 5-HIAA level; consequently, the ratio of 5-HIAA/5-HT was then tended to be lower (\( p = 0.0803 \)). The dopaminergic system was not altered. In the hippocampus, the NE content was significantly lower in noni group compare to the control group (\( p = 0.0335 \)) while the metabolite, MHPG or the ratio of MHPG/NE was not affected. The DOPAC, the DA metabolite was significantly lower in the noni group compared to the control group (\( p = 0.0027 \)). The other neurotransmitters were not affected. In the substantia nigra, the NE metabolite, MHPG level of noni group was significantly higher than the control group (\( p = 0.0281 \)) with a trend of higher ratio of MHPG/NE (\( p = 0.0503 \)). The DA metabolite, DOPAC tend to be lower in the noni group compared to the control group (\( p = 0.0652 \)); while another metabolite, HVA was significantly lower than the control group (\( p = 0.0281 \)).

Discussion

In this study, we found that when noni juice was given orally for 15 days, the rats spent more time exploring the aversive opened-arms as indicated by more time spent in these arms, as well as a higher time per entry in these arms, while the control rats avoid it and stayed in a protected closed-arm. Because the motor activity of the rats, indicated by the total number of entries into both arms and the rearing behavior were not different between treatments, we can conclude that noni juice reduced anxiety-related behavior in rats in the EPM. The study on the effect of noni on nervous system was scarce, only few studies had been done. In 1990, Younos et al. demonstrated the sedative and analgesic activity of noni root extract. Later in 2004, Kalandakanond et al. had shown that noni juice produced from different sources i.e. French Polynesia (Tahitian Noni juice®) and Thailand (Siam

![Figure 1](image)

Figure 1 Effects of noni on percentage of time spent in the opened-arm (A) and on the time spent per entry into the opened-arm in the elevated plus-maze test. *, **significantly different from control at \( p < 0.05 \) and \( p < 0.001 \), respectively (n= 6 per group). Data are presented as mean±SEM.

Table 1 Effects of noni on behavioral responses in the elevated plus-maze test.

<table>
<thead>
<tr>
<th>Behavioral Parameters</th>
<th>Control</th>
<th>Noni</th>
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<tr>
<td>Times spent in opened-arms (sec)</td>
<td>45.96 ± 6.58</td>
<td>98.95 ± 6.67**</td>
</tr>
<tr>
<td>Times spent in closed-arms (sec)</td>
<td>209.50 ± 7.59</td>
<td>139.48 ± 8.81**</td>
</tr>
<tr>
<td>Number of opened-arm entries (times)</td>
<td>6.33 ± 1.28</td>
<td>8.50 ± 0.67</td>
</tr>
<tr>
<td>Number of closed-arm entries (times)</td>
<td>8.67 ± 1.17</td>
<td>9.50 ± 1.31</td>
</tr>
<tr>
<td>Total arm entries (times)</td>
<td>15.00 ± 2.28</td>
<td>18.00 ± 1.13</td>
</tr>
<tr>
<td>Number of rearing (times)</td>
<td>23.50 ± 2.11</td>
<td>21.67 ± 2.36</td>
</tr>
<tr>
<td>Number of grooming (times)</td>
<td>12.50 ± 4.33</td>
<td>4.33 ± 2.35</td>
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Values are means±SEM. **\( p < 0.001 \) compared with control group by student’s t-test; n= 6 per group.
noni juice produced from different sources i.e. French Polynesia (Tahitian Noni juice®) and Thailand (Siam Noni®) had antianxiety property indifferent from benzodiazepine (diazepam), a classic anxiolytic drug. Recently, Muto et al. (2010) reported that the impaired cognitive function induced by stress in mice could be reduced by noni juice consumption. However, these researches contained no insight mechanism of such improvement.

In this current study, the neurochemical analysis was also conducted; we found alterations in the monoaminergic system in the noni-treated rats compared to the control rats. The reduction in these neurotransmitters including NE in the amygdala and the hippocampus, 5-HT in the amygdala, DOPAC in the hippocampus and the substantia nigra, and HVA in the substantia nigra of the noni group may contribute to anxiolytic effects of noni juice. It is known that central neurotransmitters play an important role in the control of anxiety with the dysregulation of serotonergic system being the major concern, while noradrenergic and/or dopaminergic systems were also believed to be partially involved. According to many studies about the 5-HT hypothesis, anxiety is usually associated with increased endogenous 5-HT, and anxiolytic tend to decrease endogenous 5-HT (Briley et al., 1990; Barnes et al., 1992; Handley, 1995). Thus, the decreased 5-HT levels in frontal cortex of noni group can partially explain its anxiolytic actions. Furthermore, Tanaka et al (2000) has revealed that NE release along with its increased metabolite, MHPG in amygdala and other brain regions were closely related with anxiogenic effect produced by α₂-antagonist, yohimbine. Similarly in our study, the decreased levels of NE in amygdala and hippocampus were observed in noni group. It might be another pathway by which noni acts to decrease anxiety-like behaviors. In term of DA activity, previously Ge et al. (1997) has shown that aversive situation increased the level of DA, DOPAC and HVA in brain regions related to anxiety (e.g. frontal cortex, amygdala, etc.). It is then likely that the lower level of anxiety in noni treated rats may be due to decreased DA and its metabolites throughout the brain with significant effect in hippocampus and substantia nigra. However, it is unlikely that only one neurotransmitter or only one region is involved in the provocation of anxiety; rather several neurotransmitters including norepinephrine, dopamine and serotonin, and several brain regions are probably involved. Moreover, the alterations of various neurotransmitters by noni may be mediated through GABAergic system since the regulation of GABA upon the monoaminergic system has been proposed through the co-localization of the GABA receptor on noradrenergic neuron (Kachidian et al., 1989), dopaminergic neuron (Waldvogel et al., 2009) and serotonergic neuron (Serrats et al., 2003). Further supported by Deng et al. (2007), they found that the methanol crude extract of noni fruit showed binding affinity to the GABAA receptors. It is, thus, possible that noni may act upon the GABAA receptor and modulate the activities of these monoaminergic neurons in various brain regions. Interestingly, Deng et al. (2007) also showed that the noni extract had no

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Table 2: Monoamines and their metabolites (nmol/mg protein) in different brain regions of control and noni treated rats.

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>Control (Mean ± SEM)</th>
<th>Noni (Mean ± SEM)</th>
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<tbody>
<tr>
<td>NE</td>
<td></td>
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<td>5-HT</td>
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<td>DOPAC</td>
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<td>HVA</td>
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<td>MHPG</td>
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Values are mean ± SEM. *p < 0.05, **p < 0.01 compared with control group, by student's t-test, n=6 per group.
binding affinity to 5-HT receptor or 5-HT transporter; noni was, thus, excluded as a non-selective ligand for 5-HT neurotransmitter receptors.

Conclusively, noni juice, a natural product of Morinda citrifolia L. contained anxiolytic effect in rat when tested with elevated plus-maze and this effect is in part mediated through the changes of monoaminergic neurotransmitter levels. Further studies are required to resolve the in depth molecular mechanism of noni mediated anxiolytic effect.

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References


