

EVALUATION OF THE ANXIOLYTIC EFFECTS OF CHRYSIN, A *PASSIFLORA INCARNATA* EXTRACT, IN THE LABORATORY RAT

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*The definitive anxiolytic effects of *Passiflora incarnata* are unknown. We studied the potential anxiolytic effects of chrysin, a *Passiflora* extract, and the purported modulation of the benzodiazepine receptor on the GABA_A receptor in laboratory rats. We hypothesized that chrysin decreases anxiety via interaction with the GABA_A receptor in laboratory rats as measured by elevated plus-maze (EPM), corticosterone, and catecholamine assays.*

We randomized 44 male Sprague-Dawley rats in a double-blind, placebo-controlled, between-subjects experimental design. Each animal received an intraperitoneal injection of (1) vehicle (DMSO 4%), (2) chrysin, 2 mg/kg, (3) midazolam, 1.5 mg/kg, or (4) flumazenil, 3 mg/kg and chrysin, 2 mg/kg. The EPM was used to evaluate the behav-

ioral component of anxiolysis, and catecholamine and corticosterone assays were examined to measure the neurohormonal effects of anxiety.

No statistical difference was found among groups in catecholamine and corticosterone levels. Midazolam significantly decreased anxiety compared with control and flumazenil plus chrysin groups ($P < .05$); there was no significant difference compared with the chrysin group. These data suggest that chrysin may have anxiolytic properties similar to midazolam but to a lesser magnitude at the 2-mg/kg dose used in this study.

Key words: Anxiety, chrysin, elevated plus-maze, *Passiflora incarnata*.

It is estimated that 19 million adults in the United States suffer from some type of anxiety disorder, with costs of more than \$42 billion a year.¹ Anxiety disorders are not relegated only to the United States, but are ubiquitous across cultures.² These anxieties range from mild, such as fear of strange situations (eg, operating room) or public speaking, to severe that may be incapacitating and result in job loss.

According to Selye,³ anxiety, along with many other physiological and psychological stimuli, can activate a stress response, resulting in the release of endocrine and neurotransmitter mediators. The hypothalamus-pituitary axis is activated, resulting in increased secretion of adrenocorticotropic hormone, which then stimulates the release of corticosteroids from the adrenal cortex. The elevated plasma corticosteroid levels are modulators in the biological response to stress. Anxiety-inducing stress also activates the sympathetic nervous system, stimulating the release of catecholamines (ie, epinephrine and norepinephrine) from the adrenal medulla as a response to stressors

(fight or flight).³ This sympathetic nervous system activation results in increased heart and respiratory rates, glycolysis, and stimulation of the release of various neurohormonal mediators. As anxiety results in activation of the stress response, prolonged anxiety and exposure to stressors may lead to fatigue and a weakened immune system,³ predisposing people to illness.

Most patients admitted to the operating room for surgery experience anxiety and subsequent activation of the stress response. Factors associated with activation of the stress response place patients at greater risk for adverse outcomes and predispose them to a more complicated anesthetic plan. Anxiety activates the stress response, resulting in increased neuroendocrine mediators (elevated catecholamines and cortisol levels), which may be deleterious to a patient with a tenuous cardiovascular status, resulting in an adverse outcome or in poor wound healing.^{4,5} Reducing preoperative anxiety by the administration of anxiolytic drugs is an important component of anesthesia because it is necessary to decrease patients' distress to

avert physiologic and emotional sequelae of the stress response. Benzodiazepines frequently reduce anxiety, pain, and cardiovascular activation in the treatment of chest pain.⁶ In addition, emotional feelings of well-being and decreased anxiety related to surgery have been demonstrated to improve patient satisfaction and may decrease recovery time.⁷

The 3 most common benzodiazepines administered preoperatively for anxiolysis are diazepam (Valium), lorazepam (Ativan), and midazolam (Versed),⁸ with up to 75% of healthy adults⁴ and children⁹ receiving midazolam for anxiolysis before surgery. Midazolam is primarily used for anxiolysis, amnesia, and to enhance patient compliance during the preinduction and induction period.^{10,11} Other medications have been used for sedation and anxiety, such as barbiturates, antihistamines, and over-the-counter supplements. Furthermore, herbal supplements, such as valerian and *Passiflora incarnata*, are gaining popularity as self-medication for anxiolysis.

A majority of drugs manufactured for anxiolysis have undesirable side effects, such as hypotension, sedation, and a high potential for addiction and abuse. One major benefit of most herbal substances is that they do not have a large potential for abuse and addiction.¹² Many Americans are using herbal supplements in lieu of pharmaceuticals to treat their maladies as public enthusiasm for alternative herbal medications has increased significantly in recent years. In 1997, 12% of US consumers reported using herbal medications, representing a 380% increase since 1990.¹³ Although there is a huge increase in herbal supplement use, controls and standardization are lacking. According to the Dietary Supplement Health and Education Act of 1994, there is no requirement for evidence of efficacy, safety, or quality control standards for supplements, increasing the risk of adverse effects related to herbals.¹⁴ 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.¹³

Passiflora incarnata (*P. incarnata*), also known as passionflower, is an herbal supplement that has been used throughout history for purported anxiolytic and sedative effects; however, there is a lack of research that defines the actual chemical or chemicals in *P. incarnata* that results in its anxiolysis. In addition, there is a large void of data that definitively describe the pharmacodynamic site that produces the mechanism of action of *P. incarnata* and its resultant behavioral and physiologic effects. Two independent labora-

tories^{15,16} reported that the flavonoid extract, chrysin (5,7 dihydroxyflavone), is responsible for the anxiolytic properties of *P. incarnata* in rodents, with the site of action being the γ -aminobutyric acid (GABA)_A receptor.

Although 1 study in humans showed anxiolytic effects of *P. incarnata* using an observatory tool to score anxiety,¹⁷ most of the limited investigation of the anxiolytic effects of *P. incarnata* has been conducted in the rodent model.^{15,16,18,19} The limited number of *P. incarnata* and chrysin studies regarding anxiety have solely examined the behavioral component of anxiolysis without physiologic confirmation or corroboration of these behavioral data. There are no studies in the review of literature about the neurohormonal stress response (corticosteroid or catecholamine release) in rodents receiving *P. incarnata* or chrysin.

We investigated the anxiolytic efficacy of chrysin, a *Passiflora incarnata* extract, in rats with the added critical examination of neurohormonal modulation and the interactions with the benzodiazepine receptor site on the GABA_A receptor.

Methods and materials

For the study, 44 male Sprague-Dawley rats (Harlan Sprague Dawley Laboratories) weighing 200 to 250 g were randomized in a double-blind, placebo-controlled, between-subjects experimental design. All methods were performed in accordance with Brooke Army Medical Center Department of Clinical Investigations and approved by the Institutional Animal Use Committee. Each animal received an intraperitoneal injection of one of the following: (1) control (vehicle, dimethyl sulfoxide [DMSO] 4%), (2) chrysin (Sigma Chemical Co, St Louis, Missouri), 2 mg/kg solubilized in DMSO 4%, (3) midazolam, 1.5 mg/kg, or (4) flumazenil (Sigma Chemical Co), 3 mg/kg, and chrysin (Sigma Chemical Co), 2 mg/kg solubilized in DMSO 4%. The elevated plus-maze (EPM) apparatus was used to evaluate the behavioral component of anxiolysis, and catecholamine and corticosterone assays were used to measure the neurohormonal effects of anxiety.

All rats were injected intraperitoneally 30 minutes before behavioral evaluation with the EPM because previous studies demonstrated an anxiolytic effect between 20 and 30 minutes.^{15,16,19} Between the time of injection and testing, each rat was placed back into its cage to reduce any confounding influences. Animals were placed on the EPM facing the open arm, and each experimental session was recorded for 5 minutes, tracking animal movements via MotorMonitor software (Hamilton-Kinder, Poway, California). Each

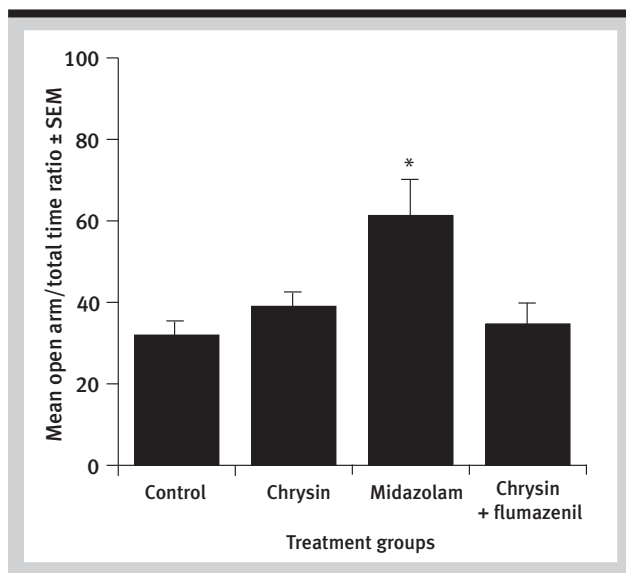
Table. Ratio open arm time–total maze time on elevated plus-maze per group

	Ratio open arm time–total maze time
Control (n = 11)	31.98 ± 2.6
Chrysin (n = 11)	39.04 ± 3.6
Midazolam (n = 11)	61.34 ± 8.7*
Flumazenil + chrysin (n = 11)	34.72 ± 5.0

Data are presented as mean ± standard error of the mean and represent the ratio of time spent on the open arm to total time on maze (5 minutes).

* Indicates significant statistical difference of $P < .05$.

Figure. Ratio of open arm time–total time on elevated plus-maze



Data are presented as mean ± standard error of the mean and represent the ratio of time spent on the open arm to total time on maze (5 minutes). Each group was composed of 11 rodents. Drugs were injected 30 minutes before testing on the elevated plus-maze.

* Indicates significant statistical difference of $P < .05$.

session was also recorded via a video camera, and the videotapes were analyzed by an investigator blinded to the group to validate the MotorMonitor data. The EPM is a widely used instrument to measure anxiety in the rodent model²⁰⁻²⁴ and has been validated by Pellow et al²⁵ based on the previous work by Montgomery.²⁶ Research on this instrument has supported its use as a standard measurement of anxiety, specifically benzodiazepine-induced anxiolysis in rodents.²⁷ Between testing of each animal, the EPM was cleansed

with soap and water and dried. All experimentation occurred on a timed schedule between the hours of 1:00 PM and 4 PM to ensure that each treatment group was exposed to similar variability of corticosterone release related to the animals' circadian rhythm. After testing on the EPM, rodents were decapitated, and trunk blood was obtained and collected in heparinized tubes for plasma corticosterone and catecholamine (epinephrine and norepinephrine) levels and sent to Esoterix Laboratory (Austin, Texas). A 2-tailed analysis of variance, with an α of .05 was used via SPSS 11.0 (SPSS, Chicago, Illinois) to determine if there were any significant differences among the groups for EPM, corticosterone, and plasma catecholamine data. When statistical significance was found, a posthoc Tukey was used to determine the location of significance.

Results

Only the midazolam group demonstrated a statistically significant difference in decreased anxiety ($P < .05$) when analyzing the ratio of open arm time–total maze time in the rodent model when compared with the control and flumazenil plus chrysin groups. However, no statistical difference was found between the midazolam and chrysin groups (Table). No statistical difference was found among the groups regarding the catecholamine and corticosterone levels. Although chrysin did not show a statistically significant difference compared with the control or flumazenil plus chrysin groups, the data were suggestive of an anxiolytic effect (Figure).

Midazolam showed a statistically significant difference compared with the control and flumazenil plus chrysin groups, but when compared directly with the chrysin group, there was no statistically significant difference between the 2 groups in open arm time and ratio of open arm time–total time on the EPM. These data suggest chrysin may have an anxiolytic effect similar to that of midazolam.

Discussion

The behavioral data suggest that the rats prefer the closed arms to the open arms of the maze. An increase in the percentage of time spent in the open arms reflects an anxiolytic effect.^{25,28} Our findings support that benzodiazepines (midazolam) increase open arm time in the EPM, reflecting a decrease in anxiety.²⁵ However, our statistical analysis between the control and chrysin groups did not show a significant difference. These results do not support the original hypothesis that the chrysin flavonoid derived from the *P incarnata* plant has significant anxiolytic effects at

the dose of 2 mg/kg. It was interesting to note that there was no significant difference in the time spent in the open arms of the EPM between the chrysin and midazolam groups. These findings suggest that although chrysin does not have the same anxiolytic response as midazolam, there is a possible trend toward an anxiolytic effect compared with the control and flumazenil groups. A dose of 2 mg/kg was used in our chrysin group, while previous research studies used doses ranging from 1 to 10 mg/kg.¹⁶ This may indicate that a higher chrysin dose may show significant anxiolytic effects.

It was further hypothesized that there would be differences found between the chrysin group and the flumazenil plus chrysin group, speculating that chrysin may modulate the benzodiazepine receptor site of the GABA_A receptor. This is the same site at which midazolam and, in theory, all other benzodiazepines exert anxiolytic effects.¹⁵ Our findings did not show a statistically significant difference between the chrysin and flumazenil plus chrysin groups, indicating that the site of action may not be the benzodiazepine receptor site of the GABA_A receptor.

From this work, future research can be designed to answer several questions that have arisen as a result of these findings. One design would add a group that combines midazolam and chrysin to evaluate the potential synergistic or additive anxiolytic effects vs either medication alone. This combined group could then suggest how a patient currently self-medicating with herbal medications containing chrysin may respond when given midazolam in the preoperative setting.

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