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Efficacy and Safety of *Crocus sativus* L. in Patients with Mild Cognitive Impairment: One Year Single-Blind Randomized, with Parallel Groups, Clinical Trial

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Abstract. There is evidence to suggest the efficacy of Crocus (saffron) in the management of cognitive decline. This study examined the efficacy of Crocus in patients with amnesic and multi domain MCI (aMCImd). The participants included 17 patients on Crocus and 18 on a waiting list, who were examined with a short neuropsychological battery, MRI 3T, while some patients were examined via 256-channel electroencephalogram (HD-EEG) at baseline and after 12 months. The results showed that patients on Crocus had improved Mini-Mental State Examination scores ($p = 0.015$), while the control group deteriorated. Also, MRI, EEG, and ERP showed improvement in specific domains. This led us to conclude that Crocus is a good choice for management of aMCImd.

Keywords: *Crocus sativus*, electroencephalography, magnetic resonance imaging, mild cognitive impairment, saffron

INTRODUCTION

The continuously growing elderly population is leading to an increased prevalence of age-related disorders, such as mild cognitive impairment (MCI). No conventional or alternative therapy is currently available and approved to treat MCI patients, so it is important to study the benefit of alternative medicines or plants on cognitive and functional performance.

The discovery of new natural compounds with neuroactive properties has gained much attention recently for the management of neurodegenerative diseases. An overview of the preclinical and clinical literature regarding the effects of *Crocus sativus* and its constituents on memory is provided by Pitsikas [1]. Significant atrophy of the hippocampus and entorhinal cortex in magnetic resonance imaging (MRI) have been considered a hallmark of Alzheimer's disease (AD), while hippocampal volume has been frequently considered as a reliable biomarker in AD [2]. Electroencephalography-derived event-related potentials (ERPs), and more specifically the P300

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component, indicate electrical brain activity, and ERPs are considered to be free from cultural and educational influence, so they can give reliable results about the cognitive process [3]. The most commonly used stimulus discrimination task is the auditory oddball.

Our study was designed to follow up MCI patients of amnestic and multiple domains subtype (aMCImd) with and without Crocus for one year.

METHODS

The study participants with cognitive complaints were chosen from the outpatient Memory and Dementia Clinic of 3rd University Department of Neurology, Aristotle University of Thessaloniki. Participants were diagnosed with aMCImd according to the Petersen and Winblad criteria [4] by a consensus of specialized health professionals. Neurological examination, neuropsychological and neuropsychiatric assessment, medical/social history, neuroimaging examination, and ERPs were performed to support the diagnosis of aMCImd.

The initial study sample ($n = 102$) was recruited from February 2013 to February 2014 and all patients finished their last examination in March 2015. Only 35 were examined after 12 months, 17 from the experimental group (patients on Crocus) and 18 from the control group. Participants had to meet inclusion and exclusion criteria, which are used for the clinical studies of MCI. The demographic characteristics of this study (age, gender, and education) are illustrated in Table 1.

The effectiveness of therapeutic condition was evaluated with neuropsychological assessment performed at baseline and after a period of 12 months. All participants were examined at the same time and place and by different psychologists, who were not aware of the classification of the participants. Neuropsychological assessment included a battery of psychometric tests assessing mood (Geriatric Depression Scale, GDS [5]), activities of daily living (Functional Rating Scale of Symptoms of Dementia, FRSSD [6]), behavior (Neuropsychiatric Inventory, NPI [7]), and general cognitive function (Montreal Cognitive Assessment, MoCA [8], and Mini-Mental State Examination, MMSE [9]).

Table 1
Demographic characteristics of the participants ($n = 35$)

	Intervention Group Mean (SD)	Control Group Mean (SD)	p
Education	8.17 (4.91)	10.1 (4.00)	0.17
Gender	Women: 12 Men: 5	Women: 14 Men: 4	0.92
Age	71.47 (6.73)	69.72 (7.33)	0.32

Four patients on Crocus and four patients of control group accepted to be examined by high resolution 3Tesla MRI, using the SPM12 and VBM12 toolbox. The data was analyzed with the VBM12 pipeline and then smoothed with a 12 mm FWHM across every dimension. Three patients on Crocus and three patients of the control group accepted to be examined by a special team before and after 12 months by using an EEG recorder with high density and accuracy. HD-EEG data were collected with the EGI 300 Geodesic EEG system (GES 300) using a 256-channel HydroCel Geodesic Sensor Net (HCGSN) and a sampling rate of 250 Hz (EGI Eugene, OR). Electrodes were positioned according to the '256 HCGSN adult 1.0' montage system. HD-EEG signals were recorded relative to a vertex reference electrode (Cz) and with AFz as the ground electrode.

RESULTS

Statistical analysis was conducted using SPSS v21.0 (IBM Corp., Armork, NY) statistical software. Although there was deterioration in almost all tests in the control group, there was no significant difference in MMSE ($p = 0.17$), NPI ($p = 0.89$), FRSSD ($p = 0.33$), GDS ($p = 1.00$), and MOCA ($p = 0.86$). In participants who were on Crocus, there was improvement in almost all tests but only in MMSE was significant ($p = 0.05$). In the FRSSD, GDS, and MOCA, the improvement was not significant ($p = 0.88$, $p = 0.20$, and $p = 0.12$, respectively). The change in MMSE was positive and significantly higher in Crocus patients after 12 months than in the control group, which had a negative change ($p = 0.01$) (Table 2).

MRI results with the exploratory threshold of the cluster containing a fair amount of voxels (21) were found for case 'a', corresponding to the treatment much more than in controls after the administration of the Crocus and showed a difference in the Temporal_Inf_L, on the AAL Atlas (Table 3). Figure 1 shows the local maxima of the first (a) case of the statistical analysis as denoted by SPM12.

HD-EEG data were analyzed offline and digitally filtered (Butterworth IIR filter: high cut-off 30 Hz; low cut-off 0.1 Hz) using the Net Station 4.3 software (EGI). The continuous HD-EEG signals were

Table 2
Means and standard deviations of intervention and control groups in neuropsychological tests

	Intervention Group M (SD)		Control Group M (SD)		p
	PRE	POST	PRE	POST	
MMSE	27.41 (1.70)	28.18 (1.24)	27.89 (1.84)	27.11 (2.82)	**0.015**
FRSSD	7.059 (14.10)	3.81 (2.48)	4.00 (2.14)	4.94 (4.29)	0.65
MoCA	22.94 (3.00)	23.75 (3.70)	22.81 (2.64)	22.27 (4.23)	0.62
NPI	0.11 (0.33)	3.06 (7.65)	4.10 (8.90)	6.43 (7.55)	0.32
GDS	2.29 (2.87)	3.06 (3.96)	3.06 (3.21)	2.76 (2.84)	0.66

MMSE, Mini-Mental State Examination; FRSSD, Functional Rating Scale of Symptoms of Dementia; MoCA, Montreal Cognitive Assessment; NPI, Neuropsychiatric Inventory; GDS, Geriatric Depression Scale.

Table 3
Structural differences from baseline to 12 months between control and treatment group

	Voxels	T	p	MNI coordinates	Region	Anatomical Position
a	21	14.3415	<0.0001	–57 –54 –15	sROI_MNI_V4	*Temporal_Inf_L*
	9	*10.0496*	<0.0001	*27 54 –15*	*sROI_MNI_V4*	*Frontal_Mid_Orb_R*
b	*3*	*10.9057*	<0.0001	*–40.5 –54 –21*	*sROI_MNI_V4*	*Cerebelum_Crus1_L 2*
c	*4*	*9.0672*	<0.0001	*34.5 54 27*	*sROI_MNI_V4*	*Frontal_Mid_R*
	1	8.2715	<0.0001	–22.5 –63 –25.5	sROI_MNI_V4	*Occipital_Sup_L*

segmented using the task triggers and event stimulus timestamp. In the second assessment, great performance was detected in people who took part in clinical trial N200 ($p = 0.11$), latency N200 ($p = 0.85$), P300 ($p = 0.77$), time spend before activation -latency P300- ($p = 0.01$) (Fig. 2). There was also a significant difference in sound error task of two-tone oddball experiment between the two groups ($p = 0.03$) in the second assessment, with worse performance of people who did not take Crocus.

DISCUSSION

Until today, there was no other study proving that a medication is effective for patients with MCI. The experimental group had better performance and a statistical significant difference in the test results, compared to the MMSE of the control group. Although better performance was detected in other scales, it was not statistically significant, so we only take the MMSE scores into account. These results are similar with those in others studies [10, 11], which used non pharmacological interventions in MCI patients. The results in other studies, confirming the effectiveness of cognitive training in MCI patients, are similar to our results. Apart from the study by Tsolaki et al. [12], these studies have not shown that cognitive training can also improve activities of daily living. There are no other studies with natural products used as medications that result in statistically significant improvements in MCI patients. Herbal medicines have been used in the treatment of dementia but with variable responses. For the abovementioned reason we compare our study results with non-pharmacological interventions. *Crocus sativus* (saffron) may inhibit the progress of dementia and may therefore be useful in AD. Although it contains pharmacological molecules, it is considered as a natural compound, which contains pharmacologically active and important volatile agents [13]. Numerous studies have shown that crocins or crocetins (which are the active ingredients of saffron) are capable of a variety of pharmacologically protective effects, which have been attributed to their antioxidant capacities. To our knowledge, there are no existing studies for efficacy of Crocus in MCI patients.

Moreover, the statistical analysis of the intervention's group results in the MMSE of the first and second assessment indicates better performance of this group after one year, which is very important if we consider that the majority of people with MCI have a progressive deterioration of their cognition during the time, as we notice also in our control group.

Results in MRIs showed a small volume difference on the left inferior temporal gyrus, in favor of the treatment group after 12 months of the Crocus powder administration. Though promising, the correct interpretation is limited by the small number of participants. Left inferior temporal gyrus is close to medial temporal lobe and hippocampus. P300 measures can detect early cognitive decline and show how Crocus can change brain activation and cognitive processing in MCI patients. In our EEG research, we found that even a small amount of MCI patients can be

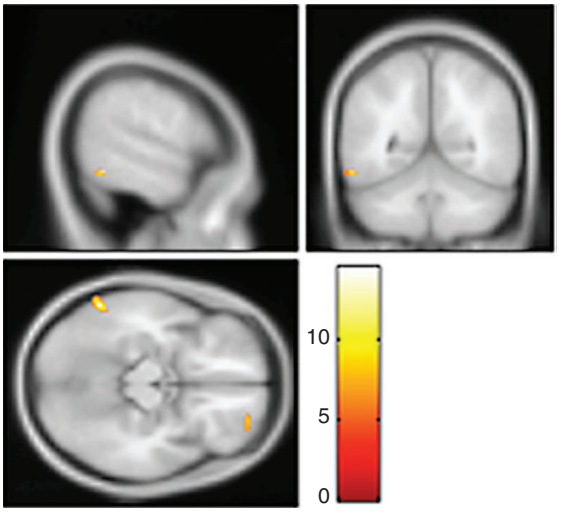

Fig. 1. Global maxima of case a, corresponding to the Temporal_Inf_L region as denoted by the AAL Atlas.

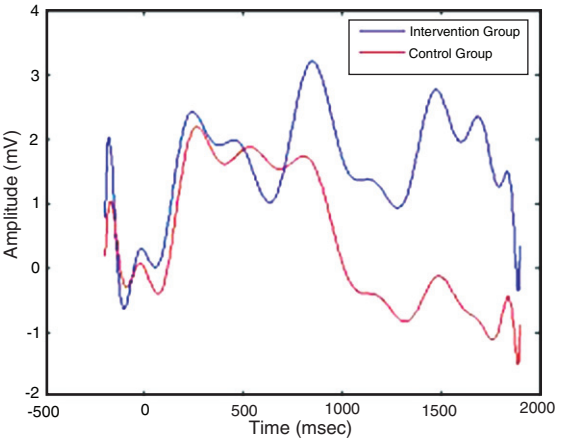

Fig. 2. N200, latency of N200, P300, and latency of P300 in both groups.

improved after the implementation of *Crocus sativus* and we could detect statistical significant differences in latency of P300. In comparison with two previous studies which have demonstrated that the anticholinergic drug scopolamine affects both memory and P300 latency and amplitude in healthy subjects [14], whereas the acetylcholinesterase inhibitor physostigmine restores these P300 changes [15], our study comes to add to the literature that changes in the brain and more specifically in latency of P300 can be detected after Crocus implementation as well.

Why does Crocus have such results in MCI patients? A recent review by Pitsikas [1] showed that *Crocus sativus* crude extracts and purified chemicals in preclinical pharmacological studies have demonstrated that it possess: 1) anti-tumor effects, 2) display anti-nociceptive and anti-inflammatory properties, 3) reduce atherosclerosis and hepatic damage, 4) counteract hyperlipidemia, 5) protect from myocardial injury, and 6) display antihypertensive action. Also it is characterized by 7) inhibitory activity on amyloid-beta aggregation [16] and acetylcholinesterase [17], radical scavenging activity [18], and finally it is safe and effective in mild to moderate AD [19]. The effects exerted by saffron and its components in the aforementioned pathologies were also extensively discussed in different reviews.

Both preclinical and clinical studies suggest that Crocus has a beneficial effect in cognition. The mechanism(s) underlying effects of saffron and its active constituents on cognition is still a matter of investigation. Among the potential mechanisms, the promotion of long-term potentiation, the anti-amyloidogenic activity, its inhibitory action on the AChE activity, their potent antioxidant and anti-inflammatory properties are proposed to explain their action on cognition.

Recently, but also in very old research interested in CNS, active drug discovery projects have been very negligent in using existing knowledge on herbal remedies. Such a situation is surprising if we consider that herbal remedies remain the ultimate hope for many patients with mental health problems.

Hence, in the present study, the activity of a natural product that possesses biological properties, an extract of *Crocus sativus* L. (saffron), grown in Crocos, Kozani (Greece) was studied. As a result, saffron grown in Greece can be used promisingly in functional foods, drinks with antioxidant activity, and in pharmaceutical preparations for their antioxidant activity and probably for the antiaging activity. Saffron can also be used internally with antioxidant properties and in other diseases (e.g., cancer).

Finally, this study has limitations. Being a single-blind, parallel control group pilot study, participants were not randomly assigned to the intervention and control arms and no placebo control was performed. Although the baseline characteristics of the two groups were similar in terms of age, gender, and neuropsychological measures, it is likely that other risk factors were different between the two groups. There are also published phase I and phase II studies with the same design as our study, which examined the efficacy and safety of new molecules [20–24]. All studies on new pharmaceutical products are first tested on a small number of patients, similar to the study by Summers et al. for tacrine [25], the first inhibitor of

cholinesterases. After testing their hypothesis, they conducted a study with more patients. After this first success, we are preparing a large, double-blind randomized study with a greater number of patients.

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