

Effect of an herbal extract of *Sideritis scardica* and B-vitamins on cognitive performance under stress: A pilot study

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Abstract

Chronic stress can impair cognitive functions including learning and memory. The current study investigated the reduction of (mental) stress and improvement of stress tolerance in 64 healthy men and women after six weeks intake of a dietary supplement containing an extract of *Sideritis scardica* and selected B-vitamins.

Mental performance and visual attention were measured by Trail-Making Test (TMT) and Colour-Word-Test (CWT) before/after an acute stress stimulus (noise, CW-Interference).

TMT improved upon product intake. The CWT reaction time accelerated upon product intake in situations of CW-Congruence (overall) ($p=0.014$), CW-conflict (overall) ($p=0.024$), CW-conflict (with noise) ($p=0.001$), CW-Congruence (without noise) ($p=0.004$) and CW-conflict (without noise) ($p=0.017$). CWT-changes upon product intake, differentiated for noise and CW-interference, showed (i) a bisection of CW-interference-related impairment of the reaction time in the presence of noise from 27 ms to 13.5 ms, (ii) a bisection of noise-related impairment of the reaction time in the presence of CW-conflict from 34 ms to 17 ms, (iii) an improvement of the impairment of the reaction time due to combined stress (noise plus CW-conflict) by 14.5 ms from 66 ms to 51.5 ms, (iv) despite of the improvement of the reaction time, no increase of the error rate. Safety blood parameters and the reporting of no adverse events argue for the product's safety.

These results may be relevant for persons solving cognitive tasks under conflict and/or noise (e.g. open-plan offices or car-driving) and support that the tested product alleviates stress-induced impairment of executive functioning (working memory, cognitive flexibility, controlled behavioural inhibition).

Keywords: *Sideritis scardica*, B-vitamins, mental stress, cognitive performance, trail making test, colour word test, Stroop test, stress tolerance, stress resilience

Introduction

Stress is a common public health problem of the 21st century. It is defined as a "constellation of events, consisting of a stimulus (stressor) that precipitates a reaction in the brain (stress perception) and activates physiological fight or flight systems in the body (stress response)" [1]. Exposure to various forms of stress is a common daily occurrence in the lives of most individuals, with both positive and negative effects on – beyond other body systems – cognitive function. The impact of stress is influenced by the type and duration of the stressor. In its acute form, stress may be a necessary adaptive mechanism for survival and with only transient changes within the brain [2]. Studies confirm that severe and/or

long-term mental stress impairs cognitive function and mental performance in humans [2].

In south-eastern parts of Europe (e.g. Greece, Turkey), the plant *Sideritis scardica* is originally cultured and traditionally used as tea for alleviating common cold and for calming down after work [3,4], i.e. for stress relief. In studies in rat synaptosomes, a fluid extract of *Sideritis scardica* dose-dependently inhibited the reuptake of serotonin, dopamine and norepinephrine which leads to an increased amount of these neurotransmitters in synaptic clefts [5, 6]. These effects made the authors hypothesize benefits against mental impairment [6] with stress-induced interference being an example. A *Sideritis scardica* extract affected brain function (electroencephalograms) in rats, similarly to products known for



their capability to improve cognitive impairment [7]. All this promising preclinical evidence asked for verification in humans.

In addition, vitamins such as B₁, B₆ and B₁₂ are of great importance for the stress perception and stress tolerance. They act as cofactors in the synthesis of hormones and neurotransmitters (including adrenaline, noradrenaline and serotonin) [8-10]. This may explain why several studies suggest an association between an inadequate supply of nutrients and stress symptoms: The vitamin B₁ (thiamine) and acetylcholine metabolism in the cholinergic nerve endings are closely related. Thiamine triphosphate has a regulating effect on certain proteins that are involved in the grouping of acetylcholine receptors. Those receptors are located in the nerve cells responsible for the impulse conduction [9]. In epidemiological studies, a marginal to deficient thiamine status is associated with depressed mood [11]. Vitamin B₆ (pyridoxine), in the form of pyridoxal phosphate, is the central coenzyme in the metabolism of amino acids and neurotransmitters involved as part of the regulation of mental function and mood [8]. In a cross-sectional study with 1371 adults (<65 years) low vitamin B₆ plasma levels (≤ 20 nmol/l) were significantly associated with depressive symptoms [12]. Vitamin B₁₂ (cobalamin) deficiency due to an insufficient dietary intake are associated with permanent stress [13]. Stress-related Vitamin B₁₂ deficiency is associated with disturbances in the methyl-metabolism of the nerve cell, which affects the neurotransmitter metabolism adversely [10]. Health consequences may include fatigue and weakness, irritability, depressed mood, loss of concentration, mental confusion and disorientation [14-16]. In animal models, folic acid deficiency was associated with low serotonin levels in the central nervous system [17]. Also a simultaneous deficiency of folic acid and vitamin B₁₂ further inhibits S-adenosyl-methionine-dependent methylation reactions and increases homocysteine levels. The consequences are neurotoxic effects and depression [10, 17].

So far, no human studies have examined the reduction of (mental) stress or its negative effects by using *Sideritis scardica* alone or in combination with B-vitamins. The current study investigated the reduction of (mental) stress and improvement of stress tolerance in healthy humans after intake of a dietary supplement containing such combination.

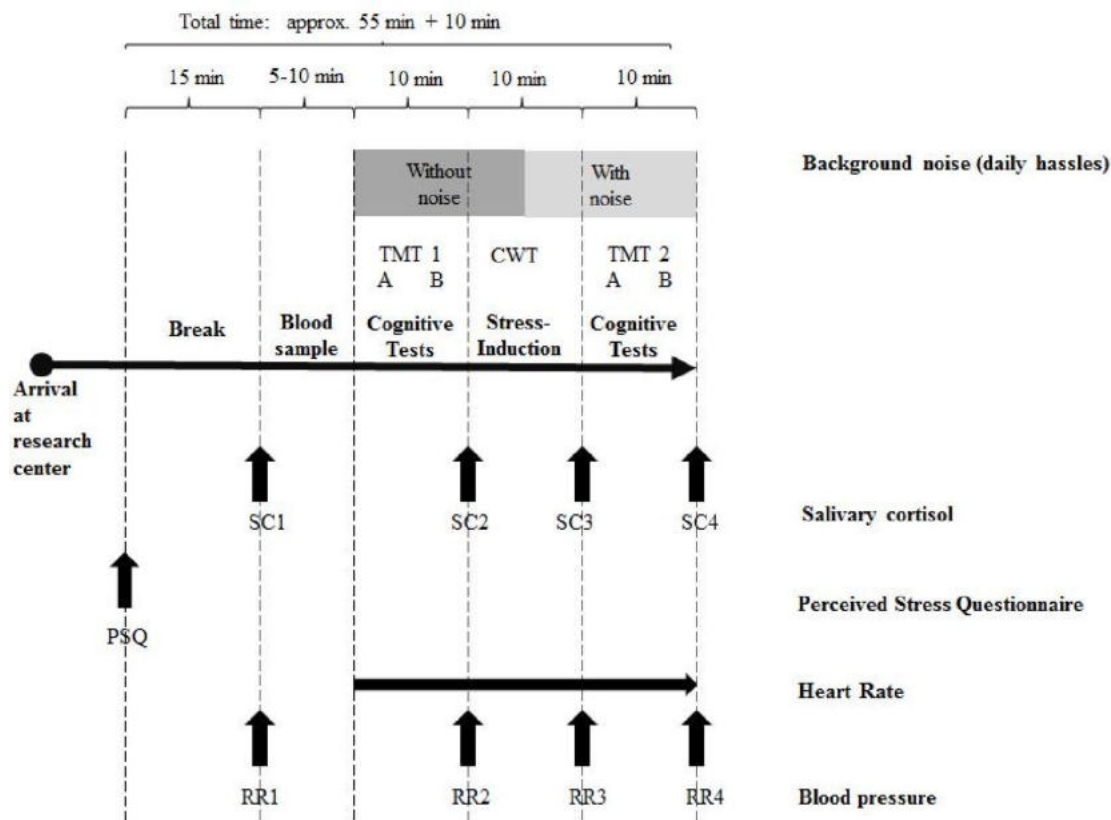
Materials and Methods

The study was approved by the ethical committee of the State Medical Chamber of Lower Saxony, Hannover (Germany). Study participants received oral and written information about the study purpose and its procedures and gave written consent. The study was conducted in the research centre of the Gottfried Wilhelm Leibniz University, Institute for Food Science and Human Nutrition, Hannover, Germany, from February till July 2014. Healthy, non-smoking women and men, aged between 25-60 years and living in the metropolitan area around Hannover were included. The participants were instructed to ingest one capsule of a dietary supplement containing an herbal extract of *Sideritis scardica* (330 mg of an alcoholic aqueous extract), vitamin B₁ (0.55 mg), vitamin B₆ (0.7 mg), Vitamin B₁₂ (1.25 µg), folic acid (100 µg) twice daily (in the morning and in the evening; postprandial) for six weeks. Compliance was acceptable if the number of forgotten capsules did not exceed 10% during the whole intervention period.

Study procedure

At baseline (week 0) and at week 6, the study participants took part in a stress-tolerance tests and various examinations (Figure 1): after arrival, the study participants rested for 15 minutes. Then they filled in a general questionnaire (current health status, general stressors, current stressors, current stress symptoms, anti-stress-measures) and the Perceived Stress Questionnaire (PSQ). Afterwards, computer-based cognitive tests started, i.e. the Trail-Making-Test (TMT) and the Colour-Word-Test (CWT; syn. Stroop-Test). The CWT features two functions: stress-induction (by CW-interference) and measurement of stress-induced impairment of mental performance. During the second half of the CWT, background noise was added as second stressor. Finally, a TMT was conducted again by using the same tasks as the first time, but accompanied by same background noise like during the second half of the CWT. To determine physiological effects of the stress induction, salivary cortisol and blood pressure were assessed in blood samples at four time points per visit. Additionally, heart rate was recorded continuously during TMT and CWT.





Legend: CWT: Colour-Word-Test, TMT: Trail Making Test with Part A and Part B, PSQ: Perceived Stress Questionnaire, SC: Salivary cortisol, RR: Riva Rocci (blood pressure)

Figure 1: Study procedure at baseline (week 0) and after product intake for six-weeks (week 6)

Stress induction, assessment of cognitive function and subjectively perceived stress

Cognitive Performance was assessed by the Trail-Making-Test (TMT) and the Colour-Word-Test (CWT; syn. Stroop-Test). Participants had to link randomly scattered numbers (1-2-3-...-25; TMT-A) or randomly scattered numbers and alphabetic characters (1-A-2-B-...-13; TMT-B). The CWT featured two functions: (i) stress-induction by CW-interference; (ii) to measure a person's selective attention capacity and skills in terms of processing speed (reaction time) and error rate. The CWT requires the participants to identify the name of a color (e.g., "blue," "green," or "red") independently of the color in which the word is printed. Color and Word were shown in CW-interference, i.e. randomly either in CW-congruence (e.g. "red" in red ink) or CW-conflict (e.g. "red" in blue instead of red ink). CW-interference was supplemented by standardized background noise (daily hassles such as pneumatic hammer, alarm-clock, etc.) during the second half of the ten minutes lasting CWT. TMT and CWT are standardized and validated tools of neuropsychological test batteries [18-21].

The validated Perceived Stress Questionnaire (PSQ) [22] was used to assess the subjective chronic stress perception on emotional and cognitive level in the study participants.

Data analysis

Primary hypothesis was that the dietary supplement intake increases the stress tolerance, the mental performance and the visual attention under an acute stress stimulus. Primary target variable was defined as change in stress tolerance in week 6 compared to week 0, measured as a change in mental performance and visual attention before/after an acute stress stimulus (CW-interference, noise). The intended primary parameter (TMT) did not prove to be feasible for measuring stress-relieving or attention-promoting properties of any intervention, because the intra-assay controls did not confirm the assumptions concerning the functionality of this test. In contrast, however, the other test systems (the intended secondary parameter CWT and the PSQ) did prove to be feasible for providing reliable results and were therefore used to assess the product properties, primarily.

SPSS version 22.0 was used for data analysis. Data were described by mean ± SD, median and its 95%-confidence interval (95%-CI). Normally distributed data (all except CWT and TMT) were analysed by t-test for paired samples. Not normal distributed data (CWT and TMT) were analysed by Wilcoxon test for paired samples (p≤0.05).

Results

Table 1 displays general characteristics of the participants. In the questionnaire on individual patterns of stressors, the participants reported that within the family context they can be stressed mostly by bad news (60 %), missing recovery time (52 %), persistent worries (45 %) or conflicts with family members (43 %). Within everyday life, shopping during rush hour (31 %), driving car during rush hour (29%), noise (29%) or crowds (22 %) were the most frequent possible stressors. Time or deadline pressure (70%), severe criticism (48%), conflicts with the chief or colleagues (45 %) were the most frequent possible stressors within the job.

Table 1: General characteristics of the study population

Characteristic		n	%
Gender	Males	24	38
	Females	40	62
		Mean	SD
Age (years)		38.70	10.30
Body Weight (kg)		71.90	13.5
Body Height (m)		1.74	0.12
BMI (kg/m ²)		23.74	3.71

The practice time in the Trail Making Test (TMT), decreased in TMT-A and TMT-B (Table 2) between week 0 and 6, independently of the presence or absence of noise. Also the error rate of both TMT parts with and without noise decreased. However, only the changes in the absence of noise were statistically significant,

except for the error rate of TMT-B without noise. Unexpectedly, noise did not impair the TMT speed but accelerated it. This excluded its suitability for investigating an alleviation of an impairment.

Table 2: Practice time and error rate (median [95%-confidence interval]) in the Trail Making Test part A and B (n=64)

	Practice time (sec)		Error rate (numbers)	
	week 0	week 6	week 0	week 6
Part A without noise	85 [69...109]	61 [51...70]	2 [2...4]	1 [0...1]
	p < 0.001		p = 0.008	
Part A with noise	50 [45...59]	48 [40...56]	1 [0...2]	0 [0...1]
	p = 0.079		p = 0.222	
Part B without noise	60 [54...70]	53 [47...59]	0 [0...0]	0 [0...0]
	p = 0.002		p=0.295	
Part B with noise	56 [49...62]	51 [43...60]	1 [0...2]	0 [0...1]
	p = 0.148		p = 0.139	

In contrast, stress-induced cognitive impairment was provoked in the Colour-Word-Test (CWT) (Table 3).

Table 3: Suitability of colour-word-interference (CW-congruence, CW-conflict) and noise (no, yes) as stressors for inducing impairment of cognitive performance in the CWT. Medians of the changes in reaction time at week 0 are shown.

Stressor	Impairment
Only noise	+ 39 msec
CW-conflict in the presence of noise	+ 27 msec
Only CW-conflict	+ 32 msec
Noise in the presence of CW-conflict	+ 34 msec
Noise and CW-conflict simultaneously	+ 66 msec

After six weeks of dietary supplement intake, the CWT showed a faster reaction time than at baseline in situations of CW-congruence (total) ($p=0.014$), CW-conflict (total) ($p=0.024$), CW-conflict (with noise) ($p=0.001$), CW-congruence (without noise) ($p=0.004$) and on CW-conflict (without noise) ($p=0.017$) (Figure 2). The median total error rate in the CWT significantly decreased from week 0 (6.5 errors) to week 6 (5 errors; $p=0.028$). CWT-changes, differentiated for noise, CW-inference and product intake (Figure 3), showed that product intake resulted in

- (i) a bisection of CW-interference-related impairment of the reaction time in the presence of noise from 27 ms to 13.5 ms,
- (ii) a bisection of noise-related impairment of the reaction time in the presence of CW-conflict from 34 ms to 17 ms,
- (iii) an improvement of the impairment of the reaction time due to combined stress (noise plus CW-conflict) by 14.5 ms from 66 ms to 51.5 ms,
- (iv) despite of the improvement of the reaction time, no increase of the error rate.

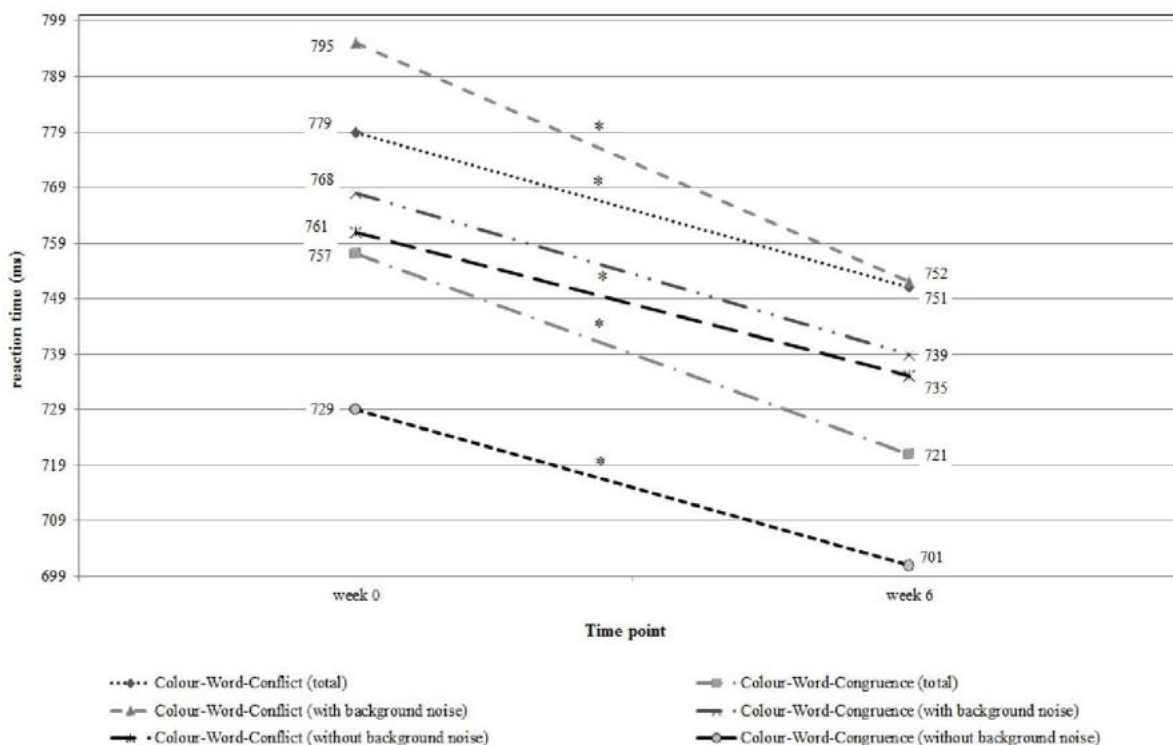


Figure 2: Reaction time (median) in the Colour Word Test with colour-word-conflict or colour-word-congruence with or without background noise at week 0 (n=64) and week 6 (n=64)

* Statistical significant difference between week 0 and week 6 ($p_{wilt} \leq 0.05$)

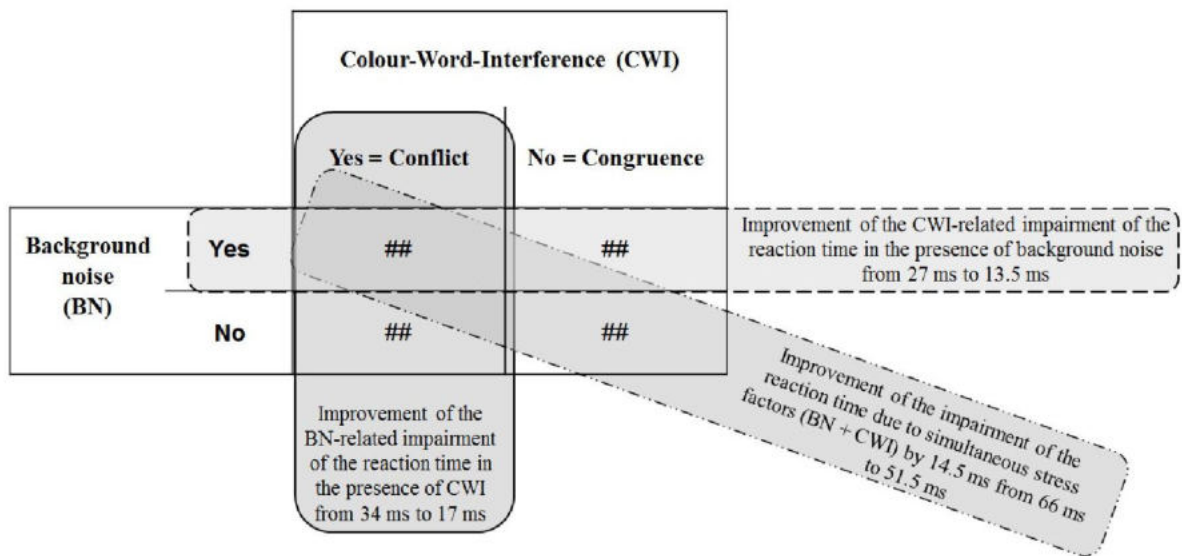


Figure 3: Product effects shown as two-dimensional projections of the three-dimensional constellation of influences to the performance in the Colour-Word-Test (Stroop-test).

The Perceived Stress Questionnaire (PSQ) index decreased significantly from week 0 (2.31 points) to week 6 (2.22 points) ($p=0.005$) (detailed data not shown).

The analysis of several physiological parameters (salivary cortisol, blood pressure, heart rate) did not show changes (Table 4).

Table 4: Physiological stress-related parameters (salivary cortisol, blood pressure and heart-rate) (mean ± SD) at week 0 and week 6 (n=64)

Measurement	1	2	3	4	1	2	3	4
	week 0				week 6			
Salivary cortisol ¹ (µg/dl)	0.58 ± 0.26	0.51 ± 0.24	0.47 ± 0.24	0.47 ± 0.23	0.56 ± 0.24	0.50 ± 0.27	0.49 ± 0.33	0.48 ± 0.32
Blood pressure ¹ (mmHg)	120.8 ± 12.0 : 75.6 ± 10.0	120.9 ± 16.8 : 75.6 ± 9.6	120.0 ± 15.5 : 75.4 ± 8.8	120.5 ± 16.3 : 76.6 ± 10.1	117.6 ± 14.0 : 72.6 ± 9.7	118.3 ± 15.7 : 74.3 ± 10.0	115.9 ± 14.5 : 74.2 ± 9.5	116.1 ± 14.0 : 74.9 ± 9.1
Heart rate ¹ (beats/min)	69.3 ± 8.9	70.4 ± 10.1	69.3 ± 9.0	-	66.8 ± 9.1	68.4 ± 9.0	68.8 ± 9.8	-

¹ All pair-wise comparisons of the single measurements did not differ significantly ($p>0.05$)

The compliance rate of the current study was 80%. Gender-related influences on the compliance rate between men and women were statistically not significant ($p=0.474$).

The safety results were exonerative, i.e. participants reported that no adverse events occurred, and safety laboratory parameters (leukocytes, erythrocytes, thrombocytes, haemoglobin, haematocrit, creatinine, serum glucose, HbA1c, uric acid, ASAT, ALAT, γ-GT, cholesterine, triglycerides, HDL, LDL, HDL/LDL-ratio, CRP) did not change significantly.

Discussion

The study showed evidence in favor of the tested product. The intended primary parameter (TMT) did not prove to be feasible for measuring stress-relieving or attention-promoting properties of any intervention, because the intra-assay controls did not confirm the assumptions concerning the functionality of this test. In contrast,

however, the other test systems (CWT and PSQ) did prove to be feasible for providing reliable results and were therefore used primarily to assess the product properties. CWT reaction time after six weeks product intake was better than at baseline, without increase of the error rate. Stressor-induced, i.e. noise-induced and color-word-interference-induced impairments of CWT reaction times improved upon product intake.

The TMT is a neuropsychological test of visual attention and task switching providing information about visual search speed, scanning, speed of processing, mental flexibility and executive functioning [23]. Concerning the experimental setup we expected an increased practice time and error rate in the second run of the TMT-tests due to stress-induction and ongoing background noise compared to the first TMT run. However, practice time and error rate were reduced in the second TMT-tests. This might be explained by the Yerkes-Dodson rule, whereby performance increases with physiological or mental arousal, but only up to a cut-off point. When levels of arousal become too high, performance decreases [24]. With regard to the current study results this would mean, that the background noise pushes the performance of the study participants instead of acting as a negative stressor. Irrespective of the Yerkes-Dodson law, learning effects as a cause for the observed improvement in conducting TMT-A and TMT-B, could not be excluded as well. Longitudinal studies demonstrated, in particular, learning effects for the TMT-B [25]. Those exercise effects are shown according to intervals of one to six weeks of intervention [26] and, therefore, might be another reason why our study participants reached better TMT results at week 6. Overall, these findings indicate that the intended primary parameter did not prove to be feasible for measuring any intervention's capability to relieve stress-induced impairments (which did not occur). Thus, the intra-assay controls did not confirm the assumptions concerning the functionality of this test.

In contrast, the CWT proved to be suitable for assessing influence on stress-induced impairments (which did occur in the CWT, Table 3). The disadvantage that the CWT was predefined only as secondary variable may be outweighed by the objectivity of the test itself and particularly of the test-intrinsic comparisons. CWT revealed a significant improvement of the reaction times at week 6 in comparison to week 0 in the respective word-colour combinations and stressor-constellations. Also the CWT error rate decreased statistically significantly. The interpretation of the CWT-results indicating that the investigated dietary supplement influenced the executive functions of the participants positively at week 6 in comparison to week 0 may include the following aspects:

- The CWT (Stroop-Test) is used for investigation of brain-activities that are involved in planning, decision-making, and managing real-world interference (e.g. texting and driving) [27].
- *Working memory* is one of the executive functions providing those information which humans need to survive [1]. The better reaction time (shorter overall and alleviated stress-induced impairment) and less errors during the CWT may be explained by a more focused attention on the changing tasks.
- *Cognitive flexibility* is the second component of the human executive function, necessary to get used to the changes of daily life[1]. With regard on better reaction time and less errors, it could be hypothesized that the investigated dietary supplement may have led to a faster and more flexible information processing in the brain.
- *Behaviour inhibition* is the third executive function in humans, necessary to avoid behaviour which inhibits goal-directed action [1]. The intake of the investigated dietary supplement may have caused increased goal-directed attention and behaviour which finally led to less errors and better reaction time during the CWT.

Due to the objectivity of the CWT-assessments, the study results may support the efficacy of the investigated dietary supplement, although a study design without control group is often deemed insufficient for formal proof which can be achieved e.g. by a double-blind randomized controlled trial.

Moreover, the results from the PSQ test suggest that the intake of the product decreases the subjectively perceived chronic stress, although the chronic stress of the study participants could be classified as high at both time points. The significant decrease during the study period is only statistically based and do not show relevant changes of the subjectively perceived chronic stress. As mentioned before, stress is measured subjectively, and could differ individually and according to certain circumstances [28, 29].

The physiological biomarkers salivary cortisol, blood pressure or heart rate did not show a stress-related increase. Missing reactions could be due to individual differences in the physiological stress response [28,29] or due to an only subliminal stress-level induced by the stressors used in our study, not yet intensive enough to be reflected in these biomarkers' response.

With a value of 80%, the compliance was within an acceptable range for studies with dietary supplements. This might be due to the participant's awareness of stress and high believe in the stress-reducing efficacy of the dietary supplement. Interestingly, women who generally seem to be more compliant than men, didn't fulfil this expectation.

Study limitations

Confounding variables could not be excluded. For example, interactions with other stress-reducing strategies cannot be avoided. Nearly all study participants (98%) admitted to use common stress-reducing activities before and/or during the study period such as general stress education (72%), sports and wellness (69%), yoga (39%), massages or physiotherapy (34%), etc., with high variability over time due to trends or currently recommended methods. The high variability did not allow for detailed confounder-analysis, adequately. Subjects were explicitly advised to avoid additional dietary supplements or tea preparations being prescribed for stress-reduction. With the high compliance rate in mind, it could be assumed that the participants abode by the rules. Last, the absence of a placebo group or blinding complicates

the interpretation to what extent the present study results are clearly related to the dietary supplement.

Conclusion

The study results support that the investigated dietary supplement combining an herbal extract of *Sideritis scardica* and selected B-vitamins can alleviate stress-induced impairment of executive functioning in regard to working memory, cognitive flexibility and controlled behavioural inhibition after an intake period of six weeks.

These results may be relevant for individuals who have to solve cognitive tasks in the presence of conflict and/or noise, e.g. in an open-plan office or in complex, potentially hazardous traffic situations during car driving.

Conflict of interest

Study was partly supported by Schaper & Brümmer GmbH & Co. KG, Salzgitter, Germany

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