Investigation of the Anxiolytic and Antidepressant Effects of Curcumin, a Compound From Turmeric (*Curcuma longa*), in the Adult Male Sprague-Dawley Rat

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As the use of herbal medications continues to increase in America, the potential interaction between herbal and prescription medications necessitates the discovery of their mechanisms of action. The purpose of this study was to investigate the anxiolytic and antidepressant effects of curcumin, a compound from turmeric (*Curcuma longa*), and its effects on the benzodiazepine site of the γ-aminobutyric acid receptor A ($GABA_A$) receptor. Utilizing a prospective, between-subjects group design, 55 male Sprague-Dawley rats were randomly assigned to 1 of the 5 intraperitoneally injected treatment groups: vehicle, curcumin, curcumin + flumazenil, midazolam, and midazolam + curcumin. Behavioral testing was performed using the elevated plus maze, open field test, and forced swim test. A 2-tailed multivariate analysis of variance and least significant difference post hoc tests were used for data analysis. In our models, curcumin did not demonstrate anxiolytic effects or changes in behavioral despair. An interaction of curcumin at the benzodiazepine site of the $GABA_A$ receptor was also not observed. Additional studies are recommended that examine the anxiolytic and antidepressant effects of curcumin through alternate dosing regimens, modulation of other subunits on the $GABA_A$ receptor, and interactions with other central nervous system neurotransmitter systems. **KEY WORDS:** *anxiolysis*, *curcumin*, *elevated plus maze*, *open field test*, *forced swim test*, *Sprague-Dawley rat* *Holist Nurs Pract* 2017;31(3):193–203

INTRODUCTION

Anxiety and depressive disorders are highly prevalent and often debilitating. In the United States, these 2 disorders affect nearly 23% of the adult population, with anxiety disorders alone costing more than $33 billion annually.[1,2] Anxiety is a known result of fear and stress; it involves both subjective emotions, such as tension, apprehension, and worry, and physiologic effects involving activation of the sympathetic nervous system.[3,4] Anxiety is a normal adaptive process that enables the body to respond to threats in both acute and chronic situations. Psychological and physiological responses to anxiety are mediated by hormones and neurotransmitters associated with the stress response.[5] Corticotropin-releasing hormone, neuropeptides,

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cortisol, and catecholamines—all contribute to these responses.[3] Only 33% of those suffering from anxiety disorders receive treatment, although these conditions are readily treatable.[6]

The Anxiety and Depression Association of America (ADAA) describes major depression as feelings of hopelessness, discouragement, lack of pleasure in things, and an overall lack of interest in life itself.[1] Depression has been shown to involve altered levels of serotonin, norepinephrine, and other neurotransmitters in addition to abnormally low levels of cortisol associated with disrupted circadian rhythms.[7] The lifetime risk of experiencing depression is 17%, and it is the leading cause of disability worldwide.[1,8] Although anxiety and depression are often addressed with separate treatment modalities, they present as comorbid diseases as much as 50% of the time and together can have a significant detrimental effect on psychological well-being.[1]

Many patients suffering from anxiety and depression seek alternative pharmacological treatments and turn to the $34 billion industry of herbal medications.[9] A 2007 National Health Interview Survey found that 55.1 million American adults used herbal medications or supplements, an increase of nearly 5 million from the previous survey in 2002.[10] Identifying and understanding herbal medication use are vital for a health care provider. In fact the Joint Commission's National Patient Safety Goals call for a thorough medication reconciliation to include all prescribed, over-the-counter, herbal and nutriceutical substances.[11] The National Health and Nutrition Examination Survey in 2007 examined the motivations of adult participants in taking dietary supplements, which included vitamins, minerals, herbals, and other supplements. This study found that 45% of participants reported taking supplements to "improve overall health" and that 4% reported taking supplements "for mental health."[12] The reason for taking herbal medications, vitamins, minerals, and other remedies that generally fall under the designation of "supplements" varies more when particular communities or demographics are examined in isolation. For example, the US military showed different patterns in motivation for supplement use. In a 2004 study, service members, retirees, and their family members most commonly reported taking complementary and alternative medicine remedies such as herbal medications for anxiety, depression, and stress.[13]

Curcumin is a widely used Chinese and Indian herbal medication. It is the active ingredient in turmeric, a common flavoring and preservative added to foods, and is generally considered to be nontoxic.[14] Curcumin has been used extensively to treat a wide variety of ailments, ranging from minor wounds to rheumatism and the common cold.[15] Recent attention has been paid to its reported ability to treat inflammation, stress, anxiety, and depression.[16] Many mechanisms have been proposed regarding the effectiveness of curcumin as an anti-inflammatory and antioxidant.[17] In addition to its postulated anti-inflammatory properties, several studies have also shown that curcumin affects the activity of multiple neurotransmitters including dopamine, norepinephrine, and serotonin.[18,19] Furthermore, curcumin's reduction of inducible nitric oxide synthase (iNOS) may play a role in its anxiolytic properties via the γ-aminobutyric acid receptor A ($GABA_A$).[20] Gilhotra and Dhingra[20] propose that curcumin may inhibit iNOS by preventing the downregulation of $GABA_A$ receptors by nitric oxide, resulting in anxiolysis. Of the many mechanisms of action of curcumin that have been studied, no research has been carried out examining the direct effects of curcumin on the benzodiazepine-binding site of the $GABA_A$ receptor in relation to treating anxiety and depression. This particular mechanism is important to understand when examining patients and reviewing their preoperative anesthesia report, as herbal medications may potentially interact with anesthetic medications.

Patients about to undergo surgery often experience anxiety and subsequent activation of the stress response. The surgical patient's stress response triggers an increase in cortisol release, resulting in many of the physiological responses anesthesia providers strive to attenuate, such as immunosuppression, increased plasma glucose levels, and delayed wound healing.[21] Reducing preoperative anxiety by administering anxiolytic drugs is an essential component in physiologically optimizing a patient for surgery and minimizing the risk of perioperative complications.[22] Traditional medications used in the perioperative period for anxiolysis, such as benzodiazepines, modulate the $GABA_A$ receptor.[23] A study by Norred[24] found that 27% of presurgical patients take 1 or more herbal supplements.

Basic science using animal models builds the framework and foundation for evidence-based practice that nurses and health care providers utilize in daily care of patients. To conduct experiments and scientific investigation in human subjects, empirical data

provided by animal research, such as this study, provide levels of safety and effectiveness to avoid toxicities or dangerous outcomes. Thus, it is imperative that nurses and health care providers are aware of synergism, interactions, and/or antagonism of prescribed medications with herbal supplements to safely prescribe pharmaceutical treatments. Therefore, it is important to identify any herbal medications patients may be taking that could potentiate or inhibit the anxiolytic effects of commonly used anesthesia medications, such as midazolam. The purposes of this study were to determine whether curcumin has anxiolytic and/or antidepressant effects in rat models of anxiety and behavioral despair and to examine the effects of curcumin at the benzodiazepine site on the $GABA_A$ receptor in the rat central nervous system.

MATERIALS AND METHODS

Fifty-five male Sprague-Dawley rats (Harlan Sprague Dawley Laboratories), each of which weighed between 242 and 298 g, were obtained in 1 shipment. All rats were housed in polycarbonate "shoebox" cages lined with bedding in groups of 3. The animals went through a 14-day adaptation period in a temperature-controlled environment (22° ± 1°C, 60% humidity) with a modified 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 food and water ad libitum. The animals were handled only for the purposes of drug administration, cage cleaning, and obtaining daily weights. All protocols used in this study were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at the US Army Institute of Surgical Research, San Antonio, Texas.

This study used the elevated plus maze (EPM) and open field test (OFT) as measures of anxiety and locomotion. The forced swim test (FST) was used to measure behavioral despair (depression). All 3 of these methods are standard instruments used in the evaluation of anxiolytic and antidepressant effects of herbal supplements and various other substances in the rat model.

The rats were randomly assigned to 1 of the 5 groups (n = 11 per group). Each group of rats received a single intraperitoneal injection of 1 the following: (1) 0.5% dimethyl sulfoxide (DMSO); (2) curcumin 20 mg/kg[20]; (3) curcumin 20 mg/kg + flumazenil 3 mg/kg; (4) midazolam 1.5 mg/kg; or (5) midazolam 1.5 mg/kg + curcumin 20 mg/kg. DMSO, commonly used as a solvent for water-insoluble drugs, was used as a vehicle for flumazenil and curcumin. Curcumin and flumazenil were purchased from Selleckchem, Houston, Texas. DMSO was purchased from Sigma-Aldrich, St Louis, Missouri. DMSO alone was used for the control group. All groups were compared with the control group and each other to determine whether there were changes in anxiety or behavioral despair. The group receiving curcumin 20 mg/kg with flumazenil was used to evaluate the anxiolytic effects of curcumin pertaining to the $GABA_A$ receptor site. Flumazenil was given 10 minutes before curcumin. A potential interaction between midazolam and curcumin was evaluated using the midazolam + curcumin group.

Elevated plus maze

The EPM is a previously validated instrument that measures anxiety in rats.[25] The EPM is 50 cm above ground level and consists of 2 open arms and 2 closed arms at right angles to each other. Each arm is 50 cm in length and 10 cm wide. The 2 open arms and the 2 closed arms are arranged directly opposite from one another, forming the shape of a (+) intersecting at a 10 × 10 cm open square. The EPM, made of black plastic, which provides a waterproof surface and decreases visual stimulation to the rodent, was performed in a darkened room (Figure 1). Footage taken during the testing period was then analyzed by an automated tracking system (AnyMaze® software) for the following parameters: time spent in predefined zones, time mobile, distance traveled, and speed. Increased time spent in the closed arms and decreased time spent in the open arms is associated with increased anxiety in the rat model.[26]

Open field test

After evaluation in the EPM, the investigators carried the animals to a separate, enclosed area and they were evaluated in the OFT, a valid test to measure anxiety and mobility in the rodent model. The OFT was conducted in a square arena (the open field) composed of a square, plastic box, 40 × 40 × 13.5 inches to contain the rat (Figure 2). After the subject was placed in the center of the apparatus, the rat instinctively

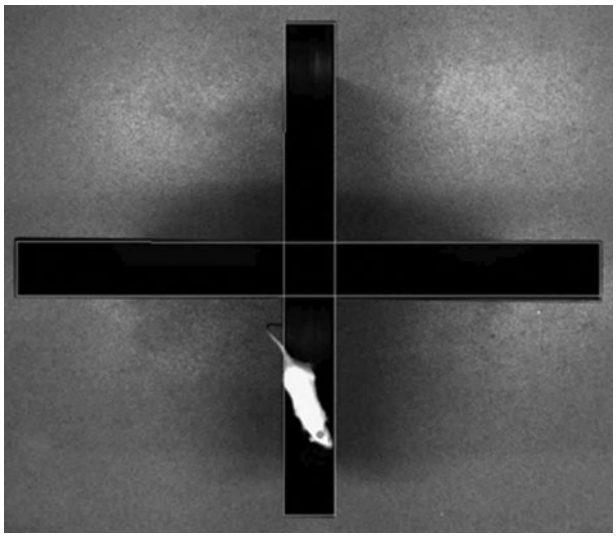

FIGURE 1. Photograph of the elevated plus maze used to collect ratio of open-arm time to total time, mean time mobile, mean distance traveled, and mean speed.

preferred the periphery of the open field. Most often rodents exhibited thigmotaxis, defined as walking close to walls.

The OFT indicates anxiety-like behavior on the basis of a natural tendency of rats to avoid unprotected spaces, such as the center of the open field. Because rats are habituated to tight spaces, including the laboratory cage, exposure to a large area causes agoraphobia and consequent anxiety-like behavior. Therefore, any reaction observed is an effect of the unlearned, stressful event. Reduced anxiety is quantified by center entries in the OFT. The OFT also yields a valuable measure of time mobile, distance traveled, and speed. As with the EPM, open field behavior is sensitive to anxiolytic treatments, which decreases stress-induced inhibition of exploratory behavior.[27]

The OFT is also useful for evaluating the effects of novel chemical agents on general activity. A high frequency of these behaviors indicates increased mobility and exploration and/or a lower level of anxiety. The number of central entries and the duration of time spent in the central square are measures of exploratory behavior and decreased anxiety. The quantification of center entries, time mobile, distance traveled, and speed was captured during the 5-minute testing period via another AnyMaze software tracking system. These data were statistically analyzed between groups.

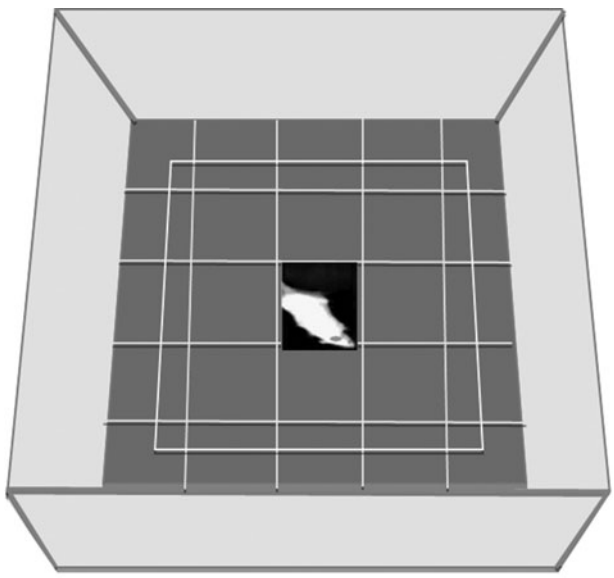

FIGURE 2. Diagram of the open field test used to collect center entries, mean time mobile, mean distance traveled, and mean speed.

Forced swim test

Since its inception, the FST has been a valid assessment of behavioral despair and stress in the rodent model.[28] In 2 sessions separated by 24 hours, the rats were forced to swim in a narrow, transparent cylinder in which there was no escape. The cylinders, measuring 20 cm diameter × 40 cm high contained water (25° ± 2°C) filled to a depth of 13 cm (Figure 3). The water was changed after each subject. The first immersion session, lasting 15 minutes, was the desensitization session and was conducted 24 hours before medication administration and without behavioral recording. After 15 minutes, the rodent was removed from the water, dried, and returned to its cage. This desensitization session acclimated the rats to the FST, thus providing a stable, high level of immobile behavior during the 5-minute test session 24 hours later.[28]

Immediately after the OFT, the rodents were placed in the FST for the 5 minute testing session, which was videotaped. Measured indices were time mobile and fecal pellet output (FPO). Time mobile was reported as counted by 2 investigators who were blinded to treatment groups. Increased immobility time reflects a depressive state, and increased FPO is a direct indicator of increased stress.[29]

Statistical analysis

Data were collected from 55 rats, 2 of which were subsequently removed when they failed to meet the

FIGURE 3. Photograph of forced swim test used to collect forced swim test mean time mobile and fecal pellet output.

criteria of the study. All data were analyzed using a 2-tailed multivariate analysis of variance and a least significant difference post hoc test. A Pearson correlation was computed to analyze the similar variables between the EPM and the OFT: time mobile, distance traveled, and mean speed.

RESULTS

The rats weighed an average of 268.2 ± 10.3 g, without significant difference between the groups (Table 1).

Elevated plus maze

The EPM evaluated the following variables: ratio of open-arm time to total EPM time, time mobile, distance traveled, and mean speed. Ratio analysis comparing open-arm time to total EPM time showed a significant increase in time spent in the open arms of the EPM by the rats in the midazolam group when compared with the curcumin group ($P = .023$), the flumazenil + curcumin group ($P = .012$), and the vehicle group ($P = .023$) (Table 1 and Figure 4).

The midazolam group exhibited a significantly decreased mean time mobile compared with the curcumin group ($P = .031$), the flumazenil + curcumin group ($P = .024$), and the vehicle group ($P = .011$) (Table 1 and Figure 5).

No statistically significant differences were found between the groups in mean distance traveled or in mean speed (Table 1).

TABLE 1. Data Collected for Mean Ratio of Open-Arm Time to Total Time, Mean Time Mobile, Mean Distance Traveled, and Mean Maximum Speed on Elevated Plus Maze

	Treatment Group				
	Vehicle Mean ± SEM	Curcumin Mean ± SEM	Midazolam Mean ± SEM	Flumazenil + Curcumin Mean ± SEM	Midazolam + Curcumin Mean ± SEM
Weights	269.9 ± 3.6	269.1 ± 3.3	267.6 ± 2.3	267.5 ± 3.6	267.1 ± 2.9
EPM—mean ratio open-arm time, %	29.7 ± 3.4[a]	29.6 ± 4.2[a]	47.2 ± 7.8[a]	27.7 ± 3.5[a]	35.3 ± 6.3
EPM—mean time mobile, s	239.3 ± 7.2[a]	230.3 ± 8.6[a]	180.5 ± 24.8[a]	232.68 ± 9.7[a]	196.1 ± 21.0
EPM—mean distance traveled, m	12.9 ± 0.9	12.6 ± 1.0	9.9 ± 1.7	12.0 ± 1.2	9.4 ± 1.4
EPM—mean speed	0.04 ± 0.003	0.04 ± 0.003	0.03 ± 0.006	0.04 ± 0.004	0.03 ± 0.005

	Post Hoc Analysis (LSD)			*P*
EPM—mean ratio open-arm time, %	Vehicle	vs	Midazolam	.023
	Midazolam	vs	Curcumin	.023
			Flumazenil + curcumin	.012
EPM—mean time mobile, s	Curcumin	vs	Midazolam	.031
	Vehicle	vs	Midazolam	.011
	Midazolam	vs	Flumazenil + curcumin	.024

Abbreviations: EPM, elevated plus maze; LSD, least significant difference; SEM, standard error of the mean.
[a]Significant statistical difference of $P < .05$.

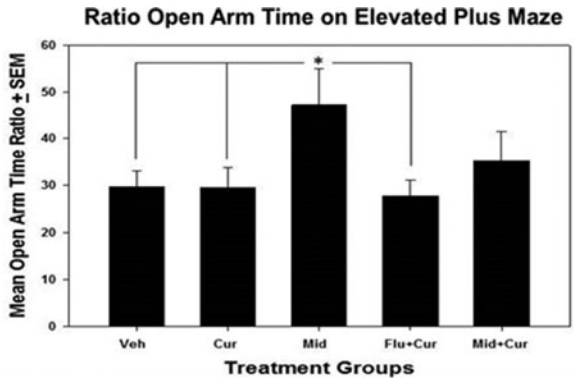

FIGURE 4. Ratio of open-arm time to total time on elevated plus maze. Each group was composed of 11 rodents, with the exception of the midazolam group that had 10 after 1 rat fell from the maze during testing. Drugs were injected 30 minutes before testing on the elevated plus maze. *Significant statistical difference of $P < .05$. SEM indicates standard error of the mean.

The open field test

The OFT evaluated the following variables: center entries, time mobile, distance traveled, and mean speed. The number of center entries was significantly higher in the curcumin group compared than in the midazolam group ($P = .029$), the flumazenil + curcumin group ($P = .029$), and the midazolam + curcumin group ($P = .048$) (Table 2 and Figure 6).

The curcumin group exhibited significantly higher mean time mobile compared with the midazolam group ($P = .015$) and the midazolam + curcumin group ($P = .001$) (Table 2 and Figure 7). The midazolam + curcumin group and the midazolam group had higher mean time mobile when compared with the vehicle group ($P = .001$ and $P = .012$, respectfully) (Table 2 and Figure 7). The flumazenil + curcumin group exhibited significantly higher mean time mobile compared with the midazolam + curcumin group ($P = .015$) (Table 2 and Figure 7).

The mean distance traveled was significantly less in the midazolam + curcumin group than in the curcumin group ($P = .002$) and the vehicle group ($P = .003$) (Table 2 and Figure 8). Mean speed analysis indicated that the midazolam + curcumin group exhibited a significantly slower mean speed compared with the curcumin group ($P = .002$) and the vehicle group ($P = .003$) (Table 2 and Figure 9).

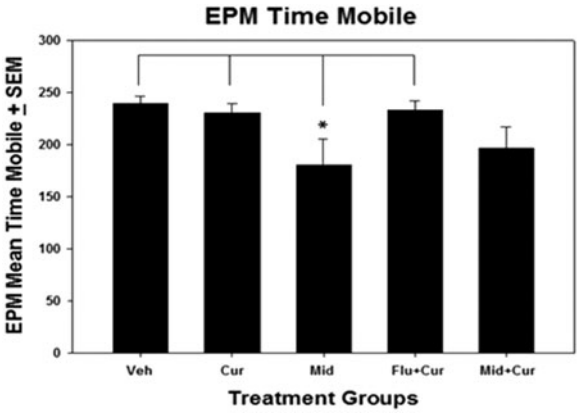

FIGURE 5. Mean time mobile (in seconds) on elevated plus maze. Each group was composed of 11 rodents, with the exception of the midazolam group that had 10 after 1 rat fell from the maze during testing. Drugs were injected 30 minutes before testing on the elevated plus maze. *Significant statistical difference of $P < .05$. SEM indicates standard error of the mean.

Forced swim test

The FST data analyzed time mobile and FPO. The mean time mobile was significantly lower in the curcumin group than in the midazolam + curcumin group ($P = .021$) (Table 3 and Figure 10). Furthermore, the midazolam group had a significantly lower mean time mobile when compared with the vehicle group ($P = .028$), the flumazenil + curcumin group ($P = .026$), and the midazolam + curcumin group ($P = .001$).

Mean FPO was significantly higher in the vehicle group (5.9 ± 0.3) than in the curcumin group (4.1 ± 0.6, $P = .021$), the midazolam group (3.4 ± 0.6, $P = .002$), and the midazolam + curcumin group (4.0 ± 0.7, $P = .015$) (Table 3; Figure 11).

EPM-OFT correlation

The relationship between the variables measured in the EPM and the OFT was investigated using Pearson correlation coefficient. EPM mean distance traveled had a large positive correlation to the OFT mean time mobile, mean distance traveled, and mean speed ($r = 0.61$, $r = 0.65$, $r = 0.65$, respectfully; $P < .001$). EPM mean speed had a large positive correlation to the OFT mean time mobile, mean distance traveled, and mean speed ($r = .61$, $r = .65$, $r = .65$, respectfully; $P < .001$). A large positive correlation coefficient is defined by Cohen as an r more than 0.5.[30]

DISCUSSION

When combined, anxiety and depression affect nearly a quarter of adult Americans, with annual treatment

TABLE 2. Data Collected for Mean Center Entries, Mean Time Mobile, Mean Distance Traveled, and Mean Speed on Open Field Test

	Treatment Group				
	Vehicle Mean ± SEM	Curcumin Mean ± SEM	Midazolam Mean ± SEM	Flumazenil + Curcumin Mean ± SEM	Midazolam + Curcumin Mean ± SEM
OFT—mean ratio center entries	2.1 ± 0.6	2.7 ± 0.7[a]	0.9 ± 0.3[a]	0.9 ± 0.6[a]	1.1 ± 0.5[a]
OFT—mean time mobile, s	203.9 ± 12.4[a]	201.9 ± 8.1[a]	142.6 ± 22.8[a]	173.8 ± 15.4[a]	114.1 ± 20.4[a]
OFT—mean distance traveled, m	23.2 ± 2.0[a]	23.6 ± 1.7[a]	16.9 ± 3.2	18.6 ± 2.3	12.6 ± 2.5[a]
OFT—mean speed	0.077 ± 0.01[a]	0.079 ± 0.01[a]	0.056 ± 0.01	0.062 ± 0.01	0.042 ± 0.01[a]

	Post Hoc Analysis (LSD)			*P*
OFT—mean ratio center entries	Curcumin	vs	Midazolam	.029
			Midazolam + curcumin	.029
			Flumazenil + curcumin	.048
OFT—mean time mobile, s	Curcumin	vs	Midazolam	.015
			Midazolam + curcumin	.001
	Vehicle	vs	Midazolam + curcumin	<.001
	Vehicle	vs	Midazolam	.012
	Flumazenil + curcumin	vs	Midazolam + curcumin	.015
	Curcumin	vs	Midazolam + curcumin	.002
OFT—mean distance traveled, m	Vehicle	vs	Midazolam + curcumin	.003
	Curcumin	vs	Midazolam + curcumin	.002
OFT—mean speed	Vehicle	vs	Midazolam + curcumin	.003

Abbreviations: LSD, least significant difference; OFT, open field test; SEM, standard error of the mean.
[a]Significant statistical difference of $P < .05$.

costs in the billions of dollars.[2] Although anxiety can be a normal response to stress, it has the potential to become deleterious. The lifetime risk of developing depression is approximately 17%, and half of those people present with anxiety as a comorbid condition. Thus, it is incumbent upon the health care provider to understand the different treatments currently in use for these conditions and how these treatments affect the plan of care.

More than 55 million Americans use herbal medications,[10] and in certain populations, such as the military, a majority of them are being used for anxiety and depression.[13] Curcumin is a popular herbal

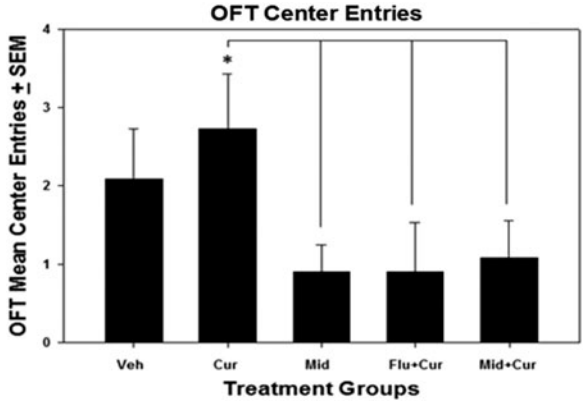

FIGURE 6. Number of center entries in the open field test. Each group was composed of 11 rodents. After evaluation in the elevated plus maze, rodents were tested in the open field test.*Significant statistical difference of $P < .05$. SEM indicates standard error of the mean.

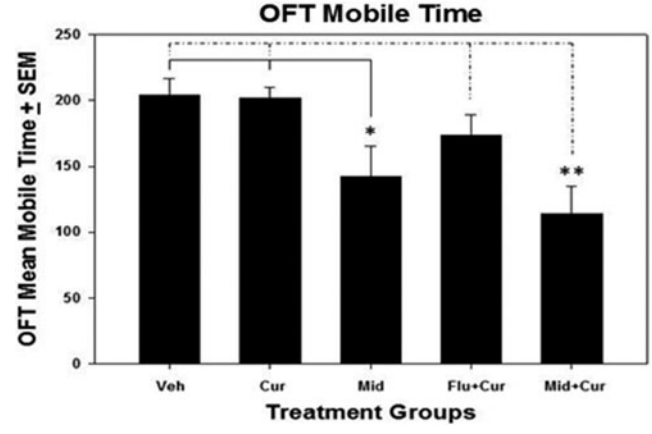

FIGURE 7. Mean time mobile (in seconds) in open field test. Each group was composed of 11 rodents. After evaluation in the elevated plus maze, rodents were tested in the open field test.*Significant statistical difference of $P < .05$. SEM indicates standard error of the mean.

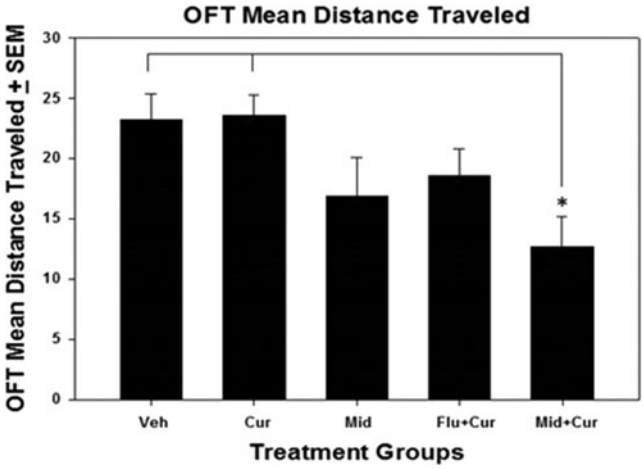

FIGURE 8. Mean distance traveled (in meters) in open field test. Each group was composed of 11 rodents. After evaluation in the elevated plus maze, rodents were tested in the open field test.*Significant statistical difference of $P < .05$. SEM indicates standard error of the mean.

medication that has been reported to contain anxiolytic and antidepressant effects.[16] Several studies cite curcumin as having an effect on dopamine and norepinephrine transmission, as well as serotonin and iNOS.[18-20] Although curcumin's inhibition of iNOS may attenuate the downregulation of the $GABA_A$ receptor[20] resulting in anxiolysis, it is unknown whether curcumin directly affects the benzodiazepine-binding site of the $GABA_A$ receptor.

The goal of this study was to evaluate curcumin as an anxiolytic and antidepressant in a rat model while determining whether its effects are modulated at the benzodiazepine site on the $GABA_A$ receptor. On the basis of statistical analysis of data obtained from the EPM and the OFT, curcumin did not demonstrate any significant anxiolytic effects in our experimental model. Analysis of the data obtained from the FST showed that curcumin did not prevent behavioral despair in our model.

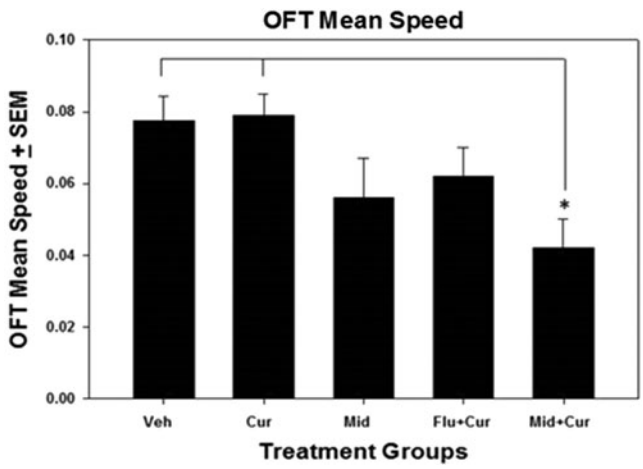

FIGURE 9. Mean speed in open field test. Each group was composed of 11 rodents. After evaluation in the elevated plus maze, rodents were tested in the open field test.*Significant statistical difference of $P < .05$. SEM indicates standard error of the mean.

In the EPM, curcumin showed no significant anxiolytic effects when compared with midazolam, our positive control. Similarly, only the midazolam group showed significant changes in mean time mobile, with the curcumin group having no significant differences from the vehicle group, our negative control. On the basis of these results, midazolam behaved as expected in the EPM by showing anxiolytic activity. With no significant differences found between the curcumin group and the curcumin + flumazenil group, there is no evidence of direct modulation of the benzodiazepine site on the $GABA_A$ receptor.

Center entries in the OFT demonstrated mixed results, with the curcumin group showing no significant difference in center entries compared with the vehicle group. This data suggest curcumin did not have anxiolytic effects in this measure, as we would expect an anxiolytic to produce more center entries than our negative control.[27] Interestingly, the midazolam group showed significantly decreased center entries, as did the flumazenil + curcumin and the midazolam + curcumin groups. We speculate that it may be because of the sedative effects of midazolam.[26] It is also interesting that the flumazenil + curcumin group behaved differently from the curcumin group. We would expect to see this type of result if flumazenil antagonized curcumin at the benzodiazepine site on the $GABA_A$ receptor as we originally hypothesized, but our other tests do not support this conclusion. It may be possible that the 2 substances interact in an unknown manner, or that flumazenil actually induces anxiety or possibly sedation in rats by acting at some other site or receptor in the central nervous system.

In analysis of OFT mean time mobile, the midazolam and midazolam + curcumin groups were not significantly different from each other. Midazolam was only significant when compared with the vehicle and curcumin groups, whereas the midazolam + curcumin group was significantly different from the vehicle, curcumin, and curcumin + flumazenil groups. The midazolam + curcumin group's additional significance may suggest some interaction between curcumin and midazolam; however, these effects may be the result of midazolam alone, which has known

TABLE 3. Data Collected for Mean Time Mobile and Mean Fecal Pellet Output on Forced Swim Test

	Treatment Group				
	Vehicle	Curcumin	Midazolam	Flumazenil + Curcumin	Midazolam + Curcumin
FST—mean time mobile, s	107.5 ± 24.3[a]	67.4 ± 25.7[a]	26.2 ± 9.4[a]	109.0 ± 28.7[a]	151.4 ± 29.6[a]
FST—mean fecal pellet output	5.9 ± 0.3[a]	4.1 ± 0.6[a]	3.4 ± 0.6[a]	4.8 ± 0.5	4.0 ± 0.7[a]

	Post Hoc Analysis (LSD)			*P*
FST—mean time mobile, s	Curcumin	vs	Midazolam + curcumin	.021
	Midazolam	vs	Flumazenil + curcumin	.026
			Midazolam + curcumin	.001
			Vehicle	.028
FST—mean fecal pellet output	Vehicle	vs	Curcumin	.021
			Midazolam	.002
			Midazolam + curcumin	.015

Abbreviations: FST, forced swim test; LSD, least significant difference; SEM, standard error of the mean.
[a]Significant statistical difference of $P < .05$.

anxiolytic, and sedative effects.[26] Curcumin demonstrated a possible interaction with midazolam in the OFT, where the midazolam + curcumin group showed significantly less mean distance traveled and decreased mean speed, whereas midazolam alone had no significant findings.

The large positive correlation found between the EPM and OFT suggests that higher EPM distance traveled was associated with higher OFT time mobile, distance traveled, and mean speed.[30] This suggests validity and consistency between these 2 instruments evaluating mobility, distance traveled, and speed.

The FST was used to evaluate behavioral despair in this model. In the FST, a longer duration of immobility indicates greater behavioral despair.[28] We hypothesized that groups receiving curcumin may have an increased mean time mobile. However, our study did not demonstrate statistical significance in the mean time mobile between the negative control and curcumin groups. The midazolam group had the lowest mean time mobile; this is consistent with the known mechanism of action of midazolam as an anxiolytic and sedative.[26] Although the curcumin group did not perform as expected, it had significantly

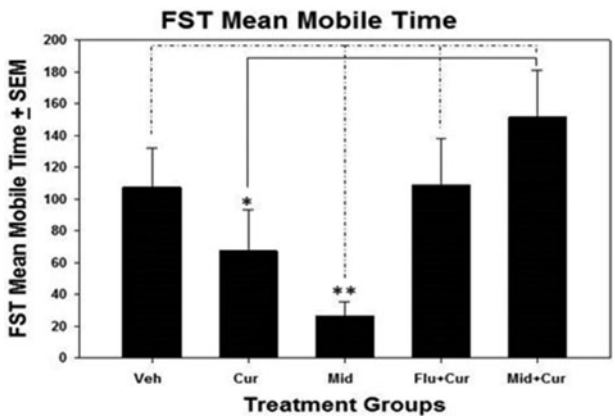

FIGURE 10. Mean time mobile (in seconds) in forced swim test. Each group was composed of 11 rodents, with the exception of the midazolam group that had 10 after 1 rat escaped the forced swim test during testing. Each rodent performed the forced swim test immediately after the open field test. Asterisks (*) and (**) indicate significant statistical difference of $P < .05$. SEM indicates standard error of the mean

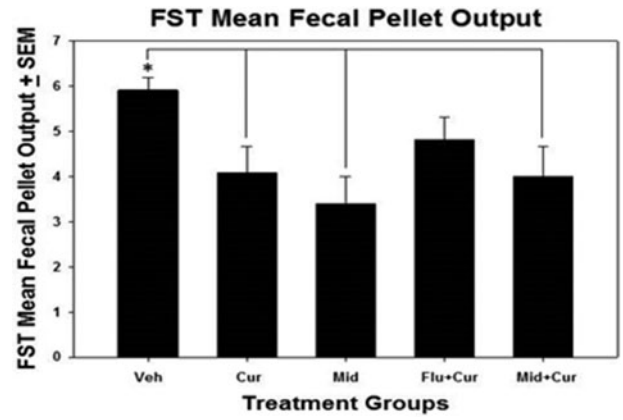

FIGURE 11. Fecal pellet output in the forced swim test. Each group was composed of 11 rodents, with the exception of the midazolam group that had 10 after 1 rat escaped the forced swim test during testing. Each rodent performed the forced swim test immediately after the open field test. *Significant statistical difference of $P < .05$. SEM indicates standard error of the mean.

different results when compared with the midazolam and curcumin group. The curcumin group had a higher mean time mobile than the midazolam group. Furthermore, the midazolam + curcumin group had the highest mean time mobile, suggesting that curcumin may decrease the sedative effects of midazolam in this instrument. The lack of significant findings in the flumazenil + curcumin group once again suggests that curcumin has little to no effect at the benzodiazepine site on the $GABA_A$ receptor.

In the rat model, stressors have shown increased stimulation of colonic motor function and an increase in FPO.[29] The vehicle group had significantly more FPO when compared with the other groups. It is known that stressful situations cause an increase in corticotrophin-releasing factor (CRF) in both human and rat models during periods of anxiety and depression.[29] Increased CRF levels are associated with increased peristaltic activity and defecation.[29,31] These findings suggest that the midazolam group experienced decreased distress or despair as indicated by FPO.

As this study evaluated a 1-time intraperitoneal dose of 20 mg/kg of curcumin without finding significant results, future research should focus on multidosing regimens, larger doses, or alternative routes of administration, all of which could affect the bioavailability and potentially result in different or more stable plasma levels of curcumin. We recommend further investigation of the mechanism of action of curcumin, specifically other receptors and neurotransmitter systems, to include serotonergic, dopaminergic, or nitric oxide.

As chemicals and compounds from herbal medications may be dangerous and potentially toxic, research investigating potential herbal medications begins with basic science in animal models. Once these empirical data are obtained, they can provide support for hypotheses to further pursue scientific inquiry in humans. Even data that do not show statistical significance are important to science, as these results may assist the scientific community in directing their future research, avoiding unnecessary use of resources, and circumvent potential toxicities or side effects. In addition, understanding the biochemical and pharmacological effects of this herbal medication will enable patients to be better informed consumers and health care providers to understand and anticipate potential medication interactions.

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