The purpose of this study was to investigate the anxiolytic effects of luteolin and its potential interaction with the gamma-aminobutyric acid (GABA_A) receptor in male Sprague-Dawley rats. Lemon balm has traditionally been used as an herbal remedy in the treatment of many medical conditions, including anxiety. Luteolin is a major component of the essential oil lemon balm.

We divided 55 rats into 5 groups: (1) control (negative control), (2) luteolin, (3) midazolam (positive control), (4) flumazenil and luteolin, and (5) midazolam and luteolin. The behavioral component of anxiety was examined by using the elevated plus-maze (open arm time/total time) and motor movements.

Data analyses were performed using a 2-tailed multivariate analysis of variance and Sheffe post hoc test. Our data suggest that luteolin does not produce anxiolysis by modulation of the GABA_A receptor; however, luteolin may modulate motor movements and locomotion.

Keywords: Anxiolysis, elevated plus-maze, lemon balm, luteolin, rat.

It is estimated that 19 million adults in the United States have some form of anxiety disorder, which is speculated to cost more than $42 billion a year in care and treatment of patients with anxiety.¹ The 1-year prevalence rate for all anxiety disorders exceeds 16% among adults ages 18 to 54 years and includes significant overlap with substance abuse and mood disorders.² Various physiological and psychological stimuli, including anxiety, can activate the stress response resulting in endocrine and neurotransmitter release.³ Corticosteroid levels are elevated to modulate the biologic response to stress. The sympathetic nervous system is also activated by stress-producing anxiety, resulting in release of catecholamines (ie, epinephrine and norepinephrine) from the adrenal medulla.³ This “flight or fight” response includes elevated heart rate and respiratory rate, glycolysis, and the release of various neurohormonal mediators. As the exposure to these mediators and neurotransmitters continues, fatigue and a weakened immune system may result.³

Many Americans have anxiety and attempt to treat themselves in numerous ways, including the use of herbal medications. Enthusiasm for herbal medications as alternative treatments has increased rapidly in America. In 1997, 12% of US consumers reported using herbal medications, representing a 380% increase since 1990.⁴ In the United States alone, between 1993 and 1998, the Food and Drug Administration documented approximately 2,600 adverse events, including 100 deaths, related to herbal medications.⁴

Patients are admitted for anesthesia and surgery with the belief that herbal remedies are benign and, therefore, often fail to report use to anesthesia providers. Herbal medications have become a significant alternative to medicines; however, limited information is available regarding their effects during the perioperative period.⁴ The steep rise in herbal medication use may be associated with an increase in morbidity and mortality in the perioperative period as a consequence of herbal interactions with other prescribed medications (polypharmacy) or herbally induced alterations in physiology.⁵ Cardiovascular effects (myocardial infarction and stroke), altered hemostasis, prolonged or insufficient anesthesia, organ transplantation rejection, and drug interactions have been reported as complications of herbal supplements.⁵ Up to 70% of herbal users do not reveal their use to healthcare providers, which may exacerbate the problem.⁶ While herbal medications have numerous purported benefits, very little scientific research has been conducted concerning their pharmacologic properties.

Lemon balm has been used in traditional medicine from ancient times for the medicinal treatment of the following maladies: anxiety, insomnia, insect bites, muscle spasms, and viral infections.⁷ Luteolin, a flavonoid of lemon balm, is considered to have sedative and anti-in-
flammatory effects, but little is known about its biochemical targets. It has been suggested that lemon balm components modulate nicotinic and muscarinic receptors on human cerebral cortical cell membranes. Although these data suggest luteolin and lemon balm modulate various biochemical and physiologic functions, there are no data specifically describing the anxiolytic effects of luteolin.

The purposes of this study were 2-fold: to determine if the lemon balm flavonoid, luteolin, has anxiolytic effects in the rat model and to investigate possible modulation of the gamma-aminobutyric acid (GABA<sub>A</sub>) receptor by luteolin in the rat central nervous system.

### Materials and methods

For the study, 55 male Sprague-Dawley rats (Harlan Sprague Dawley Laboratories, Indianapolis, Indiana) weighing 200 g to 250 g were used. They were housed in groups of 4 in polycarbonate “shoebox” cages lined with bedding. The animals went through 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 (6:00 AM to 6:00 PM) and 12 hours of dark (6:00 PM to 6:00 AM). They were allowed free access to food and water. The animals were handled only for weighing, drug administration, and cleaning of cages and were naive to the elevated-plus-maze (EPM) apparatus. The use of laboratory rats in this protocol was in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and received Institutional Animal Care and Use Committee approval.

Rats were divided into 5 treatment groups (11 rats per group). Each animal received an intraperitoneal injection of one of the following: (1) vehicle (Tween 1%, Sigma Chemical Co, St Louis, Missouri, solvent for luteolin); (2) luteolin (Sigma), 50 mg/kg, dissolved in Tween 1%; (3) midazolam (Roche, Basel, Switzerland), 1.5 mg/kg; (4) flumazenil (Sigma), 3 mg/kg, dissolved in dimethyl sulfoxide and luteolin, 50 mg/kg (dissolved in Tween 1%); or (5) midazolam 1.5 mg/kg, and luteolin, 50 mg/kg (dissolved in Tween 1%). The group that received flumazenil (a known benzodiazepine receptor antagonist) and luteolin was used to evaluate the potential modulation of the benzodiazepine receptor site on the GABA<sub>A</sub> receptor by luteolin. The group receiving midazolam and luteolin was used to evaluate the potential additive or synergistic effect of luteolin with a known benzodiazepine agonist. All animals received two 1-mL total volume intraperitoneal injections. Experimentation occurred between the hours of 8:00 AM and 2:00 PM during 2 consecutive days.

After the 30-minute period following drug administration, each animal was placed in the center of the EPM in a lighted room. The EPM is a widely used instrument to measure anxiety in the rodent model and has been validated by Treit et al<sup>13</sup> and Pellow and colleagues<sup>14</sup> based on previous work by Montgomery.<sup>15</sup> Research on this instrument has supported its use as a standard measurement of anxiety and, specifically, benzodiazepine-induced anxiolysis in rodents.<sup>16</sup>

Each rat was initially placed on the EPM oriented facing an open arm. Rats innate desire to explore a novel

### Table. Ratio of Open Arm Time to Total Maze Time in Seconds and Number of Short-Duration Crossings and Total Motor Movements on the Elevated Plus-Maze Per Group<sup>a</sup>

<table>
<thead>
<tr>
<th>Group</th>
<th>Vehicle</th>
<th>Luteolin</th>
<th>Midazolam</th>
<th>Flumazenil + luteolin</th>
<th>Midazolam + luteolin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratio open/total</td>
<td>20.72 ± 9.72</td>
<td>16.95 ± 8.22</td>
<td>34.32 ± 33.35</td>
<td>19.80 ± 15.09</td>
<td>23.56 ± 33.24</td>
</tr>
<tr>
<td>Short-duration crossings</td>
<td>7.45 ± 4.741</td>
<td>4.73 ± 4.027</td>
<td>3.64 ± 3.957</td>
<td>4.0 ± 4.69</td>
<td>1.45 ± 2.948&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total movement</td>
<td>14.52 ± 4.76</td>
<td>12.01 ± 4.44</td>
<td>7.91 ± 7.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.86 ± 5.28</td>
<td>4.5 ± 4.35&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>P</td>
<td>.037</td>
<td>&lt;.000</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Data are presented as mean ± SD.

<sup>b</sup> Indicates significant statistical difference of P < .05.

### Figure 1. Ratio of Open Arm Time/Total Time (in seconds) on the Elevated Plus-Maze

Each group was composed of 11 rodents. Drugs were injected 30 minutes before testing on the elevated plus-maze.
environment but simultaneously avoid well-lighted and exposed areas. Time spent in the open arm represents decreased anxiety exhibited in the rat model. Time spent in the enclosed arm represents increased anxiety. Each test was video recorded for verification of data validity. The video tapes were blindly reviewed by 4 researchers (T.R., P.J., N.M., R.D.) to track time spent in the open arms expressed as a ratio of the total time on EPM (5 minutes). Short-duration crossings measured the number of crossings from closed arm to closed arm. In addition, each appearance from the enclosed arms and a simple count of observable movement in the open arms of the EPM were analyzed. These movements reveal a gross measure of locomotion but do not distinguish what type of activity (fine or gross movement) is being performed. Each observed appearance from enclosed arms, ie, gross or fine motor movements such as sniffing, head dipping, grooming, or rearing, were quantified on blind reviews. The EPM was cleaned with soap and water and dried between each animal run to limit variability. Immediately following the 5-minute test on the EPM, the animals were removed from the testing room.

Results
Data analyses were performed using a 2-tailed multivariate analysis of variance and Sheffé post hoc test. Data were collected from 55 subjects. Analysis of the ratio of open arm time to total time spent in the EPM (5 minutes) revealed no statistically significant difference between groups (Table and Figure 1).

The total number of crossings from closed arm to closed arm observed during time in the EPM were analyzed. Analysis showed a significant decrease in movement of rats in the midazolam and midazolam and luteolin groups compared with the control group (Table and Figure 2). The midazolam group ($P = .037$) and the midazolam and luteolin group ($P < .000$) showed statistical significance for decrement of movements. The midazolam and luteolin group also demonstrated a significant decrease in short-duration crossings ($P = .011$). Our data suggest that luteolin does not produce anxiolysis by modulation of the $\text{GABA}_A$ receptor; however, luteolin may modulate motor movements and locomotion.

Discussion
Lemon balm and its extracts have a long history of suspected central nervous system actions such as sedation and calming effects. This study investigated the purported anxiolytic properties of luteolin and its potential interaction with the $\text{GABA}_A$ receptor site.

A study by Coleta and colleagues suggested that a luteolin dose of 50 mg/kg would be appropriate to examine anxiolysis in our study. These data suggested a clear sedative effect from aqueous extracts of lemon balm at doses ranging from 10 to 100 mg/kg dose in mice. However, no deficiency in motor activity was noted with doses in these ranges.

The behavioral measurements from our research comparing the ratio of open arm time to total time spent (5 minutes) in the EPM suggest that luteolin is not an anxi-
iolytic. In addition, there was no difference between the flumazenil (benzodiazepine receptor antagonist) and luteolin group, which suggests it does not modulate the benzodiazepine receptor site, and the hypothesis that luteolin modulates the GABA_A receptor is not supported.

These data demonstrated that midazolam and midazolam and luteolin significantly decreased motor movements, and the motor activity also trended downward for the luteolin group. Significance was found between the luteolin and midazolam plus luteolin groups, suggesting a possible additive or synergistic effect. Although our data did not support anxiolytic effects of luteolin in the rat model, they suggest that luteolin degrades motor movement potentially by modulation of the central nervous system or neuromuscular system. The motor movement results in the midazolam plus luteolin group additionally suggest that luteolin may have additive or synergistic effects with midazolam, a known GABA_A benzodiazepine agonist. This may be the result of the modulation of another neurotransmitter site in the central nervous system or neuromuscular junction. Possible explanations for our findings may be based on studies by Wake and colleagues and Jiang et al. Wake and colleagues suggested that lemon balm components modulate nicotinic and muscarinic receptors on human cerebral cortical cell membranes. Jiang et al suggested that luteolin inhibits sarcoplasmic calcium channels and intracellular calcium release in the aorta of the rat model. Hence, it is important to determine the molecular site of action of luteolin to understand the biochemical effects of this herbal extract.

Future investigations should explore the motor effects of luteolin using other balance and locomotion instruments such as the roterod. Additional studies should be designed to determine potential interactions of luteolin at glutamatergic receptors (eg, N-methyl-D-aspartate), GABA receptors (other than the benzodiazepine site), dopaminergic receptors, and receptors at the neuromuscular junction. Once the molecular mechanism of action is clearly defined, work may then focus on studying possible clinical interactions of luteolin and other pharmaceuticals. This future research could then further explore the efficacy of luteolin as an anxiolytic adjunct or whether it has adverse interactions with anesthesia medications in the perioperative period.

REFERENCES


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