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Effect of Medicinal Syrup Made from Silkworm Cocoon on Mixed Anxiety-Depression Disorder: A Triple-blind Randomized Clinical Trial

Yahya Zeinalpour[1], Mahdi Kabiri[2], Roja Rahimi[2], Rahim Khalilzadeh[3] and Mehrdad Karimi[1,*]

[1] Department of Traditional Medicine, School of Persian Medicine, Tehran University of Medical Sciences, Tehran, Iran
[2] Department of Traditional Pharmacy, School of Persian Medicine, Tehran University of Medical Sciences, Tehran, Iran
[3] Department of Psychiatry, Orumiyeh University of Medical Sciences, Orumiyeh, Iran

*** *Corresponding author:*** Mehrdad Karimi, Department of Traditional Medicine, School of Persian Medicine, Tehran University of Medical Sciences, Tehran, Iran. Tel: +98 9127905439; Email: mehrdadkarimi@yahoo.com

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Abstract

Background: Silkworm cocoon produced by silkworms with the scientific name of Bombyx mori L. is a well-known medicinal agent mainly composed of proteins.
Objectives: This study was designed to assess the efficacy of syrup made from this natural agent on mild to moderate depression.
Methods: The study was conducted based on a triple-blind randomized clinical trial. A total of 60 patients with mixed anxiety-depressive disorder were randomized to receive either intervention (silk syrup + sertraline) or control (placebo syrup + sertraline) for 12 weeks. Depression and anxiety were assessed using the Beck Depression and Anxiety inventories at weeks 0, 6, and 12.
Results: Out of the subjects, 54 patients completed the trial in two groups of intervention (n=30) and control (n=24) and entered the final analysis. According to the results of this study, the mean of anxiety and depression scores in weeks 6 and week 12 was significantly lower in the intervention group than in the control group (P<0.001).
Conclusion: Silk syrup can be beneficial for the management of anxiety and depression in mild to moderate MADD; therefore, it is suggested as an adjuvant treatment to increase the efficacy of conventional medications adopted for the treatment of MADD patients.

Keywords: Anxiety, Bombyx mori, Cocoon, Depression, Silk, Silkworm, Sericin

1. Background

One of the most common complaints among patients visiting primary healthcare units is mixed anxiety-depression disorder (MADD) (1). The World Health Organization defines MADD as a disorder with both depression and anxiety symptoms of limited and equal intensity together with some autonomic features (2). The prevalence rate of this disorder has been reported variably in studies; however, researchers recognize MADD as one of the most prevalent psychiatric disorders (3). Approximately 85% of patients suffering from depression also report symptoms of anxiety. Likewise, depression symptoms are present in more than 90% of cases of an anxiety disorder (4). Increased risk of significant distress and inability to carry out daily activities can complicate untreated cases of MADD (5). Moreover, this disorder can lead to reduced self-care (6), maladaptive behaviors (e.g., drug/alcohol abuse) (7), reduced mental function, declined quality of life (8), and increased morbidities and mortality (9).

Considering the substantial effect of treatment on the quality of life, promotion of safety, and improvement of personal and social functions (10), it is of high priority to prompt diagnosis and intervention to reduce symptoms in MADD patients (11). A variety of pharmacological and non-pharmacological interventions have been recommended to reduce depression and anxiety in this disorder (4).

Benzodiazepines are used as the first-line treatment of anxiety symptoms (12), while depression is cured using tricyclic antidepressants, monoamine oxidase inhibitors, and selective serotonin reuptake inhibitors (SSRIs) (13). Despite being able to alleviate the symptoms of depression and anxiety, pharmacological treatments are commonly accompanied by side effects, including gastrointestinal symptoms (especially nausea), headache, reduced appetite, and sexual dysfunction (14). Meanwhile, both developing resistance to treatment and discontinuation syndrome following interruption or reduction of antidepressant medication may complicate treatment (15). Therefore, regarding the MADD treatment plan, it is highly necessary to perform research to introduce natural compounds that have fewer side effects and are generally well-tolerated by the patients.

According to Traditional Persian Medicine (TPM) literature, silkworm cocoon is one of the most important drugs in treating psychiatric disorders (16). This natural medicine is produced by the domestic silkworm Bombyx mori and has long been used as both food and drug in east Asian countries (17). Silkworm cocoon is a rich source of proteins, amino acids, and vitamins B1, B2, and E that are necessary for the optimal function of the nervous system (18). Deficiencies in the B vitamins and amino

acids, as precursors of neurotransmitters, are one of the most common nutritional deficiencies in mental and mood disorders, including MADD.

2. Objectives

This study was designed to investigate the efficacy of a medicinal syrup made from silkworm cocoon on patients with mild to moderate MADD.

3. Methods

3.1. Study Design and participants

This study was designed as a triple-blind randomized clinical trial. The statistical population consisted of out-patients diagnosed with MADD, visiting a psychiatry clinic in Orumiyeh, Iran. The inclusion criteria were being at the age range of 18-60, having depression and anxiety symptoms for a minimum of 2 weeks, lacking a history of other mental disorders (approved by a psychiatrist), lacking a history of physical illnesses (including cancer, AIDS, diabetes, and liver failure), not using anti-depressant or anxiolytic medications in the past 2 months, lacking a history of drug addiction, not attending psychotherapy sessions simultaneous with treatment, not being pregnant (for female participants), and having a depression score of 14-28 and an anxiety score of 8-25 in Beck Depression and Anxiety inventories. On the other hand, participants who were unwilling to continue treatment, did not return for a second visit at week 6 to receive medications, used anti-depressant or anxiolytic drugs during the trial period, or experienced a mental crisis/physical illness were excluded from the study.

3.2. Blocked randomization and blinding

Participants were allocated to intervention and control groups by block randomization. A randomized sequence was prepared by a data analyst; accordingly, the participants were assigned to four groups (A, B, C, D) of either intervention (A, C) or control (B, D) based on a random number table. The medications were dispensed in labeled containers. The patients, healthcare professionals, and the researcher performing data analysis were all blinded.

3.3. Intervention

The protocol of this clinical trial conformed to the SPIRIT 2013 Statement. The intervention group received silk syrup (5 ml, twice daily) and sertraline (50 mg, daily), while the control group was administered placebo syrup (5 ml, twice daily) and sertraline (50 mg, daily). All participants were subjected to treatment for 12 weeks and received the medications in two visits (at the initiation of treatment and week 6).

Patients who had not consumed more than 15% of the administered medications were excluded from the study. Silk and placebo syrup were prepared with similar taste and color and dispensed coded without name tags. Weekly phone calls were conducted to follow the patients for compliance, any side effects, or symptom exacerbation.

3.4. Drug and placebo preparation

The recommended daily dose for silk cocoon in TPM is 10 g. The extractive value of the aqueous extract of the cocoon is 5%. In this regard, 500 mg of dried extract corresponds to 10 g of cocoons. The daily dose of syrup was considered 10 ml. Therefore, the syrup was formulated to contain 500 mg extract in every 10 ml of syrup.

Cocoons were carefully cleared from caterpillars and cut into tiny pieces using scissors. The cocoons were subsequently washed, placed in 20-times their weight distilled water, and autoclaved for 1 h. After 24 h, they were boiled for 2 h and then sieved. Afterward, silk syrup was prepared with 80% of the weight of sugar. Sodium benzoate and potassium sorbate 0.1% were used as preservatives. Placebo syrup was prepared using sugar and 2% gelatin. Lowry Protein Assay was used to standardize the syrup. Total protein content was calculated after the construction of the calibration curve. The protein concentration of the syrup was set at 50 mg/mL. Physicochemistrical and microbial control were also performed (21, 22).

Drug and placebo preparation and standardization were performed by the Department of Traditional Pharmacy, Tehran University of Medical Sciences, Tehran, Iran. Sertraline was purchased from Sobhan Pharmaceutical Co., Iran, and was administered to both groups at a dose of 50 mg daily.

3.5. Assessment of primary outcome

Study variables were assessed using Beck Depression Inventory, Second Edition (BDI-II) and Beck Anxiety Inventory (BAI). Beck Depression Inventory, Second Edition, is a 21-item multiple-choice inventory, designed to assess the severity of depression in adolescents and adults. Items are rated on a 4-point Likert scale ranging from 0 to 3 based on severity. The total score lies in a range of 0-63, with higher scores indicative of more severe depression (23). Beck Anxiety Inventory is also a 21-item multiple-choice inventory, with the items rated on a 4-point Likert scale (0-3), and a total score range of 0-63. This instrument is used to measure the degree of anxiety in adolescents and adults, with higher scores showing higher levels of anxiety.

3.6. Validity and reliability

Studies have constantly reported high internal consistency and test-retest reliability of the BDI-II (24). The internal consistency of BAI, as examined in

various studies, is also high and reasonable with test-retest coefficients and at the range of 0.62-0.93 (25). Correspondingly, the Persian version of both BDI (26) and BAI (27) have been examined and shown good validity and reliability.

3.7. Ethical considerations

This trial was conducted in compliance with the principles of Good Clinical Practice and the Declaration of Helsinki. The study was approved by the Ethical Committee of Tehran University of Medical Sciences (IR.TUMS.VCR.REC.1397.739), and registered in the Iranian Registry of Clinical Trials (IRCT20190724044320N1). All participants signed an informed consent form.

3.8. Statistical analysis

All statistical analysis tests were performed using the Statistical Package of Social Science (SPSS) version 16. Differences in demographic data between intervention and control groups were analyzed by a two-tailed t-test and Chi-square. Moreover, mixed-model analysis of variance was employed to compare mean depression and anxiety scores between the intervention and control groups. P-values of less than 0.025 (0.05/2) were considered statistically significant.

4. Results

4.1. Recruitment and participant flow

A total of 60 male and female out-patients were recruited gradually from April 2019 to September 2019 in a psychiatric clinic in Orumiyeh. All patients were diagnosed with MADD by a specialized psychiatrist. Participants were assessed for eligibility regarding the mentioned inclusion and exclusion criteria, and subsequently, randomized and allocated to intervention and control groups by block randomization.

Out of the 60 total cases, 6 patients discontinued intervention due to surgery, became pregnant during the treatment, and declined to return for follow-up visits. Therefore, the samples of this study consisted of 54 patients in two groups of silk syrup (n=30) and control (n=24) in the final analysis (Figure 1).

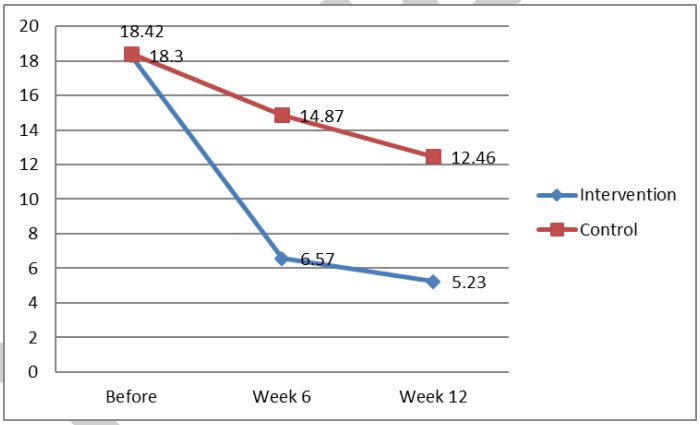

Figure 1. Mean depression score at 0, 6, and 12 weeks in the intervention versus control group

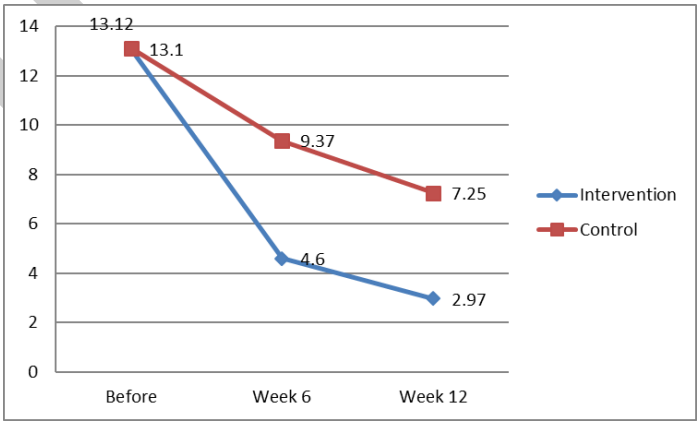

Figure 2. Mean anxiety score at 0, 6, and 12 weeks in the intervention versus control group

Table 1. Baseline demographic and clinical characteristics of the intervention and control groups based on variables

Variable		Group		P-value
		Intervention	Control	
Gender	Male	18 (64.3)	10 (35.8)	0.180*
	Female	12 (46.2)	14 (53.8)	
Age		43.70±13.26	42.33±12.05	0.697**
Marital status	Single	6 (60.0)	4 (40.0)	0.754*
	Married	24 (54.5)	20 (45.5)	
Education	<High school graduate	20 (52.6)	18 (47.4)	0.677*
	Associate's degree	2 (50.0)	2 (50.0)	
	Bachelor's degree	8 (66.7)	4 (33.3)	
Occupation	Employed	14 (63.6)	8 (36.4)	0.322*
	Unemployed	16 (50.0)	16 (50.0)	
Economic status	Low	10 (50.1)	10 (50.1)	0.591*
	Medium	20 (58.8)	14 (41.2)	
Smoking cigarette	Yes	7 (70.0)	3 (30.0)	0.529*
	No	22 (52.4)	20 (47.6)	
History of disease	Yes	8 (66.7)	4 (33.3)	0.380*
	No	22 (52.4)	20 (47.6)	
Family history of psychiatric disorder	Yes	5 (55.6)	4 (44.4)	1.000*
	No	25 (55.6)	20 (44.4)	

Data are reported as mean±SD or frequency (%).
*Chi-squared test; **Independent t-test

4.2. Baseline data

The baseline demographic and clinical characteristics of participants are presented in Table1. There was no statistically significant difference between the two groups in terms of demographic data, including age, gender, marital status, education level, and occupational and economic status or clinical background characteristics, including smoking and personal or familial history of psychiatric disorder.

4.3. Outcome measurements

4.3.1. Effect of silk syrup on depression and anxiety scores

The primary outcomes were the scores obtained from the BDI and BAI. According to Table2, BDI and BAI scores improved significantly from baseline to week 6 (P<0.001). The main effect of the group (excluding the effect of time) showed that the mean of depression score (according to BDI) and anxiety score (according to BAI) were lower in the group receiving silk syrup (5 ml, twice daily) and sertraline (50 mg, daily) than in the control group receiving placebo syrup (5 ml, twice daily) and sertraline (50 mg, daily) (P<0.001). Furthermore, the significance of the main effect of time (regardless of group effect) indicated the difference between the mean of depression and anxiety scores between the three times (P<0.001). In addition, the significance of the interaction showed that except for the baseline time (week 0), the mean of depression and anxiety scores in each of the other two times (week 6 and week 12) was lower in the silk receiving group than in the control group (P<0.001).

4.4. Safety and tolerability

During the study, the safety and tolerability of drugs were monitored. Headaches (n=1), loss of appetite (n=1), palpitations (n=2), and malaise (n=1) were reported by patients in the intervention group. There was one complaint of a sense of pressure in teeth (n=1) in the placebo group. All of the mentioned symptoms were temporary and resolved in the first and second week of treatment.

Table 2. Effect of silk syrup on anxiety and depression in the intervention and control groups

Variable		Week 0	Week 6	Week 12	Effect	F-test	P-value	Partial η^2
Depression score	Intervention	18.30±4.10	6.57±3.76	5.23±4.17	Time	224.345	<0.001	0.812
	Control	18.42±4.90	14.87±5.13	12.46±5.95	Group	20.600	<0.001	0.284
					Time × group	43.741	<0.001	0.457
Anxiety score	Intervention	13.10±3.65	4.60±2.80	2.97±2.07	Time	205.628	<0.001	0.798
	Control	13.12±3.97	9.37±3.51	7.25±4.50	Group	13.827	<0.001	0.210
					Time × group	20.021	<0.001	0.278

Data are reported as mean±SD

5. Discussion

This research was carried out to study the effect of a medicinal syrup made from silkworm cocoon on mild to moderate MADD. The results demonstrated that co-administration of silk syrup with sertraline led to a significant decrease in depression and anxiety scores in patients suffering from mild to moderate MADD after 6 and 12 weeks of treatment. To the best of our knowledge, no study has been conducted investigating the efficacy of silk on depression or anxiety.

According to TPM, some diseases, such as depression, involve both the heart and the brain. The function of the heart is not limited to pumping blood, and therefore, its diseases are not only restricted to cardiovascular disease. Other disorders, such as depression, involve the heart according to TPM resources. Persian scholars, especially Ibn Sina, believe that depression and anxiety do not occur without the involvement of the heart and emphasize the heart-brain axis in this disease. In this respect, strengthening the heart and the brain are considered one of the pillars of treating depression. Persian medical books have specified the impacts of silk on both the brain and heart, which promote the effectiveness of this medication in depression. Silk is an important component of medications with elating properties and being heart and brain tonics (28). According to Ibn Sina, silk is a powerful tonic of the heart and a blood clarifier that is elating and resolves sadness and palpitations (28).

The results of a number of studies have indicated the accompaniment of heart disease with depression and anxiety and have provided evidence for simultaneous occurrence of these diseases (11, 29, 30) In line with these findings, in a study conducted on Wistar rats, Mahmoud et al. (2015) demonstrated that silk can have a protective effect against heart toxicity induced by isoproterenol (31). Khan et al. (2014) also reported a protective effect of silk on heart cells attributed to the antioxidant properties of silk and its flavonoid and amino acid content (21). In another study, Ali et al. (2011) induced hyperlipidemia and atherosclerosis in male rabbits, and following the administration of silk for 6 weeks, found a significant difference between atherosclerosis and cholesterol levels in the intervention group, compared to the control group (18). Wattanathorn et al. (2012) provided evidence that silkworm pupae increased cognitive function in rats with Alzheimer's disease (32).

The results of previous research have shown that silk is a rich source of proteins, amino acids, and vitamins B1, B2, and E, which are necessary for the optimum function of the nervous system (33, 34). Based on the findings of several studies, tryptophan, tyrosine, phenylalanine, and methionine are effective in treating mood disorders, including depression and anxiety (35), with all of the mentioned amino acids being present in silk. The National Institute of Mental Health has mentioned tryptophan as a precursor of serotonin. This amino acid has sedative and anxiolytic properties and reduces depression and anxiety resulting from serotonin deficiency (36).

The mechanisms by which silk decreased depression and anxiety symptoms in the present study were possibly due to the antioxidant and estrogenic properties of silk (37). Considering the demonstrated effects of oxidative stress and estrogen in the pathophysiology of depression and anxiety (38-40), silk may affect the nervous system via the mentioned mechanisms to improve depression and anxiety symptoms in MADD. A limitation of this study was low trust in participating the patients, which prevented timely visits to receive the medications. This was eliminated by long talks with the patients to gain their trust. another limitation of the present study was that the intent-to-treat analysis was not performed.

6. Conclusion

According to the results of this study, accompanying silk syrup with selective serotonin reuptake inhibitors can be beneficial in reducing depression and anxiety symptoms of mild to moderate MADD. This prevalent mental disorder imposes a high burden due to the complications resulting from the disease itself and treatment with pharmacological agents. Complementary medicine can provide low-cost, convenient, and safe treatment methods with minimum side effects and drug resistance. The present research, performed based on a TPM approach to manage depression, recommended silk syrup as a complementary medicine that could increase the efficacy of SSRIs in the management of anxiety and depression without any serious side effects.

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Footnotes

Conflicts of Interest: The authors declare that they have no conflicts of interest.
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Ethical considerations: This trial was conducted in compliance with the principles of Good Clinical Practice and the Declaration of Helsinki. The study was approved by the Ethical Committee of Tehran University of Medical Sciences (IR.TUMS.VCR.REC.1397.739), and registered in the Iranian Registry of Clinical Trials (IRCT20190724044320N1). All participants signed an informed consent form.

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