Herbal medication use continues to rise and interactions with existing medications propose risks and may have significant effects and consequences on the administration of anesthesia. The purpose of this study was to investigate the anxiolytic and antidepressant effects of asiatic acid and its potential modulation of the \(\gamma\)-aminobutyric acid (GABAA) receptor. Fifty-five male Sprague Dawley rats were divided into 5 groups: vehicle (DMSO), asiatic acid (AA), midazolam, or a combination of flumazenil + AA or midazolam + AA, and injected intraperitoneally 30 minutes prior to testing. The rats were tested on the Elevated Plus Maze (EPM) and the Forced Swim Test (FST). Data were analyzed using a two-tailed multivariate analysis of variance (MANOVA). Significance was found regarding the ratio of open arm time, maximum speed, and time spent mobile in the AA group and the midazolam + AA group (\(P < .05\)). Flumazenil decreased the anxiolytic effects, suggesting that AA modulates the benzodiazepine site on the GABA\(_A\) receptor. Further studies are recommended to determine the efficacy of prolonged treatment for anxiety and depression.

Keywords: Anxiolysis, asiatic acid, elevated plus maze, forced swim test, Sprague Dawley rat.
Depression is hypothesized to be a result of decreased concentrations of neurotransmitters in the brain, such as norepinephrine and serotonin, and that these patients may also secrete excessive amounts of cortisol and have alterations in their circadian rhythms.6 Medications used to attenuate the symptoms of anxiety, such as benzodiazepines, have also been shown to have efficacy in improving postoperative outcomes, such as nausea/vomiting and pain.7 As early as 1964, Egbert8 in his landmark study on preoperative anxiety found that patients with decreased anxiety required less pain medication and were discharged an average of 2.7 days sooner. Because of these and other positive outcomes, the use of anxiolytics has become common with preoperative administration of midazolam to 75% of adults <65 years of age.9 Additionally, many of the medications used to treat anxiety disorders may also be used for depressed patients; however, current antidepressant therapies are limited because of a significant time lapse between initiation of medication and improvement of symptoms. A recent study found that 28% of patients take between 10–14 weeks to have remission of their symptoms.10

In addition to traditional medications used in a controlled environment, anesthesia providers should be aware that many patients self-medicate for anxiety or depression using herbal preparations. Recent studies have found that there continues to be significant use of herbal medications in the United States. Prevalence of herbal therapy increased from 12% in 1997 to over 18% in 2002.11 This trend continues, as evidenced by the 2007 National Health Interview Survey (NHIS), which found that 17.7% of Americans reported using nonvitamin/non-mineral natural products in the past year.12 Furthermore, a 2004 study of active duty enlisted personnel found a much higher rate of supplement use among that population, with over 60% of survey participants using supplements at least once per week.13 McPherson14 found that pain, stress, and anxiety were the most common reasons for using Complementary and Alternative Medicine (CAM) among active duty soldiers, military retirees, and family members at a military hospital. Finally, a 2000 survey by Tsen and colleagues15 found that 22% of presurgical patients reported the use of herbal remedies.

The increasing trend in the use of herbal medications is not an issue in and of itself; rather it is the lack of reporting by patients that is of clinical significance. Since 2007, The Joint Commission has required documentation of any patient use of herbal remedies, vitamins, and nutraceuticals. The Joint Commission National Patient Safety Goals (NPSG.03.06.01) stress the importance of documentation of all current prescription, over-the-counter (OTC), and herbal medications,16 but this persists as a challenge for anesthesia providers because the rate of failure to disclose the use of alternative or herbal medications may be as high as 77%.17 Furthermore, the safety, efficacy, and quality control of supplements are not regulated by the FDA, which makes it difficult to generalize study outcomes. The concern surrounding herbal self-medication and lack of disclosure by patients is that these preparations may have side effects or mechanisms of action that interfere with anesthetic medications.18 Little research exists as to efficacy, side effects, or interactions of most herbal therapies.

Gotu kola, or *Centella asiatica*, a popular Indian herbal medication, has been used to treat several microcirculatory problems and gastrointestinal disorders, improve cognitive impairment, and decrease anxiety.19 Additionally, the purported neuroprotective properties of *Centella asiatica* have been investigated as a possible therapy for patients with memory loss20 and for GABA_A modulation activity.21 Asiatic acid (AA), a triterpenoids isolated from *Centella asiatica*, was shown to have an inhibitory effect on acetylcholinesterase and selective GABA_A receptor agonist activity.22 Additionally, AA at 30 mg/kg was demonstrated to improve memory in the rat model.23 *Centella asiatica* may be useful in the treatment of generalized anxiety disorder and it has been suggested that it may be used as a promising anxiolytic or antidepressant agent in the near future.24 However, there are no in vivo data describing the potential anxiolytic or antidepressant effects of AA as the active component of *Centella asiatica*.

The purpose of this study was 2-fold: to determine if AA has anxiolytic and/or antidepressant effects in the rat model; and to investigate possible modulation of the benzodiazepine site on GABA_A receptors in the rat central nervous system.

**Material and Methods**

This study used the Elevated Plus Maze (EPM) to evaluate anxiety and the Forced Swim Test (FST) to examine depression. The EPM and FST have been used extensively in evaluating anxiolytic and antidepressant effects of herbal medications. For example, valerian extracts demonstrated significant decreases in anxiety and depression in the rat model25 and the combination of St John’s wort and Passiflora significantly enhanced the antidepressant effects of St John’s wort in the FST.26

Fifty-five male Sprague Dawley rats (Harlan Sprague-Dawley Laboratories) weighing 250–300 grams were used. They were housed in groups of 3 in polycarbonate “shoebox-sized” cages lined with bedding. The animals went through a 7-day adaptation period in a temperature-controlled environment (22 ± 1°C, 60% humidity) with a reverse light-dark cycle where they received 12 hours of light (12:00 AM to 12:00 PM) and 12 hours of darkness (12:00 PM to 12:00 AM). The rats were allowed free access to food and water. The animals were handled for weighing, drug administration, and cleaning of cages only. The use of laboratory rats in this protocol was in accordance
with the NIH Guide for the Care and Use of Laboratory Animals and received approval from the Institutional Animal Care and Use Committee at the US Army Institute of Surgical Research (USAISR), San Antonio, Texas.

The 55 rats were randomly assigned to 5 groups (n = 11 per group). Each animal received intraperitoneal injections of the following: 1) vehicle 0.5% dimethyl sulfoxide (DMSO); 2) AA 30 mg/kg; 3) midazolam 1.5 mg/kg; 4) flumazenil 3 mg/kg + AA 30 mg/kg; or 5) midazolam 1.5 mg/kg + AA 30 mg/kg. DMSO is commonly used as a solvent for water-insoluble drugs and was used to prepare flumazenil and AA. Group number 3 (midazolam 1.5 mg/kg) had significant importance as midazolam, a known benzodiazepine, was used for comparison between the other groups because of its established anxiolytic effects. Group number 4 (flumazenil + AA) was used to evaluate the effects of AA modulated at the benzodiazepine site on the GABA<sub>A</sub> receptor because flumazenil is an antagonist at this site. Group number 5 (midazolam 1.5 mg/kg + AA 30 mg/kg) was used to evaluate if there were interactive effects with the coadministration of these drugs. All animals received equivalent intraperitoneal volumes consisting of 2 separate 1-mL injections, for a total volume of 2 mL. All experimentation occurred on a timed schedule between 3:00 pm and 9:00 pm over 4 consecutive days to control for the circadian rhythm of the animals.

**Elevated Plus Maze (EPM).** The EPM is an instrument utilized to measure anxiety in the rodent model and has been validated in previous research studies. The EPM consists of 2 open arms and 2 closed arms 50 centimeters (cm) in length and 10 cm in width. The open arms are directly opposite of each other; similarly, the closed arms oppose one another. The EPM is shaped like a cross (+) with the intersection of the 4 arms measuring 10 x 10 cm. The open arm was covered with 1-cm high Plexiglas on the side to prevent the rodent from falling. The maze was placed 50 cms above the floor. The EPM, as well as the interior floor, was painted a dark color to minimize rodent stimulation, and provided a waterproof surface when exposed to urine and feces (Figure 1).

All rats were injected intraperitoneally 30 minutes before behavioral evaluation with the EPM. The rationale was that previous studies have demonstrated anxiolytic effects between 20–30 minutes. Between the time of injection and testing, the rat was placed back into its cage in order to reduce any confounding influences or exposure to any additional unfamiliar environments. A testing period of 5 minutes was used on the EPM. Each rat was placed in the center of the EPM facing an open arm. During the 5-minute test, the number of entries into each type of arm and the time spent in each arm was scored, as well as speed and mobility of each rat. A rat was considered to have entered an arm via the AnyMaze programmed software. The time spent on the open arms was also expressed as a percentage of the time spent on both the open and the closed arms. Rodents are animals of prey that innately avoid the exposure of the open arms of the EPM; therefore, an increase in the percentage of time spent in the open arms reflected an anxiolytic effect. Between testing each animal, the EPM was cleansed with nonfragrant soap and water and dried. Each experimental session was recorded for 5 minutes by the AnyMaze software and was analyzed by an investigator blinded to the treatment received by each animal.

**Forced Swim Test (FST).** After evaluation in the EPM, animals were carried to a separate room and evaluated in the Forced Swim Test (FST). Since its development, the FST has been a valid assessment of depression in the rodent model. In 2 sessions separated by 24 hours, rats were forced to swim in a narrow cylinder from which they could not escape (Figure 2). The transparent cylinders (20 cm diameter x 40 cm high) contained water (25°C ± 2°C) to a depth of 13 cm, and the water was changed for each animal. The first session, lasting 15 minutes, was the training session and was conducted 24 hours prior to drug administration and without behavioral recording. After 15 minutes, the animal was removed, dried, and placed in its cage. This training session acclimated the rats to the test situation, thereby providing a stable, high level of immobile behavior during the 5-minute test session 24 hours later that was video recorded. Two trained research assistants blindly reviewed videotapes, and the amount of time the rats were mobile was calculated.

The duration of immobility when rodents are exposed to an inescapable situation (FST) is a reflection of depression. In this model of depression, the longer the duration of immobility, the greater the behavioral despair or depression. In addition to depression, stress was measured in the FST by fecal pellet output (FPO) of the rodents.
Stress alters gastrointestinal motility by delaying gastric emptying and accelerating colonic transit. The amount of mobility time and FPO were calculated by the blind review of the videos.

Data were collected from 54 rodent subjects because 1 subject was withdrawn from the study because of inadvertent, inaccurate medication administration. This error was realized immediately and the subject was removed from the study, decreasing the number of subjects from which data analysis was performed to 54. The rats weighed an average of 253.43 grams, without significant difference between groups (Table 1). All data were analyzed using a two-tailed multivariate analysis of variance (MANOVA) and a LSD post-hoc test.

**Results**

- **Elevated Plus Maze.** Analysis of data collected from the EPM, as tracked by the AnyMaze software, revealed statistically significant differences in ratio of open-arm time, mobility, and maximum speed of movement exhibited by the various subject groups \((P < .05)\).

  Analysis of ratio open-arm time to total time in the EPM showed significant increases in time spent in the open arms of the EPM by the rodents in the midazolam + AA group. The subjects in the midazolam + AA group

![Figure 2. Photograph of Forced Swim Test (FST) Used to Collect FST Mean Time Mobile and Fecal Pellet Output (FPO)](image)

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Vehicle</th>
<th>Asiatic acid</th>
<th>Midazolam</th>
<th>Flumazenil + Asiatic acid</th>
<th>Midazolam + Asiatic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weights</td>
<td>252.20 ± 2.28</td>
<td>252.09 ± 3.17</td>
<td>254.09 ± 3.12</td>
<td>250.36 ± 2.88</td>
<td>258.27 ± 4.10</td>
</tr>
<tr>
<td>EPM – Time mobile (seconds)</td>
<td>162.04 ± 11.82a</td>
<td>106.96 ± 10.81a</td>
<td>102.96 ± 16.39a</td>
<td>131.91 ± 18.01a</td>
<td>66.97 ± 17.18a</td>
</tr>
<tr>
<td>EPM – Mean max speed (velocity)</td>
<td>0.59 ± 0.03a</td>
<td>0.48 ± 0.03a</td>
<td>0.50 ± 0.03</td>
<td>0.55 ± 0.03</td>
<td>0.47 ± 0.05a</td>
</tr>
<tr>
<td>EPM – Ratio open-arm time</td>
<td>8.52 ± 1.75</td>
<td>8.94 ± 1.70</td>
<td>10.55 ± 1.74</td>
<td>788 ± 1.41</td>
<td>17.23 ± 2.75a</td>
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<thead>
<tr>
<th>Post-hoc analysis (LSD)</th>
<th>(P = )</th>
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<tbody>
<tr>
<td>EPM – Time mobile (seconds)</td>
<td>Vehicle vs AA (.015)</td>
</tr>
<tr>
<td></td>
<td>M (.010)</td>
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<tr>
<td></td>
<td>M+AA (.000)</td>
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<tr>
<td></td>
<td>AA vs M+AA (.067)</td>
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<tr>
<td></td>
<td>F+AA vs M+AA (.004)</td>
</tr>
<tr>
<td>EPM – Mean max speed (velocity)</td>
<td>Vehicle vs AA (.017)</td>
</tr>
<tr>
<td></td>
<td>M+AA (.014)</td>
</tr>
<tr>
<td>EPM – Ratio open-arm time</td>
<td>Vehicle vs M+AA (.003)</td>
</tr>
<tr>
<td></td>
<td>AA vs M+AA (.004)</td>
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<tr>
<td></td>
<td>M vs M+AA (.017)</td>
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<tr>
<td></td>
<td>F+AA vs M+AA (.001)</td>
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</tbody>
</table>

**Table 1. Data Collected for Ratio Open-Arm Time to Total Time, Mean Time Mobile and Mean Maximum Speed on Elevated Plus Maze (EPM)**

Note: Data are presented as mean ± SEM.

\(^{a}\)Indicates significant statistical difference of \(P < .05\).
spent significantly more time in the open arms of the EPM compared to the vehicle group \( (P = .003) \), AA group \( (P = .004) \), midazolam \( (P = .017) \), and flumazenil + AA group \( (P = .001) \) (Table 1; Figure 3).

Analysis of mobility in the EPM showed significant decreases in the mobility of rodents in the AA \( (P = .015) \), midazolam \( (P = .010) \), and the midazolam + AA groups \( (P < .000) \) compared to the vehicle group. In addition, significant differences in levels of mobility were found between the flumazenil + AA group (131 seconds) and the midazolam + AA group (66 seconds), \( (P = .004) \). Differences between the AA group (106.9 seconds) and the midazolam + AA group (66.9 seconds) approached significance \( (P = .067) \); however, they were not statistically significant (Table 1; Figure 4).

Analysis of mean maximum speed in the EPM showed significant decreases in the speed of rodents in the AA \( (P = .017) \) and midazolam + AA groups \( (P = .014) \) compared to the vehicle group (Table 1; Figure 5).

• **Forced Swim Test (FST).** The FST data included mobility time and fecal pellet output (FPO). Mobility time was reported as mean time mobile counted between 2 blinded investigators. The mobility times did not yield statistically significant results; however, significance was found in the FPO. The FST data show an increased time mobile in all groups compared to the vehicle, but no statistical significance was found (Table 2; Figure 6).

Significance in the FPO was found between the vehicle group (4.6) and the AA group (1.8), \( (P = .001) \) and the midazolam + AA group (1.5), \( (P < .000) \); and the flumazenil + AA group (3.3) and the midazolam + AA group (1.5), \( (P = .022) \) (Table 2; Figure 7).

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**Discussion**

Herbal medications may result in significant herbal-drug interactions that pose a risk and may predispose a patient for harm.\(^{38}\) As herbal medication use continues to rise, the lack of standardization, unmonitored effects, and interactions with existing medications propose risks and warrant effective monitoring of their safety.\(^{39}\)

Furthermore, 22% of presurgical patients reported using herbal supplements.\(^{15}\)

*Centella asiatica* extracts have demonstrated GABA\(_A\) modulation activity\(^{40}\) and have been used as an Indian herbal medication to improve cognitive impairment and to decrease anxiety.\(^{19,40}\) AA, isolated from *Centella asiatica*, has also demonstrated an inhibitory effect on acetylcholinesterase and selective GABA\(_B\) receptor agonist activity.\(^{22}\) Neuroprotective properties of AA were purported as a means of therapy for memory loss.\(^{20}\) AA at 30 mg/kg demonstrated the ability to improve memory in the rat model.\(^{23}\) *Centella asiatica* may be useful in the treatment of generalized anxiety disorders and may be used as a promising anxiolytic agent in the future;\(^{24}\) however, there have been no in vivo studies describing the potential anxiolytic and antidepressant effects of AA as the active component of *Centella asiatica*. This study was the first to investigate the anxiolytic and antidepressant effects of AA in the rat model.

Behavioral studies suggest that rats inherently prefer the closed arms of the maze as opposed to the open arms.\(^{31}\) Increases in the percentage of time spent in the open arms of the maze indicate anxiolytic effects, which was our proposed hypothesis in the AA groups. Our current research findings, comparing the ratio of open
arm time to total time spent in the EPM, suggest that AA alone at the 30 mg/kg dose is not statistically significant; however, these data show that AA in combination with midazolam significantly increased open arm time. This suggests a possible interaction producing an anxiolytic effect in combination with midazolam. These data also show a statistically significant decrease in time mobile in the AA, midazolam, and the midazolam + AA groups. The decrease in mean maximum speed of the rodents in the AA and the midazolam + AA groups also was statistically significant. This suggests a possible synergistic or additive effect between the two, with AA presumably acting at unidentified receptor sites, such as in the central motor centers or peripheral neuromuscular junctions.

The FST was used to evaluate the depressive phenotype of rats. In the FST, a longer duration of immobility indicated greater behavioral despair or depression. We hypothesized that groups receiving AA would have an increased mean movement time; however, our study showed no statistically significant difference in the mean movement time between the control and experimental groups. Although the data were not statistically significant, there was significant variance between the mean movement time of the experimental and control groups. The AA group and the midazolam + AA experimental groups had an increased mean movement time compared to the vehicle. The mean movement time demonstrates an increasing trend, suggesting possible clinical significance warranting further investigation with a prolonged dosing regimen.

In rat models, stressors such as the FST have shown increased stimulation of colonic motor function and an increase in FPO. Statistical significance was found in FPO. The AA and the midazolam + AA groups had significantly less FPO compared to the vehicle group.

The link between the brain and the intestines has been intensely researched, and studies reveal that both human and animal models show an increase in corticotropin-releasing factor (CRF) during periods of anxiety and depression. CRF levels directly correlate with colonic motility and defecation.

Furthermore, the molecular mechanism of action of AA should be defined in order to understand the biochemical and pharmacological effects of this herbal extract. Future studies should focus on the antidepressant effects of repeated doses of AA over a period of weeks to mimic the onset of desired results of antidepres-
sants. Additional studies might include those designed to determine effects of AA at various other CNS receptor sites, such as cholinergic, dopaminergic, or glutamatergic receptors (eg, NMDA) and peripherally at the neuromuscular junction. Once the molecular mechanism of action is clearly defined, work may then focus on studying the significant clinical interactions of AA and other pharmaceuticals. Future research of the anxiolytic effects of AA should be explored and validated using additional anxiety tests such as light-dark exploration and open field tests in the rat model. Continued research of the antidepressive effects of AA using the tail suspension behavioral despair test may also give additional insight and verify learned helplessness. These data provide a foundation for continued investigation of AA and its potential modulation of the central nervous system and neuromuscular junction, and its possible interactions with anesthesia.

REFERENCES


### Table 2. Data Collected for Forced Swim Test (FST) Mean Time Mobile and Fecal Pellet Output (FPO)

<table>
<thead>
<tr>
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<th>Vehicle</th>
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<th>Midazolam</th>
<th>Flumazenil + Asiatic acid</th>
<th>Midazolam + Asiatic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FST – Mean time mobile</strong></td>
<td>38.05 ± 12.48</td>
<td>61.59 ± 23.34</td>
<td>48.14 ± 16.67</td>
<td>53.77 ± 15.89</td>
<td>62.77 ± 17.57</td>
</tr>
<tr>
<td><strong>FST – Fecal pellet output</strong></td>
<td>4.60 ± 0.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.82 ± 0.65&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.00 ± 0.57</td>
<td>3.27 ± 0.65&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.46 ± 0.43&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Indicated significance

Note: Data are presented as mean ± SEM.

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


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