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Improved Cognitive Performance after Dietary Supplementation with a *Pinus radiata* Bark Extract Formulation

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Dietary interventions may have the potential to counter age-related cognitive decline. Studies have demonstrated an improvement in age-related cognitive impairment in animals after supplementation with plant extracts containing flavonoids but there are few human studies. This double-blind, controlled study examined the effects on cognitive performance of a 5 week supplementation with Enzogenol® *Pinus radiata* bark extract containing flavonoids, in 42 males aged 50–65 years, with a body mass index >25. Participants were supplemented for 5 weeks either with Enzogenol® plus vitamin C, or with vitamin C only. A battery of computerized cognitive tests was administered, and cardiovascular and haematological parameters were assessed prior to and following supplementation. The speed of response for the spatial working memory and immediate recognition tasks improved after supplementation with Enzogenol® plus vitamin C, whereas vitamin C alone showed no improvements. A trend in a reduction of systolic blood pressure was observed with Enzogenol® plus vitamin C, but not with vitamin C alone. The blood safety parameters were unchanged. The findings suggest a beneficial effect of supplementation with Enzogenol® on cognition in older individuals. Larger studies are needed to ascertain its potential as a preventive treatment for age-related cognitive decline.

Keywords: Pinus radiata; pine bark extract; Enzogenol; vitamin C; cognition; cognitive decline.

INTRODUCTION

Our cognitive abilities decline with age (Christensen, 2001) and age-related neurodegenerative disorders such as Alzheimer's dementia or Parkinson's disease can greatly exacerbate this decline (Savla and Palmer, 2005). A number of studies indicate that performance in memory tests is predictive of the later development of dementia (Small *et al.*, 2000), even up to 10 years prior to onset (Elias *et al.*, 2000), suggesting that the pathological process begins early. If cognitive decline can be treated in the early stages more serious cognitive impairment may be prevented or delayed. Concerns about such age-related decline in cognitive function have led to a growing public interest in dietary measures such as antioxidants and plant extracts that hold promise to both preserve cognitive abilities with age and to improve cognitive performance (Martin *et al.*, 2002; Weinreb *et al.*, 2004).

There is an increasing number of human intervention studies examining the effects of dietary supplements on cognition. For example, cognitive benefits have been demonstrated in older individuals after supplementation with vitamin E (Sano *et al.*, 1997), soya extract (Duffy *et al.*, 2003) and *Ginkgo biloba* extract (Mix and Crews, 2002). Recently there has been interest in the possible nootropic effects of the many different flavonoids that are consumed as part of our diet. Flavonoids show very high antioxidant activities, exert neuroprotective effects *in vitro* and may play a role as neuroprotective agents *in vivo* (Youdim *et al.*, 2002). Feeding studies in rats have demonstrated that supplementation with extracts high in flavonoid compounds can delay or even reverse age-related cognitive deficits in animals (Joseph *et al.*, 1998, 1999). However, few human studies have investigated the effect of flavonoids on cognition.

The present study investigated the effects of supplementation with a commercially available flavonoid antioxidant formula, containing a *Pinus radiata* bark extract branded Enzogenol®, which is particularly rich in proanthocyanidins, plus vitamin C; compared with vitamin C alone, on cognitive performance, blood pressure and standard haematological safety parameters in a group of older adults. Previous studies using the same pine bark extract in combination with vitamin C have shown potential improvements in the parameters of oxidative stress and cardiovascular health. An uncontrolled, open label study showed potential benefits for endothelial function, systolic blood pressure and plasma

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viscosity. This study also found reductions in plasma protein carbonyl concentration and leukocyte DNA strand breakage, indicating possibly reduced levels of oxidation (Senthilmohan *et al.*, 2003). A second study in smokers that included a vitamin C control group confirmed the reduction of protein carbonyls, reported reduced fibrinogen levels in a subset of heavy smokers and noted a possible trend to lower systolic blood pressure (Young *et al.*, 2006).

For the present study, it was hypothesized that performance in cognitive tasks that are most sensitive to age related cognitive impairment would improve with the present flavonoid supplementation. Specifically, the primary outcome measures were performance in the spatial working memory and immediate recognition tasks. Spatial working memory was previously shown by this group to be the most sensitive measure of age-related cognitive decline comparing young, middle and older age individuals (Tournier *et al.*, 2004). More recently, regression analysis applied to the same data set indicated that both spatial working memory and immediate recognition measures showed the greatest decline in performance with increased age (unpublished observation, A. Pipingas, 2006). Therefore these two tasks were considered the most likely to improve with supplementation.

METHODS

Study population. Participants were recruited by way of newspaper advertisements, posters and e-mails. Advertisements asked for male non-smokers aged 50–65 years, who were right handed and did not exercise much. Interested participants were screened via telephone and invited to participate if they met the more specific inclusion and exclusion criteria. Criteria included having a sedentary occupation, a body mass index (BMI) greater than 25, not taking any form of vitamin or herbal supplementation and not suffering from any neurological disorder or epilepsy. These criteria were chosen to select for participants that are potentially at a higher risk of cognitive decline due to their age and sedentary lifestyle. The age span was restricted to 15 years in order to avoid too great a variation in the cognitive performance measures.

One hundred and six prospective interested participants responded to advertisements. Of those, 45 participants met the telephone screening criteria and underwent a medical examination to obtain medical history and to ensure that they were generally in good health and fit to participate. Forty two prospective participants passed the examination and gave written informed consent to be enrolled into the program. Approval for the study was obtained from the Swinburne University Human Research Ethics Committee.

Treatment. The study was a randomized, double-blind, vitamin C controlled study. Participants were randomly allocated to one of two groups, hereafter referred to as the treatment group or the control group. Participants were assigned to either the treatment or control groups using a random permuted block procedure with block size of four, by a study investigator who was not involved in any aspect of recruitment, testing or analysis of the data. Participants assigned to the treatment group received a daily dose of four capsules of a commercially available supplement containing in total 960 mg of Enzogenol® and 120 mg of vitamin C. Enzogenol® is an aqueous extract from the bark of New Zealand grown *Pinus radiata* trees containing approximately 80% total proanthocyanidins and other water-soluble flavonoids, flavonoid-conjugates and phenolic acids. Participants assigned to the control group received four capsules daily containing in total 120 mg of vitamin C only. Both capsule types were identical in appearance. Both groups were supplemented for 5 weeks.

Study measures. Participants were assessed at the beginning and end of the 5-week supplementation period. Participants were asked not to consume alcohol within 24 h, or tea or coffee within 2 h prior to their scheduled appointment. During the testing session personal details were collected, followed by blood pressure measurement, computerized cognitive testing, brain electrical activity measurement and blood sampling. This study reports the results of computerized cognitive testing, blood pressure measurements and standard blood safety parameters.

Diastolic and systolic blood pressure was measured whilst the participants were seated upright and relaxed in a chair. Blood pressure was measured using an electronic, self-inflating sphygmomanometer cuff.

Blood sampling via venous puncture was conducted at local pathology clinics. In accordance with a previous open label study (Shand *et al.*, 2003), and as part of the safety assessment, the blood markers measured in this trial included liver function tests, urea and electrolytes, a full blood cell and differential count and blood lipid profiles.

Participants performed eight computer-based cognitive tasks designed to test aspects of spatial and object memory, executive processes, attention and processing speed using tasks similar to those used in a previous study by this group (Tournier *et al.*, 2004), with the exception of the contextual memory task, which was a new version. Participants made responses to trials for each task using a hand-held button box. For both testing sessions, the participants were given instructions on how to perform each task and completed a practice task prior to performing the main task used for analysis. Each of the eight cognitive tasks is described in turn, in the order that they were administered to participants.

Contextual memory. This task used a variation on a previous task, which used everyday images. Participants initially watched a series of abstract images presented in one of four locations on the computer screen (top, bottom, left and right). In the second part of the task the same abstract images were again presented in the centre of the screen. Participants were required to recollect the location where each image was initially presented and thus recall the context of presented information. Participants responded by pressing one of four buttons corresponding to the four screen locations.

Immediate recognition. Participants initially viewed a series of abstract images presented in the centre of the screen. On completion of the series, a second series of abstract images were presented. Half of these images

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were identical to the original images presented, the other half were new images. Participants indicated with a left or right button press whether the image was old or new. This task is designed to probe cognitive processes involved in visual recognition memory. Abstract images were used to reduce the likelihood of verbalizing the information and to make this task more challenging.

Simple reaction time. Participants responded as quickly as possible by pressing the right button to a series of white squares that appear at the centre of the screen at random intervals. This task is designed to probe speed of information processing and speed of motor response.

Choice reaction time. Participants responded as quickly as possible to a series of blue triangles and red squares that appeared at the centre of the screen at random intervals. This task is designed to probe the speed of an accurate choice and subsequent motor response.

Visual vigilance. Participants were required to respond as quickly as possible to the appearance of a target digit in a series of rapidly presented digits. This task tests the ability to maintain visual vigilance during the course of a task as assessed through speed of response.

Complex visual vigilance. This task is a more challenging version of the previous visual vigilance task. Participants responded to a sequence of three numbers in a row, requiring maintained focused attention and rapid speed of response.

Spatial working memory. A 4 × 4 grid was presented on a black background with some of the locations in the grid filled with a white square. Participants memorized which grid locations were filled. They were then presented with a series of images of the same grid, however, with only one square filled. Participants responded with a left or right button press to indicate whether the filled grid location matched any of the previously presented filled grids. Four locations were tested in each of 14 trials. The process of holding the set of filled grid squares in memory, spanning the time that judgments of individual grid locations had to be made, was termed spatial working memory.

Delayed recognition memory. This task is a repeat of the second task, using the abstract images studied approximately 30 min earlier. This task is designed to probe cognitive processes involved in visual recognition memory and is considered to be a test of short to longer-term memory.

Statistical analysis. For each of the eight cognitive tasks, the mean accuracy and response time scores were calculated for each participant for Session 1 and Session 2 (prior to and following supplementation). The accuracy was calculated as the percentage of correct responses. The mean response time was calculated as the average response time in milliseconds for all correct responses.

The primary outcome measures were performance on the spatial working memory and immediate recognition tasks. These tasks were analysed individually using repeated measures analysis of variance (ANOVA), with the aim of detecting group (treatment, control) by time (Session 1, Session 2) interactions. Accuracy and response time were analysed separately. Secondly, with the aim of investigating the effects of supplementation on cognition in a more general sense, the remaining tasks were included in two multivariate analysis of variance (MANOVA) models, for accuracy and response time data.

All statistical analyses were performed using SPSS software (SPSS for Windows, 2004).

RESULTS

Demographic characteristics for the treatment and control groups are shown in Table 1. The two groups appeared to be well matched on all measures. There were no significant differences between the treatment groups in age, years of education, height, weight or BMI ($p > 0.1$). Seven participants in the control group were taking medication for hypertension compared with four participants in the treatment group. There were no significant differences between the groups in haematological indices at the start of the treatments ($p > 0.1$). Similarly, there were no significant differences between the groups in performance on the cognitive tasks at baseline ($p > 0.1$).

Means and standard deviations of the cognitive measures for the treatment and control groups for Session 1 and Session 2 are shown in Table 2. For the spatial working memory task, analysis of response time data using repeated measures ANOVA revealed a significant group by time interaction ($F(1,40) = 4.59$, $p = 0.038$, partial $\eta^2 = 0.103$). This reflected a decrease in response time of 64 ms or 6.4% for participants in the treatment group, with a small increase in the control group (Fig. 1). Although the mean score for the treatment group was higher than the control group at baseline, this difference was not significant ($p > 0.1$). Post hoc

Table 1. Demographic characteristics of participants assigned to treatment and control groups

	Treatment group			
	Treatment (n = 22)		Control (n = 20)	
Characteristic	M	SD	M	SD
Age	58.2	4.2	58.4	4.0
Years education	13.2	2.9	14.5	3.4
Height (cm)	173.1	8.4	177.3	8.1
Weight (kg)	93.1	20.5	92.5	13.8
Body mass index (BMI)	31.2	7.1	29.4	3.8

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Table 2. Means and standard deviations of cognitive task measures for treatment and control groups

	Treatment				Control			
	Pre-treatment		Post-treatment		Pre-treatment		Post-treatment	
	M	SD	M	SD	M	SD	M	SD
Accuracy (%)								
Simple reaction time	99.9	0.4	99.8	0.6	100.0	0.0	99.9	0.44
Choice reaction time	97.5	3.1	97.4	2.7	96.9	2.4	97.1	3.64
Immediate recognition	64.0	8.4	62.7	10.0	66.4	8.4	67.5	7.56
Visual vigilance	99.8	0.6	98.5	2.9	100.0	0.0	99.0	2.00
Complex visual vigilance	51.7	15.3	56.0	15.9	53.8	20.8	61.9	21.43
Spatial working memory	73.7	10.7	76.6	14.9	72.2	11.9	76.4	10.83
Contextual memory	26.3	11.2	37.8	13.5	30.9	12.3	35.4	11.21
Delayed recognition	59.6	7.7	61.5	8.8	62.4	6.7	64.3	6.54
Response time (ms)								
Simple reaction time	252	37	266	44	245	41	253	42
Choice reaction time	424	45	434	44	422	44	423	47
Immediate recognition	1107	113	1047	106	1050	134	1057	141
Visual vigilance	400	22	405	34	395	19	401	38
Complex visual vigilance	446	70	437	69	454	75	427	49
Spatial working memory	1018	145	953	111	946	133	947	166
Contextual memory	1300	245	1176	173	1214	339	1163	176
Delayed recognition	1080	105	1045	115	1082	120	1072	130

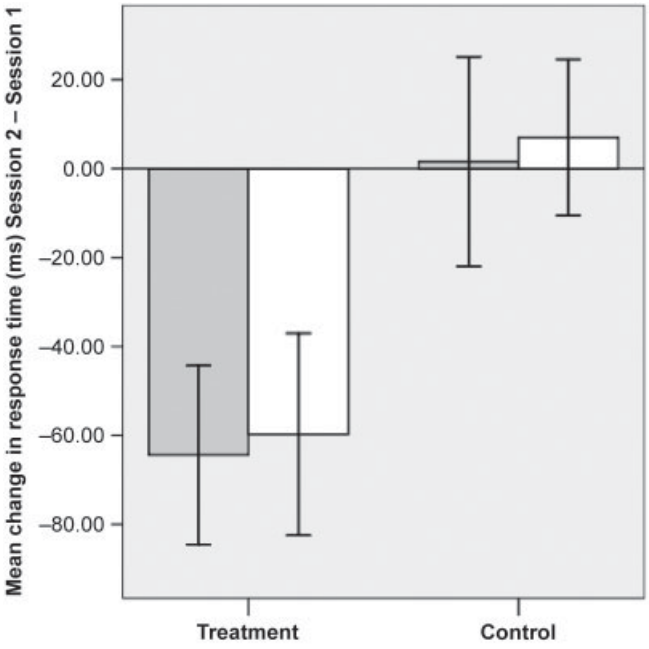

Figure 1. Mean change in response time (ms) for spatial working memory (■) and immediate recognition (□) tasks for treatment and control groups. Error bars show ± 1 standard error.

t-tests were conducted for each group separately and revealed that the change in response time from Session 1 to Session 2 was significant in the treatment group ($t(21) = 3.21$, $p = 0.004$) but not in the control group ($t(19) = -0.07$, $p = 0.95$).

For the immediate recognition task, repeated measures ANOVA indicated there was a significant group by time interaction ($F(1,40) = 5.25$, $p = 0.027$, partial $\eta^2 = 0.116$) reflecting an improvement in the response time of 60 ms or 5.4% in the treatment group, compared with a small increase in the control group (Fig. 1). Although the mean score for the treatment group was higher than the control group at baseline, this difference was not significant ($p > 0.1$). Post hoc *t*-tests showed that the change was significant in the treatment group ($t(21) = 2.63$, $p = 0.016$) but not in the control group ($t(19) = -0.396$, $p = 0.696$).

Repeated measures ANOVA revealed that there was no group by session interaction for performance accuracy on either task. However, for the spatial working memory task both treatment and control groups were significantly more accurate in Session 2 ($F(1,39) = 11.99$, $p = 0.001$) reflecting either a general learning effect in all participants from Sessions 1 to 2, or an effect of vitamin C.

The new version of the contextual memory task demonstrated a baseline performance close to chance and was excluded from further analyses. Analysis of the remaining tasks together using MANOVA showed no group by time interaction for either response time or accuracy measures, indicating either that the treatment effect was specific to the spatial working memory and immediate recognition tasks, or that the other tasks are less sensitive in detecting changes in cognitive performance.

There was no significant change in systolic or diastolic blood pressure measures for either group. However, there was a trend of reduced systolic blood pressure (Table 3). Interestingly, a reduction of systolic blood pressure had previously been reported in an open label study using the same pine bark extract (PBE) with vitamin C (Shand *et al.*, 2003). Furthermore, a recent vitamin C controlled study by Young *et al.* (2006) also found a trend of reduced systolic blood pressure using the same PBE with vitamin C (Table 3). As a post hoc measure, the systolic blood pressure data from the present study and the study by Young *et al.* were pooled and repeated measures ANOVA conducted for the combined group. Although Young *et al.* were studying smokers, the treatment was very similar to the present study, but used half the dose and supplemented for 12 weeks. The results from this meta-analysis indicated

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Table 3. Individual and combined analyses of systolic blood pressure data from the present study and a double-blind, controlled trial by Young *et al.* (2006), comparing supplementation with Enzogenol® and vitamin C against vitamin C alone

Study *n* = treatment/control	Change in systolic blood pressure PBE + Vitamin C Mean (95% CI)	 Vitamin C Mean (95% CI)	Repeated measures ANOVA *F*	 *p*
Present study *n* = 18/13	−6.9 (−14.3, 0.4)	−0.9 (−7.4, 5.4)	1.63	0.213
Young *et al.* 6 weeks *n* = 22/22	−2.1 (−5.3, 1.1)	+2.9 (−2.1, 7.9)	3.07	0.087
Young *et al.* 12 weeks *n* = 22/22	−3.5 (−7.3, 0.3)	+1.4 (−3.7, 6.5)	2.55	0.118
Present study and Young *et al.* 6 weeks *n* = 39/35	−4.2 (−7.8, −0.6)	+1.5 (−2.3, 5.2)	5.06	0.029
Present study and Young *et al.* 12 weeks *n* = 39/35	−5.0 (−8.7, −1.3)	+0.5 (−3.3, 4.3)	4.94	0.038

there was a significant decrease in systolic blood pressure in the treatment group compared with the control group, regardless of whether the present 5 week data were combined with the 6 or 12 week time points by Young *et al.* (Table 3).

The biochemical and haematological analyses of the venous blood samples, including liver function tests, full blood cell and differential counts, and blood lipid profiles showed no significant changes in either treatment or control groups. No adverse events were reported by participants in this study.

DISCUSSION

In the present study, a short term supplementation over 5 weeks with combined PBE and vitamin C improved the speed of response on spatial working memory and immediate recognition tasks, whereas supplementation with vitamin C alone showed no improvements. A trend towards reduced systolic blood pressure was noted in the current study, and while not statistically significant, this decrease was of the same magnitude as demonstrated in a previous open-label study (Shand *et al.*, 2003). A combined analysis of data from the present study and a recent vitamin C controlled study in smokers (Young *et al.*, 2006) indicated that systolic blood pressure may indeed be reduced with this combined PBE and vitamin C treatment. However, this conclusion has to be taken with caution, and needs verification in a larger study since the two studies are not of identical design and the study by Young *et al.* was in smoking subjects. The supplementation was notably safe without adverse events or any indications of changes in any blood safety parameters.

Our previous research has indicated that the spatial working memory and immediate recognition tasks are the most sensitive to the effects of aging compared with the other tasks used in this test battery. It was consequently hypothesized that in this group of cognitively intact older males, these tasks would be the most likely to reveal any cognitive benefits that the PBE supplementation may afford, and this was demonstrated in the study.

Improvements in cognition after supplementation with PBE plus vitamin C were demonstrated for speed of response but not for performance accuracy, which is consistent with cognitive aging studies which suggest that accuracy is preserved at the expense of speed of response, both with age (Brebion, 2001) and in mild cognitive impairment (Nicholl *et al.*, 1995). Furthermore, a recent study that also used a computerized battery to assess cognitive effects following supplementation with plant extracts found that 'speed of memory' improved with supplementation (Tildesley *et al.*, 2005). Thus the findings of the current study advocate speed of response as an important measure when assessing outcomes of interventions that are designed to counteract cognitive decline or improve cognition.

The present treatment used a *Pinus radiata* bark extract, Enzogenol®, with high antioxidant capacity (Wood *et al.*, 2002), in combination with vitamin C. PBEs are rich in proanthocyanidins and contain a range of flavonoids including catechin, epicatechin, quercetin, dihydroquercetin, taxifolin and phenolic acids (Wood *et al.*, 2002). Here we discuss a number of possible mechanisms by which the treatment may exert its positive effects on cognitive performance including antioxidant action, improvements of cerebral blood supply and circulation, and influences on neuronal signal transduction.

Oxidative damage has been observed in the aging brain, and antioxidants may play a role in protecting the brain from reactive oxygen species (Halliwell, 2001). However, accumulation of oxidative damage in the brain is likely to manifest in cognitive decline only over long periods of time. The present antioxidant supplementation was able to improve significantly cognitive performance over a short period of only 5 weeks, suggesting that other mechanisms may at least in part account for the observed effect.

Improved blood circulation to the brain might also have contributed to enhanced cognition and would also be consistent with our findings of improved systolic blood pressure. Previous research indicates that hypertension (Brady *et al.*, 2005) and cardiovascular risk factors (Barnes *et al.*, 2006) are associated with cognitive decline. Furthermore, bioflavonoid consumption has been reported to improve cardiovascular risk factors (Caccetta *et al.*, 2001) and to promote relaxation of vascular smooth muscle (Stein *et al.*, 1999) indicating a possible contribution of improvements in blood circulation to better cognitive functions. Previous studies using the same PBE plus vitamin C supplementation have shown the potential for cardiovascular benefits including improved endothelial function, reduced plasma fibrinogen concentrations (Young *et al.*, 2006), and reduced plasma viscosity and systolic blood pressure (Shand *et al.*, 2003). An effect of lowering systolic blood pressure has been corroborated here by our pooled analysis, which indicated a significant reduction

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with the PBE plus vitamin C treatment over vitamin C alone.

Another mechanism that may play a role in the effects of the present treatment on cognitive performance is the direct influence of flavonoids or their metabolites in the brain. Although only a few studies have produced evidence of flavonoid uptake into the brain, one study found epicatechin metabolites formed in rat brains after oral ingestion of epicatechin, indicating the possibility of PBE constituents entering the brain and directly affecting neurological signalling (Abd El Mohsen *et al.*, 2002). Studies of dietary supplementation with berry and spinach extracts in rats have been shown to prevent onset (Joseph *et al.*, 1998) and reverse (Joseph *et al.*, 1999) age-related deficits in cognitive behaviour, and to normalize cognitive performance deficits in an amyloid plaque transgenic mouse model (Joseph *et al.*, 2003). Interestingly, blueberry supplementation in these studies caused significant increases in indices of neuronal signalling in the brains of the animals, indicating mechanistic actions beyond the antioxidant activity of the flavonoids. In addition, a study using the same PBE supplement as the present study has demonstrated a reduction in the duration and severity of migraines in human migraine sufferers, possibly suggesting that this supplementation may have effects that affect the brain (Chayasirisobhon, 2006).

There are a number of limitations to this study. Although there was no response time effect in the controls taking vitamin C only, it is unclear whether the PBE alone would have demonstrated the same positive effects or whether there is a synergistic benefit when combined with vitamin C. Furthermore, accuracy measures for spatial working memory demonstrated an improvement in both treatment and control groups and it cannot be certain whether this improvement was due to practice or to a beneficial effect of vitamin C. However, the vitamin C dosage was 120 mg, a much lower dosage than has been used in other studies which have used, for example, 500 mg (Yaffe *et al.*, 2004) or 1500 mg (Baker *et al.*, 1999) with no demonstrated cognitive benefit. Given that with age, the response time tends to decline before accuracy and that there was no benefit in the response time for the vitamin C group, it is likely that the improvement reflects practice effects rather than the benefits of vitamin C. Nevertheless, the above limitations should be assessed in a larger study that includes a treatment group receiving only the pine bark extract.

This randomized, double-blind, vitamin C controlled study has demonstrated the potential to improve both cognitive and cardiovascular functions prone to decline with age after a relatively short duration of supplementation with Enzogenol® plus vitamin C in a group of older men that may be at risk of cognitive decline. Larger studies in men and women of different age groups are necessary to ascertain its potential as a preventive treatment to fight age-related or neurodegenerative loss in brain function.

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