JOURNAL OF COMPLEMENTARY MEDICINE RESEARCH
RESEARCH ARTICLE WWW.JOCMR.COM

Investigating the effect of Zataria multiflora essential oil on reducing anxiety caused by opioid withdrawal syndrome: a double-blind clinical trial

Zohreh Hedaryan[1*], Behzad Jaybashi [2],Roya Hemmatpour [3],Mehrdad Fojlaley[4] , Fernando Maldonado Lopes[4]

1- Doctorate in traditional medicine ,department of medicine ,Technofest institute of technology university (TITU) ,Belgium
2- PhD in traditional medicine ,Technofest institute of technology university (TITU) ,Belgium
3- PhD in traditional medicine ,Technofest institute of technology university (TITU) ,Belgium
4- Professor at Technofest institute of technology university (TITU) ,Belgium
5- Corresponding author : Zohreh Hedaryan

Abstract:
Objective: Drug addiction is a chronic disease. Its withdrawal causes unfavorable and averse symptoms such as anxiety. The objective of this study was to investigate the effect of Zataria multiflora on reducing anxiety caused by opioid withdrawal syndrome.
Methods: The present study is a randomized and double-blind clinical trial conducted on 40 people with opioid withdrawal syndrome. They were selected from Isar Rehabilitation Clinic of Semnan City. They were randomly placed in two groups of Zataria multiflora essential oils (n = 21) and placebo (19). Beck Anxiety Inventory (BAI) was completed in the pre-test, post-test, and follow-up stages by subjects to measure their level of anxiety. Data were analyzed using SPSS 21 software.
Results: The results revealed no significant difference between Zataria multiflora and placebo in the pre-test stages ($p > 0.05$). In the post-test and follow-up stages, Zataria multiflora could significantly reduce the anxiety of people with opioid withdrawal syndrome compared to the placebo ($p < 0.05$, $f = 5.064$). The results of the LSD test also revealed a significant difference between the pre-test and post-test, and follow-up stages ($P < 0.05$). However, no significant difference was observed between the post-test and follow-up stages ($P > 0.05$).
Conclusion: The results revealed that Zataria multiflora can reduce the anxiety of people with opioid withdrawal syndrome. However, considering the limitations of the present study and a few studies conducted in this field, it is necessary to conduct similar randomized clinical trials.

Keywords: Essential oil, Zataria multiflora, Anxiety, Opioid withdrawal syndrome
DOI: 10.5455/jcmr.2023.14.05.15

Introduction

Addiction is a progressive and destructive chronic disease (1), leading to death in the affected person (2). It also harms the family and society in which the addicted person lives and endangers the health of the people in society (3, 4). It is a strong and constant desire that significantly reduces control and leads to significant harm (3, 5). Based on the definition of DSM5, addiction is a state in which a person continues to use drugs despite their adverse conditions (6). These adverse conditions include mood and behavioral changes (7) and anxiety disorders (8, 9), mental health problems (10), suicide (11, 12), legal problems (13), and job problems (14). The prevalence of addiction in Iran has been fluctuating in recent years, so 4 national plans for epidemiology and rapid assessment suggest that it is necessary to repeat the plans for epidemiology every two years. Based on the last assessment in Iran, its rate is estimated at 5.4% (15).

Opioid withdrawal syndrome is a symptom associated with reduced blood levels in people who have used drugs for a long time (16, 17). However, the primary symptoms of withdrawal symptoms are muscle cramps, bone, and muscle pains, shivering, sweating (18), dizziness, cardiac arrhythmia (19), seizures (20), and anxiety (21). Methadone is one of the long-acting opioid-based drugs used for opioid withdrawal (22). The reason for the high use of this drug is that it reduces the symptoms of withdrawal syndrome (23). The primary effect of methadone is on the central nervous system, which causes a state of relaxation (24). The Welfare Organization has announced that about 90% of drug addicts who withdraw will use drugs again and this withdrawal does not last more than a few weeks (25). The rate of recurrence in another study conducted in Iran was reported at 30% (26). Drug addiction is a destructive habit (27) and its withdrawal is associated with anxiety (28). Anxiety is one of the reasons for its recurrence (29, 30, and 31). The recurrence rate will be high if anxiety is not treated. The therapeutic methods used to reduce anxiety include meditation (32, 33), yoga (34, 35), music therapy (36, 37), psychotherapy (38, 39), benzodiazepines (40, 41), SSRIs (42) and herbal medicines (43, 44).

Studies have shown that Zataria multiflora is one of the herbal medicines playing a role in reducing anxiety (45). Zataria multiflora is a plant from the Lamiaceae family that grows in Iran, Pakistan, and Afghanistan (46) Zataria multiflora Boiss is a plant with circular and oval leaves that have round dense white buds. This aromatic shrub with a height of 60 to 90 cm has woody branches without leaves, while the young branches have dense and scattered leaves that are 5 to 10 mm in size (47). The effective compounds of Zataria multiflora include terpenes, phenols, aliphatic alcohols, flavonoids, saponins, and tannins. Among them, the biologically active compounds of thymol (48, 49, and 50) and carvacrol (48, 51, and 52) have antioxidant properties (53). Several studies have reported that Zataria multiflora has analgesic (54) and anti-inflammation (55) properties and is effective in reducing digestive disorders (56), cardiac disorders (57), and anxiety disorders (58, 59). Carvacrol is a monoterpene phenolic compound whose structure is 5-isopropyl-2-methylphenol and its chemical formula is C10H14O (60). Thymol is similar to carvacrol. The major difference between them is the position of the hydroxyl group in its structure. In other words, carvacrol is an isomer of thymol and they have a similar smell (61). Thymol and carvacrol can downregulate inflammatory diseases and reduce cell death (61). Due to their anti-anxiety (58, 59) and anti-inflammatory (55, 61, 62) properties, thymol and carvacrol in Zataria multiflora reduce inflammation in the nervous system (63) and vascular system (64), reducing the negative effects of anxiety on the human body.

Very few studies have been conducted in this area. We here refer to some of the studies consistent with our study. The study by Ghaneialvar et al. showed that T. spicata extract extracted from thymol and thymol nano-polymer can reduce the symptoms of morphine withdrawal syndrome (65). Another study by Mohamed ME et al. showed that Zataria multiflora extract can improve the negative effects of tramadol on Purkinje cells of the cerebellar cortex of rats (66). Another study by Khodayar MJ et al. showed that the administration of 200 mg/kg and 400 mg/kg of Zataria multiflora extract and essential oil can reduce morphine withdrawal behaviors in rats and may be effective in reducing signs and symptoms of opiate withdrawal syndrome in humans (67). Another study by Komaki et al. showed that Zataria multiflora extract can reduce anxiety during stressful activities in rats (68). Zataria multiflora can affect opioid withdrawal syndrome and studies have revealed that it can reduce anxiety caused by opioid withdrawal syndrome. However, no study was found to investigate the effect of Zataria multiflora on reducing anxiety caused by opioid withdrawal. Thus, the present study seeks to fill this gap.

Methods

The present study was a randomized double-blind clinical trial with a pretest-posttest and a follow-up design. The statistical population of this study included all addicts who were referred to the Isar Rehabilitation Clinic of Semnan City. The sample size was considered at 20 people for each group according to the study by Sayyah et al., who examined the effect of Zataria multiflora essential oil and anxiety caused by opioid withdrawal (69). Using a purposeful sampling method, they were divided into two groups: a Zataria multiflora group (21 people) and a placebo group (19 people). Diagram 1 shows this process. The inclusion criteria included getting a consent form, using methadone, having complete knowledge during filling the form of consent, diagnosis of DSM5 criteria, the lack of psychological and pharmaceutical treatments, having an age between 18 and 60 years, having reading and writing skills, and lack allergies to Zataria multiflora. Also, the exclusion criteria included unwillingness to continue participating in the study, pregnancy for women, use of anti-anxiety and sedative drugs, and presence of concomitant diseases such as depression, schizophrenia, sleep disorder, digestive diseases, and attending psychotherapy sessions during the study. They entered the research process after a diagnostic interview by the clinic psychiatrist and confirming the anxiety diagnosis. All subjects were given a BAI before allocating the samples into groups. The subjects who received scores of 8 and above were studied. All samples were in the treatment stage and received a proper dose of their methadone,

and the methadone dose was reduced by 5 mg per week to compare and compare the drugs.

Zataria multiflora essential oil was given to the experimental group for 3 days, every eight hours for 30 minutes through the nebulizer. Accordingly, 4 drops of Zataria multiflora essential oil with 10 ccs of distilled water were prepared for each nebulization (45). The placebo group received capsules containing 50 mg of starch. This study was double-blind, so the subjects and the researcher were unaware of the type of drug they used, and the secretary of the clinic had coded the drugs but had no interference in the study during the treatment. If the subject was not willing to continue treatment, he or she could be removed from the research process. After the third day of intervention with Zataria multiflora, the subjects were again given the BAI. After one month, the same inventory was given to the intervention and placebo group again. For quantitative data analysis, the mean of standard deviation and repeated analysis of variance and Mann-Whitney U were used.

Beck anxiety inventory (BAI)

The BAI was used to investigate the level of anxiety of patients. It was developed by Beck et al. in 1990 (70). The goal of this inventory is to evaluate clinical symptoms and the severity of anxiety by focusing on physical symptoms in individuals. It was a criterion for distinguishing anxiety and depression (71). Beck et al. reported the internal consistency of this inventory between 0.3 and 0.92 (70). In Iran, Cronbach's alpha coefficient of this inventory was reported at 0.92 (72). It is a self-reporting measurement tool that measures the intensity of anxiety of a person during the last week (72). It is scored in the range of 0 to 3 for each item, and the total score can be from 0 to 63. In this inventory, the scores of 0 to 7 indicate the lowest level of anxiety, the scores of 8 to 15 indicate mild level of anxiety, the scores of 16 to 25 indicate moderate level of anxiety, and the scores of 26 to 63 indicate severe or the highest level of anxiety (73). Each item of this inventory measures one of the factors of anxiety in two groups with anxiety and panic (72).

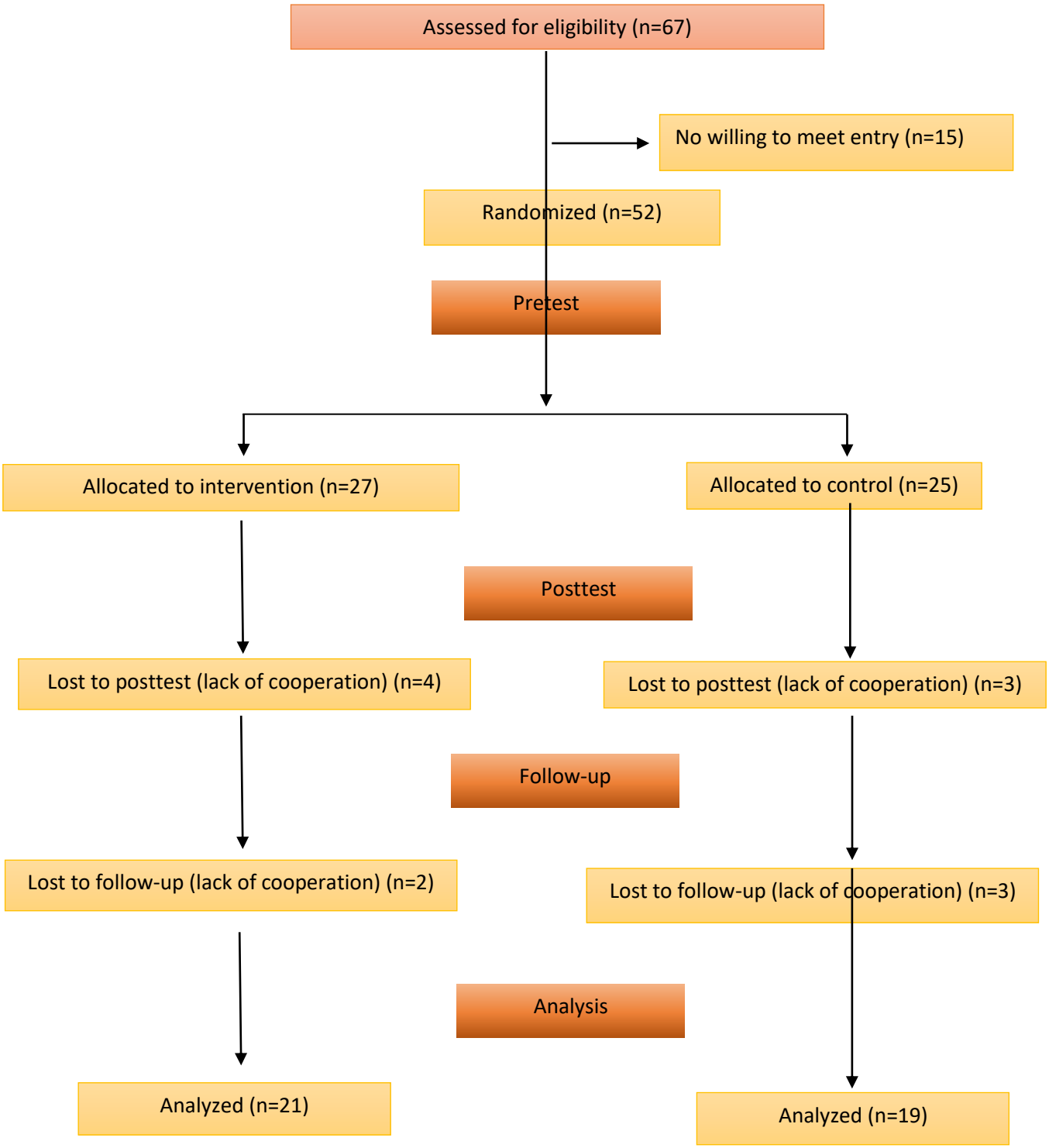

Diagram1: Random allocation of samples to experimental and control groups

Results

The results of the study revealed that both control and experimental groups were homogenous regarding demographic variables such as sex, marital status, education, and age. Its details are summarized in Tables 1 and 2. The mean and standard deviation of age in the control and experimental groups are 37.26 (15.17) and 34.91 (9.52), respectively. Based on Table 2, the Mann-Whitney test is not significant, indicating that the age distribution of the subjects is the same.

Table 1: Demographic and medical information of subjects (chi-square tests)

variable	levels	group		Chi-Square	P
		control	experimental		
Sex	male	13	10	0.184	1.766
	female	6	11		
Marital status	married	8	6	0.370	0.803
	single	11	15		
education	Under diploma	9	6	0.131	7.089
	diploma	3	11		
	AD	3	3		
	BA	3	1		
	MA	1	0		

Table 2: Mann-Whitney test results

	age
Mann-Whitney U	169.000
Wilcoxon W	359.000
Z	-.827
Asymp. Sig. (2-tailed)	.408
Exact Sig.	.421[b]

Table3: Descriptive statics

	group	Mean	Std. Deviation	N
pretest	placebo	36.5263	14.67564	19
	Zataria multiflora	35.0952	14.17711	21
	Total	35.7750	14.24778	40
posttest	placebo	35.5263	13.09948	19
	Zataria multiflora	24.7143	8.82691	21
	Total	29.8500	12.20876	40
Follow-up	placebo	34.5263	13.14605	19
	Zataria multiflora	23.4762	10.63776	21
	Total	28.7250	13.00096	40

Before analyzing the data by parametric statistics, the normal distribution of the data should be examined. It was examined by the Kolmogorov-Smirnov test, and its results are reported in Table 4. As seen, the value obtained for the variable of Zataria multiflora essential oil in the pretest, posttest, and follow-up is not significant at the P<0.05 level.

Table 4: Kolmogorov Smirnov test results

	group	Kolmogorov-Smirnov		
		Statistic	df	Sig.
pretest	placebo	.066	19	.200
	Zataria multiflora	.069	21	.200
posttest	placebo	.117	19	.200
	Zataria multiflora	.084	21	.200
Follow-up	placebo	.078	19	.200
	Zataria multiflora	.186	21	.055

The results of Tables 5, 6, and 7 indicate that the assumptions of the homogeneity of the covariance matrix, the homogeneity of error variance, and the equality of covariances have been fulfilled (p> 0.05). Thus, the Sphericity Assumed test is used for data analysis, the results of which are shown in Table 8. Based on the results obtained from repeated measure, the scores of the effects of Zataria multiflora essential oil (Table 8), time (P <0.05, F = 8.973), and interaction of groups and time (P <0.05, F= 5.064) are significant. These results show that Zataria multiflora essential oil scores change at the pretest, posttest, and follow-up stages.

To identify these changes, the LSD test was used. Its results are reported in Table 9 and Chart 2. They indicate that there is a significant difference between the pretest, posttest, and follow-up in the scores of the effect of Zataria multiflora essential oil (P<0.05). However, no significant difference was observed between the post-test and follow-up stages regarding the effect of Zataria multiflora essential oil (P>0.05). The results suggest that aromatherapy with Zataria multiflora essential oil significantly reduced the anxiety caused by opioid withdrawal syndrome in the experimental group compared to the control group (P<0.05). Table 10 presents the results.

To identify these changes, the LSD test was used, the summary of which is presented in Table 11 and Chart 3. Also, the interaction effect of group and time showed that aromatherapy with Zataria multiflora essential oil can significantly reduce anxiety scores caused by opioid withdrawal syndrome in the experimental groups compared to the control in the posttest and follow-up stages compared to the pretest stage (P<0.05, F =5.064). Chart 4 shows its details.

Table 5- Results of Box's M test

Box's M	F	Df1	Df2	Sig
6.020	0.917	6	10159.196	0.482

Table6: Levene's Test of Equality of Error Variances

		Levene statistic	Df1	Df2	sig
Pretest	Based on mean	0.054	1	38	0.818
Posttest	Based on mean	2.048	1	38	0.161
Follow-up	Based on mean	0.932	1	38	0.340

Table7: Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b]		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
time	.852	5.929	2	.052	.871	.933	.500

Table8: Tests of Within-Subjects Effects (Sphericity Assumed)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
time	1064.045	2	532.023	8.973	0.000	0.191
time * group	600.445	2	300.223	5.064	0.009	0.118
Error(time)	4505.905	76	59.288			

Table 9: **Pairwise Comparisons**

(I) time	(J) time	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
pretest	posttest	5.690*	1.467	.000	2.720	8.661
	Follow-up	6.810*	2.018	.002	2.724	10.895
posttest	pretest	-5.690*	1.467	.000	-8.661	-2.720
	Follow-up	1.119	1.640	.499	-2.201	4.439
Follow-up	pretest	-6.810*	2.018	.002	-10.895	-2.724
	posttest	-1.119	1.640	.499	-4.439	2.201

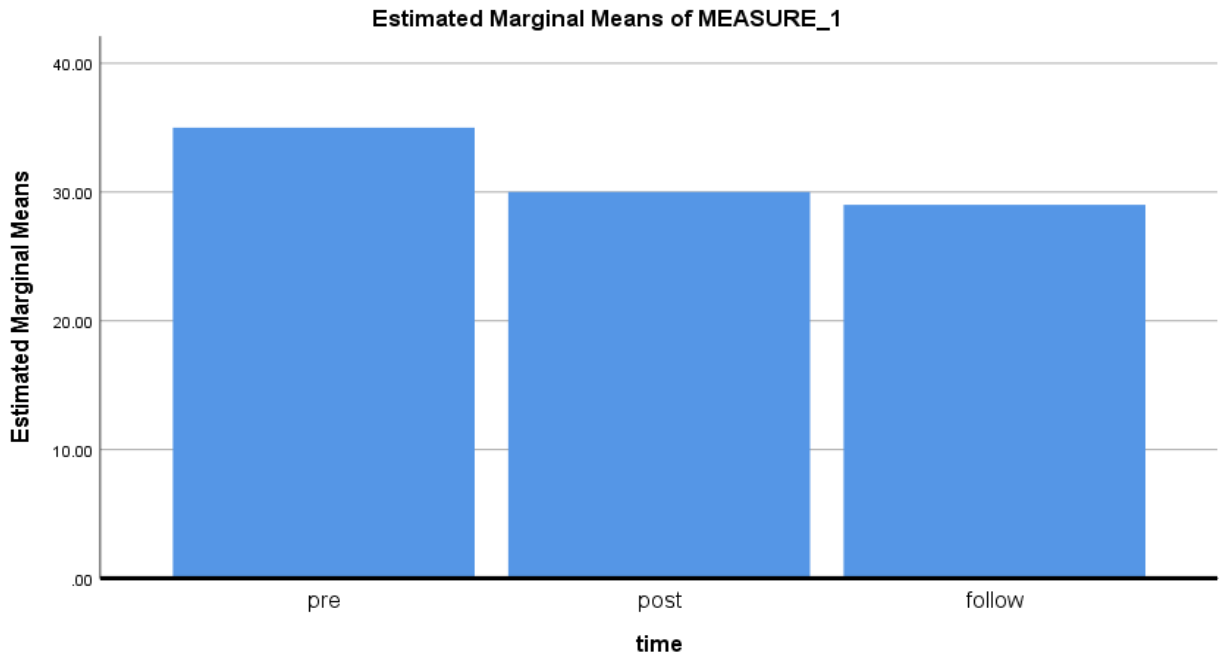

Chart 2: The effect of aromatherapy with Zataria multiflora essential oil on anxiety caused by opioid withdrawal syndrome in pre –test., post –test, and follow -up

Table 10: Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Intercept	119861.561	1	119861.561	339.611	.000	.899
group	1804.061	1	1804.061	5.112	.030	.119
Error	13411.639	38	352.938			

Table11: Pairwise Comparisons

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
placebo	Zataria multiflora	7.764*	3.434	.030	.812	14.717
Zataria multiflora	placebo	-7.764*	3.434	.030	-14.717	-.812

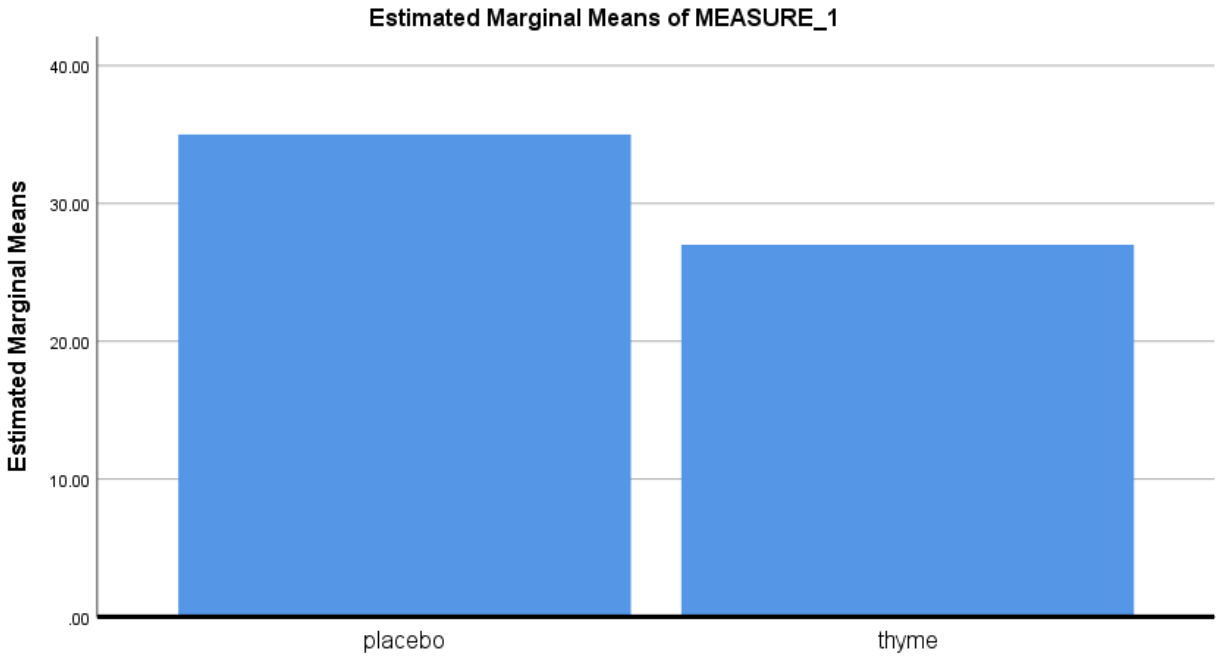

Chart 3: The effect of aromatherapy with Zataria multifloraessential oil on anxiety caused by opioid withdrawal syndrome in control and experimental groups

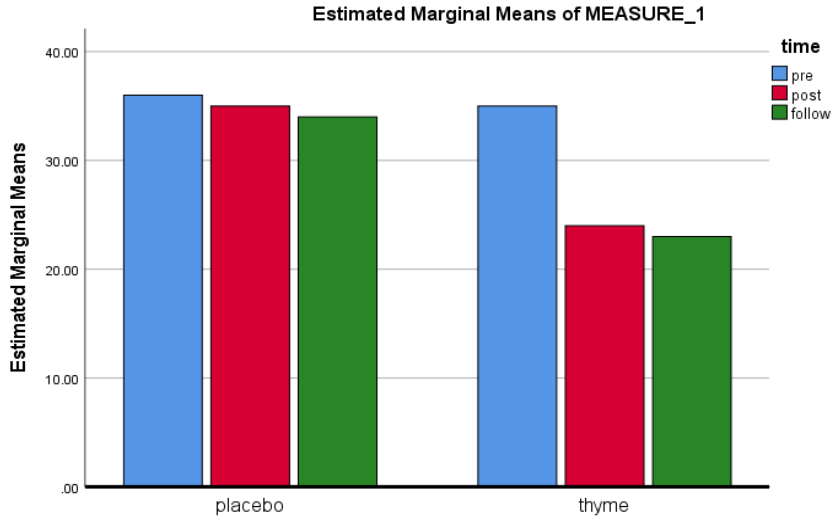

Chart 4: Interaction effect of group and time on reducing anxiety caused by opioid withdrawal syndrome

Discussion

The results revealed that the use of Zataria multiflora essential oil reduces the anxiety caused by opioid withdrawal syndrome. The present study showed that the anxiety scores decreased in the experimental group in the post-test and follow-up stages compared to the control group. A study conducted to investigate the effect of inhalation of Zataria multiflora essential oil compared to a placebo on the anxiety level of type 2 diabetic patients showed that aromatherapy with Zataria multiflora essential oil can significantly reduce the anxiety level compared to a placebo (45). Another study conducted to investigate the anxiety of women in labor and the effect of aromatherapy with Zataria multiflora essential oil on these women showed that aromatherapy with Zataria multiflora essential oil can reduce anxiety in these subjects (74). Another review study reviewed previous studies conducted to investigate the protective effects of carvacrol and thymol, as one of the primary polyphenols of Zataria multiflora. Results showed that carvacrol and thymol have strong anti-anxiety and anti-inflammation effects (61). A review study on various medicinal plants investigated their effects on anxiety. Its results showed that Zataria multiflora can be effective in reducing anxiety thanks to its carvacrol compounds (75). In another review study on the effects of medicinal plants on anxiety, results revealed that the flavonoid compounds found in medicinal plants can significantly reduce anxiety (76).

An animal study investigated the anti-inflammatory effect of Zataria multiflora extract on inflammation caused by lipopolysaccharides in rats. It showed that Zataria multiflora essential oil can reduce the inflammation caused by lipopolysaccharides. The reduction in this inflammation caused a significant reduction in the anxiety of the rats in the elevated maze, open field, and forced swimming tests (59). Another animal study on rats showed that a diet containing Zataria multiflora can reduce anxiety reactions in rats (77). Another study showed that Zataria multiflora extract can improve the negative effects of tramadol on the Purkinje cells of the cerebellar cortex in rats (66). Another study revealed that the administration of 200 mg/kg and 400 mg/kg of Zataria multiflora extract and essential oil can reduce morphine withdrawal behaviors in rats and may be useful in reducing the signs and symptoms of opioid withdrawal syndrome in humans (67). Another study showed that T. spicata extract extracted from thymol and thymol nano-polymer can reduce symptoms of morphine withdrawal syndrome (65).

In explaining the results, it can be stated that the ingredients of Zataria multiflora include terpenes, phenols, aliphatic alcohols, flavonoids, saponins, and tannins. Among them, biologically active compounds of thymol (48, 49, and 50) and carvacrol (48, 51, and 52) have antioxidant properties (53). Several studies have reported that Zataria multiflora has analgesic (54) and anti-inflammation (55) properties and is effective in reducing digestive disorders (56), cardiac disorders (57), and anxiety disorders (58, 59). Carvacrol is a monoterpene phenolic compound whose structure is 5-isopropyl-2-methylphenol and its chemical formula is C10H14O (60). Thymol is similar to carvacrol. The major difference between them is the position of the hydroxyl group in its structure. In other words, carvacrol is an isomer of thymol and they have a similar smell (61). Thymol and carvacrol can downregulate inflammatory diseases and reduce cell death (61). Due to their anti-anxiety (58, 59) and anti-inflammatory (55, 61, 62) properties, thymol and carvacrol in Zataria multiflora reduce inflammation in the nervous system (63) and vascular system (64), reducing the negative effects of anxiety on the human body.

Conclusion:

Inhalation of Zataria multiflora essential oil for 3 days and every 8 hours in the form of a combination of 4 drops of pure Zataria multiflora essential oil with 10 cc of distilled water can significantly reduce anxiety scores in people with opioid withdrawal syndrome. This drug with very few side effects can be effective as a complementary drug during treatment. However, more studies are needed to examine the long-term effect of this treatment.

Research limitations:

The primary limitation of this study was the researcher's lack of control over the samples while taking the drugs. In other words, the subjects might not take all the drugs timely and based on the guidelines. Another limitation of this study was the lack of control of the subjects in the laboratory environment. It might increase the possibility of errors in the anxiety questionnaire, which was a self-reporting tool. Another limitation of this study was the small size of the sample, which may increase the possibility of bias in the results. This study results can be generalized to the patients of the Isar RehabilitationClinic in the Semnan City and different results might be obtained in other clinics and cities.

References

1. Cheron J, Kerchove d'Exaerde Ad. Drug addiction: from bench to bedside. Translational Psychiatry. 2021;11(1):424.
2. Madras BK. The President's Commission on combating drug addiction and the opioid crisis: origins and recommendations. Clinical Pharmacology & Therapeutics. 2018;103(6):943-5.
3. Darcq E, Kieffer BL. Opioid receptors: drivers to addiction? Nature Reviews Neuroscience. 2018;19(8):499-514.
4. Liang M, Chen G, Xi Z, Qian H, Shang Q, Gao B, et al. The roles of K+-dependent

Na+/Ca2+ exchanger 2 (NCKX2) in methamphetamine-induced behavioral sensitization and conditioned place preference in mice. Neuroscience Letters. 2023;792:136952.
5. Sinnott-Armstrong W, Pickard H. 851What is Addiction? In: Fulford KWM, Davies M, Gipps R, Graham G, Sadler JZ, Stanghellini G, et al., editors. The Oxford Handbook of Philosophy and Psychiatry: Oxford University Press; 2013. p. 0.
6. O'Brien C. Addiction and dependence in DSM-V. Addiction. 2011;106(5):866-7.
7. Moustafa AA, Tindle R, Cashel S, Parkes D, Mohamed E, Abo Hamza E. Bidirectional relationship between heroin addiction and depression: Behavioural and neural studies. Current Psychology. 2020:1-17.
8. Grados MA, Rizwan B. A Common Mechanism for Anxiety Disorders and Drug Addiction: Implications for Current and Novel Pharmacological Treatments. Handbook of Child and Adolescent Anxiety Disorders: Springer; 2022. p. 357-64.
9. Cui J, Liu F, Liu X, Li R, Chen X, Zeng H. The impact of Qigong and Tai Chi exercise on drug addiction: a systematic review and meta-analysis. Frontiers in Psychiatry. 2022;13.
10. Saadati H, Tavakoli Ghouchani H, Asghari D, Gholizadeh N, Rahimi J, Valizadeh R. Comparison of the quality of life and general health in opium and non-opium users referred to the addiction treatment centers. Journal of Substance Use. 2021;26(4):356-62.
11. Fallah S, Yadollah M, Derakhshanpour F, Bay F, Pordanjani SR, Nazari SSH. Impact of Addiction in Family Members in Women's Suicide Attempts: A Case-control Study. Middle East Journal of Rehabilitation and Health Studies. 2023(In Press).
12. Choflet A, Davidson J, Lee KC, Ye G, Barnes A, Zisook S. A comparative analysis of the substance use and mental health characteristics of nurses who complete suicide. Journal of clinical nursing. 2021;30(13-14):1963-72.
13. Hieu DM, Gray B, Tuan DM, Colman BP. Facing drug addiction: Vietnam's struggle with opioids. Drug Science, Policy and Law. 2021;7:20503245211034934.
14. Kanwal N, Nizam Bin Isha AS, Salleh RB, Kanwal N, Al-Mekhlafi A-BA. Paradoxical effects of social media use on workplace interpersonal conflicts. Cogent Business & Management. 2023;10(1):2200892.
15. Sarrami H, Ghorbani M, Minooei M. Survey of Four Decades of Addiction Prevalence Researches in Iran. Research on Addiction. 2013;7(26):29-52.
16. Cosci F, Chouinard G. Acute and persistent withdrawal syndromes following discontinuation of psychotropic medications. Psychotherapy and psychosomatics. 2020;89(5):283-306.
17. Fontaine R, Chouinard G, Annable L. Rebound anxiety in anxious patients after abrupt withdrawal of benzodiazepine treatment. Am J Psychiatry. 1984;141(7):848-52.
18. Nestler EJ. Molecular basis of long-term plasticity underlying addiction. Nature reviews neuroscience. 2001;2(2):119-28.
19. Behzadi M, Joukar S, Beik A. Opioids and cardiac arrhythmia: a literature review. Medical principles and practice. 2018;27(5):401-14.
20. Korolenko C, Ovchinnikov A, Sultanova A, Sycheva TY, Shpiks T, Tagiltseva E. Severity of Neurotic Disorders and Social Masochism in Chemical Addicts. Neuroscience and Behavioral Physiology. 2021;51:596-9.
21. Giovannetti C, Garcia Arce S, Rush B, Mendive F. Pilot evaluation of a residential drug addiction treatment combining traditional Amazonian medicine, ayahuasca and psychotherapy on depression and anxiety. Journal of Psychoactive Drugs. 2020;52(5):472-81.
22. Lugo RA, Satterfield KL, Kern SE. Pharmacokinetics of methadone. Journal of Pain & Palliative Care Pharmacotherapy. 2005;19(4):13-24.
23. Su MK, Lopez JH, Crossa A, Hoffman RS. Low dose intramuscular methadone for acute mild to moderate opioid withdrawal syndrome. The American Journal of Emergency Medicine. 2018;36(11):1951-6.
24. Keane H. Categorising methadone: Addiction and analgesia. International Journal of Drug Policy. 2013;24(6):e18-e24.
25. M. S, Sn M. Factor influences on return to substance abuse in clients referred to addiction treatment centers in Maragheh City in 1390. Advances in Nursing & Midwifery. 2014;24(86):57-64.
26. Kassani A, Niazi M, Hassanzadeh J, Menati R. Survival analysis of drug abuse relapse in addiction treatment centers. International journal of high risk behaviors & addiction. 2015;4(3).
27. Everitt BJ, Robbins TW. Drug addiction: updating actions to habits to compulsions ten years on. Annual review of psychology. 2016;67:23-50.
28. Shokrgozar S, Kheshtmasjedi MF, Soleimani R, Abdollahi E. Frequency of depression and anxiety in patients undergoing methad one maintenance therapy at drug rehabilitation centers of Rasht. International Journal of Pharmaceutical Research. 2018;10:63-71.
29. Wu P, Goodwin RD, Fuller C, Liu X, Comer JS, Cohen P, et al. The relationship between anxiety disorders and substance use among adolescents in the community: specificity and gender differences.

Journal of youth and adolescence. 2010;39:177-88.
30. Ohannessian CM. Anxiety and substance use during adolescence. Substance abuse. 2014;35(4):418-25.
31. Pelloux Y, Costentin J, Duterte-Boucher D. Anxiety increases the place conditioning induced by cocaine in rats. Behavioural brain research. 2009;197(2):311-6.
32. Zheng Y, Yan L, Chen Y, Zhang X, Sun W, Lv J, et al. Effects of Loving-Kindness and Compassion Meditation on Anxiety: A Systematic Review and Meta-Analysis. Mindfulness. 2023:1-17.
33. Burgstahler MS, Stenson MC. Effects of guided mindfulness meditation on anxiety and stress in a pre-healthcare college student population: a pilot study. Journal of American College Health. 2020;68(6):666-72.
34. Nanthakumar C. Yoga for anxiety and depression–a literature review. The Journal of Mental Health Training, Education and Practice. 2020.
35. Gallagher A, Kring D, Whitley T. Effects of yoga on anxiety and depression for high risk mothers on hospital bedrest. Complementary Therapies in Clinical Practice. 2020;38:101079.
36. Lu G, Jia R, Liang D, Yu J, Wu Z, Chen C. Effects of music therapy on anxiety: A meta-analysis of randomized controlled trials. Psychiatry Research. 2021;304:114137.
37. Lieber AC, Bose J, Zhang X, Seltzberg H, Loewy J, Rossetti A, et al. Effects of music therapy on anxiety and physiologic parameters in angiography: a systematic review and meta-analysis. Journal of neurointerventional surgery. 2019;11(4):416-23.
38. Kelson J, Rollin A, Ridout B, Campbell A. Internet-delivered acceptance and commitment therapy for anxiety treatment: systematic review. Journal of medical Internet research. 2019;21(1):e12530.
39. Otte C. Cognitive behavioral therapy in anxiety disorders: current state of the evidence. Dialogues in clinical neuroscience. 2022.
40. Rickels K, Moeller HJ. Benzodiazepines in anxiety disorders: Reassessment of usefulness and safety. The World Journal of Biological Psychiatry. 2019;20(7):514-8.
41. Gupta A, Bhattacharya G, Farheen SA, Funaro M, Balasubramaniam M, Young J, et al. Systematic review of benzodiazepines for anxiety disorders in late life. Ann Clin Psychiatry. 2020;32(2):114-27.
42. Jakubovski E, Johnson JA, Nasir M, Müller-Vahl K, Bloch MH. Systematic review and meta-analysis: Dose–response curve of SSRIs and SNRIs in anxiety disorders. Depression and anxiety. 2019;36(3):198-212.
43. Koohpayeh SA, Hosseini M, Nasiri M, Rezaei M. Effects of Rosa damascena (Damask rose) on menstruation-related pain, headache, fatigue, anxiety, and bloating: A systematic review and meta-analysis of randomized controlled trials. Journal of Education and Health Promotion. 2021;10.
44. Yarnell E. Herbs for anxiety. Alternative and Complementary Therapies. 2018;24(2):91-8.
45. Jaybashi B, Hemmatpour R, Heydarian Z, Fojlaley M, Lopes FM. The Effect Of Zataria multiflora Aroma Inhalation Treatment On Anxiety In Type 2 Diabetic Patients: A Clinical Trial With Control And Follow-Up Groups. Journal of Pharmaceutical Negative Results. 2023:3490-7.
46. Ali MS, Saleem M, Ali Z, Ahmad VU. Chemistry of zataria multiflora (lamiaceae). Phytochemistry. 2000;55(8):933-6.
47. Simbar M, Azarbad Z, Mojab F, Majd HA. A comparative study of the therapeutic effects of the Zataria multiflora vaginal cream and metronidazole vaginal gel on bacterial vaginosis. Phytomedicine. 2008;15(12):1025-31.
48. Abyari M. Synergistic effect of UV-A and Methyl Jasmonate on the Biosynthesis of Thymol and Carvacrol Metabolites in Thymus. Cell and Tissue Journal. 2023;13(4):285-97.
49. Babaee K, Amini Dehaghi M, Modares Sanavi S, Jabbari R. Water deficit effect on morphology, prolin content and thymol percentage of Zataria multiflora (Thymus vulgaris L.). Iranian Journal of Medicinal and Aromatic Plants Research. 2010;26(2):239-51.
50. Kowalczyk A, Przychodna M, Sopata S, Bodalska A, Fecka I. Thymol and Zataria multiflora essential oil—new insights into selected therapeutic applications. Molecules. 2020;25(18):4125.
51. Mirzaei F, Fatehi F, Ismaili A, Yaghotipoor A, Qaderi A. Expression of genes 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR), sabinen synthase (Tctps1) and α-terpineol synthase (Tctps5) and its relation to thymol and carvacrol biosynthesis in Thymus vulgaris under water deficit stress. Journal of Medicinal Plants. 2017;16(64).
52. Mohamadi Farsani M, Ghasemi Pirbalouti A, Bakhshi Khaniki G, Momtaz H. Effect of Paclobutrazol and Brassinosteroid elicitors on expression gene 1-deoxy xylulose-5-phosphate reductoisomerase and its relationship with the biosynthesis of monoterpene carvacrol and thymol in Thymus daenenesis Celak under drought stress. Journal of Iranian Plant

Ecophysiological Research. 2016;11(43):76-89.
53. Sharififar F, Moshafi M, Mansouri S, Khodashenas M, Khoshnoodi M. In vitro evaluation of antibacterial and antioxidant activities of the essential oil and methanol extract of endemic Zataria multiflora Boiss. Food control. 2007;18(7):800-5.
54. Sardari S, Mobaiend A, Ghassemifard L, Kamali K, Khavasi N. Therapeutic effect of Zataria multiflora (Thymus vulgaris) essential oil on patients with COVID19: A randomized clinical trial. Journal of Advances in Medical and Biomedical Research. 2021;29(133):83-91.
55. Ahmed OM, Galaly SR, Mostafa M-A, Eed EM, Ali TM, Fahmy AM, et al. Zataria multiflora oil and thymol counter doxorubicin-induced hepatotoxicity via modulation of inflammation, apoptosis, and oxidative stress. Oxidative Medicine and Cellular Longevity. 2022;2022.
56. Khattab M, Kholif A, Abd El Tawab A, Shaaban M, Hadhoud F, El-Fouly H, et al. Effect of replacement of antibiotics with Zataria multiflora and celery seed mixture on the feed intake and digestion, ruminal fermentation, blood chemistry, and milk lactation of lactating Barki ewes. Food & function. 2020;11(8):6889-98.
57. Guesmi F, Khantouche L, Mehrez A, Bellamine H, Landoulsi A. Histopathological and biochemical effects of Zataria multiflora essential oil on H2O2 stress in heart tissues. Heart, Lung and Circulation. 2020;29(2):308-14.
58. Salmani H, Hakimi Z, Arab Z, Marefati N, Mahdinezhad MR, RezaeiGolestan A, et al. Carvacrol attenuated neuroinflammation, oxidative stress and depression and anxiety like behaviors in lipopolysaccharide-challenged rats. Avicenna Journal of Phytomedicine. 2022;12(5):514-26.
59. Arab Z, Hosseini M, Mashayekhi F, Anaeigoudari A. Zataria multiflora extract reverses lipopolysaccharide-induced anxiety and depression behaviors in rats. Avicenna journal of phytomedicine. 2020;10(1):78.
60. White SB. Antibacterial efficacy of phosvitin, carvacrol, or nisin alone or combined against foodborne human enteric pathogens: Iowa State University; 2011.
61. Kaeidi A, Rahmani MR, Hassanshahi J. The Protective Effect of Carvacrol and Thymol as Main Polyphenolic Compounds of Zataria multiflora on Some Biologic Systems in Disease Condition: A Narrative Review. Journal of Rafsanjan University of Medical Sciences. 2020;19(1):81-96.
62. Melo FHC, Venâncio ET, De Sousa DP, de Franca Fonteles MM, De Vasconcelos SMM, Viana GSB, et al. Anxiolytic-like effect of Carvacrol (5-isopropyl-2-methylphenol) in mice: involvement with GABAergic transmission. Fundamental & clinical pharmacology. 2010;24(4):437-43.
63. Noshy PA, Elhady MA, Khalaf AAA, Kamel MM, Hassanen EI. Ameliorative effect of carvacrol against propiconazole-induced neurobehavioral toxicity in rats. Neurotoxicology. 2018;67:141-9.
64. Kulišić T, Kriško A, Dragović-Uzelac V, Miloš M, Pifat G. The effects of essential oils and aqueous tea infusions of oregano (Origanum vulgare L. spp. hirtum), Zataria multiflora (Thymus vulgaris L.) and wild Zataria multiflora (Thymus serpyllum L.) on the copper-induced oxidation of human low-density lipoproteins. International journal of food sciences and nutrition. 2007;58(2):87-93.
65. Ghaneialvar H, Abbasi N. Thymol Nano Polymer Synthesis and its Effects on Morphine Withdrawal Syndrome in Comparison with Clonidine in Rats. Frontiers in Behavioral Neuroscience. 2022:222.
66. Mohamed ME-B, Mohamed HA, Ghait GR, Mohamed MH. Effects of the Co-Administration of Zataria multiflora and Tramadol on The Postnatal Development of Purkinje Neurons of The Albino Rat. Egyptian Academic Journal of Biological Sciences C, Physiology and Molecular Biology. 2022;14(2):129-51.
67. Khodayar MJ, Taherzadeh E, Siahpoosh A, Mansourzadeh Z, Tabatabaei SAH. Thymus daenensis extract and essential oils effects on morphine withdrawal signs in mice. Jundishapur Journal of Natural Pharmaceutical Products. 2014;9(3).
68. Komaki A, Hoseini F, Shahidi S, Baharlouei N. Study of the effect of extract of Thymus vulgaris on anxiety in male rats. Journal of traditional and complementary medicine. 2016;6(3):257-61.
69. Sayyah M, Rahim ASF. The role of alcoholic extract of Zataria multiflora Boiss in controlling opium withdrawal symptoms: a randomized, double-blind study. Archives of Psychiatry and Psychotherapy. 2017;3:27-33.
70. Beck AT, Epstein N, Brown G, Steer RA. An inventory for measuring clinical anxiety: psychometric properties. Journal of consulting and clinical psychology. 1988;56(6):893.
71. Julian LJ. Measures of anxiety. Arthritis care & research. 2011;63(0 11).
72. Kaviani H, Mousavi A. Psychometric properties of the Persian version of Beck Anxiety Inventory (BAI). Tehran University Medical Journal. 2008.
73. Sandin K, Shields G, Gjengedal RG, Osnes K, Bjørndal MT, Reme SE, et al. Responsiveness to change in health status of the EQ-5D in patients treated for

depression and anxiety. Health and Quality of Life Outcomes. 2023;21(1):35.
74. Munafiah D, Lestari WOS, Hastuti W. Manfaat Essential Zataria multiflora Terhadap Penurunan Kecemasan Ibu Bersalin Kala I Fase Aktif. Jurnal SMART Kebidanan. 2019;6(2):118-21.
75. Agatonovic-Kustrin S, Kustrin E, Gegechkori V, Morton D. Anxiolytic terpenoids and aromatherapy for anxiety and depression. Reviews on New Drug Targets in Age-Related Disorders. 2020:283-96.
76. Hosein Farzaei M, Bahramsoltani R, Rahimi R, Abbasabadi F, Abdollahi M. A systematic review of plant-derived natural compounds for anxiety disorders. Current topics in medicinal chemistry. 2016;16(17):1924-42.
77. Anaraki M, Akbar Tabar M, SepehrAra L. Effect of diet containing Zataria multiflora leaves on anxiety behavior in rats. Armaghan E-danesh. 2015;19(10):841-51.