Complementary Therapies in Medicine 46 (2019) 29–35

Contents lists available at ScienceDirect

Complementary Therapies in Medicine

journal homepage: www.elsevier.com/locate/ctim

The effect of *Delphinium denudatum* (Jadwar) on fatigue: A randomized double blind placebo-controlled clinical trial

Babak Daneshfard[a], Nafiseh Hosseini Yekta[a,*], Alireza Khoshdel[b], Alireza Heiran[c], Roya Cheraghi[d], Hassan Yarmohammadi[c]

[a] Department of Persian Medicine, Faculty of Medicine, AJA University of Medical Sciences, Tehran, Iran
[b] Epidemiology Research Center, Faculty of Medicine, AJA University of Medical Sciences, Tehran, Iran
[c] Student Research Committee, Shiraz University of Medical Sciences, Shiraz, Iran
[d] R&D manager, Shefanegar Nazari Pharmaceutical corporation, Qom, Iran

ARTICLE INFO

Keywords:
Complementary and alternative medicine
Chronic fatigue syndrome
Delphinium denudatum
Fatigue
Herbal medicine
Jadwar
Persian medicine

ABSTRACT

Objectives: Fatigue is a common problem in modern-day life. The aim of this study was to evaluate the effect of *Delphinium denudatum* (Jadwar) on fatigue.

Methods: This study was a randomized double-blind placebo-controlled clinical trial between healthy normal university students. In each group, participants were given one capsule of either WEACURE® (containing 500 mg of Jadwar root powder) or placebo for 15 consecutive days. Multidimensional Fatigue Inventory (MFI) questionnaire was used before and after the intervention to evaluate different aspects of fatigue.

Results: A total number of 64 participants completed the study. Data analysis showed decrease in the scores of all five domains of fatigue in Jadwar group (13.31 ± 3.05–7.75 ± 2.66, 12.31 ± 3.55–7.63 ± 2.62, 12.22 ± 4.26–6.97 ± 2.06, 11.56 ± 4.21 to 7.28 ± 2.37, 12.91 ± 3.09–7.34 ± 2.13 in general fatigue, physical fatigue, reduced activity, reduced motivation, and mental fatigue domains, respectively) which was statistically significant (P value < 0.0001). This situation was significantly superior to the placebo group. Prescribed dosage of WEACURE® capsule was well tolerated.

Conclusion: As a complementary tonic agent, Jadwar have a potential to reduce fatigue in normal population. However, objective evaluation of its anti-fatigue effect should be further evaluated.

1. Introduction

Fatigue is a serious problem of modern societies. It is the feeling of extreme tiredness or sleepiness resulting from high physical/mental workload, disturbed sleep, or prolonged stressful state. Fatigue could be either acute or chronic.[1] Chronic Fatigue Syndrome (CFS), also known as myalgic encephalomyelitis, is a long-term challenging disorder with fluctuating symptoms including prolonged unexplained fatigue, musculoskeletal pain, disturbed memory and cognition, headache, and poor sleep.[2,3]

This relatively common condition is an inevitable consequence of modern-day life, affecting all races and socioeconomic classes. CFS prevalence rate varies 1.5–3% in the general population.[3,4] It is estimated that 836,000–2.5 million Americans have had CFS in 2015, most of which remained undiagnosed.[5] Depression and anxiety disorders have a high prevalence rate amongst CFS patients, which in turn increases the disease burden.[6]

CFS is heterogeneous in clinical presentations and there is no tool for its diagnosis. It can be identified through having a history of fatigue for at least 6 months by excluding other etiologies.[7] Its pathogenesis is not clearly understood although different infectious, endocrine, and immunologic causes have been hypothesized.[3,8]

Although there is no treatment for CFS, cognitive behavioral therapy and graded exercise have shown to be effective.[9,10] CFS patients similar to those with chronic diseases of unknown etiology use complementary and alternative medicines (CAM) since the conventional treatments have not been beneficial. Some of these therapies including Traditional Chines Medicine (TCM), qigong, and massage therapy have shown to have positive effects.[4,11]

Herbal medicine is one of the most popular branches of CAM, used for various ailments since the ancient times.[12] Many herbs have been used to treat fatigue in traditional and folkloric medicines.[13] Studies have revealed that some medicinal herbs such as ginseng,[14] pumpkin[15] and water hyssop[16] can reduced fatigue symptoms. However, more

* Corresponding author.
E-mail address: nhyekta@yahoo.com (N.H. Yekta).

https://doi.org/10.1016/j.ctim.2019.05.027
Received 30 January 2019; Received in revised form 23 May 2019; Accepted 29 May 2019
Available online 30 May 2019

investigations are warranted to provide sufficient evidence.

Delphinium denudatum Wall. (Ranunculaceae) is a medicinal herb known as *Jadwar* in Iran, India and Pakistan.[17] Its root has antibacterial,[18] antifungal,[19] anticonvulsant,[20,21] hepato-protective,[22] antioxidant,[23] anxiolytic,[24] analgesic,[25] and morphine de-addiction[26,27] properties. Regarding its toxicity, an animal study found LD_{50} of *Delphinium denudatum* at 16,100 mg/kg.[28]

Jadwar also known as *Mah-Parvin* in Persian Medicine (PM), accounts for numerous clinical effects including detoxification, pain relief, and mental and physical reinforcement. It has also been introduced as an aphrodisiac and tonic agent.[17,29,30] However, many of its properties have not been scientifically evaluated through clinical trials. The objective of this study was to evaluate the effect of Jadwar on fatigue in adult normal population.

2. Materials and methods

2.1. Study design

This study was a randomized placebo-controlled clinical trial. It was double-blinded as the participants and the investigators were unaware of the group allocation. Participants were enrolled in two groups with 1:1 allocation ratio. No change was happened regarding the study protocol until the end of trial.

2.2. Ethical considerations

The study was approved by the local Ethics Committee of AJA University of Medical Science (ID: IR.AJAUMS.REC.1396.84). It was also registered in the Iranian Registry of Clinical Trials (IRCT) (IRCT ID: IRCT20130216012486N3). All the participants signed the written informed consent form before their enrollment. We followed CONSORT guideline in conducting this study.

2.3. Preparation of drug and placebo

We purchased WEACURE® (*Habb-e-Jadwar*) capsules manufactured by *Shefanegar Nazari* pharmaceutical corporation, Qom, Iran. Each capsule contains 500 mg of Jadwar root powder. The WEACURE® capsules were approved as *Delphinium denudatum* Wall. (family: Ranunculaceae) in Herbarium Center at School of Pharmacy, Shiraz University of Medical Sciences (Voucher no. PM1153). Placebo capsules contained 500 mg of starch powder manufactured by and purchased from the same corporation.

2.4. Drug analysis

We measured total flavonoid content of the drug based on the amount of quercetin. The acceptable concentration of 67 ± 0.5 μg/mg of quercetin was detected using ultraviolet spectrophotometer. Weight concentration of total flavonoid and total phenol was also 0.78%W/W and 11.7%W/W, respectively. Moreover, high-performance liquid chromatography (HPLC) method was applied to provide fingerprints of the drug in three different wavelengths (Fig. 1). For this purpose, Smartline model apparatus (Knauer, Germany) was used with the following features: Ultraviolet (UV) detector wavelengths: 254 nm, 300 nm, and 350 nm; injection volume: 20 μL; C18 column with maintained temperature of 25 degrees Celsius; flow rate: 1 ml/min. For sample preparation, maceration extraction method using methanol (MeOH) 100% was applied. Then, we dissolved 2 mg of dried extract in 2 milliliters of dimethyl sulfoxide (DMSO) yielded in final solution with the concentration of 1 mg/ml. A binary gradient elution system using water and methanol solvents was applied as shown in Table 1.

2.5. Microbiologic tests

We carried out microbiological evaluations including Total Aerobic Microbial Count (TAMC), Total combined Yeast and Mold Count (TYMC), *Staphylococcus aureus*, *Salmonella* spp., *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans*. Cultures were prepared in appropriate mediums e.g., Tryptic Soy Agar (TSA) for TAMC and Sabouraud Dextrose Agar (SDA) for TYMC tests, and kept in incubator. All of the results were acceptable based on the United States Pharmacopeia 41 (USP 41) reference values.[31]

2.6. Inclusion and exclusion criteria

AJA University of Medical Science students who did not have any acute or chronic disease (including diabetes, hypertension, hyperlipidemia, and hypothyroidism) based on the data gathered in the interview and were willing to participate, signed the written informed consent before the enrolment. They were also asked about the history of any kind of allergic reaction to Jadwar. Students who were sensitive to Jadwar or refused to participate were excluded.

2.7. Randomization, blinding and concealment of allocation

We used computer generated random numbers to make a list of two groups (A and B sequences). Participants were allocated sequentially based on the list. Researchers and participants were blinded to the allocation until the end of study. To conceal group allocation, we used similar containers and capsules in shape and color.

2.8. Intervention

Drug and placebo capsules were prescribed for 15 consecutive days. Participants had to consume one capsule per day according to their determined allocation group. They were followed during the study regarding proper usage of capsules.

2.9. Outcome measure

Persian version of Multidimensional Fatigue Inventory (MFI) questionnaire was used in this study. This self-report instrument consists of 20 statements (Table 2). MFI evaluates fatigue through five domains of general, physical and mental fatigue as well as reduced activity and motivation. Each domain has 4 statements scoring 1-5. Thus, summation of each domain score varies from at least 4 up to maximum of 20. It is worth mentioning that higher scores indicate worse state of fatigue. Three levels of fatigue were defined based on the total score: low (20–40), moderate (40–60), and high (60 ≤). This questionnaire that can be used for both patients and normal participants, was invented by Smets et al.[32] Reliability, validity, and internal consistency of its Persian version was approved in a previous study.[33]

2.10. Statistical analysis

The statistical analysis was applied by R programming language (version 3.3.1 for Windows) with deducer Graphical User Interface (GUI) package. Wilcoxon signed-rank test, Wilcoxon rank-sum test, and Chi-squared test were used for group comparison. P value less than 0.05 was considered to be statistically significant.

3. Results

3.1. Study flow

Out of 102 interviewed students, 80 were enrolled and 64 completed the study as shown in Fig. 2. Table 3 contrasts the baseline characteristics in Jadwar and placebo groups. At the baseline,

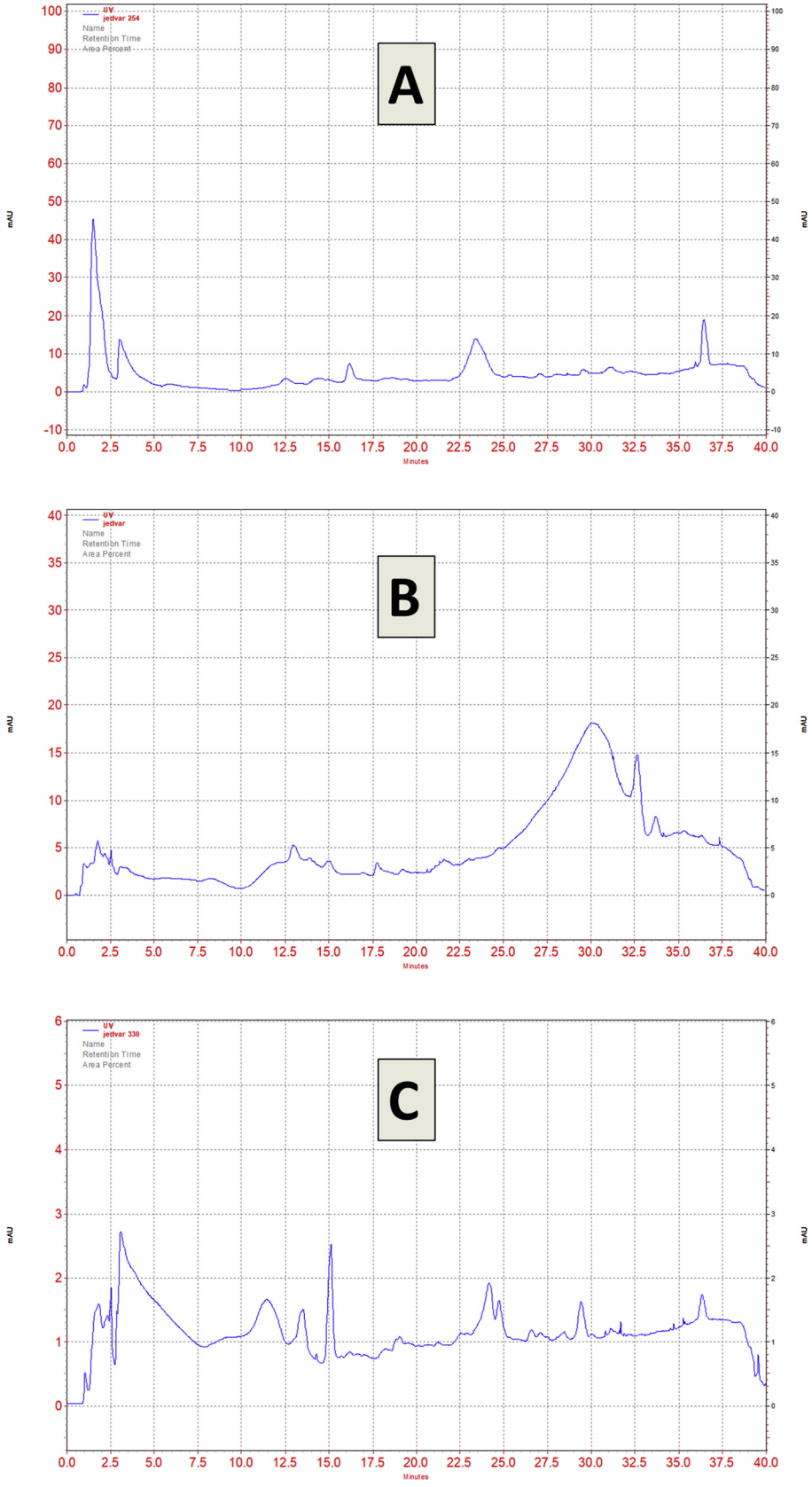

Fig. 1. HPLC fingerprints of Jadwar in three wavelengths of A) 254 nm, B) 300 nm, and C) 350 nm.

Table 1
Binary gradient elusion system used in HPLC analysis.

Time (min)	Flow rate (ml/min)	Solvent A (Water) (%)	Solvent B (Methanol) (%)
0	1	95	5
5	1	95	5
30	1	0	100
35	1	0	100
36	1	95	5
40	1	95	5

demographic variables were not different between the two groups. Despite random allocation, groups were mismatched in total score, general fatigue, reduced activity, and mental fatigue domains of MFI. In addition, these scores indicated worse condition for Jadwar group.

3.2. Multidimensional fatigue inventory (MFI) questionnaire

Data analysis revealed significant decrease in MFI domain scores in the Jadwar group (P value < 0.0001). However, no significant change was seen in the placebo group except for reduced motivation domain which had a significant increase (P value = 0.03) (Fig. 3).

Moreover, we compared MFI score differences (Δ) between the groups. The proposed intervention was found to be significantly superior to the placebo in improving all MFI domains (P value < 0.0001). As it is shown in Fig. 4, all of the scores decreased [meaning improvement] in the Jadwar group while they increased in the placebo group.

Participants tolerated the WEACURE® capsules and they did not report any serious side effects.

4. Discussion

To the best of our knowledge, this is the first study to investigate the effect of *Delphinium denudatum* (Jadwar) on fatigue in a human clinical trial. According to our findings, this medicinal herb has a potential to be used as a tonic agent with a protective effect against general fatigue. Results of this research showed that 15-day supplementary consumption of WEACURE® capsules (containing 500 mg of Jadwar root powder) can significantly improve the perception of healthy individuals regarding their physical, mental, and emotional functionality. Such a considerable subjective effect of Jadwar should be further evaluated on the performance of different populations.

Delphinium denudatum possesses numerous active ingredients including diterpenoid alkaloids (such as talatizidine, isotalatizidine, condelphine, and denudatine), sterols (such as ß-sitosterol, stigmasterol, and campesterol), flavonoids, and fatty acids.[23,34] Previous studies have revealed anti-fatigue potential of some of these chemical compounds.

Reactive Oxygen Species (ROS) production is considered to have a crucial role in pathophysiology of fatigue.[35,36] Many researches have focused on deleterious action of ROS and free radicals for discovering anti-fatigue drugs. Studies have shown that antioxidants have beneficial effects in chronic fatigue due to their radical scavenging properties.[37] Moreover, an especial group of alkaloids i.e., marine indole alkaloids, could be effective in reducing anxiety and depression.[38] Other groups of plant-derived alkaloids such as *Actinidia arguta* crude alkaloids have shown to have anti-fatigue activities.[39,40] Although other kinds of alkaloids have been found in *Delphinium denudatum*, it is worthy to investigate their possible anti-fatigue properties.

Antioxidant and anti-inflammatory effects are common features of flavonoids. Previous investigations revealed that plant-derived flavonoids have anti-fatigue activity through different mechanisms such as decreasing lactic acid production.[41–43]

Quercetin is a unique flavonoid content of Jadwar with various therapeutic applications, which can promote performance and health..[44] This polyphenolic compound possesses antioxidant,[45] anti-inflammatory,[46] anticancer,[47] anti-aging,[48] neuroprotective,[49] nephroprotective,[50] and cardioprotective properties.[51] It has an exceptional free radical scavenging activity[52] which seems to play an important role in its anti-fatigue effect. Moreover, it has shown antidepressant and anxiolytic activities[53,54] which can explain the positive mental and emotional effects of Jadwar. Regarding the physical strengthening property of Jadwar, it has been proven that quercetin could increase the VO2max and endurance capacity.[55] However, more human trials regarding the clinical effects of quercetin are required to rigorously confirm its properties.[56]

Fatty acids are other important ingredients of Jadwar. Previous investigations have shown anti-fatigue effects of different fatty acids. A placebo-controlled trial revealed that essential fatty acids can be used as safe effective treatment for post-viral fatigue.[57] ω-3 and ω-6 fatty acid intake has also shown to be effective in decreasing fatigue in breast cancer survivors.[58] Other studies have mentioned that polyunsaturated fatty acids are involved in pathophysiology of CFS and could have a therapeutic effect on fatigue.[59,60] Both saturated and unsaturated fatty acids which have been detected in *Delphinium denudatum* root[17] could

Table 2
Multidimensional Fatigue Inventory (MFI) questionnaire. For each statement, score 1 indicates full agreement and score 5 indicates complete disagreement. Other situations are scored between 2-4. It is to be noticed that statements number 2, 5, 9, 10, 13, 14, 16, 17, 18, and 19 have to be scored reversely.

Statement	Yes, it is completely right	1	2	3	4	5	No, it is completely wrong
1. I have a feeling full of energy and goodness.							
2. I am physically able to do little things.							
3. I feel very active.							
4. I like all kinds of good things to do.							
5. I feel I need to sleep and rest.							
6. I do a lot of work during the day.							
7. At the time of working, I can focus on it.							
8. I have the ability to do a lot of physical tasks.							
9. I'm afraid of doing the tasks.							
10. I think I'm working very little during the day.							
11. I can focus well.							
12. I can rest well.							
13. It is a hard work to focus on things.							
14. I think I'm physically in a bad situation.							
15. I have a lot of ideas and plans to do.							
16. I get tired very soon.							
17. My activities are low.							
18. I do not want to do anything.							
19. My thoughts simply goes scattered.							
20. I'm in a very good physical situation.							

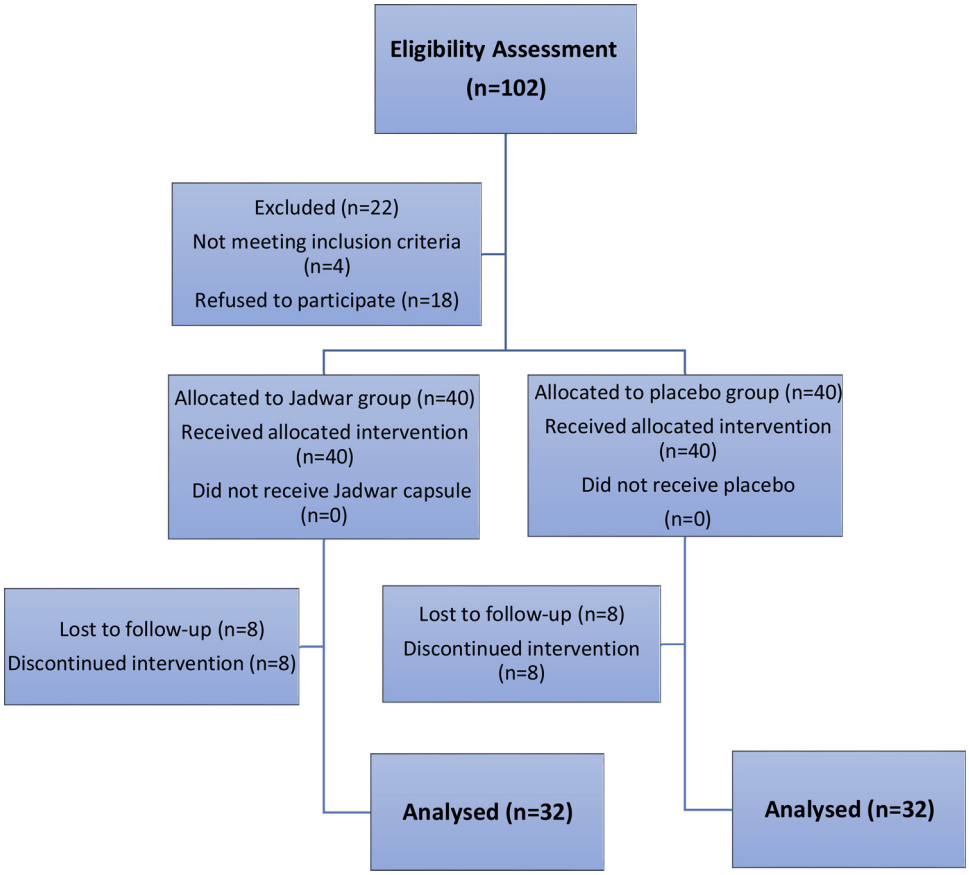

Fig. 2. The CONSORT chart of the study.

Table 3
Comparing Jadwar and placebo groups in baseline characteristics.

	Jadwar Group (n = 32)	Placebo Group (n = 32)	*P*-value
Age (years), Mean (± SD)	22.12 (± 2.32)	23.74 (± 5.83)	0.586
BMI (kg/m^2), Mean (± SD)	22.06 (± 3.45)	24.36 (± 4.77)	0.164
Married, N (%)	3 (9.37)	8 (25)	0.098
Baseline MFI scores	13.31 (± 3.05)	11.37 (± 3.09)	0.014
General fatigue, Mean	12.31 (± 3.55)	11.16 (± 3.31)	0.13
(± SD)	12.22 (± 4.26)	10.31 (± 3.09)	0.048
Physical fatigue, Mean	11.56 (± 4.21)	9.91 (± 3.54)	0.104
(± SD)	12.91 (± 3.09)	11.12 (± 3.27)	0.035
Reduced activity, Mean	62.31	53.87	0.021
(± SD)	(± 15.87)	(± 13.44)	
Reduced motivation, Mean			
(± SD)			
Mental fatigue, Mean (± SD)			
Total score			

BMI: Body Mass Index; MFI: Multidimensional Fatigue Inventory; SD: Standard Deviation.

be possibly another explain for anti-fatigue effect of Jadwar. However, this hypothesis should be investigated in future studies.

Results of our study showed that 15-day consumption of Jadwar (500 mg per day) significantly reduced all MFI scores including general, physical and mental fatigue as well as reduced activity and motivation. Not only no significant improvement was seen in the placebo group, but also reduced motivation domain was significantly worsened. Considering total scores, the Jadwar group fatigue level significantly decreased from high level (total score = 62.31 ± 15.87) to low level (total score = 36.97 ± 9.44). However, placebo group remained in moderate level of fatigue without any significant change. It is also worth mentioning that although Jadwar group was worse at the baseline, it dramatically improved after the intervention.

According to the abovementioned, some of the active ingredients of Jadwar have shown anti-fatigue potentials. In addition to its efficacy, Jadwar seems to be a safe herbal medicine; however, more evaluations are needed in future studies. Moreover, as it has been proven that quercetin has positive effects on anxiety and depression disorders, Jadwar might also be beneficial for those psychiatric patients who suffer from mental/physical fatigue.

4.1. Study limitations

One of the main limitations of this study was that we had only male participants. Other limitations were the relatively small sample size and having no follow up period. Some of the baseline MFI scores did not matched in the groups, which might be due to the method of randomization. In addition, although we used a standard questionnaire as a subjective assessment, we did not benefit from objective outcome measures which could help to evaluate the clinical effect of Jadwar on functionality and find out the possible mechanisms of action.

5. Conclusions

In conclusion, this study showed anti- fatigue potential of WEACURE® capsule containing Jadwar root powder. This natural product significantly improved perception of the individuals regarding their physical, mental, and emotional functionality. Further investigations with the objective evaluation of functionality, larger sample sizes, and follow-up are recommended. Our suggestions for future experimental studies are to consider objective outcome measures to explore underlying mechanisms of action in addition to evaluating Jadwar efficacy in patients with chronic fatigue syndrome and other physical/psychological ailments.

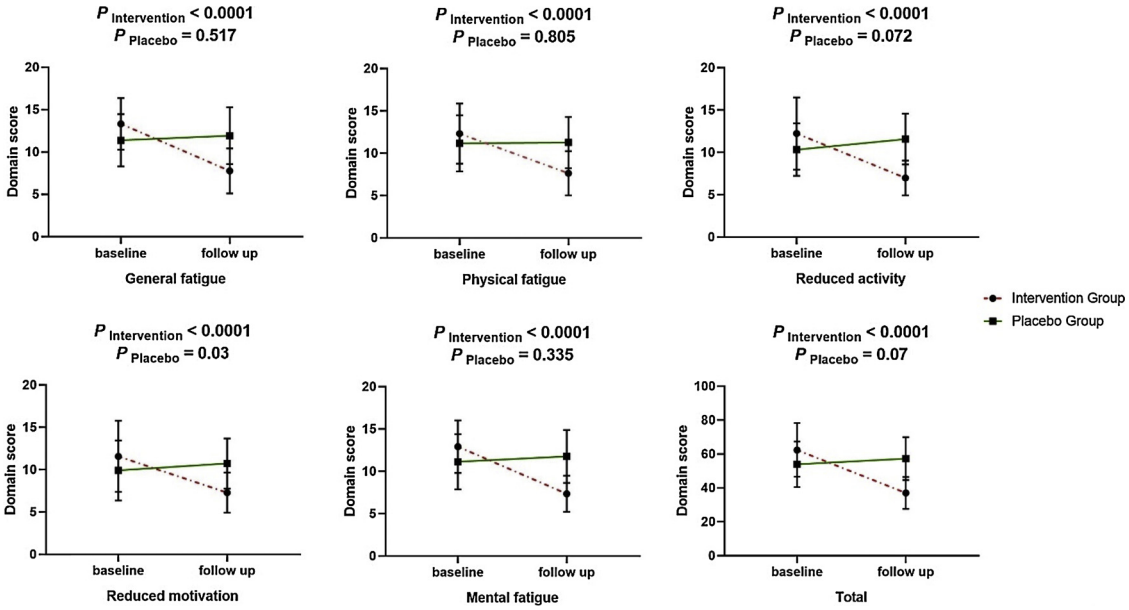

Fig. 3. Comparing the intervention (Jadwar) group with the placebo group on the baseline-follow up trend of MFI scores.

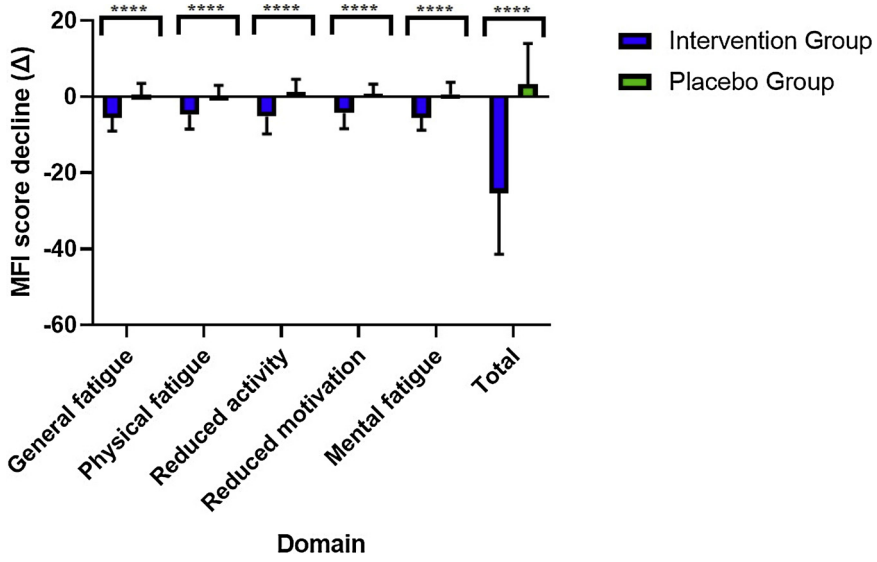

Fig. 4. Comparing the intervention (Jadwar) group with the placebo group on MFI score decline (Δ) [*Wilcoxon rank-sum test, ****: $P < 0.0001$*].

Conflict of interest

The authors declare no conflict of interest in this study.

Acknowledgements

This study has been approved and financially supported by Research Council of AJA University of Medical Sciences (grant number: 91000203). The authors would like to thank Mr. H. Argasi at the Research Consultation Center (RCC), Shiraz University of Medical Sciences for his linguistic editing of this manuscript. Moreover, all of the participants are appreciated for their cooperation.

References

1. Caldwell JA, Caldwell JL, Thompson LA, Lieberman HR. Fatigue and its management in the workplace. *Neurosci Biobehav Rev.* 2018;96(2019):272–289.
2. Roberts D. Chronic fatigue syndrome and quality of life. *Patient Relat Outcome Meas.* 2018;9:253–262.
3. Cleare AJ, Reid S, Chalder T, Hotopf M, Wessely S. Chronic fatigue syndrome. *BMJ Clin Evid.* 2015;2015 pii: 1101.
4. Wang Y-Y, Li X-X, Liu J-P, Luo H, Ma L-X, Alraek T. Traditional Chinese medicine for chronic fatigue syndrome: A systematic review of randomized clinical trials. *Complement Ther Med.* 2014;22(4):826–833.
5. Clayton E. Beyond myalgic encephalomyelitis/chronic fatigue syndrome: An iom report on redefining an illness. *JAMA.* 2015;313(11):1101–1102.
6. Daniels J, Brigden A, Kacorova A. Anxiety and depression in chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME): Examining the incidence of health anxiety in CFS/ME. *Psychol Psychother Theory Res Pract.* 2017;90(3):502–509.
7. Bjørklund G, Dadar M, Pen JJ, Chirumbolo S, Aaseth J. Chronic fatigue syndrome (CFS): Suggestions for a nutritional treatment in the therapeutic approach. *Biomed Pharmacother.* 2019;109:1000–1007.
8. Blomberg J, Gottfries C-G, Elfaitouri A, Rizwan M, Rosén A. Infection elicited autoimmunity and Myalgic encephalomyelitis/chronic fatigue syndrome: an explanatory Model. *Front Immunol.* 2018;9:229.
9. Larun L, Brurberg KG, Odgaard-Jensen J, Price JR. Exercise therapy for chronic fatigue syndrome. *Cochrane Database Syst Rev.* 2016(2).
10. Price JR, Mitchell E, Tidy E, Hunot V. *Cognitive behaviour therapy for chronic fatigue syndrome in adults.* The Cochrane Library; 2008.
11. Alraek T, Lee MS, Choi T-Y, Cao H, Liu J. Complementary and alternative medicine for patients with chronic fatigue syndrome: a systematic review. *BMC Complement Altern Med.* 2011;11(1):87.
12. Jabbari M, Daneshfard B, Emtiazy M, Khiveh A, Hashempur MH. Biological effects and clinical applications of dwarf elder (Sambucus ebulus L): a review. *J Evid Based*

Complementary Altern Med. 2017;22(4):996–1001.
13. Semalty A, Semalty M, Panda VS, Asrani KH, Ashar HD. Herbal drugs in chronic fatigue syndrome: an overview. *Schweizerische Zeitschrift für Ganzheitsmedizin/Swiss Journal of Integrative Medicine.* 2012;24(3):155–168.
14. Etemadifar M, Sayahi F, Abtahi S-H, et al. Ginseng in the treatment of fatigue in multiple sclerosis: a randomized, placebo-controlled, double-blind pilot study. *Int J Neurosci.* 2013;123(7):480–486.
15. Wang S-Y, Huang W-C, Liu C-C, et al. Pumpkin (Cucurbita moschata) fruit extract improves physical fatigue and exercise performance in mice. *Molecules.* 2012;17(10):11864–11876.
16. Anand T, Phani Kumar G, Pandareesh M, Swamy M, Khanum F, Bawa A. Effect of bacoside extract from Bacopa monniera on physical fatigue induced by forced swimming. *Phytother Res.* 2012;26(4):587–593.
17. Nizami Q, Jafri M. Unani drug, Jadwar (Delphinium denudatum Wall.)—a review. *Indian Journal of Traditional Knowledge.* 2006;5(4):463–467.
18. Suresh G, Gunasekar PH, Kokila D, et al. Green synthesis of silver nanoparticles using Delphinium denudatum root extract exhibits antibacterial and mosquito larvicidal activities. *Spectrochim Acta A Mol Biomol Spectrosc.* 2014;127:61–66.
19. Atta-ur-Rahman, Nasreen A, Akhtar F, et al. Antifungal diterpenoid alkaloids from Delphinium denudatum. *J Nat Prod.* 1997;60(5):472–474.
20. Raza M, Shaheen F, Choudhary M, et al. Anticonvulsant activities of ethanolic extract and aqueous fraction isolated from Delphinium denudatum. *J Ethnopharmacol.* 2001;78(1):73–78.
21. Raza M, Shaheen F, Choudhary M, Sombati S, Rahman A-u, DeLorenzo R. Inhibition of sustained repetitive firing in cultured hippocampal neurons by an aqueous fraction isolated from Delphinium denudatum. *J Ethnopharmacol.* 2004;90(2-3):367–374.
22. Hussain MA, Suresh D, Gupta RMV, Gouda S, Srivani V. Hepatoprotective activity of ethanolic extract delphinium root, against alcohol induced hepatotoxic rats. *Adv Pharmacol Toxicol.* 2012;13(1):75–78.
23. Mohanapriya S, Vijaiyansiva G. Bioactive constituent of Delphinium denudatum wall. And their antioxidant efficacy. *J Acad Ind Res.* 2013;2(2):138–141.
24. Abid M, Gosh AK, Khan NA. In vivo psychopharmacological investigation of Delphinium denudatum and Amaranthus spinosus extracts on wistar rats. *Basic Clin Neurosci.* 2017;8(6):503–512.
25. Zaheer I, Sz Rahman, Ra Khan, Parveen M, Ahmad M. Evaluation of analgesic activity of extracts of Delphinium denudatum in animal models: A dose dependent preclinical trial. *J Clin Diagn Res.* 2018;12(12).
26. Rahman S, Khan RA, Kumar A. Experimental study of the morphine de-addiction properties of Delphinium denudatum Wall. *BMC Complement Altern Med.* 2002;2(1):6.
27. Zafar S, Ahmad MA, Siddiqui TA. Protective role of Delphinium denudatum (Jadwar) against morphine induced tolerance and dependence in mice. *J Ethnopharmacol.* 2001;78(1):95–98.
28. Zafar S, Ahmad M, Siddiqui T. Acute toxicity and antinociceptive properties of Delphinium denudatum. *Pharm Biol.* 2003;41(7):542–545.
29. Alavi S, Al-adviyah Makhzan. *(The storehouse of medicaments).* Tehran: Intisharat va Amoozesh Enghelab Press; 2009.
30. Alam MA, Ahmed Z, Ansari AH, Ahmed S, Mand D, Alam MT. Iksir-e-Badan (Elixir): Unique influence from unani medicine–a review. *Int J Herb Med.* 2014;1(6):37–42.
31. Convention USP. *U.S. Pharmacopeia national formulary 2018: UPS41-NF36.* United States Pharmacopeial Convention Incorporated; 2017.
32. Smets E, Garssen B, Bd Bonke, De Haes J. The Multidimensional Fatigue Inventory (MFI) psychometric qualities of an instrument to assess fatigue. *J Psychosom Res.* 1995;39(3):315–325.
33. Hafezi S, Zare H, Mehri SN, Mahmoodi H. *The multidimensional fatigue inventory validation and fatigue assessment in Iranian distance education students. Distance Learning and Education (ICDLE), 2010 4th International Conference on. IEEE.* 2010; 2010:195–198.
34. Mohanapriya S, Vijaiyansiva G. Phytochemical analysis and antioxidant potential of Delphinium denudatum wall. *J Mod Biotechnol.* 2013;2(3):53–58.
35. Reid MB. Free radicals and muscle fatigue: Of ROS, canaries, and the IOC. *Free Radic Biol Med.* 2008;44(2):169–179.
36. Bruton JD, Place N, Yamada T, et al. Reactive oxygen species and fatigue-induced prolonged low-frequency force depression in skeletal muscle fibres of rats, mice and SOD2 overexpressing mice. *J Physiol.* 2008;586(1):175–184.
37. Lee J-C, Kao J-Y, Kuo D-H, et al. Antifatigue and antioxidant activity of alcoholic extract from Saussurea involucrata. *J Tradit Complement Med.* 2011;1(1):64–68.
38. Kochanowska-Karamyan AJ, Hamann MT. Marine indole alkaloids: Potential new drug leads for the control of depression and anxiety. *Chem Rev.* 2010;110(8):4489–4497.
39. Liu Y, Liu C. Antifatigue and increasing exercise performance of Actinidia arguta crude alkaloids in mice. *J Food Drug Anal.* 2016;24(4):738–745.
40. Zhou S-S, Jiang J-G. Anti-fatigue effects of active ingredients from traditional Chinese medicine: A review. *Curr Med Chem.* 2017.
41. Li W, Li T, Tang K. Flavonoids from mulberry leaves by microwave-assisted extract and anti-fatigue activity. *Afr J Agric Res.* 2009;4(9):898–902.
42. Hu Q-L, Zhang L-J, Li Y-N, Ding Y-J, Li F-L. Purification and anti-fatigue activity of flavonoids from corn silk. *Int J Phys Sci.* 2010;5(4):321–326.
43. Su K-Y, Yu CY, Chen Y-W, et al. Rutin, a flavonoid and principal component of Saussurea involucrata, attenuates physical fatigue in a forced swimming mouse model. *Int J Med Sci.* 2014;11(5):528.
44. Davis JM, Murphy EA, Carmichael MD. Effects of the dietary flavonoid quercetin upon performance and health. *Curr Sports Med Rep.* 2009;8(4):206–213.
45. D'Andrea G. Quercetin: a flavonol with multifaceted therapeutic applications? *Fitoterapia.* 2015;106:256–271.
46. Wang W, Sun C, Mao L, et al. The biological activities, chemical stability, metabolism and delivery systems of quercetin: a review. *Trends Food Sci Technol.* 2016;56:21–38.
47. Zhang H, Zhang M, Yu L, Zhao Y, He N, Yang X. Antitumor activities of quercetin and quercetin-5′, 8-disulfonate in human colon and breast cancer cell lines. *Food Chem Toxicol.* 2012;50(5):1589–1599.
48. Zhu Y, Tchkonia T, Pirtskhalava T, et al. The Achilles' heel of senescent cells: From transcriptome to senolytic drugs. *Aging Cell.* 2015;14(4):644–658.
49. Dajas F. Life or death: Neuroprotective and anticancer effects of quercetin. *J Ethnopharmacol.* 2012;143(2):383–396.
50. Siddique N, Ahmad S, Mujeeb M. Mechanism based protective effect of *Delphinium denudatum* on cisplatin induced nephrotoxicity. *World J Pharm Pharm Sci.* 2014;3(3):2063–2077.
51. Egert S, Bosy-Westphal A, Seiberl J, et al. Quercetin reduces systolic blood pressure and plasma oxidised low-density lipoprotein concentrations in overweight subjects with a high-cardiovascular disease risk phenotype: A double-blinded, placebo-controlled cross-over study. *Br J Nutr.* 2009;102(7):1065–1074.
52. Bors W, Michel C, Saran M. Flavonoid antioxidants: Rate constants for reactions with oxygen radicals. *Meth Enzymol.* 1994;234:420–429 Elsevier.
53. Holzmann I, da Silva LM, da Silva JAC, Steimbach VMB, de Souza MM. Antidepressant-like effect of quercetin in bulbectomized mice and involvement of the antioxidant defenses, and the glutamatergic and oxidonitrergic pathways. *Pharmacol Biochem Behav.* 2015;136:55–63.
54. Joshi D, Naidu P, Singh A, Kulkarni S. Protective effect of quercetin on alcohol abstinence-induced anxiety and convulsions. *J Med Food.* 2005;8(3):392–396.
55. Davis JM, Carlstedt CJ, Chen S, Carmichael MD, Murphy EA. The dietary flavonoid quercetin increases VO2max and endurance capacity. *Int J Sport Nutr Exerc Metab.* 2009;20:1–13.
56. Miles SL, McFarland M, Niles RM. Molecular and physiological actions of quercetin: Need for clinical trials to assess its benefits in human disease. *Nutr Rev.* 2014;72(11):720–734.
57. Behan PO, Behan WM, Horrobin D. Effect of high doses of essential fatty acids on the postviral fatigue syndrome. *Acta Neurol Scand.* 1990;82(3):209–216.
58. Alfano CM, Imayama I, Neuhouser ML, et al. Fatigue, inflammation, and ω-3 and ω-6 fatty acid intake among breast cancer survivors. *J Clin Oncol.* 2012;30(12):1280–1287.
59. Puri BK. Long-chain polyunsaturated fatty acids and the pathophysiology of myalgic encephalomyelitis (chronic fatigue syndrome). *J Clin Pathol.* 2007;60(2):122–124.
60. Ruxton C, Reed SC, Simpson M, Millington K. The health benefits of omega-3 polyunsaturated fatty acids: A review of the evidence. *J Hum Nutr Diet.* 2004;17(5):449–459.