Anxiety and depression are debilitating, costly psychological disorders that account for more than $133 billion annually in direct medical expenses in the United States. Finding alternative treatments to reduce the personal and financial burden for patients with these disorders, while maintaining patient safety, is vital. The purposes of this study were to determine if crocin, a compound from saffron (*Crocus sativus L*), produces anxiolytic and/or antidepressant effects using rat models for anxiety and behavioral despair and to determine the effects of crocin at the benzodiazepine site on the $\gamma$-aminobutyric acid type A receptor. Fifty-five male Sprague Dawley rats were randomly assigned to 1 of 5 groups: vehicle (dimethyl sulfoxide), crocin, midazolam, flumazenil plus crocin, and midazolam plus crocin. Behavioral analyses were conducted in the elevated plus-maze and the forced swim test. Data were analyzed using multivariate analysis of variance and a least significant difference post hoc test. Data from the elevated plus-maze suggested crocin may attenuate the anxiolytic effects of midazolam, while not affecting psychomotor activity. Data from the forced swim test showed a significant increase in mean time mobile in the midazolam plus crocin group, suggesting a decrease in behavioral despair because of the interaction between crocin and midazolam.

**Keywords:** Anxiolysis, crocin, depression, saffron, Sprague-Dawley rat.
sure in daily activities, depressed mood, and additional symptoms such as lack of appetite, energy, concentration and self-worth. Depression has been attributed to brain neurotransmitter imbalance including serotonin, dopamine, norepinephrine, and γ-aminobutyric acid (GABA). Persistent anxiety and depression can trigger a physiological stress response. The anterior pituitary secretes adrenocorticotropic hormone which stimulates cortisol to be released from the adrenal cortex. In addition, the sympathetic nervous system is activated, releasing catecholamines, epinephrine and norepinephrine, from the adrenal medulla. These neuroendocrine mediators trigger the flight-or-fight response, inducing an increase in heart rate, blood pressure, serum glucose levels, and water retention. If sustained, these effects can compromise the immune system. This physiological response to anxiety and depression may increase the risk of illness, impair the ability to function at work, school or with family.

Pharmaceutical treatment for depression includes selective serotonin reuptake inhibitors (SSRIs), tricyclic antidepressants, monoamine oxidase inhibitors, and for the treatment of anxiety, SSRIs and GABA_A agonists, such as benzodiazepines, have shown efficacy. Compliance with anxiolytic and antidepressant medications is effected by their undesirable side effects, which include the following: depressed cognitive or motor abilities, affected by their undesirable side effects, which include

- **Complementary and Alternative Medicine.** Individuals seek to avoid unpleasant side effects, and prompted by the desire to use more natural products, routinely opt for CAM. Complementary and alternative medicine therapies include, but are not limited, to herbal supplements, yoga, tai chi, meditation, deep-breathing exercises, energy healing therapy, and acupuncture, among others. The National Health Interview Survey reported that herbal therapy is the most commonly used CAM approach in the United States. Most individuals use alternative medicine to complement standard medical treatment rather than to replace it.

Among the military population, CAM therapies are commonly reported in treatment of anxiety and depression. McPherson and Schwenka surveyed 291 active-duty soldiers, retirees, and spouses and found that 81% used some type of CAM therapy. Herbal supplements were the second most used CAM treatment. Anxiety and depression were among the reasons identified by the participants for using CAM. Further investigation regarding the use of herbal supplements among service personnel is necessary to assess the effectiveness of herbal therapy and avoid potentially dangerous interactions with other traditional medications that could pose harm to the patient.

There are serious challenges with the demand for herbal supplements in the general and military populations. For instance, the industry lacks quality control standards, and the side effects and potential interactions with other medications remain largely unknown. In many instances, self-medication with herbal products is common, and the individuals may not disclose their use to primary care providers. Furthermore, The Joint Commission mandates that all medications and herbal supplements taken by patients be verified for potential interactions. Additional investigations are critical to keep healthcare providers informed regarding the pharmacodynamics, side effects, and potential for interactions of herbal supplements to ensure patient safety.

- **Saffron.** Saffron is a popular herb that has been used for centuries as a spice and for its medicinal properties. Originally cultivated in the Middle East, saffron has been used as an antispasmodic, thymoletic, cognitive enhancer, antitumor, and expectorant as described in studies by Dwyer et al and Pitsikas. Saffron has been associated with anxiolytic and antidepressant effects and has been found to ameliorate obsessive-compulsive disorders, memory disorders, antiepileptic activity, and smooth-muscle relaxation. Many compounds have been identified in the chemistry analysis of C sativus, but the main nonvolatile compounds consist of crocins, crocin, crocetin, pierocin, and flavonoids. Flavonoids have been found to exert anxiolytic action by interacting with GABA_A receptors.

Recent investigations in both humans and rat models indicate that crocin, a prominent compound in saffron, may have antidepressant and anxiolytic properties. In a rat model, Pitsikas et al investigated the anxiolytic properties of crocin compared with a vehicle and diazepam using the light/dark test. These investigators reported that crocin demonstrated anxiolytic-like effects on rats. The crocin rat group had significant latency to enter the dark chamber, similar to the diazepam group. However, no difference in locomotion was noted. Several studies suggest that the anxiolytic properties induced by crocin are not related to changes in locomotor activity. In the forced swim test (FST) and tail suspension test, Wang and colleagues found antidepressant effects of crocin in a rodent model, using fluoxetine as the positive control. In a systematic review of 6 prospective human trials involving the use of C sativus, Dwyer and colleagues found antidepressant effects compared with a placebo, fluoxetine, or imipramine using the Hamilton Depression Rating Scale, with better tolerability found in crocin compared with imipramine.

The mechanism of action by which crocin exerts its anxiolytic and antidepressant effects is not well understood. In some studies, anxiolytic properties of crocin have been shown to be similar to diazepam, hypothesizing the possible modulation of the GABA_A receptor by crocin. Benzodiazepines, such as diazepam, bind to the benzodiazepine binding site, modulating the GABA_A receptor, which reduces excitability of neurons and results...
in an anxiolytic effect.\textsuperscript{18,22} In other investigations, crocin has been shown not to increase plasma corticosterone levels in stress-induced mice models, which may indicate that crocin interacts with the hypothalamus-pituitary-adrenal axis to decrease the stress-induced release of corticosterone.\textsuperscript{26} Georgiadou and colleagues\textsuperscript{25} found crocin to antagonize the effects of the noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist ketamine in a rat model. Many studies hypothesize about the effects of crocin, but none have been confirmed. All studies recommend further research to elucidate the mechanism (or mechanisms) of action of crocin.

Patients scheduled for surgical procedures often experience anticipatory anxiety that activates the sympathetic nervous system stress response, causing undesirable effects and adverse patient outcomes. Detrimental effects of stress during the perioperative period include immunosuppression, increased risk of infection, elevated glucose levels, and delayed wound healing.\textsuperscript{27,28} Anesthesia providers often administer midazolam, a benzodiazepine and allosteric agonist to the GABA\textsubscript{A} receptor, to ameliorate anxiety and the stress response.\textsuperscript{9} It is unknown if crocin causes anxiolytic and/or antidepressant effects by the modulation of the benzodiazepine binding site on the GABA\textsubscript{A} receptor. Therefore, the purposes of this study were to determine if crocin produces anxiolytic and/or antidepressant effects using rat models that evaluate anxiety and behavioral despair; to examine the effects of crocin at the benzodiazepine site on the GABA\textsubscript{A} receptor in the rat central nervous system; and to investigate potential interactions when crocin is administered in conjunction with midazolam.

**Materials and Methods**

- **Animals.** Fifty-five male Sprague-Dawley rats, each weighing between 150 and 175 g, were obtained from Envigo RMS Inc. (Indianapolis, IN; formerly Harlan Laboratories Inc). Rats were housed in groups of 3, in clear plastic rodent containers, with 109.6 to 451.5 cm (17-70 sq in) of floor space per rat containing appropri-
ate bedding material and environmental enrichment. Over a 21-day period, light/dark cycles were used to simulate circadian rhythms in a controlled environment (temperature [standard error of the mean, SEM] of 22°C [1°C] and 60% humidity). The light cycle was between 12:01 AM and noon, and the dark cycle was between 12:01 PM and midnight. Water and food were provided ad libitum. To prevent any confounding variables, the same investigator performed all gentling of the rodents over the 21-day experimental period: cage cleaning, obtaining daily weights, drug administration, and movements of rats to and from testing procedures. All experiments were conducted in accordance with the Institutional Animal Care and Use Committee at the US Army Institute of Surgical Research, Joint Base San Antonio–Fort Sam Houston, Texas, and according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals guidelines.

**Drugs.** Crocin (99%), flumazenil (> 99%), and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (St Louis, MO). Midazolam was obtained from the US Army Institute of Surgical Research pharmacy and met pharmaceutical-grade standards. A 0.5% concentration of DMSO was used as the solvent for flumazenil, and normal saline was used as the diluent for crocin and midazolam.

**Experimental Design.** Fifty-five rats were randomly assigned to 1 of 5 groups (n = 11 per group). Each of the 5 groups received 2 intraperitoneal injections as follows: group 1: DMSO plus normal saline; group 2: DMSO plus crocin (50 mg/kg); group 3: DMSO plus midazolam (1.5 mg/kg); group 4: flumazenil (3 mg/kg) plus crocin (50 mg/kg); and group 5: midazolam (1.5 mg/kg) plus crocin (50 mg/kg). Midazolam, flumazenil, and crocin solutions were prepared fresh daily. All drugs were administered using 1-mL latex-free syringes with a 25-gauge, 1.27-cm (0.5-in) needle 30 minutes before the first test. Group 1 was used as the negative control. Group 2 was used to assess for anxiolytic and/or antidepressant effects of crocin. Group 3 was used as the positive anxiolytic control. Group 4 was used to determine if any effects were seen in the crocin group that could be attributed to the benzodiazepine binding site of the GABA<sub>A</sub> receptor; the flumazenil was given 10 minutes before the crocin. Group 5 was used to assess for any interactions between midazolam and crocin.

**Elevated Plus Maze.** The EPM is a valid and reliable assessment tool for anxiety in the rat model. For protection from predators, rodents prefer dark and enclosed spaces, such as the dark enclosed arms of the EPM; thus, increased open-arm time exploration indicates decreased anxiety. The EPM consists of 2 open arms and 2 closed arms, with each arm measuring 50 cm × 10 cm. The closed arms had walls that spanned the length of the arm and did not have a roof. The open arms were positioned perpendicular to the closed arms. The maze was elevated to a height of 50 cm to prevent the rats from escaping (Figure 1). All rats were injected with study medications 30 minutes before the behavioral test on the EPM. Following the injections, the rats were placed back into their housing unit until the start time of the EPM. All movements were recorded with a video recorder placed directly above the center of the EPM, and data were digitalized using AnyMaze software (San Diego Instruments, San Diego, CA). Thirty-seven individual data points were recorded, including number of entries into the closed or open arms, distance traveled, and time spent mobile or immobile. Mean speed (centimeters per second), mean time mobile (seconds), and open-arm time ratio (percent) were the main data points investigated. Open-arm time ratio was calculated by taking the time spent in the open arm divided by the total time and multiplying by 100 to get a percentage. The EPM test lasted 5 minutes, at which point it was cleaned with soap and water and allowed to dry between experiments.

**Forced Swim Test.** The FST is a valid instrument in the assessment of behavioral despair in the rodent model. Rats will stop swimming in an attempt to escape from the water in the cylinder if they sense it is futile. Thus, a decrease in swimming is associated with despair. Following the EPM, the investigator carried the rats to a separate room for evaluation in the FST. Each
A rat was placed into an individual cylinder measuring 20 cm in diameter × 40 cm high (Figure 2). The water was maintained at a temperature (SEM) of 25°C (2°C) and at a depth sufficient to prevent the rat from touching the bottom of the cylinder. After each video-recorded FST test, the water was changed. Twenty-four hours before testing, an FST habituation session lasting 15 minutes was conducted with each rat. Habituation acclimated the rats to the test, providing a reliable level of immobility behavior during the 5-minute test 24 hours later. The investigators counted fecal pellet output (FPO) after each experiment during the water change. Two investigators, blinded to treatment groups, measured time mobile and FPO. For the purposes of this experiment, time mobile was recorded as any time other than when the rat was passively floating and exhibiting movements necessary to keep its head above water. Decreased time mobile reflected behavioral despair, and increased FPO indicated increased stress.

**Statistical Analysis.** Data were collected from 55 rats; all data were analyzed using a 2-tailed multivariate analysis of variance and a least significant difference post hoc test.

**Results**

There was no statistically significant difference in the weight of the rats by group, with the mean weights ranging between 288 and 291 g (Table 1).

**Elevated Plus-Maze.** The EPM was used to evaluate the following variables: mean speed (centimeters per second), mean time mobile (seconds), and mean open-arm time ratio (percentage). In a comparison of mean speed, the crocin group was significantly increased compared with the midazolam plus crocin group (P = .002); and the midazolam group was significantly decreased compared with the crocin group (P = .001), flumazenil plus crocin (P = .006), and vehicle (P = .025). Additionally, the flumazenil plus crocin group was significantly increased compared with the midazolam plus crocin group (P = .013). No other statistically significant difference was found between groups regarding mean speed (Table 1, Figure 3A). For the mean time mobile, the crocin group was significantly increased compared with the midazolam group (P = .037). No other statistically significant difference was found between groups regarding mean mobile time (Table 1, Figure 3B). In a comparison of the open-arm time ratio, the midazolam group was significantly increased compared with crocin (P < .01), vehicle (P < .01), flumazenil plus crocin (P < .01), and midazolam plus crocin (P < .01). No other statistically significant difference was found between groups regarding open-arm time ratio (Table 1, Figure 3B).

Table 1. Data From Elevated Plus-Maze for Mean Weight, Speed, Time Mobile, and Open-Arm Time Ratio (and SEM)a

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Vehicle</th>
<th>Crocin</th>
<th>Midazolam</th>
<th>Flumazenil + Crocin</th>
<th>Midazolam + Crocin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean weight, g</td>
<td>291 (5)</td>
<td>288 (4)</td>
<td>288 (2)</td>
<td>290 (4)</td>
<td>294 (3)</td>
</tr>
<tr>
<td>Mean speed, cm/s</td>
<td>4.6 (0.4)</td>
<td>5.6 (0.6)</td>
<td>2.9 (0.5)</td>
<td>5.0 (0.4)</td>
<td>3.1 (0.6)</td>
</tr>
<tr>
<td>Post hoc analysis (LSD) mean speed, cm/s</td>
<td>Vehicle</td>
<td>Crocin</td>
<td>Flumazenil + Crocin</td>
<td>Midazolam + Crocin</td>
<td>P = .028b</td>
</tr>
<tr>
<td></td>
<td>Midazolam vs</td>
<td>Crocin</td>
<td>Flumazenil + Crocin</td>
<td>Midazolam + Crocin</td>
<td>P = .001b</td>
</tr>
<tr>
<td></td>
<td>Crocin vs</td>
<td>Midazolam + Crocin</td>
<td>P = .002b</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Flumazenil + Crocin vs</td>
<td>Midazolam + Crocin</td>
<td>P = .013b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean time mobile, s</td>
<td>233.9 (19.6)</td>
<td>260.4 (14.1)</td>
<td>187.9 (34.7)</td>
<td>237.7 (21.2)</td>
<td>229.6 (25.3)</td>
</tr>
<tr>
<td>Post hoc analysis (LSD) mean time mobile, s</td>
<td>Vehicle</td>
<td>Crocin</td>
<td>Flumazenil + Crocin</td>
<td>Midazolam + Crocin</td>
<td>P = .180</td>
</tr>
<tr>
<td></td>
<td>Midazolam vs</td>
<td>Crocin</td>
<td>Flumazenil + Crocin</td>
<td>Midazolam + Crocin</td>
<td>P = .037b</td>
</tr>
<tr>
<td></td>
<td>Crocin vs</td>
<td>Midazolam + Crocin</td>
<td>P = .147</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Flumazenil + Crocin vs</td>
<td>Midazolam + Crocin</td>
<td>P = .223</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Open-arm time ratio, %</td>
<td>44.5 (3.2)</td>
<td>40.9 (4.9)</td>
<td>77.6 (4.2)</td>
<td>42.5 (4.0)</td>
<td>53.2 (5.9)</td>
</tr>
<tr>
<td>Post hoc analysis (LSD) open-arm time ratio, %</td>
<td>Vehicle</td>
<td>Crocin</td>
<td>Flumazenil + Crocin</td>
<td>Midazolam + Crocin</td>
<td>P &lt; .01b</td>
</tr>
<tr>
<td></td>
<td>Midazolam vs</td>
<td>Crocin</td>
<td>Flumazenil + Crocin</td>
<td>Midazolam + Crocin</td>
<td>P &lt; .01b</td>
</tr>
<tr>
<td></td>
<td>Crocin vs</td>
<td>Midazolam + Crocin</td>
<td>P &lt; .01b</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Flumazenil + Crocin vs</td>
<td>Midazolam + Crocin</td>
<td>P = .001b</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: cm, centimeters; g, gram; LSD, least significant difference; +, plus; s, seconds; SEM, standard error of the mean.

Fifty-five Sprague Dawley rats were randomly assigned to 1 of 5 groups (11 per group). Each group received 2 intraperitoneal injections. Post hoc analysis (LSD) is shown in the row below its respective descriptive data.

Statistical significance P < .05.
• Forced Swim Test. The FST was used to evaluate mean time mobile (seconds) and FPO. In a comparison of mean time mobile, the midazolam plus crocin group was significantly increased compared with the crocin (P = .028), vehicle (P = .047), and midazolam groups (P = .005). No other statistically significant difference was found between groups regarding mean time mobile (Table 2, Figure 4A). Mean FPO was significantly increased in the crocin group compared with the midazolam (P = .002) and midazolam plus crocin group (P = .012). Additionally, the midazolam group showed a significant decrease in FPO compared with the vehicle group (P = .01) and the flumazenil plus crocin group (P = .001). Also, the midazolam plus crocin group resulted in significantly decreased FPO compared with the vehicle group (P = .003) and flumazenil plus crocin group (P = .009). No other statistically significant difference was found between groups regarding FPO (Table 2, Figure 4B).

Discussion

Mental disorders, such as depression and anxiety, occur frequently in the United States. These diseases are both incapacitating and costly to the US healthcare resources.1-3 The additive cost of anxiety and depression to the US healthcare system is billions of dollars every year.4,5 Traditional treatment modalities include both pharmaceutical and psychotherapeutic, preferably used in conjunction with one another.2 The undesirable side effects of the anxiolytic and antidepressant medications affect treatment compliance rates.10,12 Consequently, there is an increased use of CAM modalities to supplement or even replace traditional methods as individuals strive to avoid unpleasant side effects.12,14,15

The World Health Organization estimates that 70% to 80% of the world’s inhabitants rely on CAM as part of their primary care.16 Dietary supplements, a subset of CAMs, are not classified as drugs in the United States and, therefore, are not required to be vetted through the rigorous testing process that all traditional medications must complete before being sold to consumers.16 Many patients and healthcare providers are unaware of the potential interactions or consequences of taking these supplements in conjunction with allopathic medical treatments commonly prescribed.16 The Joint Commission mandated, as one of the 2017 National Patient Safety Goals, that patient medications are to be reviewed by healthcare providers because drug-drug and herbal-drug interactions are a serious safety risk.17

Noncompliance with medication regimens can be precipitated by patient intolerance to the side effects of medications prescribed.10-12 One particularly undesirable side effect of anxiolytics is the depression of psychomotor abilities.10-12 During the EPM, mean speed and mean mobile time were assessed to determine if crocin directly affects motor function. The crocin group showed significantly increased mean mobile time compared with midazolam, while showing no statistical difference compared with the vehicle. The midazolam group was significantly slower in mean speed compared with the crocin, vehicle, and flumazenil plus crocin groups. These findings indicate that crocin does not depress psychomotor activity.
Research conducted by Pitsikas et al\textsuperscript{22} demonstrated similar results. The midazolam plus crocin group was significantly slower in mean speed compared with the crocin and flumazenil plus crocin groups. However, there was no significant difference noted between the crocin group and the flumazenil plus crocin group, which suggests that crocin does not exert its effects solely on the benzodiazepine site of the GABA\textsubscript{A} receptor.

Anxiety-like behavior in rodent research can be observed through several different models.\textsuperscript{36} The EPM “uses the conflict between exploration and fear of open and/or elevated places” to enable researchers to study anxiety-like behavior in rodents, with increased time on the open arm indicating decreased anxiety.\textsuperscript{33,36} Our research found a significant difference in the open-arm time ratio between midazolam and all the other groups. However, the crocin group had the least open-arm time ratio of all the groups tested. Since the midazolam plus crocin group showed a significant decrease in the open-arm time ratio compared with the midazolam group, it would suggest that crocin may attenuate the anxiolytic effects of midazolam.

Assessment of behavioral despair in the rodent model was conducted using the FST, with decreased mean time mobile indicating behavioral despair.\textsuperscript{31,34} The data showed that the mean time mobile for the midazolam plus crocin group was significantly slower compared with the crocin and flumazenil plus crocin groups. However, there was no significant difference noted between the crocin group and the flumazenil plus crocin group, which suggests that crocin does not exert its effects solely on the benzodiazepine site of the GABA\textsubscript{A} receptor.

Additionally, increased output of fecal pellets indicates increased stress levels in the rodent model.\textsuperscript{35,36} Measuring FPO, our data showed statistical significance

\begin{table}[h]
\centering
\begin{tabular}{|l|c|c|c|c|c|}
\hline
Parameter & Vehicle & Crocin & Midazolam & Flumazenil + Crocin & Midazolam + Crocin \\
\hline
Mean time mobile, s & 40.2 (12.4) & 35.9 (10.6) & 20.5 (5.7) & 42.8 (14.3) & 79.9 (20.8) \\
Post hoc analysis (LSD) & & & & & \\
midazolam + crocin & vs & & & & \\
\hline
Mean fecal pellet output & 4.5 (0.5) & 4.1 (0.6) & 1.4 (0.6) & 4.2 (0.6) & 2.0 (0.6) \\
Post hoc analysis (LSD) & & & & & \\
midazolam & vs & & & & \\
\hline
\end{tabular}
\caption{Data From Forced Swim Test for Mean Time Mobile and Fecal Pellet Output (and SEM)\textsuperscript{a}}
\textsuperscript{a}Fifty-five Sprague Dawley rats were randomly assigned to 1 of 5 groups (11 per group). Each group received 2 intraperitoneal injections. Post hoc analysis (LSD) is shown in the row below its respective descriptive data.
\end{table}

\textsuperscript{a}Abbreviations: cm, centimeters; g, gram; LSD, least significant difference; +, plus; s, seconds; SEM, standard error of the mean.

\textsuperscript{b}Statistical significance \( P < .05 \).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{FST.pdf}
\caption{Forced Swim Test (FST) Data and Standard Error of the Mean (SEM)\textsuperscript{c}: (A) Mean Time Mobile (seconds) and (B) Mean Fecal Pellet Output. \textsuperscript{c}Fifty-five Sprague Dawley rats were randomly assigned to 1 of 5 groups (11 per group). Each of the 5 groups received 2 intraperitoneal injections: Vehicle (Veh) dimethyl sulfoxide (DMSO) plus (+) normal saline; crocin (Cro) + DMSO; midazolam (Mid) + DMSO; flumazenil (Flu) + Cro; and Mid + Cro.}
\end{figure}
between the midazolam group and the vehicle, crocin, and flumazenil plus crocin groups. The data also showed significance between the midazolam plus crocin group compared with the vehicle, crocin, and flumazenil plus crocin groups. We hypothesize that reduced stress from the administration of midazolam may be responsible for the decrease in FPO. Our data from the FST also suggest that crocin alone lacks antidepressant effects with a single intraperitoneal injection of 50 mg/kg.

Analysis of this study found that crocin alone showed neither anxiolytic nor antidepressant effects as tested. Potential limitations of this study include one-time administration of the medications and lower doses of crocin (50 mg/kg) compared with other studies such as by Wang et al.19 According to Basic and Clinical Pharmacology by Katzung and Trevor,9 to achieve desired effects, many antidepressant medications may require an extended dosing regimen of up to 1 or 2 months. While testing for antidepressant properties, Wang et al.19 administered crocin at much higher doses than we used for our study, with doses including 150 mg/kg, 300 mg/kg, and 600 mg/kg. Additionally, we administered a single injection vs recommendations by Castagné et al.31 who stated that “a minimum of two, but preferably three, pretest administrations provide more stable pharmacological results than a single administration.” Furthermore, investigators should consider an experimental design using multiple dosing. Our results were inconclusive as to the effect of crocin at the benzodiazepine site of the GABA_A receptor. However, a study by Georgiadou et al.31 showed that crocin attenuated side effects of ketamine. Further studies should investigate the possible modulation of various other receptor sites by crocin.

Conclusion
The results of this study, compared with other studies, demonstrated differences in the anxiolytic and antidepressant effects of crocin. Our study suggested that crocin may attenuate the anxiolytic effects of midazolam, while sparing psychomotor activity. In addition, crocin showed a possible drug-drug interaction with midazolam, leading to a decrease in behavioral despair. To elucidate a better understanding of crocin and its potential anxiolytic or antidepressant properties, further studies should continue to investigate its effects at various receptor sites.

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DISCLOSURES

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