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Effect of Ustukhuddus Alavi, a multi-herbal product, on the cognitive performance of adolescent female students

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ABSTRACT

Ethnopharmacological relevance: Ustukhuddus Alavi is a polyherbal formula which is introduced by Persian medicine scholars. It is traditionally used to treat brain disorders and is claimed to do so by preprocessing and cleansing the waste products from the brain. According to Persian medicine, the disposal of brain waste products is necessary for optimal cognitive performance.

Aim of the study: Sustaining optimal cognitive performance is crucial for ideal quality of life and higher academic achievements in high school students. The objective of this study was to determine the effects of this multi-component herbal product on the cognitive performance and salivary cortisol levels of adolescent female students.

Materials and methods: The effect of a 6-week randomly assigned Ustukhuddus Alavi versus placebo administration on cognitive performance was assessed by the paced auditory serial addition test (PASAT) at the baseline and after the 3- and 6-week intake of Ustukhuddus Alavi or placebo and the one-month follow-up in 86 healthy female high school students in grades 10 and 11. Additionally, we measured the levels of salivary cortisol of the students pre- and post-intervention.

Results: Significant mean difference between the Ustukhuddus Alavi and placebo groups in three of the paced auditory serial addition test (PASAT) subscales, namely mental health (p-value = 0.006), sustained attention (p-value = 0.001) and mental fatigue (p-value = 0.001), were observed after six weeks. We also found a significant difference between the mean salivary cortisol level of the two groups after the intervention (p-value = 0.047).

Conclusions: These findings reveal that the intake of the multi-ingredient herbal product Ustukhuddus Alavi for six weeks can be helpful for cognitive function and cortisol levels in female high school students. These positive effects seem to be related to the increase in sustained attention and the decrease in mental fatigue.

1. Introduction

Cognition consists of several different mental processes such as thought, experience, and the senses through which knowledge emerges (Puttarak et al., 2017). Cognitive function can be defined as a wide variety of mental abilities ranging from memory, language, executive function, self-regulation, speed of information processing, attention, and concentration, which are essential skills for learning, to psychomotor speed and visuospatial skill. Proper cognitive function is necessary for awareness of situation, planning, setting one's goals, and optimal functioning in problem-solving along with several other domains in the daily life such as physical and mental health, academic success, and employment (Borson, 2010; Wagner et al., 2020).

During the ages of 6–18 years, different levels of cognitive function are positively correlated with varying levels of psychological status ranging from undesirable emotions like sadness, anxiety, or depression and negative perception of the surroundings, others, and self to desirable feelings like self-concept and self-confidence which lead to better

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physical function in later ages (Mezcua-Hidalgo et al., 2019). The structure of the different regions of brain, for example, the prefrontal cortex, parietal and temporal regions, and their functions such as working memory, inhibitory control, and cognitive performance keep developing during late adolescence (Carbia et al., 2018).

Enhancement of cognition in adolescents is linked to neurophysiological brain maturation and cognitive disturbance results in different cognitive outcomes in adulthood, all-cause mortality, and cardiovascular disease risk according to large-scale longitudinal studies in Europe and the US (Agha et al., 2019). Dysfunction of cognition in students can result in a range of unwanted academic outcomes including detachment from school, poor behavior control, discord with teacher or classmates, educational failures, and interrupted learning for classmates (Wagner et al., 2020). These evidences show the key importance of enhancing adolescent students' cognitive capabilities.

Students misuse pharmaceutical stimulants to increase productivity, particularly to improve attention and concentration, which may lead to major health complications (Batistela et al., 2016). Safe alternative medications can be recommended to the students who are looking for cognition enhancement in a short period and especially during exam seasons (Philip et al., 2019). In this context, herbal cognitive enhancers which are medically proven to be safe can be a suitable replacement for high-risk stimulants to improve the academic performance of students.

Over the ages, a great number of herbal medications have been proposed to improve both cognition and memory in normal subjects and also those with dementia (Fernandes et al., 2014). For the review of prior related works, we refer the interested reader to the supplementary material.

In Persian medicine, some herbs and herbal preparations are recommended for the improvement of cognitive performance (Shojaii et al., 2016). "Ustukhuddus Alavi" is a polyherbal formulation containing Lavandula stoechas L. (*Ustukhuddus* in Persian), Anacyclus pyrethrum (L.) Lag., Polypodium vulgare L., Cuscuta epithymum (L.) L., Paeonia officinalis L., *and* Vitis vinifera L. which is processed in honey.

The formulation of this medication has been explained with detailed description of the preparation process in a Persian traditional pharmaceutical manuscript named "Qarabadin-e-Azam" (Azamkhan, 1853). In another Persian reference book named "Exir-e-Azam", this product is indicated to change the matter of excessive "balgham" (phlegm) for better excretion from the brain (Jahan, 2008). As a result of this role, these two books have suggested this product for several brain disorders. Moreover, the excretion of excessive balgham is required for the sharpness of mind according to several authoritative books (Ibn-e-Sina, 2005; Jorjani, 2008).

The purpose of the present study is to evaluate the efficacy of Ustukhuddus Alavi in the cognition enhancement of healthy adolescent students by a randomized, double-blind, placebo-controlled clinical trial study utilizing paced auditory serial addition test (PASAT) for the evaluation of cognitive capacities including mental health, reaction time, sustained attention, and mental fatigue. In accordance with previous research in this area, we measured the salivary cortisol level for biological assessment in addition to cognitive performance evaluation.

2. Materials and methods

2.1. Participants

First, four girls' schools were randomly selected from a list of candidate high schools in Yazd, Iran. This list was provided by the Yazd education organization from diverse geographical areas, however, most of the students who attended the study were of the average sociocultural status and economic level.

In each selected school, we invited all the students in grades 10 and 11 to participate in the study. Then, the volunteer students from each school were randomly partitioned into the intervention and control groups. Initially, 134 students volunteered, 86 of whom remained to the end of the study.

The study population consisted of 15–17 years old female high school students. We restricted our participants to female students in order to control for endocrinologic effects on cognitive performance (Chung et al., 2012).

Inclusion criteria for this study included not using any kind of medication, supplements, or herbal medicines within the month before participation in the trial or intention to use these medicines or herbal extracts during the course of the study. Also, any individuals with a history of neurological diseases, endocrine, gastrointestinal, bleeding disorders, or chronic illnesses were restricted from taking part in the trial. To screen for significant psychiatric problems, we used the depression anxiety stress scale (DASS) questionnaire. Students who had very severe psychiatric disorders characterized by the very severe scale in DASS were excluded at the time of testing.

The participants were randomly assigned either to the intervention (active product) or control (placebo) groups. Permuted-block randomization was performed using a software program for equal chance of allocation to each group. Fig. 1 shows consolidated standards of reporting trials (CONSORT) flowchart diagram summarizing the study participation from enrolment through analysis.

2.2. Ethical approval and consent to participate

This study was conducted in accordance with the ethical principles and the national norms and standards for conducting medical research in Iran. The study protocol was approved by the research ethics committee of Shahid Beheshti University of Medical Sciences (Ethics Code: IR.SBMU.RETECH.REC.1398.771). Prior to any data collection, all the students who voluntarily participated in this study and also their parents provided and signed their written informed consent for inclusion. All experiments were performed in compliance with the guidelines of the declaration of Helsinki and Tokyo for humans.

2.3. Interventional compound

The participating students received medication supplied in the form of 250 mg capsules containing either Ustukhuddus Alavi or an inert placebo. The Ustukhuddus Alavi formulation used in this trial was the same commercial formulation that is currently available to the public. Each capsule contained dried flowers of *L. stoechas*, roots of *A. pyrethrum*, rhizomes of *P. vulgare*, fruits of *C. epithymum*, roots of *P. officinalis*, and fruits of *V. vinifera*. This product is commercially available in the form of 250 mg capsules manufactured by "Talaye Sabze Tooba" company. We refer to the supplementary material for a complete analysis of Ustukhuddus Alavi capsule.

The placebo capsule was identical to the Ustukhuddus Alavi capsule in shape, smell, taste, and weight. It was made up of inert Avicel microcrystalline cellulose and was prepared by adding edible color identical to Ustukhuddus Alavi capsule ingredients.

2.4. Measures

In addition to the assessment of cognitive function detailed below, students completed two other instruments at the screening evaluation. These consist of a questionnaire on demographic information and a screen for depression, anxiety, and stress.

2.4.1. Measures of cognitive performance

PASAT is utilized to reliably evaluate the cognitive performance (Gronwall and Sampson, 1974; Tombaugh, 2006). This research software is an effective method to evaluate different aspects of cognitive performance. These include sustained attention, working memory, reaction time, dual processing, speed of data processing like digit retrieval, and mathematical operations like summation (Aliyari et al., 2020).

In this test, the participants heard a single-digit random number from

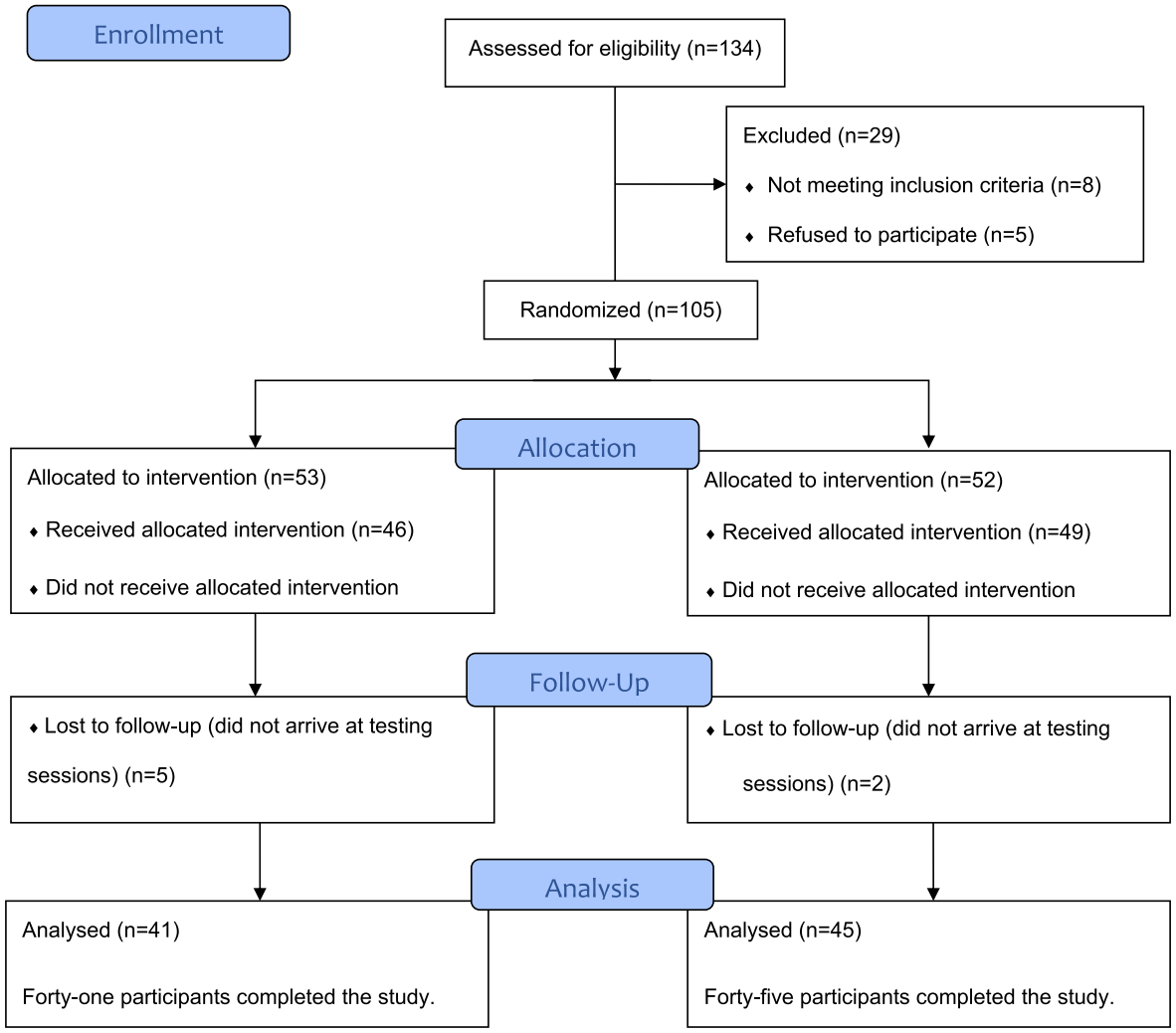

Fig. 1. Disposition of the sample through each stage.

1 to 9 every 3 s for 3 min. The subject had to add together the last pair of consecutive numbers and respond the result of summation before the next number was announced. Then the examiner calculates different performance indicators such as mental health which is defined to be the total number of correct answers, sustained attention which is defined to be the maximum number of consecutive correct answers, mental fatigue which is defined to be the maximum number of consecutive wrong answers, and reaction time which is defined to be the average response time after setting the response time for each wrong answer to 3 s even if the subject has responded earlier.

In both intervention and control groups, we have measured the cognitive function of students for the selected variables of mental health, reaction time, sustained attention, and mental fatigue at four time points, namely, baseline, third week, end-of-intervention, and after the one-month follow-up.

2.4.2. *Questionnaire*

On a basic demographic questionnaire, the students self-reported their age (in years), school year/grade, parental education level, height, and weight.

In addition, a validated Persian version of the DASS-21 questionnaire was filled by the participating students. This self-report questionnaire included 7 items in a multiple-choice format for the assessment of each one of the depression, anxiety, and stress scales. The score for each question ranged from 0 (did not apply to me at all) to 3 (applied to me very much) and each final scale was the sum of its corresponding 7 questions multiplied by a factor of 2 (Ng et al., 2007).

2.5. *Procedure*

The study design was a 10-week randomized controlled and double-blind trial. At the start date of the study, November 2019, the aim, procedure, and restrictions of the trial were described by a pediatrician and a Persian medicine specialist during a briefing session for the students in grades 10 and 11 and their parents. Thereafter, the students were invited to participate.

Preceding the start of the trial, all volunteer students filled out the DASS questionnaire and were screened to be healthy and their eligibility to participate was confirmed. Afterward, the eligible students were required to attend four study visits.

At the first visit in the schools, the participating students filled out the demographic questionnaires. Furthermore, the initial cognitive performance of participants was evaluated by the PASAT. Afterward in the medical center, the students were partitioned into intervention and control groups based on a computer-generated randomization table and received a 3-week supply sealed bottle containing twenty either Ustukhuddus Alavi or placebo capsules, respectively. The administration procedure instructed to them was to swallow one capsule with water after breakfast, and they were also given a medication administration sheet to fill out not to forget the schedule of taking the capsules. We asked the students to return after finishing this first bottle. However, they were also encouraged to make contact with the researchers if any

adverse events occurred in the meantime.

At the second visit, subjects returned to the medical center for reevaluation by the PASAT and reported adverse effects if they had any. Moreover, the students received their second bottles containing twenty more capsules of either Ustukhuddus Alavi or placebo while being instructed to follow the same routine. Once more, they were encouraged to contact the researchers if any adverse effects emerge.

Approximately three weeks later, the third visit was planned in the schools to reassess all the parameters previously evaluated including mental health, reaction time, sustained attention, mental fatigue, DASS, and any probable side effects.

The next appointment with each student was scheduled approximately one month after finishing the second bottle. At the fourth visit at the schools, the students repeated the PASAT and filled out the DASS questionnaires again.

2.6. Salivary cortisol

Saliva collection is an easy and non-invasive way of assessing the concentration of hormones like cortisol. Meanwhile, the level of free saliva cortisol is similar to the free plasma fraction (Nunes LAS, Mussavira S, 2015). Morning saliva samples were collected from 58 students for the pre- and post-intervention direct immunoenzymatic determination of cortisol concentrations. These samples were collected at the schools on the same day before the cognitive test. Students were instructed to wash their mouths and then collect 10 cc of saliva in a falcon tube. Afterward, the tubes were transferred to the laboratory in a cold box and then were kept in a freezer at −20 °C after being centrifuged for 15 min with a spinning speed of 3000 per minute. Measurements were carried out with a cortisol saliva enzyme linked immunosorbent assay (ELISA) kit with a sensitivity of 0.12 ng/mL and analytical range of 0.5–100 ng/mL (DKO020, DiaMetra srl Unipersonale, Italy). Due to the COVID-19 outbreak lockdown, the schools were closed in the midst of the study period, and we could not get the samples from all the participants.

2.7. Statistical analysis

The sample size was calculated based on a two-tailed significance level of 0.05, 80% power and effect size of 0.6. Allowing for a 10% dropout rate, the required sample size for each group was estimated to be 50 individuals.

We used the Student's t-test and Mann-Whitney test for comparing the baseline characteristics of the intervention and control groups. We performed independent samples t-tests for comparing the mean of the cognitive performance and cortisol level in the two groups. For the intervention group, we used Student's paired t-tests to test the differences between every two consecutive visits. Furthermore, we performed the repeated measures analysis of variance (ANOVA) to assess the impact of Ustukhuddus Alavi versus placebo on each subscale during different visits.

Statistical significance was defined as a p-value of less than 0.05. IBM SPSS software version 26 was used to conduct the data analysis.

3. Results

A total of 134 volunteer students enrolled in the clinical trial initially, with a total of 86 completing the study over a period of 6-week intervention and 4-week follow-up. This study was conducted on 86 healthy female students from grades 10 and 11 (55.8% and 44.2%, respectively) with a mean age of 16.3 years, ranging from 15 to 17 years, the majority (63%) of whom had normal body mass index (BMI). Demographic data of the participants that completed the study is shown in Table 1. We compared these demographic characteristics between the two groups using the Student's t-test and Mann-Whitney test for continuous and categorical variables, respectively, which were similar in both groups.

3.1. Cognitive performance

A two-sample Student's t-test was performed for each cognitive outcome measure comparing the mean of intervention (Ustukhuddus Alavi capsule) and control (placebo) groups. At baseline, the two groups of students were not significantly different from each other in any of the PASAT subscales. Results of the *t*-test for both the second and third visits indicated a statistically significant mean difference for sustained attention and mental fatigue with the Ustukhuddus Alavi capsule group demonstrating significantly improved cognitive function over the placebo control. Between-groups analysis of mental health after 3 weeks did not reach statistical significance, however, turned out to be significant after 6 weeks as reflected by a considerable increase in the number of correct answers. On the other hand, no significant difference in reaction time was found between the two groups in any of the visits (see Table 2).

Note that for all the cognitive outcome measures, students in either of the study arms tend to perform better during later visits, most likely as a result of a practice effect previously suggested in the literature (Solomon et al., 2016).

We conducted the repeated measures ANOVA utilizing the general linear model to assess the impact of Ustukhuddus Alavi versus placebo on each subscale (the tests of between-subjects effects) while accounting for the within-subjects factor of the time of visit. The significance values for mental health (0.042), sustained attention (0.003) and mental fatigue (0.005) are all less than 0.05, hence we can conclude that the intervention contributes significantly to improving these PASAT subscales. On the other hand, the significance value for the reaction time (0.867) was inconclusive.

The profile plots illustrate the model-estimated means for the two groups (namely, Ustukhuddus Alavi and placebo) for each of the four visits of the study (see Fig. 2).

Furthermore, Student's paired t-tests were performed for the intervention group comparing the mean difference for every two consecutive

Table 1
Demographic characteristics of students in the intervention and control groups.

	AGE	bmi	Mother Education					Father Education				
	Mean ± SD	Mean ± SD	Illiteracy or primary	Secondary	Diploma	Associate degree	Higher education	Illiteracy or primary	Secondary	Diploma	Associate degree	Higher education
Intervention Group (Active product) n = 45	16.27 ± 0.654	20.85 ± 3.883	11.1%	44.4%	37.8%	4.4%	2.2%	8.9%	37.8%	44.4%	6.7%	2.2%
Control Group (Placebo) n = 41	16.34 ± 0.575	21.44 ± 2.593	0.0%	43.9%	46.3%	7.3%	2.4%	0.0%	36.6%	53.7%	7.3%	2.4%
P value	0.576	0.405	0.137					0.259				
Test	**T-Test**		**Mann-Whitney Test**									

Table 2
The results of the PASAT in the intervention and control groups.

	TIME POINT	*MENTAL HEALTH (MEAN±SD)*	*REACTION TIME (MEAN±SD)*	*SUSTAINED ATTENTION (MEAN±SD)*	*MENTAL FATIGUE (MEAN±SD)*
INTERVENTION GROUP (ACTIVE PRODUCT) N = 45	Baseline	26.13 ± 9.024	2.56 ± 0.300	4.60 ± 2.903	7.13 ± 3.188
	Week 3	42.56 ± 12.398	2.34 ± 0.33	13.53 ± 7.792	3.36 ± 1.956
	Week 6	50.56 ± 8.142	2.06 ± 0.440	21.09 ± 11.602	2.09 ± 1.087
	Week 10	48.74 ± 9.274	2.24 ± 0.339	20.53 ± 13.518	2.28 ± 0.959
CONTROL GROUP (PLACEBO) N = 41	Baseline	26.83 ± 10.002	2.48 ± 0.300	5.56 ± 3.515	7.80 ± 4.026
	Week 3	38.02 ± 12.926	2.38 ± 0.276	9.71 ± 5.349	5.22 ± 4.108
	Week 6	43.84 ± 12.235	2.13 ± 0.351	13.35 ± 7.216	3.81 ± 2.767
	Week 10	41.16 ± 10.344	2.33 ± 0.287	11.92 ± 6.922	3.74 ± 2.286
SIGNIFICANE (P VALUE)	Baseline	0.735	0.204	0.169	0.392
	Week 3	0.101	0.524	0.009	0.011
	Week 6	0.006	0.444	0.001	0.001
	Week 10	0.001	0.21	0.000	0.001

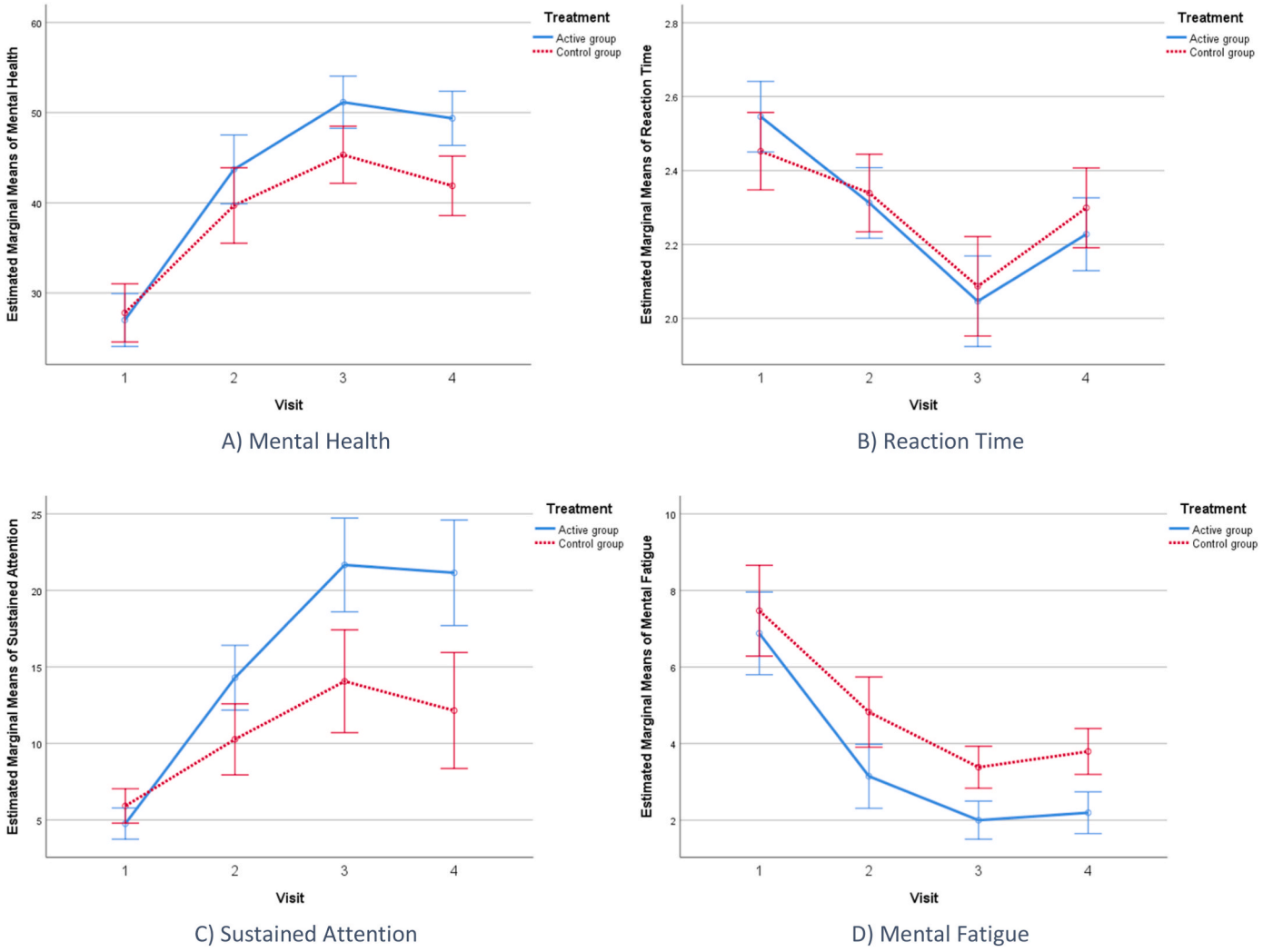

Fig. 2. The effects of Ustukhuddus Alavi vs. placebo intake on cognitive performance according to the PASAT outcomes. Error bars are ± 2 standard error.

visits. Pairwise comparisons by t-tests indicated statistically significant improvements in all the PASAT subscales for the first two periods when the product was consumed. Comparing the baseline with the end-of-intervention and one-month follow-up visits showed a very marked enhancement in all the cognitive domains tested. Mean changes from the baseline cognitive scores at the third week, sixth week, and one-month follow-up for the Ustukhuddus Alavi group are shown in Table 3.

3.2. DASS

Assessment of depression, anxiety, and stress was conducted by DASS questionnaires pre- and post-intervention and at follow-up. Statistical analysis by Mann-Whitney test showed no significant difference between the active product and placebo groups (see Table 4).

Table 3
Mean difference in cognitive scores for the students on Ustukhuddus Alavi across the study period.

Cognitive Function	Significane (Visit 1 → 2)		Significane (Visit 2 → 3)		Significane (Visit 3 → 4)		Significane (Visit 1 → 3)		Significane (Visit 1 → 4)	
	Mean Difference±SD	P value	Mean Difference±SD	P value	Mean Difference±SD	P value	Mean Difference±SD	P value	Mean Difference±SD	P value
MENTAL HEALTH	−16.422 ± 10.308	0.000	−7.488 ± 9.384	0.000	1.780 ± 4.917	0.026	−24.047 ± 8.856	0.000	−22.163 ± 10.885	0.000
REACTION TIME	0.226 ± 0.234	0.002	0.262 ± 0.257	0.000	−0.181 ± 0.266	0.000	0.494 ± 0.278	0.000	0.312 ± 0.260	0.000
SUSTAINED ATTENTION	−8.933 ± 6.257	0.000	−7.256 ± 9.152	0.000	0.512 ± 7.934	0.682	−16.419 ± 10.395	0.000	−15.860 ± 12.299	0.000
MENTAL FATIGUE	3.778 ± 3.496	0.000	1.163 ± 1.889	0.000	−0.195 ± 1.054	0.243	4.814 ± 3.057	0.000	4.837 ± 3.062	0.000

Table 4
Percentage of DASS scores in the intervention and control groups across the study period.

DASS score range cut-off		Depression				Anxiety				Stress			
		Normal 0-9	Mild 10-13	Moderate 14-20	Severe 21-27	Normal 0-7	Mild 8-9	Moderate 10-14	Severe 15-19	Normal 0-14	Mild 15-18	Moderate 19-25	Severe 26-33
Intervention Group (Active product) n = 45	**BEFORE**	26.67	17.78	37.78	17.78	24.44	11.11	28.89	35.56	26.67	24.44	17.78	31.11
	AFTER	30.23	20.93	39.53	9.30	25.58	16.28	30.23	27.91	32.56	25.58	18.60	23.26
	FOLLOW-UP	27.91	20.93	37.21	13.95	25.58	13.95	30.23	30.23	30.23	23.26	20.93	25.58
Control Group (Placebo) n = 41	**BEFORE**	31.71	29.27	19.51	19.51	41.46	9.76	17.07	31.71	26.83	21.95	24.39	26.83
	AFTER	29.73	32.43	21.62	16.22	37.84	5.41	35.14	21.62	24.32	18.92	29.73	27.03
	FOLLOW-UP	28.95	31.58	21.05	18.42	36.84	10.53	28.95	23.68	26.32	23.68	23.68	26.32
P-value	**BEFORE**	0.387				0.217				0.922			
	AFTER	0.809				0.450				0.304			
	FOLLOW-UP	0.687				0.339				0.751			

3.3. Salivary cortisol

For the biological assessment of the effect of Ustukhuddus Alavi, the mean and standard deviation of the salivary cortisol levels were calculated before and after the intervention. The mean difference between the Ustukhuddus Alavi and placebo groups was statistically significant after the intervention (see Table 5). Despite the rise of cortisol level in both groups, the mean of the active product group was still close to the normal range (3–10 ng/mL in the morning) and the increase was less than the placebo group.

3.4. Adverse effects monitoring

The study medication was well tolerated by the students. During the 6 weeks of the administration, a wide variety of adverse effects were monitored and documented (see Table 6). Throughout this study, no serious adverse event was reported by any subject. The only adverse effects which were reported by some students were facial acne and agitation.

4. Discussion

The present study analyzed the efficacy of Ustukhuddus Alavi in enhancing cognitive performance in students aged 15–17 years. To the best of our knowledge, there has been no study to verify the effectiveness of Ustukhuddus Alavi in augmenting brain functions.

Table 5
Mean and standard deviation of the cortisol levels pre- and post-intervention.

	Before	After
	Mean ± SD	Mean ± SD
Intervention Group (Active productT) n = 32	7.74 ± 3.699	10.85 ± 5.482
Control Group (Placebo) n = 26	7.88 ± 4.402	14.90 ± 6.147
P value	0.894	0.047

Table 6
Number of reported adverse effects.

	Intervention Group (ACTIVE PRODUCT)	Control Group (Placebo)
No adverse effects	42	41
Headache	0	0
Flu-like symptoms	0	0
Dry mouth	0	0
Heart palpitations	0	0
Sleep irregularities	0	0
Facial acne	1	0
Gastrointestinal complaints	0	0
Agitation	2	0
Total	45	41

Statistical analysis revealed that daily supplementation with Ustukhuddus Alavi for six weeks led to significant cognitive improvement versus placebo evaluated by the PASAT which is designed to assess mental health (p-value = 0.006), sustained attention (p-value = 0.001), and mental fatigue (p-value = 0.001). Intervention effect on reaction time did not reach significance (p-value = 0.444).

PASAT is considered to be a challenging and complex test for the general population (Brooks et al., 2011). As a result, subjects need time and practice to get accustomed to the format in order to perform well, which may explain the observed practice effect in both groups to some extent (Tombaugh, 2006).

There is a consensus in the literature that academic performance in high school students can be boosted by augmenting cognitive processes such as sustained attention (Puerta Morales, 2015). Therefore, increasing sustained attention by the consumption of Ustukhuddus Alavi has the capacity to improve the academic performance of high school students without any major side effects.

Inhalational *Lavandula angustifolia* Mill. administration has been reported to improve the quality of daytime awakeness and overnight sleep

(Hudson, 1996). Some studies have shown that the speed and precision of mathematical computations can be improved by lavender oil aromatherapy (Cavanagh and Wilkinson, 2002). In concordance with these studies, the rate of correct responses to the summation questions was significantly improved after six weeks of Ustukhuddus Alavi consumption.

Haskell-Ramsay et al. have designed a randomized, placebo-controlled, double-blind, crossover study for evaluating the cognitive effect of the administration of 230 mL purple grape juice on twenty participants aged 18–35 years. This study showed that as a result of this consumption, the reaction time for attention tasks was substantially improved (Haskell-Ramsay et al., 2017). In our study, consumption of Ustukhuddus Alavi which contains *V. vinifera* as one of its ingredients was associated with significantly better sustained attention but not reaction time.

A polyphenol-rich extract made from grape and blueberries significantly enhanced both working memory and attention in healthy students, while subjective ratings of mental fatigue did not change significantly (Philip et al., 2019). However, Ustukhuddus Alavi decreased mental fatigue significantly in our study.

A. pyrethrum root extract's antidepressant activity may be the result of elevating the levels of noradrenaline and dopamine or reducing inflammation in the central nervous system (Badhe et al., 2010). Moreover, dopamine and noradrenaline have been suggested to work together to improve learning and cognitive performance (Ranjbar--Slamloo and Fazlali, 2020). Dopamine is involved in many cognitive functions such as motivation, learning, working memory, and decision-making (Westbrook and Braver, 2016).

Although the results indicated the considerable effect of Ustukhuddus Alavi on cognitive enhancement in students, no major impact on either depression, anxiety, or stress was detected as the difference between the mean of DASS for the intervention and control group was not statistically significant. It seems that this product can be helpful as a cognition booster for the sharpness of mind but not as a therapy for psychiatric disorders.

Hypothalamic–pituitary–adrenal system responds to stress by stimulating the release of cortisol from the adrenal cortex (Lupien et al., 2005). In a large population study, higher cortisol level was linked with inferior cognitive performance in a variety of tasks (Lee et al., 2007). Moreover, cortisol administration impaired both digit span and the recall of previously learned word lists in young men (Wolf et al., 2001).

Human cognitive performance is linked to the acute and chronic changes of the cortisol level throughout life. The cognitive function of the frontal lobes is affected by the acute surge of cortisol in the young population. Moreover, their memory performance can be acutely regulated by pharmacological modification of cortisol (Lupien et al., 2005). The brain activity in important parts for the cognitive process such as the hippocampus and prefrontal cortex is sensitive to elevated cortisol levels. Furthermore, neurological development in children is negatively associated with high cortisol levels; and increased salivary cortisol levels have been observed in school children with lower cognitive performance (Teixeira et al., 2019).

Sniffing lavender aroma for 5 min in healthy subjects increases the active antioxidant metabolites and decreases cortisol level at both high and low concentrations in saliva (Atsumi and Tonosaki, 2007). In our study, the mean difference of cortisol concentration between the two groups was significant after the intervention. However, cortisol level was elevated in both groups in comparison to the baseline, which may be credited to the stress of the final exam season of the first semester during which the second cortisol samples were collected. Another possible explanation may be the stress associated with the COVID-19 pandemic.

Ustukhuddus Alavi contains L. stoechas, A. pyrethrum, P. vulgare, C. epithymum, P. officinalis, *and* V. vinifera. For each ingredient of this herbal supplement, the results of various studies have revealed the antioxidant and anti-inflammatory activities as well as potential neuroprotective properties and the regulation of the cholinergic system. The potential cognitive improvement effect of Ustukhuddus Alavi appears to be due to the components available in it, but not enough is known about the possible synergistic effects.

From the Persian medicine point of view, apart from the individual effects of the ingredients constituting a multi-ingredient medication, novel medical effects can emerge from the synergy between the ingredients that are in addition to the effects of each of them (Ibn-e-Sina, 2005). This is in accordance with Chinese medicine where multi-ingredient herbal products are much more frequently prescribed to produce specific and synergistic effects and reduce toxicities (Mohd et al., 2018).

In Persian medicine, the excretion of excessive or harmful substances (balgham) in the brain is described as the underlying mechanism of Ustukhuddus Alavi (Jahan, 2008). According to traditional medicine books, excess of balgham can lead to the development of many neuropsychological disorders such as memory dysfunction (Rahman et al., 2014). Unani scholars also believed that the main cause of Alzheimer's disease is excessive anomalous balgham in the brain which needs to be treated through the removal of these substances (Ahmer, 2015).

Considering the inclusion of the paraclinical assessment of salivary cortisol besides PASAT for the assessment of cognitive performance was the major strength of our study.

A limitation of this work is some degree of selection and sampling biases because we conducted the study in the schools recommended by the Yazd education organization. An additional restriction that we encountered was the increase in the dropout rate because of the coronavirus pandemic which broke out shortly after the study start date.

4.1. Possible mechanism of action

Multiple studies have shown an association between memory deterioration and low intake of dietary antioxidants (Meydani, 2001). Oxidative stress is associated with cognitive dysfunction, which in turn can be ameliorated by antioxidants in both humans and animals (Bin Sayeed et al., 2014). Additionally, cognitive impairment is associated with reduced cholinergic function which can be treated with the stimulation of central cholinergic activity (Suliman et al., 2016). Administration of cognitive enhancers has been suggested to raise the level of acetylcholine (ACh) by downregulating acetylcholinesterase (AChE) activity and stimulation of cholinergic receptor binding in the frontal cortex and hippocampus (Bhattacharya et al., 2000).

Lavandula can induce neuroprotective, antioxidant, anticonvulsant, and anxiolytic activity (Kabiruddin Ahmed K, Zaheer Ahmed, 2016). *L. officinalis* reduced both nitric oxide and malondialdehyde levels in the brain of the kindling animal model (Rahmati et al., 2013).

L. stoechas increases cognitive performance by improving central cholinergic neurotransmission and by defending against oxidative stress in mice brain (Mushtaq et al., 2018). Both wild plants of *Lavandula viridis* L'Hér. and their *in vitro* cultures have demonstrated a significant antioxidant defense by scavenging free radicals and protecting against lipid peroxidation, and also anticholinesterase activity (Costa et al., 2013).

Antioxidant and cholinergic effects of Lavandula essential oils have been discussed in the supplementary material.

A. pyrethrum root protects against oxidative stress and oxidative DNA damage, and also has anti-inflammatory and immunostimulating activities. Its hydroalcoholic extract considerably protected against seizure-induced oxidative damage through increasing glutathione levels and decreasing malondialdehyde levels in the whole brain. It also ameliorated the seizure-induced cognitive dysfunction and normalized cholinesterase activity in a dose-dependent way in rats (Pahuja et al., 2012). Moreover, pre-treatment by this extract has been shown to ameliorate cognitive condition by exhibiting protective activity against oxidative damage and suppressing rho kinase expression in mice (Pahuja et al., 2013). Aqueous and methanol extracts of *A. pyrethrum* roots can increase antioxidant activity which may be linked to the existence of alkaloids and phenols. No acute toxicity nor mortality was found in a

single administration of aqueous and methanol extracts of *A. pyrethrum* roots up to the highest possible dose of 5 g/kg (Manouze et al., 2017).

Additionally, *A. pyrethrum* is known as a brain tonic and has shown significant anticonvulsant effects in animal studies. Pellitorine as one of the main constituents in the leaves and roots of *A. pyrethrum* is easily absorbed and quickly goes through the blood-brain barrier, hence suggesting a possible role in the therapy of brain disorders. Ethanolic extract of *A. pyrethrum* postponed the onset and significantly decreased the length of pilocarpine-induced seizures in rats, possibly due to its effect on cholinergic receptors (Bezza et al., 2019). This extract has also been suggested to improve memory activity and as a result ameliorate cognitive capacities through elevating central cholinergic neurotransmission in scopolamine-induced amnesia in albino Wistar rats (Sujith et al., 2012).

Based on our search, there were very limited studies on the effects of *P. vulgare* on cognition at the time of writing. *P. vulgare* has antioxidative, antibacterial, and anti-inflammatory capacities due to its flavonoids and tannins components (Sofiane et al., 2015). *P. vulgare* facilitates the transmission of dopamine in the brain, which makes it beneficial in drug-induced catalepsy (Kalam et al., 2017). On the other hand, it has been shown that executive function in the young population can benefit from dopamine's role in modulating striatal circuits (Berry et al., 2018).

The methanolic extract of the whole plant of *C. epithymum* has been observed *in vitro* to protect against reactive oxygen species dose-dependently (Ganapaty et al., 2013). *C. epithymum* is a rich organic source of antioxidants for protecting the brain against oxidative stress by having flavonoid and alkaloid content, and it has been suggested for the treatment of cognitive dysfunction and neurological disorders. Additionally, this antioxidant potential reduces inflammation which is suggested to be the underlying mechanism of its neuroprotection against ischemic cell death and enhancing neuronal proliferation. Significant cognitive amelioration after eight weeks of administration with *C. epithymum* in schizophrenic patients was observed without major side effects (Parvizi et al., 2019). *Cuscuta* species protect against oxidative stress, neuroinflammation, proliferation, and also restore the cholinergic function and neuroprotective activities which may explain memory-improving effects in mice (Lin et al., 2018). The 100 mg/kg methanol:water (80:20) extract of the whole plant of *C. epithymum* has been reported to have substantial anticonvulsant effects and lower mortality in pentylenetetrazol-induced seizures in mice (Mehrabani et al., 2007).

P. officinalis can induce high levels of antioxidant activity due to being rich in polyphenolic compounds with strong antioxidant properties. Furthermore, *P. officinalis* and none of its extracts possessed cytotoxic effects (Dienaitė et al., 2019). Moreover, the root contains flavonoids, paeoniflorin, paeonin, paeonol, and tannic acid (Ahmad et al., 2012). Daily administration of paeoniflorin extracted from peony has moderated the deterioration of learning in older rats (Ohta et al., 1994). In addition, paeoniflorin has anti-inflammatory and anti-amyloidogenic effects which are preventive in neurodegeneration in Alzheimer's disease (Ghédira and Goetz, 2015).

V. vinifera is a natural source of polyphenols that serves as an immunological and anti-oxidant defense (Pazos-Tomas et al., 2020). These components which are abundant in grape-based products induce anti-oxidative, anti-inflammatory, anti-acetylcholinesterase, and anti-amyloidogenic activities which are essential for healthy brain aging. Grape-derived polyphenols can penetrate the blood-brain barrier, suppress AChE, and augment Aβ-clearance, which in turn leads to anti-neurodegenerative and memory-enhancing effects (Ibrahim Fouad and Zaki Rizk, 2019). In another study, *V. vinifera* extract has had a neuroprotective effect in aluminium-induced oxidative stress in rats brain (Lakshmi et al., 2014).

5. Conclusions

In healthy female adolescent students, the daily intake of Ustukhuddus Alavi for six weeks was efficient in enhancing cognitive function and in particular in three subscales of the tests carried out. Additionally, it has also controlled the cortisol level and maintained it in the normal range. We propose further detailed investigations to establish the exact mechanisms of cognition-enhancing action, the potential synergistic effects of this compound, and the relevant phytochemical constituents. Supplementation with Ustukhuddus Alavi in combination with a healthy lifestyle may be a safe way to boost cognitive performance, as Persian medicine has long indicated.

CRediT authorship contribution statement

Samane Tefagh: Conceptualization, Methodology, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Roshanak Mokaberinejad:** Supervision, Writing – review & editing, Project administration, Validation. **Mehrdad Shakiba:** Supervision, Project administration, Validation. **Mahdi Jafari:** Methodology. **Maryam Salehi:** Software, Resources. **Maryam Khayatkashani:** Resources, Writing – review & editing. **Nezhat Shakeri:** Formal analysis, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jep.2022.114971.

Abbreviations

PASAT	paced auditory serial addition test
DASS	depression anxiety stress scale
CONSORT	consolidated standards of reporting trials
ELISA	enzyme linked immunosorbent assay
BMI	body mass index
ANOVA	analysis of variance
iNOS	inducible nitric oxide synthase
Nrf2/HO-1	nuclear factor-erythroid 2 related factor/heme oxygenase-1

References

Agha, G., Kezios, K., Baccarelli, A.A., DuBois Bowman, F., Rauh, V., Susser, E.S., Cohn, B., Cirillo, P., Link, B.G., Factor-Litvak, P., Staudinger, U.M., 2019. Cognition level and change in cognition during adolescence are associated with cognition in midlife. Ann. Epidemiol. 35, 48–52. https://doi.org/10.1016/j.annepidem.2019.03.004 e2.

Ahmad, F., Tabassum, N., Rasool, S., 2012. Medicinal uses and phytoconstituents of *Paeonia officinalis*. Int. Res. J. Pharm. 3, 85–87.

Ahmer, S.M., 2015. Alzheimer's Disease in Perspective of Unani System of Medicine, vol. 3.

Aliyari, H., Sahraei, H., Erfani, M., Mohammadi, M., Kazemi, M., Daliri, M.R., Minaei-Bidgol, B., Agaei, H., Sahraei, M., Seyed Hosseini, S.M.A., Tekieh, E., Salehi, M., Farajdokht, F., 2020. Changes in cognitive functions following violent and football video games in young male volunteers by studying brain waves. Basic Clin. Neurosci. J. 11 https://doi.org/10.32598/bcn.9.10.335.

Atsumi, T., Tonosaki, K., 2007. Smelling lavender and rosemary increases free radical scavenging activity and decreases cortisol level in saliva. Psychiatr. Res. 150, 89–96. https://doi.org/10.1016/j.psychres.2005.12.012.
Azamkhan, M.A.K., 1853. Qarabadin-e-Azam. Tehran. Iran Intisharat va Amoozesh Enghelab Islam. Press (in Persian).
Badhe, S., Badhe, R., Ghaisas, M., Chopade, V., Deshpande, A.D., 2010. Evaluation of antidepressant activity of *Anacyclus pyrethrum* root extract. Int. J. Green Pharm. 4 https://doi.org/10.4103/0973-8258.63880.
Batistela, S., Bueno, O.F.A., Vaz, L.J., Galduróz, J.C.F., 2016. Methylphenidate as a cognitive enhancer in healthy young people. Dement. Neuropsychol. 10, 134–142. https://doi.org/10.1590/S1980-5764-2016DN1002009.
Berry, A.S., Shah, V.D., Jagust, W.J., 2018. The influence of dopamine on cognitive flexibility is mediated by functional connectivity in young but not older adults. J. Cognit. Neurosci. 30, 1330–1344. https://doi.org/10.1162/jocn_a_01286.
Bezza, K., Gabbas, Z., Laadraoui, J., Laaradia, M., Oufquir, S., Chait, A., 2019. Ameliorative potential of *Anacyclus pyrethrum* extract in generalized seizures in rat: possible cholinergic mediated mechanism. Bangladesh J. Pharmacol. 14, 188–195. https://doi.org/10.3329/bjp.v14i4.40537.
Bhattacharya, S.K., Bhattacharya, A., Kumar, A., Ghosal, S., 2000. Antioxidant activity of *Bacopa monniera* in rat frontal cortex, striatum and hippocampus. Phytother Res. 14, 174–179. https://doi.org/10.1002/(sici)1099-1573(200005)14:3<174::aid-ptr624>3.0.co;2-o.
Bin Sayeed, M.S., Shams, T., Fahim Hossain, S., Rahman, M.R., Mostofa, A., Fahim Kadir, M., Mahmood, S., Asaduzzaman, M., 2014. *Nigella sativa* L. seeds modulate mood, anxiety and cognition in healthy adolescent males. J. Ethnopharmacol. 152, 156–162. https://doi.org/10.1016/j.jep.2013.12.050.
Borson, S., 2010. Cognition, aging, and disabilities: conceptual issues. Phys. Med. Rehabil. Clin 21, 375–382. https://doi.org/10.1016/j.pmr.2010.01.001.
Brooks, J.B.B., Giraud, V.O., Saleh, Y.J., Rodrigues, S.J., Daia, L.A., Fragoso, Y.D., 2011. Paced auditory serial addition test (PASAT): a very difficult test even for individuals with high intellectual capability. Arq. Neuropsiquiatr. 69, 482–484.
Carbia, C., López-Caneda, E., Corral, M., Cadaveira, F., 2018. A systematic review of neuropsychological studies involving young binge drinkers. Neurosci. Biobehav. Rev. 90, 332–349. https://doi.org/10.1016/j.neubiorev.2018.04.013.
Cavanagh, H.M.A., Wilkinson, J.M., 2002. Biological activities of Lavender essential oil. Phyther. Res. 16 https://doi.org/10.1002/ptr.1103.
Chung, Y.C., Park, C.H., Kwon, H.K., Park, Y.M., Kim, Y.S., Doo, J.K., Shin, D.H., Jung, E. S., Oh, M.R., Chae, S.W., 2012. Improved cognitive performance following supplementation with a mixed-grain diet in high school students: a randomized controlled trial. Nutrition 28, 165–172. https://doi.org/10.1016/J.NUT.2011.05.017.
Costa, P., Gonçalves, S., Valentão, P., Andrade, P.B., Romano, A., 2013. Accumulation of phenolic compounds in in vitro cultures and wild plants of *Lavandula viridis* L'Hér and their antioxidant and anti-cholinesterase potential. Food Chem. Toxicol. 57 https://doi.org/10.1016/j.fct.2013.03.006.
Dienaitė, L., Pukalskienė, M., Pukalskas, A., Pereira, C.V., Matias, A.A., Venskutonis, P. R., 2019. Isolation of strong antioxidants from *Paeonia officinalis* roots and leaves and evaluation of their bioactivities. Antioxidants 8, 249.
Fernandes, A., Desousa, A., Prabhudesai, S., 2014. Herbal medications to enhance cognition and cognitive ability. In: Indian J Mental Health, pp. 869–871.
Ganapaty, S., Ramaiah, M., Ramakrishna, P., Reddy, D.N., 2013. Scientific validation and formulation of three Indian Folklore medicinal plants. J. Pharm. Res. 6, 823–835. https://doi.org/10.1016/j.jopr.2013.08.012.
Ghédira, K., Goetz, P., 2015. Pivoine *Paeonia officinalis* L. (Paeoniaceae). Phytothérapie 13, 328–331. https://doi.org/10.1007/s10298-015-0986-3.
Gronwall, D.M., Sampson, H., 1974. The Psychological Effects of Concussion.
Haskell-Ramsay, C.F., Stuart, R.C., Okello, E.J., Watson, A.W., 2017. Cognitive and mood improvements following acute supplementation with purple grape juice in healthy young adults. Eur. J. Nutr. 56, 2621–2631. https://doi.org/10.1007/s00394-017-1454-7.
Hudson, R., 1996. The value of lavender for rest and activity in the elderly patient. Compl. Ther. Med. 4, 52–57. https://doi.org/10.1016/S0965-2299(96)80057-4.
Ibn-e-Sina, A.H., 2005. Al-Qanun fit-tib, [The Canon of Medicine]. Lebanon: Alaalami Beirut library Press (research of Ebrahim Shamsedine), Beirut.
Ibrahim Fouad, G., Zaki Rizk, M., 2019. Possible neuromodulating role of different grape (*Vitis vinifera* L.) derived polyphenols against Alzheimer's dementia: treatment and mechanisms. Bull. Natl. Res. Cent. 43, 108. https://doi.org/10.1186/s42269-019-0149-z.
Jahan, N., 2008. Exir-e-Azam [Great Elixir]. Research Institute for Islamic and Complementary Medicine, Iran University of Medical Sciences, Tehran.
Jorjani, H., 2008. Zakhireh Kharazmshahi. The academy of medical sciences publication, Tehran.
Kabiruddin Ahmed, K., Zaheer Ahmed, A.P.A., 2016. Ustukhuddoos (*Lavandula stoechas* Linn.) - a brain scavenger drug: an overview. Int. J. Pharm. Pharmaceut. Res. 7, 618–626.
Kalam, M.A., Munshi, Y.I., Karim, S., 2017. Bisfayej (*Polypodium vulgare* L.): a review on medicinal importance of rhizome with Unani prospective and modern pharmacology. Int. J. Unani Integr. Med. 1, 4–6.
Lakshmi, B.V.S., Sudhakar, M., Anisha, M., 2014. Neuroprotective role of hydroalcoholic extract of *Vitis vinifera* against aluminium-induced oxidative stress in rat brain. Neurotoxicology 41, 73–79. https://doi.org/10.1016/j.neuro.2014.01.003.
Lee, B.K., Glass, T.A., McAtee, M.J., Wand, G.S., Bandeen-Roche, K., Bolla, K.I., Schwartz, B.S., 2007. Associations of salivary cortisol with cognitive function in the Baltimore memory study. Arch. Gen. Psychiatr. 64, 810–818. https://doi.org/10.1001/archpsyc.64.7.810.
Lin, M.-K., Lee, M.-S., Huang, H.-C., Cheng, T.-J., Cheng, Y.-D., Wu, C.-R., 2018. *Cuscuta chinensis* and C. *Campestris* attenuate scopolamine-induced memory deficit and oxidative damage in mice. Molecules 23. https://doi.org/10.3390/molecules23123060.
Lupien, S.J., Fiocco, A., Wan, N., Maheu, F., Lord, C., Schramek, T., Tu, M.T., 2005. Stress hormones and human memory function across the lifespan. Psychoneuroendocrinology 30, 225–242. https://doi.org/10.1016/j.psyneuen.2004.08.003.
Manouze, H., Bouchatta, O., Gadhi, A.C., Bennis, M., Sokar, Z., Ba-M'hamed, S., 2017. Anti-inflammatory, antinociceptive, and antioxidant activities of methanol and aqueous extracts of *Anacyclus pyrethrum* roots. Front. Pharmacol. 8 https://doi.org/10.3389/fphar.2017.00598.
Mehrabani, M., Modirian, E., Ebrahimabadi, A.R., Vafazadeh, J., Shahnavaz, S.H., Heidari, M.R., 2007. Study of the effects of hydro-methanol extracts of *Lavandula Vera* DC. and *Cuscuta Epithymum* Murr. on the seizure induced by pentylentetranzol in mice. J. Kerman Univ. Med. Sci. 14, 25–32.
Meydani, M., 2001. Antioxidants and cognitive function. Nutr. Rev. 59, S75–S82. https://doi.org/10.1111/j.1753-4887.2001.tb05505.x.
Mezcua-Hidalgo, A., Ruiz-Ariza, A., Suárez-Manzano, S., Martínez-López, E.J., 2019. 48-Hour effects of monitored cooperative high-intensity interval training on adolescent cognitive functioning. Percept. Mot. Skills 126, 202–222. https://doi.org/10.1177/0031512518825197.
Mohd, M., Sofi, G., Jahan, N., Baig, Z., 2018. Unani approaches to the method of formulating a Murakkab (compound drug formulation). J. Ayurveda Integr. Med. 10 https://doi.org/10.1016/j.jaim.2017.04.008.
Mushtaq, A., Anwar, R., Ahmad, M., 2018. *Lavandula stoechas* (L) a very potent antioxidant attenuates dementia in scopolamine induced memory deficit mice. Front. Pharmacol. https://doi.org/10.3389/fphar.2018.01375.
Ng, F., Trauer, T., Dodd, S., Callaly, T., Campbell, S., Berk, M., 2007. The validity of the 21-item version of the Depression Anxiety Stress Scales as a routine clinical outcome measure. Acta Neuropsychiatr. 19, 304–310. https://doi.org/10.1111/j.1601-5215.2007.00217.x.
Nunes, L.A.S., Mussavira, S., O, B., 2015. Clinical and diagnostic utility of saliva as a non-invasive diagnostic fluid: a systematic review. Biochem. Med. 25, 177–192.
Ohta, H., Matsumoto, K., Shimizu, M., Watanabe, H., 1994. Paeoniflorin attenuates learning impairment of aged rats in operant brightness discrimination task. Pharmacol. Biochem. Behav. 49, 213–217. https://doi.org/10.1016/0091-3057(94)90478-2.
Pahuja, M., Mehla, J., Reeta, K.H., Joshi, S., Gupta, Y.K., 2012. Root extract of *Anacyclus pyrethrum* ameliorates seizures, seizure-induced oxidative stress and cognitive impairment in experimental animals. Epilepsy Res. 98 https://doi.org/10.1016/j.eplepsyres.2011.09.006.
Pahuja, M., Mehla, J., Reeta, K.H., Tripathi, M., Gupta, Y.K., 2013. Effect of *Anacyclus pyrethrum* on pentylenetetrazole-induced kindling, spatial memory, oxidative stress and rho-kinase II expression in mice. Neurochem. Res. 38, 547–556. https://doi.org/10.1007/s11064-012-0947-2.
Parvizi, M., Fadai, F., Khodaei-Ardakani, M.R., Amin, G., Abdi, L., Noroozi, M., Ansari, I., 2019. Effect of *Cuscuta epithymum* acquainted with risperidone on the improvement of clinical symptoms and cognitive impairment in patients with schizophrenia: a triple-blind randomized placebo-controlled trial. Galen Med. J. 8 https://doi.org/10.31661/gmj.v8i0.1334, 2019.
Pazos-Tomas, C.C., Cruz-Venegas, A., Pérez-Santiago, A.D., Sánchez-Medina, M.A., Matías-Pérez, D., García-Montalvo, I.A., 2020. *Vitis vinifera*: an alternative for the prevention of neurodegenerative diseases. J. Oleo Sci. 69, 1147–1161. https://doi.org/10.5650/jos.ess20109.
Philip, P., Sagaspe, P., Taillard, J., Mandon, C., Constans, J., Pourtau, L., Pouchieu, C., Angelino, D., Mena, P., Martini, D., Del Rio, D., Vauzour, D., 2019. Acute intake of a grape and blueberry polyphenol-rich extract ameliorates cognitive performance in healthy young adults during a sustained cognitive effort. Antioxidants 8. https://doi.org/10.3390/antiox8120650.
Puerta Morales, L., 2015. Relationship between Cognitive Processes and Academic Performance in High School Students: relación entre los procesos cognitivos y el rendimiento académico en estudiantes de educación básica secundaria. Psychol. Av. la Discip. 9, 85–100.
Puttarak, P., Dilokthornsakul, P., Saokaew, S., Dhippayom, T., Kongkaew, C., Sruamsiri, R., Chuthaputti, A., Chaiyakunapruk, N., 2017. Effects of *Centella asiatica* (L.) Urb. on cognitive function and mood related outcomes: a Systematic Review and Meta-analysis. Sci. Rep. 7, 10646. https://doi.org/10.1038/s41598-017-09823-9.
Rahman, A., Ali, S.J., Zulkifle, M., Ahmad, I., 2014. Concept of Akhlat Arba (four humors) with relation to health and disease. Int. J. Herb. Med. 2, 46–49.
Rahmati, B., Khalili, M., Roghani, M., Ahghari, P., 2013. Anti-epileptogenic and antioxidant effect of *Lavandula officinalis* aerial part extract against pentylenetetrazol-induced kindling in male mice. J. Ethnopharmacol. 148 https://doi.org/10.1016/j.jep.2013.04.004.
Ranjbar-Slamloo, Y., Fazlali, Z., 2020. Dopamine and noradrenaline in the brain; overlapping or dissociate functions? Front. Mol. Neurosci. 12, 334. https://doi.org/10.3389/fnmol.2019.00334.
Shojaii, A., Ghods, R., Abdollahi Fard, M., 2016. Medicinal herbs in Iranian traditional medicine for learning and memory. Afr. J. Tradit., Complementary Altern. Med. 13, 199–209.
Sofiane, G., Nouiouawafa Ouarda, D., 2015. Antioxidant, antimicrobial and anti-inflammatory activities of flavonoids and tannins extracted from *Polypodium vulgare* L. Asian J. Biochem. Pharmaceut. Res. 114–122.
Solomon, T.M., Leech, J., deBros, G.B., Murphy, C.A., Budson, A.E., Vassey, E.A., Solomon, P.R., 2016. A randomized, double-blind, placebo controlled, parallel

group, efficacy study of alpha BRAIN® administered orally. Hum. Psychopharmacol. Clin. Exp. 31 https://doi.org/10.1002/hup.2520.

Sujith, K., Darwin, C.R., Sathish Suba, V., 2012. Memory-enhancing activity of *Anacyclus pyrethrum* in albino Wistar rats. Asian Pacific J. Trop. Dis. 2, 307–311. https://doi.org/10.1016/S2222-1808(12)60067-X.

Suliman, N.A., Mat Taib, C.N., Mohd Moklas, M.A., Adenan, M.I., Hidayat Baharuldin, M. T., Basir, R., 2016. Establishing natural nootropics: recent molecular enhancement influenced by natural nootropic. Evid. Based. Complement. Alternat. Med. 4391375. https://doi.org/10.1155/2016/4391375, 2016.

Teixeira, S.M., Reyes, A.N., da Silva, R.A., de Mattos Souza, L.D., Moreira, F.P., Wiener, C.D., Oses, J.P., de Figueiredo, V.L.M., Jansen, K., 2019. Cognitive performance and salivary cortisol levels in school children. Psychiatr. Clin. Neurosci. https://doi.org/10.1111/pcn.12904.

Tombaugh, T.N., 2006. A comprehensive review of the paced auditory serial addition test (PASAT). Arch. Clin. Neuropsychol. Off. J. Natl. Acad. Neuropsychol. 21, 53–76. https://doi.org/10.1016/j.acn.2005.07.006.

Wagner, B., Latimer, J., Adams, E., Carmichael Olson, H., Symons, M., Mazzucchelli, T. G., Jirikowic, T., Watkins, R., Cross, D., Carapetis, J., Boulton, J., Wright, E., McRae, T., Carter, M., Fitzpatrick, J.P., 2020. School-based intervention to address self-regulation and executive functioning in children attending primary schools in remote Australian Aboriginal communities. PLoS One 15, e0234895. https://doi.org/10.1371/journal.pone.0234895.

Westbrook, A., Braver, T.S., 2016. Dopamine does double duty in motivating cognitive effort. Neuron 89, 695–710. https://doi.org/10.1016/j.neuron.2015.12.029.

Wolf, O.T., Convit, A., McHugh, P.F., Kandil, E., Thorn, E.L., De Santi, S., McEwen, B.S., de Leon, M.J., 2001. Cortisol differentially affects memory in young and elderly men. Behav. Neurosci. 115, 1002–1011. https://doi.org/10.1037//0735-7044.115.5.1002.