The purpose of this study was to investigate the anxiolytic effects of myristicin, a major compound found in nutmeg, and its potential interaction with the γ-aminobutyric acid (GABA<sub>A</sub>) receptor in male Sprague-Dawley rats. Nutmeg has traditionally been used as a spice in food preparation and as an herbal remedy in the treatment of many medical conditions, including anxiety. Fifty-five rats were divided equally into 5 groups: control (vehicle); myristicin; midazolam (positive control); flumazenil and myristicin; and midazolam and myristicin. The behavioral component of anxiety was examined by using the elevated plus-maze (open-arm and closed-arm times) along with analysis of gross and fine motor movements. Data analysis was performed using a 2-tailed multivariate analysis of variance (MANOVA) and least significant difference post-hoc test.

Our data suggest that myristicin does not decrease anxiety by modulation of the GABA<sub>A</sub> receptor but may promote anxiogenesis. When myristicin was combined with midazolam, an antagonist-like effect similar to the flumazenil and myristicin combination was exhibited by a decrease in anxiolysis compared with the midazolam-only group. Myristicin may antagonize the anxiolytic effects of midazolam, increase anxiety, and affect motor movements.

Keywords: Anxiolysis, elevated plus-maze, myristicin, nutmeg, rat.

Anxiety is a physiological state characterized by cognitive, somatic, emotional, and behavioral components. These components combine to create the feelings that we typically recognize as anger and that are known as fear, apprehension, or worry. Anxiety is often accompanied by physical symptoms such as heart palpitations, nausea, chest pain, shortness of breath, stomachaches, and/or headaches. The definition can be extended to include a feeling of apprehension caused by anticipation of impending danger that enables the individual to prepare for the imminent threat. According to the Anxiety Disorders of America, 40 million adults in the United States have some form of anxiety disorder, the cost of which is estimated to be more than $42 billion a year. Additionally, anxiety is a frequently experienced emotion in the perioperative patient, which can stimulate and activate the stress response.

Activation of the stress response results in a release of both neurotransmitters and hormones. The sympathetic nervous system (SNS) is also activated, resulting in an elevated heart rate, respiratory rate, and glycolysis, and the release of additional catecholamines such as epinephrine and norepinephrine from the adrenal medulla. Prolonged activation of the SNS can lead to fatigue and a weakened immune system.

Anxiety, as frequently experienced in perioperative patients, places them at greater risk of adverse outcomes and may result in a more complicated anesthetic plan. The resulting stress response may cause harm to the patient with an already tenuous status, perhaps resulting in an adverse outcome or resulting in poor wound healing. Conversely, it has been shown that when patients have feelings of well-being and decreased anxiety related to surgery, their satisfaction is increased and they may have shortened recovery times. Additionally, anxiolysis has been shown to be effective in improving postoperative outcomes such as nausea, vomiting, and pain.

Anesthesia providers frequently administer anxiolytic medications such as midazolam as a means to reduce anxiety, pain, and catecholamine activation. Research has demonstrated that premedication with benzodiaz-
Many Americans suffer from anxiety and attempt self-medication with complementary and alternative medicine. These unregulated herbal remedies may have similar mechanisms of action as drugs manufactured for the treatment of anxiety and other disorders. Common undesirable side effects of drugs developed for anxiolysis include hypotension, sedation, and a high potential for addiction and abuse. Conversely, herbal medications are not commonly associated with abuse or addiction. Herbal medication use has increased rapidly as alternative treatments have multiplied exponentially in America. Compared with previous studies, research indicates that 40% of US consumers reported using herbal products, representing a 25% increase in a 7-year period.

According to the Dietary Supplement Health and Education Act of 1994, there is no requirement for evidence of efficacy, safety, or quality control standards for supplements, increasing the risk of adverse effects related to herbal agents. In the United States between 1993 and 1998, the Food and Drug Administration documented approximately 2,600 adverse events, including 100 deaths, related to herbal medications. Currently, there is no central repository for documenting adverse effects outcomes associated with herbal remedy interactions; therefore, the true number of adverse effects may be much higher than reported. In fact, because of this undisclosed use of herbal medications, the Joint Commission has mandated a screening for herbal medications at each healthcare visit. This lack of data demonstrates the need for scientific research concerning herbal medications and the possible adverse effects and interactions with perioperative medicines. Many herbal products can interact with frequently used medications, including anesthesia, and may cause serious unforeseen consequences or complications.

Patients present for surgery with the belief that herbal remedies are benign, and they often fail to report their use to anesthesia providers. Herbal medications have become a major alternative to traditional medicines. In the United States between 1990 and 1997 the use of herbal medicine use increased 380%, and the use of vitamins increased 130%, and the use of vitamins increased 130%. This steep rise in herbal medication use may be associated with an increase in morbidity and mortality during the perioperative period as a consequence of interactions with prescribed medications or herbal-induced alterations in pharmacodynamics. Up to 72% of 1,539 adults surveyed regarding their alternative therapies, including herbal use, do not disclose their treatment to healthcare providers, exacerbating the problem. Although herbal products have numerous purported benefits, very little scientific research has been conducted concerning their pharmacologic properties.

The use of nutmeg as a spice in food preparation and as a remedy for common ailments such as nausea, vomiting, and diarrhea is centuries old. There are also reported uses of nutmeg as an aphrodisiac and narcotic. The use of nutmeg as a spice and a remedy for common ailments dates back as far as the 12th century. Nutmeg is widely used for homeopathic remedies, as a spice for cooking, soaps, hair tonics, and perfumes, and is thought to possess aphrodisiac properties. Nutmeg has been used by various individuals for its central nervous system activity such as euphoria, giddiness, and hallucinations. This activity is postulated to result from biotransformation of its chemical components to amphetamine-like compounds. Nutmeg contains more than 15 different compounds, and myristicin is the most abundant component of the nutmeg seed. Nutmeg also contains several compounds with structural similarities to substances with known central nervous system neuromodulatory activity. A case report described nutmeg toxicity consisting of dizziness, hallucinations, ataxia, and weakness. Myristicin, the main component of nutmeg, has been implicated to have the following adverse effects: detachment from reality, tachycardia, flushing, hypotension, drowsiness, confabulation, gagging, vomiting, ileus, paresthesias, numbness, blurred vision, hyperthermia, and sweating. It is important to explore the possible effects of myristicin as a first step in understanding how ingesting large amounts of nutmeg may affect patients receiving anesthesia, since scarce data regarding this compound exist. Very little research has been conducted regarding herbal products, including nutmeg, and the possible effects of their interactions with common pharmacologic agents such as benzodiazepines.

The first objective of this study was to determine if myristicin has anxiolytic effects in the rat model. The second objective was to investigate possible modulation of the γ-aminobutyric acid (GABA_\text{A}) receptor by myristicin in the central nervous system of the rat. Because nutmeg contains multiple compounds and substances, it is nearly impossible to obtain and administer a standardized nutmeg compound. Therefore, this study evaluated a standardized myristicin extract with a validated purity greater than 97%.

A prospective, experimental design using the elevated plus-maze (EPM) in the rodent model was used to investigate the objectives of this study. The EPM is a widely used instrument to measure anxiety in the rodent model and has been validated by Treit et al and Pellow and colleagues based on the previous work by Montgomery. Research on the EPM has supported its use as a standard measurement of anxiety and specifically benzodiazepine-induced anxiolysis in rodents. Rats inherently desire to explore a new environment but simultaneously avoid well-lighted and exposed areas (open arms of EPM).
Time spent in the open arm represents decreased anxiety exhibited in the rodent model. Time spent in the enclosed arm represents increased anxiety.16-20

To evaluate the anxiolytic effects of myristicin, we studied rats divided into 5 groups: control (vehicle); myristicin; midazolam (positive control); flumazenil (a benzodiazepine antagonist) and myristicin; and midazolam and myristicin.

**Material and Methods**
Fifty-five male Sprague-Dawley rats (Harlan Sprague Dawley Laboratories, Indianapolis, Indiana) weighing 200 to 300 g were used. They were housed in groups of 3 in polycarbonate “shoebox” cages lined with bedding. The animals experienced a 14-day adaptation period in a temperature-controlled environment (22°C ± 1°C, 60% humidity) with a light-dark cycle, receiving 12 hours of light (6 am to 6 pm) and 12 hours of dark (6 pm to 6 AM). They were provided free access to food and water. The animals were handled only for weighing, drug administration, and cleaning of cages and were naive to the EPM instrument. The use of laboratory rats in this protocol was in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals21; the protocol received approval from our Institutional Animal Care and Use Committee.

Rats were assigned to 1 of 5 treatment groups (11 rats per group) by the use of computer-generated numbers. Each animal received an intraperitoneal injection of one of the following: (1) vehicle (corn oil); (2) myristicin (Sigma Chemical Co, St Louis, Missouri), 30 mg/kg (based on previous work by Sonavane et al22), dissolved in corn oil23; (3) midazolam (Roche, Basel, Switzerland), 1.5 mg/kg; (4) flumazenil, 3 mg/kg (Sigma), dissolved in dimethyl sulfoxide, and myristicin, 30 mg/kg (dissolved in corn oil); or (5) midazolam, 1.5 mg/kg, and myristicin, 30 mg/kg (dissolved in corn oil). The group receiving flumazenil (a known benzodiazepine receptor antagonist24) plus myristicin was used to evaluate the potential modulation of the benzodiazepine receptor site on the GABAA receptor by myristicin. The flumazenil dose of 3 mg/kg was used based on previous rodent research examining anxiety.25

All animals received 2 injections of 1-mL total volume intraperitoneally. Depending on the group, the first injection was either flumazenil or dimethyl sulfoxide (vehicle for flumazenil). The second injection was also group dependent according to group assignments. In addition, all experimentation occurred on a timed schedule between the hours of 9 AM and 3 PM over 2 consecutive days to ensure that each treatment group was exposed to similar variability of corticosterone release related to the circadian rhythm of the animals.

After the 30-minute period following the drug administration, each animal was placed in the center of the EPM located in a lighted room. Each rat was oriented on the EPM facing an open arm, and behavioral responses to anxiety were evaluated by the EPM for 5 minutes. The EPM was networked with software (MotorMonitor, Version 5.00 GLP, Kinder Scientific Company, Poway California) that tracked the number of entries into each type of arm (open vs closed), time spent in the open arms expressed as a percentage of the total time, and fine and basic motor movements. Basic motor movements are the simple count of beam breaks in the EPM. Each time a photo beam is interrupted, the basic movement count is increased. These movements reveal a gross measure of locomotion but do not distinguish what type of activity is being performed. Fine motor movements are a compilation of small-animal movements such as grooming or head weaves or bobs. The EPM was cleaned with soap and water and dried between each animal trial to limit variability. Immediately following the 5-minute test on the EPM, the animals were removed and placed back in their cage.

**Results**
Data analyses were conducted using a 2-tailed multivariate analysis of variance and least significant difference (LSD) post-hoc test. Analysis of the ratio of open-arm time versus total time spent in the EPM revealed statistically significant increases between the midazolam and control groups (P = .003); midazolam and myristicin groups (P < .001); midazolam group and flumazenil plus myristicin group (P = .014); midazolam group and midazolam plus myristicin group (P = .02); and midazolam plus myristicin group and myristicin group (P = .039) (Tables 1 and 2; Figure 1).

Total number of basic (gross) and fine motor movements tracked during time in the EPM were analyzed. Analysis showed a significant increase in basic motor movement of rats in the flumazenil plus myristicin group compared with the myristicin group (P = .008); flumazenil plus myristicin group compared with the midazolam group (P < .001); and the flumazenil plus myristicin group compared with the midazolam plus myristicin group (P = .001) (Tables 1 and 2; Figure 2). Conversely, a decrease in basic motor movement was observed in the midazolam group compared with the control group (P = .003); and the midazolam plus myristicin group versus the control group (P = .014).

Similarly, a significant increase in fine motor movement of rats was found in the flumazenil plus myristicin group compared with the myristicin group (P = .39); flumazenil plus myristicin group versus the midazolam group (P < .001); and the flumazenil plus myristicin group compared with the midazolam plus myristicin group (P = .001). A decrease in fine motor movements was found in the midazolam group compared with the myristicin group (P = .046); and the midazolam plus myristicin group compared with the control group (P = .002) (Tables 1 and 2; Figure 2). Although there was
a similar trend of amplified movement, no statistically significant difference in number of gross or fine motor movements was noted between the myristicin group and the control group (Table 1 and Figure 2).

**Discussion**

Nutmeg and its compounds have a long history of mixed central nervous system actions such as sedation and central nervous system modulating effects. This study examined the purported anxiolytic properties of myristicin, a major nutmeg compound, and its potential interaction with the GABA$_A$ receptor site.$^{17,22}$

Similar studies by Sonavane et al.$^{22}$ suggested that a myristicin dose of 30 mg/kg would be appropriate to examine anxiolysis in our study because this dose showed combined anxiolytic and anxiogenic properties. In another study by Sonavane et al.$^{26}$ a smaller dose of myristicin, at 10 mg/kg, showed anxiolysis, in which the rats exhibited more time in the open arm of the EPM, compared with a higher dose at 100 mg/kg, which showed anxiogenesis and more time in the closed arm. In these studies, rats remained conscious with the ability to maintain locomotion, which is essential for participation in the EPM. Thus, we determined that 30 mg/kg was the optimal dose of myristicin for rats to maintain the ability to ambulate in the EPM.

The behavioral measurements comparing the ratio of open-arm time to total time spent in the EPM suggests that myristicin does not produce anxiolysis. In addition, there was a significant difference between the flumazenil

<table>
<thead>
<tr>
<th>Measure</th>
<th>Control</th>
<th>Myristicin</th>
<th>Midazolam</th>
<th>Flumazenil + myristicin</th>
<th>Midazolam + myristicin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratio open-arm/total maze time</td>
<td>24.1 ± 12.3</td>
<td>12.8 ± 8.6</td>
<td>49.8 ± 32.6$^a$</td>
<td>28 ± 14.0</td>
<td>30.1 ± 19.0$^a$</td>
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<td>Basic motor movements</td>
<td>862 ± 144.7</td>
<td>670.3 ± 194</td>
<td>544.7 ± 366.9$^a$</td>
<td>952.4 ± 134.2$^a$</td>
<td>603.1 ± 270.6$^a$</td>
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<td>Fine motor movements</td>
<td>623.9 + 104</td>
<td>506.9 + 128.1</td>
<td>366.1 + 250.8$^a$</td>
<td>652.9 + 73.4$^a$</td>
<td>401.4 + 186.1$^a$</td>
</tr>
</tbody>
</table>

Table 1. Ratio of Open-Arm Time to Total Maze Time (in seconds) and Number of Motor Movements on Elevated Plus-Maze Per Group

Data represented as mean ± SD.

$^a$ Indicates statistically significant difference of $P < .05$.  

<table>
<thead>
<tr>
<th>Measure</th>
<th>Group</th>
<th>Post-hoc analyses LSD $P$ value</th>
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</thead>
<tbody>
<tr>
<td>Ratio open-arm time/total maze time in second group</td>
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<tr>
<td>Midazolam</td>
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<td>Basic motor movements</td>
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<tr>
<td>Midazolam + myristicin</td>
<td>Flumazenil + myristicin</td>
<td>.001</td>
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Table 2. Post-Hoc Analysis: Ratio of Open-Arm Time/Total Maze Time (in seconds) and Number of Motor Movements on Elevated Plus-Maze Per Group

LSD indicates least significant difference,
(benzodiazepine receptor antagonist) plus myristicin group and the myristicin-alone group, which suggests there may be a possible interaction between myristicin and flumazenil. Alone, myristicin does not show significant modulation of the benzodiazepine receptor; therefore, these data do not support the hypothesis that myristicin modulates the GABA<sub>A</sub> receptor resulting in anxiolysis.

Motor movement data demonstrate that midazolam decreased both basic and fine motor movements, and flumazenil and myristicin significantly increased both basic and fine motor movements. The motor activity data for the myristicin group showed no significant difference compared with the control. The data suggest that myristicin may act at sites other than the hypothesized benzodiazepine site of the GABA<sub>A</sub> receptor. These findings may be the result of the modulation of another neurotransmitter site in the central nervous system. Although our data did not support anxiolytic effects of myristicin in the rat model, it suggests that myristicin modulates the central nervous system by increasing gross and fine motor movements in combination with flumazenil.

This study can be used to guide future research in investigating the effects of myristicin. We recommend exploring the possibility that myristicin may act as a benzodiazepine GABA<sub>A</sub> antagonist, much like flumazenil. An important future study should include a flumazenil-only research group to compare these results with myristicin alone. It is also important to determine the molecular site of action of myristicin in order to understand the biochemical effects of this herbal compound and then pinpoint the site or sites of action of myristicin. Myristicin may act at sites other than the hypothesized benzodiazepine site of the GABA<sub>A</sub> receptor.

Sonavane et al<sup>26</sup> pointed out that incremental doses of myristicin produced varied responses in the rat model. This dose-response relationship should be further investigated by escalating doses of myristicin in the same model with the inclusion of a flumazenil-only group. Our review of the literature revealed various central nervous system effects. We suggest evaluating other doses of myristicin for their respective central nervous system effects to discern any dose-dependent variances in action.

Of interest to anesthesia, we recommend future investigations to explore the possible interactive relationship with flumazenil and studies designed to determine the effect of myristicin on other neurotransmitter systems, that is, the glutamatergic receptors (e.g., N-methyl-D-aspartate, or NMDA); and other known GABA<sub>A</sub> receptor

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Figure 1. Ratio of Open-Arm Time to Total Time (in seconds) on Elevated Plus-Maze
Each group was composed of 11 rodents. Drugs were injected 30 minutes prior to testing on the elevated plus-maze.
*Indicates statistically significant difference of P < .05.
SEM indicates standard error of the mean.

Figure 2. Basic and Fine Motor Movements on Elevated Plus-Maze
Each group was composed of 11 rodents. Drugs were injected 30 minutes prior to testing on the elevated plus-maze.
*Indicates statistically significant difference of P < .05.
SEM indicates standard error of the mean.
agonists. Lastly, we suggest that a prospective study may assist in determining if higher doses of benzodiazepines are required in perioperative patients who consume nutmeg.

REFERENCES


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