Journal of Ethnopharmacology ■ (■■■■) ■■■–■■■

Contents lists available at SciVerse ScienceDirect

Journal of Ethnopharmacology

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

The effect of *Nigella sativa* Linn. seed on memory, attention and cognition in healthy human volunteers

Muhammad Shahdaat Bin Sayeed [a,b,*], Md. Asaduzzaman [b,c], Helal Morshed [d], Md. Monir Hossain [a], Md. Fahim Kadir [b,c], Md. Rezowanur Rahman [c]

[a] Department of Clinical Pharmacy and Pharmacology, University of Dhaka, Bangladesh
[b] Department of Pharmacy, University of Asia Pacific, Dhanmondi, Dhaka, Bangladesh
[c] Department of Pharmaceutical Technology, University of Dhaka, Bangladesh
[d] Department of Pharmaceutical Chemistry, University of Dhaka, Bangladesh

ARTICLE INFO

Article history:
Received 3 September 2012
Received in revised form
24 April 2013
Accepted 4 May 2013

Keywords:
Nigella sativa Linn. seeds
Neuropsychological tests
Memory test
Attention test
Cognitive test
Toxicity study

ABSTRACT

Background: Experimental evidences have demonstrated that *Nigella sativa* Linn. seed (NS) has positive modulation effects on aged rats with memory impairments, prevents against hippocampal pyramidal cell loss and enhances consolidation of recall capability of stored information and spatial memory in rats. NS has neuroprotective, nephroprotective, lung protective, cardioprotective, hepatoprotective activities as established by previous studies on animals. Several clinical trials with NS on human have also demonstrated beneficial effect.

Aim of the study: The present study was designed to investigate the effects of NS on memory, attention and cognition in healthy elderly volunteers. Furthermore, safety profile of NS was assessed during the nine-week study period.

Methods: Forty elderly volunteers were recruited and divided randomly into group A and group B—each consisting of 20 volunteers. The treatment procedure for group A was 500 mg NS capsule twice daily for nine weeks and Group B received placebo instead of NS in the similar manner. All the volunteers were assessed for neuropsychological state and safety profile twice before treatment and after nine weeks. The neuropsychological tests were logical memory test, digit span test, Rey-Osterrieth complex figure test, letter cancellation test, trail making test and stroop test. Safety profile was assessed by measuring biochemical markers of Cardiac (total cholesterol, triglycerides and high density lipoprotein cholesterol, very low density lipoprotein, low density lipoprotein cholesterol, creatine kinase-MB); Liver (aspartate aminotransferase, alanin aminotransferase, alkaline phosphatase, total protein, albumin, bilirubin) and Kidney (creatinine and blood urea nitrogen) through using commercial kits.

Results: There was significant difference ($p < 0.05$) in the score of logical memory test-I and II, total score of digit span, 30 min delayed-recall, percent score in Rey-Osterrieth complex figure test, time taken to complete letter cancellation test, time taken in trail making test-A and test-B, score in part C of stroop test due to ingestion of NS for nine weeks. There were not statistically significant changes ($p > 0.05$) in any of the biochemical markers of cardiac, liver, kidney function during this nine-week study period.

Conclusions: The current study demonstrates the role of NS in enhancing memory, attention and cognition. Therefore, whether NS could be considered as potential food supplement for preventing or slow progressing of Alzheimer disease needs further investigations. However, study with Alzheimer's patients with large population size for longer period of time is recommended before using NS daily and extensive phytochemical investigations are recommended for novel drug discovery from NS for treating cognitive disorders.

1. Introduction

The seed of *Nigella sativa* Linn. (NS) (Family: Ranunculaceae), commonly known as black seed or black cumin, is employed as a spice and food additive in various parts of the world (Ali and Blunden, 2003). The nutritional and health improving properties of NS are very well known (Ramaa et al., 2006) and NS is considered as a very effective herbal food substance having diversified use in traditional treatment (Salem, 2005) as well as for reducing the risk of various maladies (Butt and Sultan, 2010). The chemical composition of NS is well studied (Ashraf et al., 2006) and the compounds found therein, especially thymoquinone (TQ), carvacol, p-cymene, t-anethol and 4-terpinol have potent

* Corresponding author at: Department of Clinical Pharmacy and Pharmacology, University of Dhaka, Dhaka-1000, Bangladesh. Tel.: +880 2 9664953; mobile: +880 171 3459747; fax: +880 2 9664950.
E-mail address: shahdaat2013@yahoo.com (M.S.B. Sayeed).

0378-8741/$ - see front matter © 2013 Published by Elsevier Ireland Ltd.
http://dx.doi.org/10.1016/j.jep.2013.05.004

antioxidant activities (El Shenawy et al., 2008; Yoruk et al., 2010; Panahi et al., 2011). Several studies have attributed this attenuation of oxidative stress by NS to the free radical scavenging properties of NS and provided evidence of increased expression of antioxidant genes (Ismail et al., 2010). NS decreases hepatic lipid peroxidation and increases activities of catalase, glutathione-S-transferase, adenosine deaminase, myeloperoxidase by normalizing glutathione (GSH) and nitric oxide (NO) levels due to TQ activity (Ilhan et al., 2005; El Gendy et al., 2007; Sogut et al., 2008). TQ has been shown to suppress the ferric-nitriloacetate-induced oxidative stress and prevent oxidative injuries (Khan and Sultana, 2005).

Many clinical studies have reported strong evidence that oxidative stress is involved in the pathogenesis of various cognitive dysfunction including memory impairment, age related dementia, Alzheimer's disease (Liu and Zhang, 2012; Xie et al., 2012; Clausen et al., 2012). Free radical oxidation of GSH and the unsaturated fatty acids is observed in cognitive diseases, e.g., Alzheimer's disease (Ali, 2004). Therefore, the progression of neurodegenerative diseases like Alzheimer's, dementia etc. was found to be inhibited by free radical scavengers and antioxidants as suggested in these cases (Gibson and Huang, 2005; Stuchbury and Münch, 2005). Studies showed that NS has positive modulation effects on aged rats with memory impairments (Azzubaidi et al., 2011b). Another study showed preventive effect of *Nigella sativa* oil against hippocampal pyramidal cell loss (Azzubaidi et al., 2012). Again, chronic oral administration of NS can enhance the consolidation and recall capability of stored information and spatial memory in rats (Jalali and Roghani, 2009).

NS is extensively studied and its safety profile for human consumption and traditional nutritional applications is well-established (Ramadan, 2007). It has neuroprotective (Ezz et al., 2011), nephroprotective (Uz et al., 2008; Yaman and Balikci, 2010; Bayrak et al., 2008), lung protective (Hossein et al., 2008; Tayman et al., 2013), cardioprotective (Ebru et al., 2008), hepatoprotective (Yildiz et al., 2008) activities in different animal models. Other studies have shown beneficial roles of NS on the cardiovascular system (e.g. improvement of heart rate), pancreatic beta-cell (Demir et al.2006; Abdelmeguid et al., 2010); serum cholesterol level (Dahri et al., 2005); serum lipid profile (Kocyigit et al., 2009); the prevention of DNA damage and initiation of carcinogenesis in colonic tissue (Kapoor, 2009) and insulin resistance syndrome (Najmi et al., 2008). Investigation by Tauseef et al. (2009) found normal level of liver, kidney and cardiac enzymes in rats after long-term administration of NS oil.

People in different parts of the world including Bangladesh usually take NS alone or oil of NS with either honey or boiled mint for various health benefits including memory improvement (Sharrif, 2011). In spite of having a long history of folklore use, no study has ever undertaken to assess the effect of NS in the improvement of memory, attention and cognition in human. In the present study, efforts were directed to assess the effectiveness of NS in the enhancement of memory, attention and cognition in healthy elderly human volunteers inspired by the results of some previous studies and reports on memory enhancement or preventive effects of NS on hippocampal pyramidal cell loss conducted on animal models (Jalali et al., 2009; Azzubaidi et al., 2012; Azzubaidi et al., 2011b); safety profile study in rats (Tauseef Sultan et al., 2009) and adult person (Qidwai et al., 2009) as well as other clinical studies in human (Dehkordi and Kamkhah 2008; Tissera et al., 1997; Tissera et al., 2000). Aside from neuropsychological study, the safety profile in healthy human volunteers was also evaluated concurrently by observing the biochemical markers of liver, kidney and cardiac functions. The result of the present investigation will be useful in recommending the utilization of this phytochemically rich nutraceutical (Butt and Sultan, 2010; Ramadan, 2007) in the amelioration of age related cognitive decline and other neurodegenerative disorders affecting memory and cognitive functions.

2. Material and methods

2.1. Participants

Forty seven healthy elderly male volunteers were recruited randomly and finally forty volunteers took part in the experiment till last. Four of the participants found the experiment tiresome and therefore did not participate in the second session. One of the participants was identified as color-blind (which was later included as a criterion for exclusion) and therefore was not eligible to take part in Stroop test. Another two participants stopped taking capsule in the middle of the trials and therefore were not qualified for this experiment. The trial (code: UAP/SBS/NS201201) was carried out in accordance with the Declaration of Helsinki and subsequent revisions (WMADH, 2008). Written informed consent was obtained from each volunteer prior to study. Before the selection of the participants, they were introduced with a complete set of medical health questions to evaluate their health conditions for the suitability of the study. All subjects were interviewed and included in the study if they met the following criteria: (1) no previous neuropathological story, (2) absence of hospitalization for psychiatric illness, (3) no history of drug or alcohol abuse and (4) normal psychomotor development. Those who had diabetes, hypothyroidism, renal disease or malignancy were on thiazide diuretics, b-blockers or corticosteroids were excluded from the study. Those who had been admitted to hospital with a severe illness within the previous 3 months were also excluded. We also excluded those with a known cardiovascular condition (ischemic heart disease, peripheral arterial disease, abdominal aortic aneurysm, and carotid artery disease). All the habitual smokers who usually smoke more than eight cigarettes per day were dropped from the selection procedure of volunteers. Subjects were advised to take standard dietary during study period and were asked for avoidance of caffeine before 12 h of the study. The selection of participants also considers the educational background and body-mass not to differ too much within the selected participants. If adverse events were observed, the intervention with NS was planned to be stopped for that particular individual even though previously it was reported about the safety profile of NS in human (Tissera et al., 2007; Dehkordi and Kamkhah, 2008; Qidwai et al., 2009). The demographic information as well as IQ as estimated from National Adult Reading Scale of the volunteers is presented in Table 1.

2.2. Preparation of Nigella sativa capsules

NS were crushed with the help of mortar and pestle (duration about 60 min). Then these crushed seeds were passed through a stainless steel screen (mesh size #30) (time required: 20 min) and filled into empty hard gelatin capsule shells (size #0) using a manual capsule filling machine (within about 20 min). Each capsule contains 500 mg powdered NS. The quality of seeds was ensured by the way of direct observation. A study team representative lead by Prof. Md. Asaduzzaman purchased the seeds from a known quality vendor personally. The identity was verified by Botanist Mr. Manzur-ul-Kadir Mia. The capsules were manufactured in a local GMP compliant pharmaceutical company (Incepta Pharmaceuticals Ltd, Dhaka-1341, Bangladesh). Husk of isabgol (Psyllium seed husk) were used as placebo and were prepared in similar fashion in similar type of hard gelatin capsule shell.

Please cite this article as: Sayeed, M.S.B., et al., The effect of *Nigella sativa* Linn. seed on memory, attention and cognition in healthy human volunteers. Journal of Ethnopharmacology (2013), http://dx.doi.org/10.1016/j.jep.2013.05.004

Table 1
Demographic information of the Volunteers.

Group	[a]Age(Year)	[a]Weight (Kg)	[a]Height (Meter)	[a]Education (Year Completed)	[a,b]BMI (Kg/m^2)	[c]Estimated IQ
Group-A	55.8 ± 0.57	67.30 ± 0.77	1.63 ± 0.013	14.4 ± 0.302	24.77 ± 0.34	105.98 ± 0.64
Group-B	55.9 ± 0.65	67.85 ± 1.00	1.65 ± 0.018	13.5 ± 0.366	24.55 ± 0.18	105.56 ± 0.67

[a] Mean ± Standard Error.
[b] BMI= Body Mass Index.
[c] IQ was estimated from National Adult Reading Scale.

2.3. Treatment procedure

The trial was conducted over a nine week period. The volunteers were randomized into two groups using a system of computer-generated table of random numbers. One group (group A) received two 500 mg capsules once daily after dinner for nine weeks. The second group (group B) received placebo for the same period of time in the similar fashion as in case of NS capsule. All the volunteers were assessed for base-line data before the administration of the first dose of either NS or placebo. The assessment was done to measure the condition of memory, attentiveness and cognition. For all the volunteers, the sequences of the tests administered were constant. To estimate health conditions during the study period, biochemical markers of cardiac (total cholesterol, triglycerides and high density lipoprotein cholesterol, very low density lipoprotein, low density lipoprotein cholesterol, creatine kinase-MB); liver (aspartate aminotransferase, alanin aminotransferase, alkaline phosphatase, total protein, albumin, bilirubin) and kidney (creatinine and blood urea nitrogen) and blood pressure were measured. After collecting the data for base-line assessment, each participant received the capsules of NS or placebo. The dose of NS was adjusted after literature search in Unani Pharmacopoeia of India (1–2 g), Ayurvedic Pharmacopoeia of India (1–3 g), Siddha Pharmacopoeia of India (0.5–4 g), the study on the effect of NS on hypertension conducted by Dehkordi and Kamkhah (2008), and the safety and efficacy profile of NS studied by Qidwai et al. (2009) in human. All of the volunteers in the current study were assessed for all the parameters measured at base-line and ninth week. The assessment was conducted by investigators who were blind (except principal investigator who knew the codes of group only and he did not participate in data collection procedure) about the NS or placebo. All participants were also blind about NS or placebo and the code was not broken before the end of the assessment of the last subject. All participants were informed to call the study center if he experienced with any adverse effects during the study. Those who would miss the doses for more than one day were planned to be excluded. All the participants were contacted over phone with one day interval in order to remind them about their doses and therefore there was very little chance of missing the doses for more than one day. It was suggested to take capsule before sleep at night and if someone was found that he had not taken capsules, he was asked to take immediately.

2.4. Test of memory

2.4.1. Logical memory

Logical memory test was used to measure immediate memory and delayed memory (Wechsler, 1997). In the Logical Memory, a subtest of the Wechsler Memory Scale, (Wechsler, 1997) the volunteers were told a brief story and then they were asked to retell the story twice: once immediately upon hearing the story (Logical Memory I, LM-I), and a second time after a 30-min delay (Logical Memory II, LM-II). During examination, story elements the participants used in each of their retellings were noted. The score was then calculated by counting the number of elements used in his retelling.

2.4.2. Digit span

The Digit Span Test (DST) (subtest of the Wechsler Intelligence Scale) (Wechsler, 1987) was used to assess simple verbal working memory (Wechsler, 1987). The test consists of two parts, digits forward (DSTF) and digits backward (DSTB). Strings of digits were read aloud (e.g., 2 4 7) to the participants at a rate of one digit per second (each string increasing in length from three to nine digits in forward test and two to eight digits in backward test). After every string, the participant was asked to repeat the string. Each subtest (forward and backward) was stopped when a participant incorrectly reproduced two successive strings or when a full digit number has been successfully repeated. The total score of the digit span test (DSTT) is the sum of the maximal digit numbers that the participant can recall from forward and backward testing.

2.4.3. Rey-Osterrieth complex figure test

Rey-Osterrieth Complex Figure Test (ROCFT) was used to assess visual-spatial constructional ability and visual memory (Strauss et al., 2006). The participant was first instructed to copy a complex figure and then initial drawing had been removed and he was asked to draw the figure again. The scores of copying the figures were documented twice: immediately after observation (ROI) and after 30 min (30-min delayed-recall (ROD)). Scoring system was based on the number of items correctly and incorrectly identified on the recognition task.

2.5. Attention test

2.5.1. Letter cancellation test

Letter Cancellation Test (LCT) used for assessing visual search, scanning and attention (Benton, 1968) consists of rows of letters randomly interspersed with a designated target letter. The participant was asked to cross out all the target letters. Performance was scored according to the number of correct responses (LCTC) and the time taken to complete the test (LCTT).

2.5.2. Trail making test

Trail Making Test (TMT) (Reitan and Wolfson, 1985) provides information on visual search, scanning, speed of processing, attention, mental flexibility, and executive functions (Reitan and Wolfson, 1985). TMT, in this investigation, consists of two parts—TMTA and TMTB. TMTA requires an individual to draw lines sequentially connecting 25 encircled numbers distributed on a sheet of paper. Task requirements are similar for TMTB except the fact that the person must alternate between numbers and letters (e.g., 1, A, 2, B, 3, C, etc.). The score on each part represents the amount of time required to complete the task.

Please cite this article as: Sayeed, M.S.B., et al., The effect of *Nigella sativa* Linn. seed on memory, attention and cognition in healthy human volunteers. Journal of Ethnopharmacology (2013), http://dx.doi.org/10.1016/j.jep.2013.05.004

2.6. Cognitive test

2.6.1. Stroop test

The Stroop Color-Word Test-Victoria version (VST) (Regard, 1981) used to measure of selective attention and cognitive flexibility which was originally developed by Stroop (Stroop, 1935). The test has three parts. In Part D (Dots) [STD], the participant was asked to tell the names of the colors of 24 dots printed in blue, green, red or yellow as quickly as possible. In Part W (Words) [STW] is similar to Part D, except that the dots are replaced by common words printed in lowercase letters. The participant was asked to tell the name of the colors in which the stimuli are printed, and to disregard their verbal content. Part C (Colors) [STC] is similar to Parts D and W, but here the colored stimuli were the color names "blue, green, red, and yellow" printed in lowercase so that the print color never corresponds to the color name. For each part, the time to complete and the number of errors were recorded. Spontaneous corrections were scored as correct. A ratio index of interference (Part C/Part D) [STI] was also counted. (MacLeod, 1991; Graf et al., 1995, Verhaeghen and De Meersman, 1998).

Parallel versions of the LM-I, LM-II, DST, ROCFT, LCT, TMT, GPT and Stroop test are available; in the present study, these were used to minimize a learning effect between the two study conditions in two groups. Throughout the study, all of the tests were performed in a pre-determined fixed sequence.

2.6.2. Cardiac function assessment

The levels of total cholesterol (TC), triglycerides (TG) and high density lipoprotein cholesterol (HDL) and creatine kinase-MB (CK-MB) in the serum were determined by enzymatic colorimetric methods using commercial kits (Linear Chemicals Ltd, Spain). Very low density lipoprotein cholesterol (VLDL) was calculated as TG/5. Low density lipoprotein cholesterol (LDL) levels were calculated using Friedewald's formula (Friedewald et al., 1972):

$$LDL = TC - HDL - TG/5$$

The atherogenic index (A.I.) was calculated using the following formula:

$$(A.I.) = (VLDL + LDL)/HDL$$

2.6.3. Liver function assessment

The functional state of the liver was determined by estimating the biochemical parameters such as Aspartate aminotransferase (AST/GOT), Alanin aminotransferase (ALT/GPT), Alkaline phosphatase (ALP), total protein (TP), Albumin, Bilirubin through using commercial kits (Linear Chemicals Ltd, Spain).

2.7. Kidney function assessment

Creatinine and blood urea nitrogen (BUN) were determined by using commercial kits (Linear Chemicals Ltd, Spain) to assess kidney function.

Systolic and diastolic blood pressure as well as the weight of the volunteers was measured during the study period.

2.8. Statistical analysis

The results were analyzed independently for each test. Repeated measures MANOVA test was conducted to test intervention effect of NS on all the parameters in participant over time using IBM SPSS Statistics 19. $p < 0.05$ was considered statistically significant.

3. Result

3.1. Test of memory

The results of logical memory test, DST and ROCFT for the effect of NS on memory are presented in Table 2. The result showed that there was significant difference between intervention and control group on memory over time, $F(7, 32)=8.72$, $p=0.000$, $\eta2=0.656$. Univariate tests also indicated there was NS effect on all of the tests $F(1, 38)=10.13$, $p=0.003$, $\eta2=0.211$ for LM-I, $F(1, 38)=3.99$, $p=0.049$, $\eta2=0.095$ for LM-II, $F(1, 38)=4.50$, $p=0.040$, $\eta2=0.106$ for DSTF, $F(1, 38)=7.30$, $p=0.010$, $\eta2=0.161$ for DSTT, and $F(1, 38)=2.52$, $p=0.000$, $\eta2=0.372$ for ROD except DSTB [$F(1, 38)=1.806$, $p=0.187$, $\eta2=0.045$] and ROI [$F(1, 38)=0.29$, $p=0.59$, $\eta2=0.008$].

3.2. Attention test

Attention test (LCT and TMT) results are presented in Table 2. The result shows that there was significant difference between group A and group B on attention over time, $F(4, 35)=12.011$, $p=0.000$, $\eta2=0.579$. Univariate tests also indicated there was NS effect on all of the tests of attention, $F(1, 38)=6.424$, $p=0.015$, $\eta2=0.145$ for LCTT, $F(1, 38)=11.199$, $p=0.002$, $\eta2=0.228$ for TMTA, $F(1, 38)=34.281$, $p=0.000$, $\eta2=0.474$ for TMTB except LCTC [$F(1, 38)=0.496$, $p=0.485$, $\eta2=0.013$].

3.3. Cognitive test

The results of Stroop test (Table 2) show that there was significant difference between group A and group B on cognition over time, $F(4,35)=6.912$, $p=0.000$, $\eta^2=0.441$. Univariate tests also indicated that there was effect of NS on all of the different parts of Stroop test, $F(1, 38)=5.46$, $p=0.025$, $\eta^2=0.126$ for STD, $F(1, 38)=20.295$, $p=0.000$, $\eta^2=0.348$ for STC, $F(1, 38)=2.203$, $p=0.046$, $\eta^2=0.055$ for STI except STW [$F(1, 38)=2.265$, $p=0.141$, $\eta^2=0.056$].

3.4. Cardiac, liver and kidney function assessment

There were no statistically significant changes ($p > 0.05$) in any of the biochemical markers of cardiac, liver and kidney function during this study period. Blood pressure and body weight of the volunteers were also not changed significantly ($p > 0.05$) (Table 3). In case of heart markers, there was no significant difference between group A and group B over time, $F(7, 32)=1.650$, $p=0.157$, $\eta^2= 0.265$. Univariate tests also indicated there was no intervention effect on individual taking NS or placebo, $F(1, 38)=1.555$, $p=0.220$, $\eta2=.039$ for Cholesterol, $F(1, 38)=0.003$, $p=0.959$, $\eta2=.000$ for HDL, $F(1, 38)=3.047$, $p=0.089$, $\eta2=.074$ for triglyceride-cholesterol, $F(1, 38)=0.481$, $p=0.492$, $\eta2=.012$ for VLDL-cholesterol, $F(1, 38)=1.082$, $p=0.305$, $\eta2=.028$ for LDL-cholesterol, $F(1, 38)=0.247$, $p=0.622$, $\eta2=.006$ for Atherogenic Index, $F(1, 38)=0.008$, $p=0.930$, $\eta2=.000$ for CK-MB. Similar is the case for liver markers: $F(6, 33)=0.855$, $p=0.538$, $\eta2=0.135$. Univariate tests also indicated that there was no intervention effect on individual taking NS or placebo, $F(1, 38)=0.846$, $p=0.364$, $\eta2=.022$ for AST/GOT, $F(1, 38)=0.853$, $p=0.362$, $\eta2=.022$ for ALT/GPT, $F(1, 38)=0.901$, $p=0.348$, $\eta2=.023$ for alkalanine phosphatase, $F(1, 38)=0.225$, $p=0.638$, $\eta2=.006$ for total protein, $F(1, 38)=4.686$, $p=0.057$, $\eta2=.110$ for albumin, $F(1, 38)=2.901$, $p=0.097$, $\eta2=.071$ for total bilirubin. Kidney markers were also similar to former two heart and liver markers, $F(2, 37)=0.025$, $p=0.975$, $\eta2=0.001$. Univariate tests also indicated that there was no intervention effect on individual taking NS or placebo, $F(1, 38)=0.013$, $p=0.911$, $\eta2=0.000$ for creatinine,

Please cite this article as: Sayeed, M.S.B., et al., The effect of *Nigella sativa* Linn. seed on memory, attention and cognition in healthy human volunteers. Journal of Ethnopharmacology (2013), http://dx.doi.org/10.1016/j.jep.2013.05.004

Table 2
Neuropsychological Test.

	Neuropsychological Tests		Group A(n=20 and values are expressed as Mean ± SE)		Group B(*n*=20 and values are expressed as Mean ± SE)		p
			Baseline	Nine weeks	Baseline	Nine weeks	
Memory Test	Logical Memory	Logical Memory-I	9.25 ± 0.38	10.50 ± 0.33[a]	9.65 ± 0.28	9.90 ± 0.25	0.003
		Logical Memory-II	7.80 ± 0.24	8.95 ± 0.29[a]	7.80 ± 0.23	8.10 ± 0.18	0.049
	Digit Span	Forward (DSTF)	3.95 ± 0.14	4.30 ± 0.13	4.05 ± 0.11	4.00 ± 0.15	0.040
		Backward (DSTB)	3.15 ± 0.08	3.45 ± 0.11	3.30 ± 0.13	3.35 ± 0.11	0.187
		Total (DSTT)	7.10 ± 0.16	7.75 ± 0.14[a]	7.35 ± 0.17	7.30 ± 0.16	0.010
	Rey-Osterreitlz Complex Figure Test	Time to copy (ROI)[a]	29.25 ± 0.65	30.15 ± 0.62	29.95 ± 0.58	30.55 ± 0.41	0.590
		30 min Recall (ROD)	12.45 ± 0.59	15.05 ± 0.56[a]	13.60 ± 0.59	14.35 ± 0.42	0.000
Attention Test	Letter Cancellation Test	Correct Response (LCTC)	116.75 ± 0.36	117.05 ± 0.41	116.30 ± 0.25	117.10 ± 0.40	0.485
		Time (second) (LCTT)	165.20 ± 1.39	159.90 ± 1.74[a]	164.85 ± 1.17	163.70 ± 1.13	0.015
	Trail Making Test (TMT)	TMTA (second)	94.45 ± 1.69	88.50 ± 1.84[a]	95.10 ± 1.79	93.60 ± 1.80	0.002
		TMTB (second)	311.20 ± 3.07	300.00 ± 3.25[a]	310.05 ± 2.44	309.50 ± 2.09	0.000
Cognition Test	Stroop Test[b]	Part D-Time (Error)	24.85 ± 0.56 (0)	23.20 ± 0.59 (0)	23.75 ± 0.62 (0)	23.05 ± 0.65 (0)	0.025
		Part W-Time (Error)	25.40 ± 0.69 (0.20 ± 0.09)	23.60 ± 0.61 (0.10 ± 0.07)	25.10 ± 0.64 (0.20 ± 0.92)	23.90 ± 0.54 (0.10 ± 0.69)	0.141
		Part C-Time (Error)	37.20 ± 0.68 (0.90 ± 0.22)	33.25 ± 0.91[a] (0.45 ± 0.11)	36.35 ± 0.74 (0.90 ± 0.22)	34.95 ± 0.80 (0.70 ± 0.15)	0.000
		Interference Ratio(PartC/PartD)	1.51 ± 0.03	1.44 ± 0.04	1.55 ± 0.04	1.54 ± 0.05	0.046

SE= Standard Error of Mean.
[a] statistically significant in comparison to baseline data where $p < 0.5$.
[b] The unit of time was second.

Table 3
Cardiovascular, Liver, Kidney markers and other Parameters.

	Markers	Group A (*n*=20 and values are expressed as Mean ± SE)		Group B (*n*=20 and values are expressed as Mean ± SE)		p
		Baseline	After nine weeks	Beginning	After nine weeks	
Cardiac	Total Cholesterol (mmol/L)	3.85 ± 0.05	3.83 ± 0.04	3.91 ± 0.03	3.93 ± 0.02	0.220
	HDL cholesterol (mmol/L)	2.94 ± 0.05	2.94 ± 0.04	2.90 ± 0.08	2.90 ± 0.07	0.959
	Triglyceride (TG) (mmol/L)	1.31 ± 0.008	1.30 ± 0.007	1.29 ± 0.006	1.30 ± 0.005	0.089
	VLDL-cholesterol (mmol/L)	0.26 ± 0.0014	0.26 ± 0.0012	0.26 ± 0.001	0.26 ± 0.0011	0.492
	LDL cholesterol (mmol/L)	0.65 ± 0.07	0.63 ± 0.06	0.75 ± 0.07	0.69 ± 0.06	0.305
	Atherogenic Index (A.I.)	0.32 ± 0.03	0.31 ± 0.02	0.37 ± 0.04	0.37 ± 0.03	0.622
	[b]CK-MB (μkat/L)	0.25 ± 0.006	0.25 ± 0.004	0.25 ± 0.008	0.25 ± 0.008	0.930
Liver	[a]AST/GOT (μkat/L)	0.233 ± 0.006	0.23 ± 0.004	0.23 ± 0.004	0.235 ± 0.005	0.364
	[a]ALT/GPT (μkat/L)	0.24 ± 0.005	0.24 ± 0.003	0.24 ± 0.003	0.24 ± 0.006	0.362
	[a]Alkalanine phosphatase (μktal/L)	2.59 ± 0.054	2.57 ± 0.046	2.57 ± 0.046	2.57 ± 0.028	0.348
	Total protein (g/L)	72.12 ± 0.31	71.84 ± 0.29	71.84 ± 0.26	72.22 ± 0.26	0.638
	Albumin (g/L)	40.30 ± 0.22	40.30 ± 0.22	40.09 ± 0.21	40.51 ± 0.21	0.057
	Total Bilirubin (μmol/L)	11.72 ± 0.10	11.82 ± 0.07	11.82 ± 0.08	11.89 ± 0.07	0.097
Kidney	Creatinine (μmol/L)	80.91 ± 0.69	81.05 ± 0.43	81.91 ± 0.49	82.00 ± 0.34	0.911
	BUN (mmol/L)	4.26 ± 0.07	4.23 ± 0.05	4.15 ± 0.07	4.11 ± 0.04	0.830
Others	Systolic BP (mm Hg)	119.50 ± 0.75	119.35 ± 0.63	120.55 ± .72	120.75 ± .84	0.773
	Distolic BP (mm Hg)	80.95 ± 0.70	78.95 ± 0.70	79.15 ± 0.73	79.75 ± 0.78	0.090
	Weight (Kg)	68.20 ± 0.72	68.30 ± 0.62	67.85 ± 1.00	67.95 ± 0.94	1.000

SE= Standard Error of Mean. $p < 0.05$ was considered statistically significant. No parameters in the table had $p < 0.05$.
[a] Measured at 30 °C.
[b] Measured at 37 °C.

F (1, 38)=0.047, p=0.830, $\eta 2$=0.001 for blood urea nitrogen. NS did not have any effect on body weight, systolic and diastolic blood pressure over time, F (1, 38)=0.000, p=1.000, $\eta 2$=0.000 for body weight over time, F (1, 38)=0.085, p=0.773, $\eta 2$=0.002 for systolic blood pressure and F (1, 38)=3.019, p=0.090, $\eta 2$=0.074 for diastolic blood pressure.

4. Discussion

The present study was investigated to assess the effect of NS on memory, attention and cognition in elderly human subjects. The times to copy a complex figure in ROCFT, number of correct response in LCT, the score of Part D and Part W in Stroop test

Please cite this article as: Sayeed, M.S.B., et al., The effect of *Nigella sativa* Linn. seed on memory, attention and cognition in healthy human volunteers. Journal of Ethnopharmacology (2013), http://dx.doi.org/10.1016/j.jep.2013.05.004

were not significant after treating with NS or placebo for nine weeks. Other tasks namely- LM-I, LM-II in logical memory test; 30 min delay recall score in ROCFT, time taken to cancel all the letters in LCT; TMT-A, TMT-B in trail making test - were relatively complex (except TMT-A) and the values become significantly different due to NS intake. On the other hand, in case of group B, these values are non-significantly different after nine weeks of placebo intake.

In this current study we used NS which contains different compounds having different effects in different parts of the body in different extent. Therefore the exact mechanism of positive modulating effect of NS (a food supplement with various types of ingredients) is difficult to explain what could have been done easily in case of study with one compound. Previously NS has been reported to have anti-inflammatory (Al-Ghamdi, 2001; Cheh et al., 2009), antioxidant (Burits and Bucar, 2000), anticholinesterase (Yassin, 2005) properties. It is known that decline of memory and cognition capacity is related to inflammatory process (Rogers et al., 1996). After collecting relevant information, Wyss-Coray and Mucke (2002) proposed that inflammatory process might trigger abnormal accumulation of ubiquitinated proteins which might lead to neurodegeneration. NS might play a role in this process or revert it but needs further investigations for confirmation. The study by El Sherbiny et al. (2003), Eun et al. (2008) reported about the association of memory impairment in animal model with increased oxidative stress within the brain. Since oxidative stress is characterized by an imbalance in radical production of reactive oxygen species (ROS) and antioxidative defense, both are considered to have a major role in the process of age-related neurodegeneration and cognitive decline (Gella and Durany, 2009) and therefore substance like NS which have antioxidant properties might prevent further neurodegeneration and play role in memory retention. It might be suggested that the amelioration of the memory, cognition and attentiveness in NS treated elderly human was due to anticholinesterase property of NS (Yassin, 2005). The crushed seeds of *Nigella sativa* L. might inhibit breakdown of acetylcholine resulting in returning the neurotransmitter acetylcholine to normal level and preventing further degeneration of neurons- and hence slowing the degeneration of cognitive ability. However, we hypothesize that NS can modulate the neuronal activity of hippocampus of basal forebrain, prefrontal area, premotor area and/or primary motor area of the frontal lobe of cerebral cortex and thereby enhance the cognitive power and memory. But a thorough research is required to elucidate the molecular mechanism and site of actions of NS.

We also investigated the gross safety profile of NS in human elderly volunteers during the study period. The serum levels of TG, TC, HDL, LDL, A.I. and CK-MB did not change significantly in both of the groups after either treated with NS for four weeks or after nine weeks of treatment with NS and placebo. These results suggest that NS possess no cardiovascular toxicity during the study period of nine weeks. The key hepatic enzymes such as AST/GOT, ALT/GPT and ALP as well as serum level of TP, albumin and bilirubin did not change significantly ($p > 0.05$). Stability of these hepatic enzymes suggests that the treatment with NS preserves the stability of liver cell membrane and biliary function. The stability of TP, albumin and bilirubin levels suggests the stabilization of endoplasmic reticulum leading to normal protein synthesis. The stability of these biomarkers leading to hepatic organ integrity, suggests a wide margin of safety for tested doses of NS during the study period. The serum concentrations of creatinine and BUN did not change significantly in both of the groups during this nine weeks treatment with NS and placebo suggesting the normal functioning of nephrons and stabilization of glomerular filtration rate (GFR) during the study period. We documented weight variation during the study period and could not find any difference among the groups. Similar result was found in case of systolic and diastolic pressure of the volunteers during the study period.

However, the results of gross toxicological study by evaluating different biochemical markers in the current study indicate cardiac, hepatic and renal safety profile of NS during this study period but this safety profile could not be used as a reference as it was a short term study and the sample size was also small. A thorough safety and toxicological study of NS in human model is recommended before using NS extensively. In this study, we had smaller number of samples and therefore proposing its use grossly in for clinical purposes and its use as cognitive enhancers would require more investigations with higher number of healthy population as well as with elderly people with cognitive disorders. On the other hand, whether NS could be a potential source for developing new drugs for treating Alzheimer's disease needs extensive study which could be done after isolating different compounds found therein and conducting study with those extensively. Therefore we propose extensive study with NS for these purposes.

Uncited references

(Ibrahim et al., (2008); Khanna et al., (1993); Kyllonen (1993); Nelson, Willison (1991); Sultan et al., (2012); Viña et al., (2004); $author1$ et al., (World Medical Association Declaration of Helsinki)).

Acknowledgment

The *Nigella sativa* L. seeds were bought and quality of the seeds was assured by Md. Asaduzzaman, Asst. Prof. in the Department of Pharmacy, University of Asia Pacific, Dhaka, Bangladesh. The seeds were identified by Botanist Mr. Manzur-ul-Kadir Mia, Principal Scientific Officer and Consultant of Bangladesh National Herbarium, Dhaka. The capsules were prepared by Md. Rezowanur Rahman, Senior Executive Officer, R & D F, Incepta Pharmaceuticals Ltd, Dewan Idris Road, Bara Rangamatia, Zirabo, Savar, Dhaka-1341, Bangladesh. Other than providing the capsule filling machine and other technical assistance, Incepta Pharmaceuticals did not have any involvement with this project. Senior psychologist Dr. Monowara Parveen Jahangiri of Dhaka *Shishu* (Children) Hospital, Dhaka, Bangladesh gave important suggestions regarding preparation and administration of different modules of Neuropsychological tests used in this experiment. Dr. Mahmudul Hasan Edison, government registered physician supervised blood aspiration process from the volunteers The study center was located at Shahjahan Monjil, House No.# 08, Road No. 4A, Dhanmondi R/A, Dhaka-1209, Bangladesh. We appreciate the important suggestions by A G M Mostofa, Current PhD student at the University of Texas, USA during the entire investigation.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.jep.2013.05.004.

References

Abdelmeguid, N.E., Fakhoury, R., Kamal, S.M., Al Wafai, R.J., 2010. Effects of *Nigella sativa* and thymoquinone on biochemical and subcellular changes in pancreatic β-cells of streptozotocin-induced diabetic rats. Journal of Diabetes 2, 256–266.

Al-Ghamdi, M.S., 2001. The anti-inflammatory, analgesic and antipyretic activity of *Nigella sativa*. Journal of Ethnopharmacology 76, 45–48.

Ali, B.H., 2004. The effect of *Nigella sativa* oil on gentamicin nephrotoxicity in rats. The American Journal of Chinese Medicine 32, 49–55.

Ali, B.H., Blunden, G., 2003. Pharmacological and toxicological properties of *Nigella sativa*. Phytotherapy Research 17, 299–305.

Ashraf, M., Ali, Q., Iqbal, Z., 2006. Effect of nitrogen application rate on the content and composition of oil, essential oil and minerals in black cumin (*Nigella sativa* L.) seeds. Journal of the Science of Food and Agriculture 86, 871–876.

Azzubaidi, M.S., Saxena, A.K., Talib, N.A., Ahmed, Q.U., 2011a. Preventive effect of *Nigella sativa* oil against hippocampal pyramidal cell loss induced by permanent bilateral common carotid artery occlusion in rats. In: International Health Conference IIUM 2011, 7 - 8 December 2011, Kuantan, Pahang. Available online at ⟨http://irep.iium.edu.my/10201/⟩, access date: 14 July, 2012.

Azzubaidi, M.S., Talib, N.A., Saxena, A.K., Ahmed, Q.U., 2011b. Mnemonic effects of Fixed Oil of Black Cumin (*Nigella sativa*) seeds on aged rats with memory impairment. In: Malaysia-Australia Research Colloquium on Exercise, Nutrition, Health and Wellness, 19th -21st september., Penang, Malaysia. available on ⟨http://irep.iium.edu.my/4350/⟩, access date 14 July, 2012.

Bayrak, O., Bavbek, N., Karatas, O.F., Bayrak, R., Catal, F., Cimentepe, E., Akbas, A., Yildirim, E., Unal, D., Akcay, A., 2008. *Nigella sativa* protects against ischaemia/reperfusion injury in rat kidneys. Nephrology Dialysis Transplantation 23, 2206–2212.

Benton, A.L., 1968. Differential behavioral effects in frontal lobe disease. Neuropsychologia 6, 53–60.

Burits, M., Bucar, F., 2000. Antioxidant activity of *Nigella sativa* essential oil. Phytotherapy Research 4, 323–328.

Butt, M.S., Sultan, M.T., 2010. *Nigella sativa*: reduces the risk of various maladies. Critical Reviews in Food Science and Nutrition 50, 654–665.

Cheh, N., Chipitsyna, G., Gong, Q., Yeo, C.J., Arafat, H.A., 2009. Anti-inflammatory effects of the *Nigella sativa* seed extract, thymoquinone, in pancreatic cancer cells. HPB (Oxford) 11, 373–381.

Clausen, A., Xu, X., Bi, X., Baudry, M., 2012. Effects of the Superoxide Dismutase/Catalase Mimetic EUK-207 in a Mouse Model of Alzheimer's Disease: Protection Against and Interruption of Progression of Amyloid and Tau Pathology and Cognitive Decline. Journal of Alzheimer's Disease 30, 01–03.

Dahri, A.H., Chandiol, A.M., Rahoo, A.A., Memon, R.A., 2005. Effect of *Nigella sativa* (kalonji) on serum cholesterol of albino rats. Journal of Ayub Medical College Abbottabad 17, 72–74.

Dehkordi, F.R., Kamkhah, A.F., 2008. Antihypertensive effect of *Nigella sativa* seed extract in patients with mild hypertension. Fundamental & Clinical Pharmacology 22, 447–452.

Demir, H., Kanter, M., Coskun, O., Uz, Y.H., Koc, A., Yildiz, A., 2006. Effect of black cumin (*Nigella sativa*) on heart rate, some hematological values, and pancreatic beta-cell damage in cadmium-treated rats. Biological Trace Element Research 110, 151–162.

Ebru, U., Burak, U., Yusuf, S., Reyhan, B., Arif, K., Faruk, T.H., Emin, M., Aydin, K., Atilla, I.I., Semsettin, S., Kemal, E., 2008. Cardioprotective effects of *Nigella sativa* oil on cyclosporine A-induced cardiotoxicity in rats. Basic & Clinical Pharmacology & Toxicology 103, 574–580.

El Gendy, S., Hessien, M., Salam, I.A., Morad, M., El Magraby, K., Ibrahim, H.A., Khalifa, M.H., El Aaser, A.A., 2007. Evaluation of the possible antioxidant effects of Soybean and Nigella sativa during experimental hepatocarcinogenesis by Nitosamine precursors. Turkish Journal of Biochemistry 32, 5–11.

El Sherbiny, D.A., Khalifa, A.E., Attia, A.S., Eldenshary, E.E.S., 2003. Hypericum perforatum extract demonstrates antioxidant properties against elevated rat brain oxidative status induced by amnestic dose of scopolamine. Pharmacology Biochemistry and Behavior 76, 523–533.

El Shenawy, N.S., Soliman, M.F., Reyad, S.I., 2008. The effect of antioxidant properties of aqueous garlic extract and *Nigella sativa* as anti schistosomiasis agents in mice. Revista do Instituto de Medicina Tropical de São Paulo (Rev Inst Med Trop S Paulo) 50, 29–36.

Eun, J.J., Ki, Y.L., Seung, H.K., Sang, H.S., Young, C.K., 2008. Cognitive-enhancing and antioxidant activities of iridoid glycosides from Scrophularia buergeriana in scopolamine-treated mice. European Journal of Pharmacology 588, 78–84.

Ezz, H.S., Khadrawy, Y.A., Noor, N.A., 2011. The neuroprotective effect of curcumin and *Nigella sativa* oil against oxidative stress in the pilocarpine model of epilepsy: a comparison with valproate. Neurochemical Research 36, 2195–2204.

Friedewald, W.T., Levy, R.I., Fredrickson, D.S., 1972. Estimation of the concentration of low- density lipoprotein cholesterol without the use of preparative ultracentrifuge. Clinical Chemistry 18, 499–502.

Gella, A., Durany, N., 2009. Oxidative stress in Alzheimer's disease. Cell Adhesion & Migration 3, 88–93.

Gibson, G.E., Huang, H.M., 2005. Oxidative stress in Alzheimer's disease. Neurobiology of Aging 26, 575–578.

Graf, P., Uttl, B., Tuokk, H., 1995. Color- and picture-word Stroop tests: Performance changes in old age. Journal of Clinical and Experimental Neuropsychology 17, 390–415.

Hossein, B.M., Nasim, V., Sediqa, A., 2008. The protective effect of *Nigella sativa* on lung injury of sulfur mustard-exposed Guinea pigs. Experimental Lung Research 34, 183–194.

Ibrahim, Z.S., Ishizuka, M., Soliman, M., ElBohi, K., Sobhy, W., Muzandu, K., Elkattawy, A.M., Sakamoto, K.Q., Fujita, S., 2008. Protection by *Nigella sativa* against carbon tetrachloride-induced downregulation of hepatic cytochrome P450 isozymes in rats. Japanese Journal of Veterinary Research 56, 119–128.

Ilhan, A., Gurel, A., Armutcu, F., Kamisli, S., Iraz, M., 2005. Antiepileptogenic and antioxidant effects of *Nigella sativa* oil against pentylenetetrazol-induced kindling in mice. Neuropharmacology 49, 456–464.

Ismail, M., Al-Naqeep, G., Chan, K.W., 2010. *Nigella sativa* thymoquinone-rich fraction greatly improves plasma antioxidant capacity and expression of antioxidant genes in hypercholesterolemic rats. Free Radical Biology and Medicine 48, 664–672.

Jalali, M.R., Roghani, M., 2009. The Effect of *Nigella sativa* on Learning and Memory in Male Diabetic Rats. Basic & Clinical Neuroscience 1, 32–34.

Kapoor, S., 2009. Emerging clinical and therapeutic applications of *Nigella sativa* in gastroenterology. World J Gastroenterol 15, 2170–2171.

Khan, N., Sultana, S., 2005. Inhibition of two stage renal carcinogenesis, oxidative damage and hyperproliferative response by *Nigella sativa*. European Journal of Cancer Prevention 14, 159–168.

Khanna, T., Zaidi, F.A., Dandiya, P.C., 1993. CNS and analgesic studies on *Nigella sativa*. Fitoterapia 64, 407–410.

Kocyigit, Y., Atamer, Y., Uysal, E., 2009. The effect of dietary supplementation of *Nigella sativa* L. on serum lipid profile in rats. Saudi Medical Journal 30, 893–896.

Kyllonen, P.C., 1993. Aptitude testing inspired by information processing: a test of the 4-sources model. The Journal of General Psychology 120, 375–405.

Liu, H., Zhang, J., 2012. Cerebral hypoperfusion and cognitive impairment: The pathogenic role of vascular oxidative stress. International Journal of Neuroscience 122, 494–499.

MacLeod, C.M., 1991. Half a century of research on the Stroop effect: An integrative review. Psychological Bulletin 109, 163–203.

Najmi, A., Nasiruddin, M., Khan, R.A., Haque, S.F., 2008. Effect of *Nigella sativa* oil on various clinical and biochemical parameters of insulin resistance syndrome. International Journal of Diabetes in Developing Countries 28, 11–14.

Nelson, H.E., Willison, J., 1991. National Adult Reading Test (NART): Test manual, 2nd Ed UK: NFER Nelson, Windsor.

Panahi, M., Namjoyan, F., Shakerin, Z., 2011. Evaluation of antioxidant effects of *Nigella sativa* extract on the ultra structure of neural tube defects in diabetic rats's offsprin. Jundishapur Journal of Natural Pharmaceutical Products 6, 16–23.

Qidwai, W., Hamza, H.B., Qureshi, R, Gilani, A., 2009. Effectiveness, safety, and tolerability of powdered *Nigella sativa* (kalonji) seed in capsules on serum lipid levels, blood sugar, blood pressure, and body weight in adults: results of a randomized, double-blind controlled trial. Journal of Alternative and Complementary Medicine 15, 639–644.

Ramaa, C.S., Shirode, A.R., Mundada, A.S., Kadam, V.J., 2006. Nutraceuticals an emerging era in the treatment and prevention of cardiovascular diseases. Current Pharmaceutical Biotechnology 7, 15–23.

Ramadan, M.F., 2007. Nutritional value, functional properties and nutraceuticals applications of black cumin (*Nigella sativa* L.): an overview. International Journal of Food Science & Technology 42, 1208–1218.

Regard, M., 1981. Cognitive rigidity and flexibility: A neuropsychological study. Unpublished Ph.D. dissertation, University of Victoria.

Reitan, R.M., Wolfson, D., 1985. The halstead-Reitan Neuropsychological Test Bettery. Neuropsychology Press, Tucson, Ariz.

Rogers, J., Webster, S., Lue, L.F., Brachova, L., Civin, W.H., Emmerling, M., Shivers, B., Walker, D., McGeer, P., 1996. Inflammation and Alzheimer's disease pathogenesis. Neurobiology Aging 17, 681–686.

Salem, M.L., 2005. Immunomodulatory and therapeutic properties of the *Nigella sativa* L. seed. International Journal of Immunopharmacology 5, 1749–1770.

Sharrif, M., 2011. *Nigella sativa* Traditional Usages (Black Seed). Advances in Environmental Biology 5, 5–16.

Sogut, B., Celik, I., Tuluce, Y., 2008. The effects of diet supplemented with black cumin (*Nigella sativa* L.) upon immune potential and antioxidant marker enzymes and lipid peroxidation in broiler chicks. Journal of Animal and Veterinary Advances 7, 1196–1199.

Strauss, E., Sherman, E.M.S., Spreen, O., 2006. A Compendium of Neuropsychological Tests: Administration, Norms, and Commentary, 3rd Ed Oxford University Press, USA pp. 811–841.

Stroop, J.R., 1935. Studies of interference in serial verbal reaction. Journal of Experimental Psychology 18, 643–662.

Stuchbury, G., Münch, G., 2005. Alzheimer's associated inflammation, potential drug targets and future therapies. Journal of Neural Transmission 112, 429–453.

Sultan, M.T., Butt, M.S., Ahmad, R.S., Pasha, I., Ahmad, A.N., Qayyum, M.M., 2012. Supplementation of *Nigella sativa* fixed and essential oil mediates potassium bromate induced oxidative stress and multiple organ toxicity. Pakistan Journal of Pharmaceutical Science 25, 175–181.

Tauseef Sultan, M., Butt, M.S., Anjum, F.M., 2009. Safety assessment of black cumin fixed and essential oil in normal Sprague Dawley rats: Serological and hematological indices. Food and Chemical Toxicology 47, 2768–2775.

Tayman, C., Cekmez, F., Kafa, I.M., Canpolat, F.E., Cetinkaya, M., Tonbul, A., Uysal, S., Tunc, T., Sarici, S.U., 2013. Protective Effects of *Nigella sativa* Oil in Hyperoxia-Induced Lung Injury. Arch Bronconeumol 49, 15–21.

Tissera, M.H.A., Serasinghe, P., Karunarathna, K.S., 1997. Effective reduction of serum cholesterol by baraka oil (oil of *Nigella sativa*). Available on-line at ⟨http://www.baraka.lk/tests-research/clinical-trials-of-baraka-black-seed-oil/⟩ accessed on 14 July, 2012.

Tissera, M.H.A., Serasinghe, P., Karunarathna, K.S. 2000. Effect of Baraka Oil (oil of *Nigella sativa*) on Blood Sugar in persons with Hyperglycemia. Available on-line at ⟨http://www.baraka.lk/tests-research/clinical-trials-of-baraka-black-seed-oil/⟩ accessed on 14 July, 2012.

Uz, E., Bayrak, O., Uz, E., Kaya, A., Bayrak, R., Uz, B., Turgut, F.H., Bavbek, N., Kanbay, M., Akcay, A., 2008. *Nigella sativa* Oil for Prevention of Chronic Cyclosporine Nephrotoxicity: An Experimental Model. American Journal of Nephrology 28, 517–522.

Please cite this article as: Sayeed, M.S.B., et al., The effect of *Nigella sativa* Linn. seed on memory, attention and cognition in healthy human volunteers. Journal of Ethnopharmacology (2013), http://dx.doi.org/10.1016/j.jep.2013.05.004

Verhaeghen, P., De Meersman, L., 1998. Aging and Stroop effect: A meta-analysis. Psychology & Aging 13, 120–126.
Viña, J., Lloret, A., Ortí, R., Alonso, D., 2004. Molecular bases of the treatment of Alzheimer's disease with antioxidants: prevention of oxidative stress. Molecular Aspects of Medicine 25, 117–123.
Wechsler, D., 1987. Manual for the Wechsler Adult Intelligence Scale-Revised. The Psychological Corporation, New York pp. 29–31.
Wechsler, D., 1997. Administration and scoring manual for the Wechsler Adult Intelligence Scale, 3rd ed. Psychological Corporation, San Antonio, TX.
World Medical Association Declaration of Helsinki. Ethical Principles for Medical Research Involving Human Subjects. Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the 59th WMA General Assembly Seoul, South Korea, October, 2008. ⟨http://www.wma.net/en/30publications/10policies/b3/index.html⟩; access date: July 14, 2012.
Wyss-Coray, T., Mucke, L., 2002. Inflammation in neurodegenerative disease–a double-edged sword. Neuron 35, 419–432.
Xie, T., Wang, W.P., Mao, Z.F., Qu, Z.Z., Luan, S.Q., Jia, L.J., Kan, M.C., 2012. Effects of epigallocatechin-3-gallate on pentylenetetrazole-induced kindling, cognitive impairment and oxidative stress in rats. Neuroscience Letter 516, 237–241.
Yaman, I., Balikci, E., 2010. Protective effects of *Nigella sativa* against gentamicin-induced nephrotoxicity in rats. Experimental and Toxicologic Pathology 62, 183–190.
Yassin, M.M., 2005. Prophylactic Efficacy of Crushed Garlic Lobes, Black Seed or Olive Oils on Cholinesterase Activity in Central Nervous System Parts and Serum of Lead Intoxicated Rabbits. Turkish Journal of Biology 29, 173–180.
Yildiz, F., Coban, S., Terzi, A., Ates, M., Aksoy, N., Cakir, H., Ocak, A.R., Bitiren, M., 2008. *Nigella sativa* relieves the deleterious effects of ischemia reperfusion injury on liver. World Journal of Gastroenterology 14, 5204–5209.
Yoruk, O., Gur, F.O., Uyanik, H., Yasar, M., Mutlu, V., Altas, E., Baysal, E., Taysi, S., 2010. Antioxidant Effects of *Nigella sativa* in the Treatment of Experimentally Induced Rhinosinusiti. Macedonian Journal of Medial Science 3, 132–137.

Please cite this article as: Sayeed, M.S.B., et al., The effect of *Nigella sativa* Linn. seed on memory, attention and cognition in healthy human volunteers. Journal of Ethnopharmacology (2013), http://dx.doi.org/10.1016/j.jep.2013.05.004