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Effect of *Nelumbo Nucifera* Extract on Anxiety Symptoms in Individuals With Moderate to Severe Anxiety: An Exploratory Study

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Introduction/Purpose/Background

Nelumbo nucifera (N. nucifera) is an aquatic lotus plant whose seed and leaf extracts have been studied in animals for their anxiolytic effects. This study aims to evaluate the anxiolytic effects in patients who consumed a commercially available *N. nucifera* leaf extract.

Methods

This investigation was an open-label, prospective, exploratory study in 20 adults. Participants with moderate to severe anxiety received 500 mg of *N. nucifera* leaf extract (Neluleaf, IN-Ingredients Inc., Spring Hill, Tenn.) orally twice daily from Day 1 to 28, then discontinued the regimen from Day 29 to 56. Primary endpoint was absolute change in GAD-7 score from baseline (Day 0) to Day 28. Secondary endpoint was absolute change from baseline to Day 28 in GHQ-28 score. Differences in GAD-7 and GHQ-28 scores from baseline to specified time points were compared using paired Student's t-test.

Results

GAD-7 (n = 18) and GHQ-28 scores significantly decreased from baseline to Day 28, (13.9 ± 3.4 to 9.0 ± 5.6, $p < 0.01$ and 30.8 ± 10.3 to 20.7 ± 11.6, $p < 0.01$, respectively). At Day 28, 72% of participants showed reduction in GAD-7 severity by one category, and 67% had decline in GAD-7 score by ≥ 5 points. Two participants were withdrawn due to pruritus, which resolved upon discontinuation of *N. nucifera*. Five patients experienced bloating and fatigue.

Conclusion

Individuals with moderate to severe anxiety who consumed *N. nucifera* leaf extract reported improvements in anxiety symptoms. Future randomized, double-blinded, controlled clinical trials are needed to verify the findings from this exploratory study and to report the safety profile of the *N. nucifera* leaf extract.

Over 40 million adults in the U.S suffer from anxiety disorders with symptoms of physical fatigue, shortness of breath and feelings of restlessness.[1] These symptoms often have a significant impact on mood and behaviors, and they can manifest in both mental and somatic illnesses.[1] Treatment options for anxiety disorders mainly include psychotherapy, such as cognitive behavioral therapy (CBT), pharmacological interventions, most commonly selective serotonin reuptake inhibitors (SSRI) or benzodiazepines or a combination of the two.[2] Both CBT and pharmacologic options can effectively treat the symptoms of anxiety; however, CBT is limited by the time and financial commitments, and pharmacologic options are limited by side effects, adherence rates and have the potential for drug abuse and dependency.[3,4] Therefore, alternative treatment options could provide improved outcomes to patients dealing with symptoms of anxiety.

Nelumbo nucifera (N. nucifera), also known as lotus, is an aquatic plant of the *Nymphaeaceae* family, indigenous to Asia, commonly used in traditional herbal medicine. Different parts of the lotus plant, such as the seeds, leaves and flowers, have been reported to have antioxidant, antiviral, antipyretic, antilipidemic and other biological activities.[5] Pharmacological effects of *N. nucifera* seed and leaf extracts have been associated with having antioxidant and anti-inflammatory properties, which are derived from the presence of flavonoids and phenols that protect from the damaging cellular effects of reactive oxygen species (ROS).[6,7]

Preclinical studies comparing diazepam, *N. nucifera* and placebo have reported that both diazepam and *N. nucifera* extracts demonstrate anxiolytic effects in mice.[8,9] Both diazepam and dose-dependent *N. nucifera* extracts also showed shorter onset of sleep and prolonged thiopental-induced sleep time. Additionally, the combination of *N. nucifera* seed extract and diazepam resulted in a potentiated anxiolytic effect compared to diazepam alone, suggesting *N. nucifera* seed extract may have a role in gamma-amino butyric acid (GABA) modulation in the brain.[8] Another study similarly compared the anxiolytic effects of diazepam, *N. nucifera* leaf extract and placebo in albino mice. Higher doses of *N. nucifera* leaf extract in mice posed similar anxiolytic activity comparable to anxiolytic doses of diazepam.[9] These studies support that *N. nucifera* extracts from seeds and leaves may have anxiolytic and sedative effects.

Although the data show an association between *N. nucifera* seed and leaf extracts and anxiolytic effects in mouse models, there have not been human studies to assess the clinical effect of *N. nucifera* extracts on symptomatic anxiety relief. To our knowledge, this is the first prospective, clinical study to explore the effect of *N. nucifera* leaf extract on validated markers assessing anxiety. This exploratory study aims to evaluate anxiolytic effects, as reported by patients who consumed a commercially available *N. nucifera* leaf extract.

Methods

Study Design and Procedure

This was an open-label, prospective exploratory study to evaluate the anxiolytic effects of *N. nucifera* leaf extract. Participants consumed *N. nucifera* leaf extract twice daily for 28 days followed by a *N. nucifera* free period for the following 28 days. The study recruited candidates from a university campus in the U.S.by sending emails to students and posting ads around campus. The study was conducted during the period from October 2020 to February 2021. The protocol was approved by the Institutional Review Board; all subjects gave informed consent prior to their participation in the study.

Study Population

Initial participant screening for study inclusion and assessment of baseline General Anxiety Disorder-7 (GAD-7) score was performed using an online virtual platform. Participants were included in the study if they were 18 to 65 years of age with moderate to severe anxiety, defined as a GAD-7 score of greater than or equal to 10 at screening. Candidates were excluded from this study if they had a history of or current physician diagnosis of major depressive disorder, panic disorder, obsessive-compulsive disorder, post-traumatic stress disorder, acute stress disorder, anxiety disorder, schizophrenia or other psychotropic disorder. Candidates were also excluded if they were critically ill or had a diagnosis of significant acute or chronic coexisting illness. Additionally, candidates currently receiving treatment with over-the-counter sleep medications, herbal supplementation, psychoactive medications (e.g., selective serotonin reuptake inhibitors, benzodiazepines, antipsychotics or anxiolytics), antiplatelets, insulin or other glucose modulators were excluded from the study. Current smokers and tobacco users were also excluded. Any candidates who were pregnant, breastfeeding or planning to become pregnant during the study were not eligible to participate in this study.

Study Intervention

Participants received 500 mg of *N. nucifera* leaf extract orally twice daily from Day 1 to Day 28 and then discontinued *N. nucifera* treatment from Day 29 to Day 56. The product used in this study was a patented, commercially available water-soluble extract from *N. nucifera* leaves, standardized to 10% flavones (Neluleaf, IN-Ingredients Inc., Spring Hill, Tenn.).

Measurement Instruments

GAD-7 is a seven-item self-administered questionnaire; each item is scored based on a 4-point Likert scale (ranging from 0 to 3), with 0 indicating anxiety symptoms occurring "not at all" and 3 indicating anxiety symptoms occurring "nearly every day." The total score is the sum of the seven questions, ranging from 0 to 21, with increasing scores indicating more severe anxiety; total scores above 10 are in the clinical range. A severity category of mild, moderate or severe anxiety was indicated by GAD-7 score range of 5-9, 10-14 or ≥ 15, respectively.[10]

The General Health Questionnaire (GHQ-28) is a patient-reported screening tool used to assess mental health and psychological well-being measuring four domains: somatic symptoms, anxiety and insomnia, social dysfunction and depression. The GHQ-28 assesses the individual's overall general health in the past two weeks, using a 4-point Likert scale (ranging from 0 to 3) per question. The range of total score is 0 to 84, with higher scores indicating greater severity of distress.[11]

Endpoints

The primary endpoint was the absolute change in GAD-7 total score from baseline (Day 0) to Day 28. The key secondary endpoint was the absolute change from baseline to Day 28 in the GHQ-28 total score. Additional secondary endpoints included absolute changes in GAD-7 total score from baseline to Day 14 and Day 56, absolute change in GHQ-28 total score from baseline to Day 56, total sleep time from baseline to Day 28 (as reported through daily questionnaires) and incidence of adverse events during the study.

Statistical Analysis

Since this was an exploratory study, 20 participants were enrolled to evaluate an initial effect size and better determine future studies. Differences in GAD-7 score, GHQ-28 score and average sleep time from baseline to specified time points during the 56-day study were compared using the paired Student's t-test.

Results

Twenty-eight candidates were screened and 20 were enrolled into the study. Two dropped out due to side effects. Eighteen (90%) participants completed the study and were included for statistical analyses. Twelve of 18 (67%) and 13 of 18 (72%) participants were females and Asian, respectively. Baseline characteristics, including GAD-7 and GHQ-28 scores, are listed in the Table. None of the participants had any comorbidities or were on any other medications, except for one person who was taking oral contraceptives prior to initiation of the *N. nucifera* leaf extract. None of the participants tested positive for COVID-19 during the study period.

Figures 1A and 1B describe changes in GAD-7 and GHQ-28 scores, respectively, from baseline over time. The GAD-7 scores significantly decreased from baseline to Day 28 (13.9 ± 3.4 to 9.0 ± 5.6; $p < 0.01$). GAD-7 scores were also significantly decreased at Day 14 when compared to baseline (13.9 ± 3.4 to 9.6 ± 4.6, $p < 0.01$), and GAD-7 severity levels improved by one category in 50% (9/18) of participants. Four weeks after *N. nucifera* discontinuation, the GAD-7 scores remained significantly lower from baseline to Day 56 (13.9 ± 3.4 to 8.8 ± 4.9, $p < 0.01$).

The number of participants who reported reduction in GAD-7 severity by one category was 72% (13/18) at Day 28 and 67% (12/18) at Day 56 (Figure 2). The ratio of participants with decreases in their GAD-7 score by ≥5 points was 67% (12/18) at Day 28 and 56% at Day 56 (10/19).

GHQ-28 scores significantly decreased from baseline to Day 28, (30.8 ± 10.3 to 20.7 ± 11.6; $p < 0.01$). However, GHQ-28 scores did not maintain statistical difference at Day 56 when compared to baseline (30.8 ± 10.3 to 23.9 ± 16.2; $p = 0.07$). There was no significant difference in sleep time from baseline to Day 28 (increased by 27 ± 111 minutes; $p = 0.33$).

Participants had a high compliance rate for *N. nucifera* with an average 2.7 ± 3.7 doses missed out of a possible 56 doses throughout the study. Because compliance to *N. nucifera* consumption may affect the participant's overall GAD-7 and GHQ-28 scores, a subgroup analysis was conducted

Table: Participant Characteristics

Characteristic	Total
Age in years, mean (SD)	24.4 (6.8)
Sex, n (%) Male Female	 6 (33) 12 (67)
Race, n (%) White Asian Hispanic/Latinx Other	 1 (6) 13 (72) 2 (11) 2 (11)
Weight, pounds, mean (SD)	148 (39.4)
Height, inches, mean (SD)	65.4 (4.9)
Baseline GAD-7, mean (SD)	13.9 (3.4)
Baseline GHQ-28, mean (SD)	30.8 (10.3)

Figure 1A: GAD-7 score over time (n = 18).

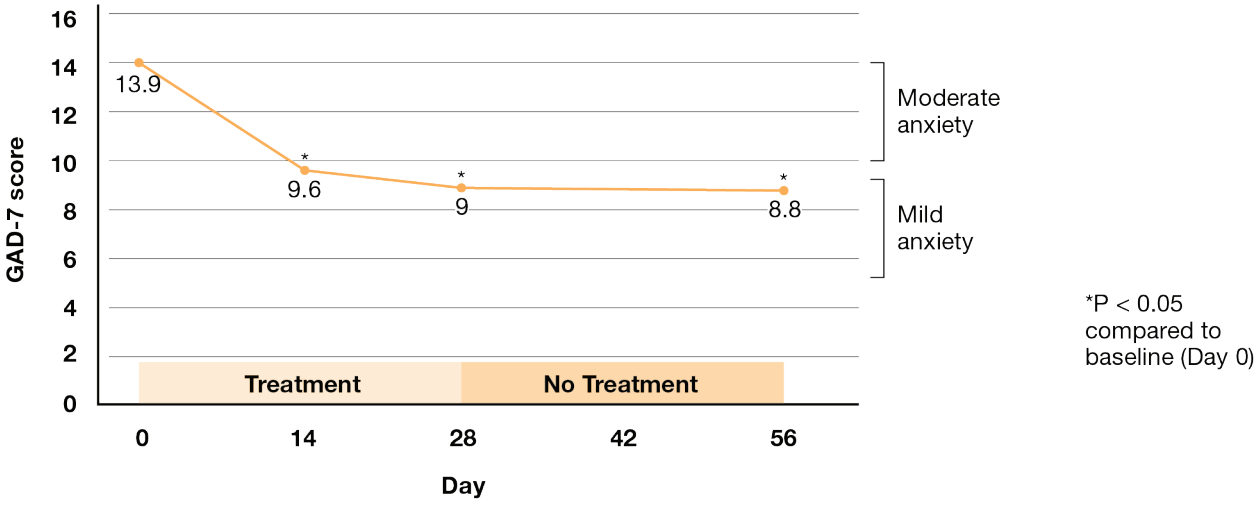


Figure 1B: GHQ-28 score over time (n = 18)

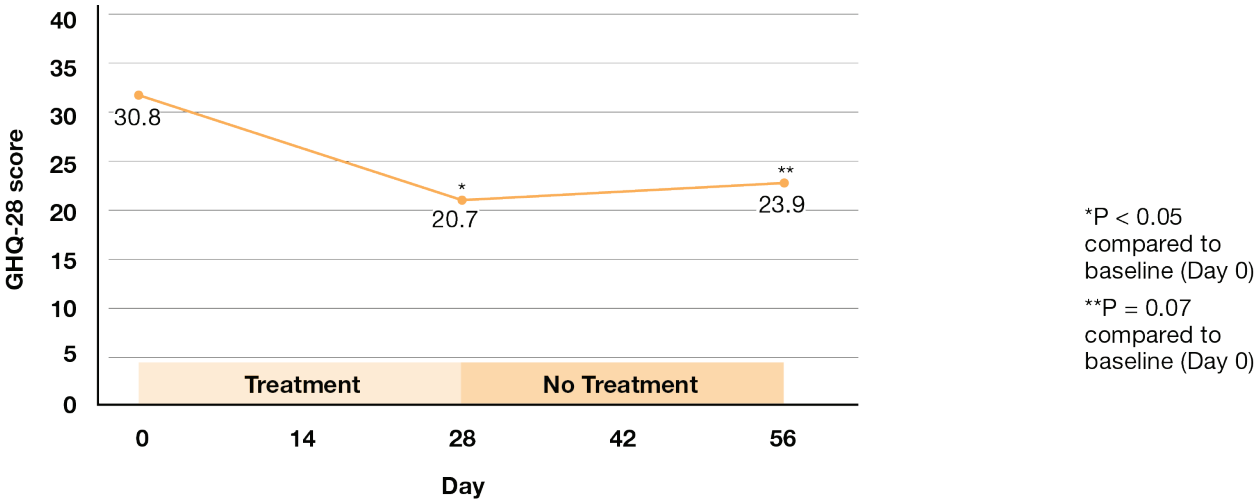
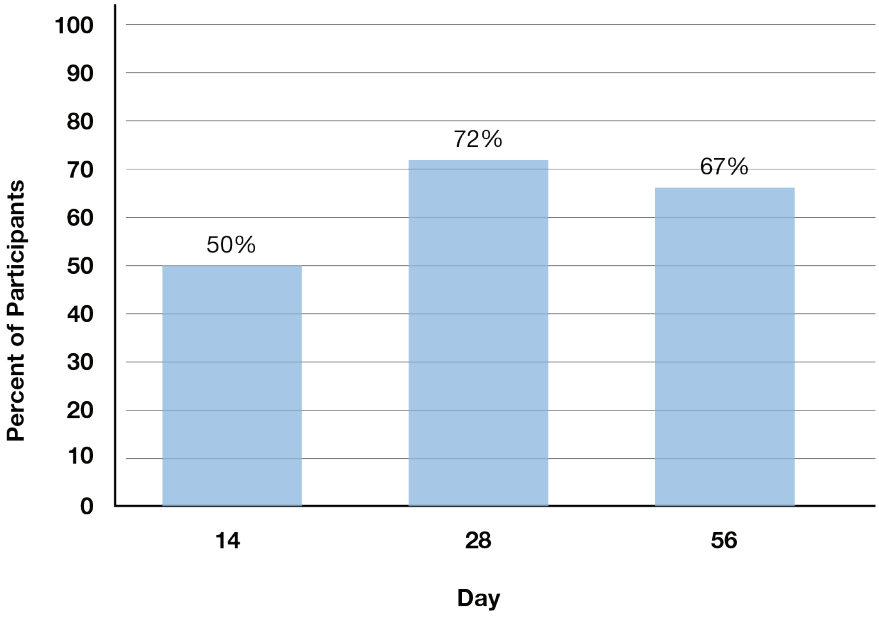


Figure 2: Percent of Patients Lowering their GAD-7 Category (n=18)



to exclude those participants who reported missing > 10% (four or more days, equivalent to eight or more doses) of *N. nucifera* consumption over the 28-day treatment period.

In the cohort of compliant patients (n = 16) significant decreases in both GAD-7 and GHQ-28 scores were maintained from baseline to Day 28 (14.0 ± 3.5 to 8.9 ± 5.4; $p < 0.01$ and 30.6 ± 10.9 to 20.8 ± 12.0; $p < 0.01$, respectively). Although GAD-7 scores were also significantly reduced from baseline to Day 56 (14.0 ± 3.5 to 8.6 ± 4.4; $p < 0.01$), GHQ-28 scores were not (30.6 ± 10.9 to 23.0 ± 15.5; $p = 0.06$). Eleven of the 16 participants (69%) showed improvement by at least one GAD-7 severity category at Day 56.

Adverse effects included pruritus (n = 2), fatigue (n = 3) and gastrointestinal symptoms (n=3), and all events were considered mild in severity. The two participants who developed mild pruritus were asked to discontinue treatment as per protocol. One participant experienced itchiness on the upper neck and lower back and was withdrawn on Day 2. The other participant experienced itchiness on the arm and stopped study participation on Day 8. Both participants' symptoms resolved after discontinuation of *N. nucifera*. Five of 18 participants reported adverse effects, two reported gastrointestinal symptoms (bloating), two reported fatigue and one reported both. Whether these effects are related to *N. nucifera* or unrelated is unknown.

Discussion

This open-label, prospective, exploratory study demonstrated that participants with moderate to severe anxiety who consumed *N. nucifera* leaf extract reported improved anxiety symptoms as measured by the reduction in GAD-7 and GHQ-28 scores. This is the first clinical study to demonstrate objective improvements in anxiety symptom severity after *N. nucifera* treatment. On average, participants improved their GAD-7 scores by nearly five points, which is an improvement of one severity category, frequently from moderate to mild anxiety. This can equate to an average reduction of 3.2 disability days and 0.5 physician visits per three months, as categorical improvements of GAD-7 translate to improvements of patient quality of life across mental health, physical functioning, pain, social functioning and general health perceptions.[10]

Improvements in GAD-7 scores were significant after 14 days and were sustained for four weeks after participants stopped taking *N. nucifera* leaf extract. Standard of care anxiety treatments, such as SSRI, often have a delayed onset of perceived effects, which can lead to noncompliance from patient perception of medication non-effect. Additionally, SSRIs often must be continued for long periods of time, which combined with the medication's side effect profile may create another barrier to anxiety treatment. *N. nucifera* leaf extract's relative short onset and residual effects after treatment discontinuation is encouraging. Limited pharmacokinetic information exists on *N. nucifera* extracts in humans; however, a study in rats found that the active components in *N. nucifera* leaf extract had a relatively long elimination half-life, rapid blood brain barrier penetration and wide distribution in the brain.[12] From our study, the 14-day improvement in GAD-7 and residual anxiety benefit observed from *N. nucifera* leaf extract may provide novel pathways to the treatment of generalized anxiety disorders. Whether these residual benefits to anxiety continue long term after treatment discontinuation should be further explored.

N. nucifera seed extract has been shown to inhibit the nuclear factor-kappa B (NF-kB) signaling activity. Stress activates the NF-kB signaling pathway, which induces the expression of pro-inflammatory markers linked with antineurogenic activity, anxiety and depression.[5,13] The anxiolytic effect of *N. nucifera* from seeds may be further explained by NF-kB inhibition. Additionally, aqueous *N. nucifera* leaf extract has been reported to potentiate the anxiolytic activity of diazepam through GABAergic modulation.[9] These studies have shown that both *N. nucifera* seed and leaf extracts may have therapeutic effects for anxiety.

While the current study demonstrates significant reductions in anxiety severity as reported by the participants, several limitations should be noted. This study did not have a control group, but rather it utilized participants as their own control versus a parallel placebo-controlled group. Neither participants nor investigators were blinded to the study intervention. Future double-blind, parallel, placebo-controlled trials are needed. Enrolled participants were mostly Asian and females, which may limit the generalizability of the reported results. Participants also did not have any comorbidities or on any medications; thus, it is unclear of the therapeutic and safety effects and potential drug interactions of *N. nucifera* leaf extract in patients with physician-diagnosed anxiety or receiving concomitant medications. This study also showed an increase of about one-half hour of sleep time after a four-week trial of the *N. nucifera* leaf extract consumption. Although this endpoint was not statistically significant, this may be driven by the small sample size and needs further exploration in larger studies.

This exploratory study provided data for future randomized, blinded, controlled clinical trials to further evaluate the effects of *N. nucifera* leaf extract in patients with moderate to severe anxiety. Safety studies of *N. nucifera* leaf extract are also warranted to determine potential adverse effects, including the possibility of pruritus.

Conclusion

Individuals with moderate to severe anxiety who consumed *N. nucifera* leaf extract reported improvements in anxiety symptoms as demonstrated by reductions in GAD-7 and GHQ-28 scores. Future randomized, double-blind, placebo-controlled studies are needed to verify the findings from this study and to report the safety profile of the *N. nucifera* leaf extract.

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Conflict of Interest

The authors have no conflicts of interest to disclose.

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References

1. Anxiety and Depression Association of America. Anxiety Disorders - Facts & Statistics. adaa.org/understanding-anxiety/facts-statistics. Accessed Sept. 14, 2020.

2. Bandelow B, Michaelis S, Wedekind D. Treatment of anxiety disorders. *Dialogues Clin Neurosci* 2017 Jun;19(2):93-107. doi:10.31887/DCNS.2017.19.2/bbandelow.

3. Glenn D, Golinelli D, Rose RD, et al. Who gets the most out of cognitive behavioral therapy for anxiety disorders? The role of treatment dose and patient engagement. *J Consult Clin Psychol* 2013 Aug;81(4):639-649. doi:10.1037/a0033403.

4. Gordon RP, Brandish EK, Baldwin DS. Anxiety disorders, post-traumatic stress disorder and obsessive-compulsive disorder. *Medicine (Baltimore)*. 2016 Nov;44(11):664-671. doi:10.1016/j.mpmed.2016.08.010.

5. Paudel KR, Panth N. Phytochemical Profile and Biological Activity of Nelumbo nucifera. *Evid Based Complement Alternat Med* 2015;2015:789124. doi:10.1155/2015/789124.

6. Park E, Kim GD, Go MS, et al. Anti-inflammatory effects of *Nelumbo* leaf extracts and identification of their metabolites. *Nutr Res Pract* 2017 Jul;11(4):265-274. doi:10.4162/nrp.2017.11.4.265.

7. Shen Y, Guan Y, Song X, et al. Polyphenols extract from lotus seedpod (*Nelumbo nucifera* Gaertn.): Phenolic compositions, antioxidant and antiproliferative activities. Food Sci Nutr 2019 Sep;7(9):3062-3070. doi:10.1002/fsn3.1165.

8. Sugimoto Y, Furutani S, Itoh A, et al. Effects of extracts and neferine from the embryo of Nelumbo nucifera seeds on the central nervous system. *Phytomedicine* 2008 Dec;15(12):1117-1124. doi:10.1016/j.phymed.2008.09.005.

9. Kulkarni M, Juvekar A. Anti-anxiety effects of leaves of Nelumbo nucifera gaertn. in mice. *Pharmacologyonline* 2009 Jan;2:292-299.

10. Spitzer RL, Kroenke K, Williams JB, et al. A brief measure for assessing generalized anxiety disorder: the GAD-7. *Arch Intern Med* 2006 May 22;166(10):1092-1097. doi:10.1001/archinte.166.10.1092.

11. Vallejo MA, Jordán CM, Díaz MI, et al. Psychological assessment via the internet: A reliability and validity study of online (vs paper-and-pencil) versions of the General Health Questionnaire-28 (GHQ-28) and the Symptoms Check-List-90-Revised (SCL-90-R). *J Med Internet Res* 2007 Jan;9(1):e2. doi:10.2196/jmir.9.1.e2.

12. Ye LH, He XX, You C, et al. Pharmacokinetics of Nuciferine and N-Nornuciferine, Two Major Alkaloids From *Nelumbo nucifera* Leaves, in Rat Plasma and the Brain. *Front Pharmacol* 2018 Aug 29;9:902. doi:10.3389/fphar.2018.00902.

13. Moon SW, Ahn CB, Oh Y, et al. Lotus (Nelumbo nucifera) seed protein isolate exerts anti-inflammatory and antioxidant effects in LPS-stimulated RAW264.7 macrophages via inhibiting NF-χB and MAPK pathways and upregulating catalase activity. *Int J Biol Macromol* 2019 Aug 1;134:791-797. doi:10.1016/j.ijbiomac.2019.05.094.

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