MEDWIN PUBLISHERS
Committed to Create Value for Researchers

Medical Journal of Clinical Trials & Case Studies
ISSN: 2578-4838

Beta-Caryophyllene, an Anti-Inflammatory Natural Compound, Improves Cognition in an Elderly Group who has Concerns about Memory

Bell SJ[1]*, Gomez-Pinilla F[2] and Ling PR[3]

[1]Scientific consultant. BrainCare, LLC, USA

[2]Professor of Neurosurgery and Integrative Biology and Physiology, UCLA, USA

[3]Consultant, Cambridge, USA

***Corresponding author:** Stacey J Bell, Scientific consultant, BrainCare, LLC, 110 Woodland Street, Reno, NV 89523, USA, Tel: (617) 999 6150; Email: stacey.bell@nutrientfoods.com

Research Article

Volume 5 Issue 5

Received Date: August 23, 2021

Published Date: September 21, 2021

DOI: 10.23880/mjccs-16000300

Abstract

Neuroinflammation is known to play a neuropathological role in cognitive decline. Beta-caryophyllene (BCP), found in many plants, is a substance that crosses the blood-brain barrier and acts on microglial cells to reduce neuro-inflammation. The purpose of this prospective, randomized study is to determine the effect of BCP on cognitive function in older individuals, who have noticed worsening of memory. In this 8-week study, 52 participants were randomized to two different doses of BCP: 90 mg (n=29) or 180 mg (n=29). Diet quality was determined at baseline. And, at baseline, week 4, and week 8, cognitive function was assessed using the Cognitive Failures Questionnaire (CFQ), which was taken online. At baseline, the average age was 67±5 years, and the subjects were obese according to mean body mass indexes.

For each of the four sub-scale scores, all measurements at week 4 and week 8 improved for both dietary intervention groups (i.e., one or two BCP capsules). Taking two BCP capsules resulted in significant improvement for all four sub-scale scores at week 4 and week 8 compared to baseline. In contrast, those taking one BCP capsule had a significant improvement only three times. The percentage improvement in the sub-scale scores for those taking two BCP capsules were about twice greater than experienced by those taking one BCP capsule. Only eight of the twenty-five individual CFQ questions significantly improved.

Pre-study dietary habits that related to cognition showed little differences between the two groups. Most participants ate a typical American diet rather than one that reduces the risk of dementia (e.g., consuming sugar-rich foods, limited seafood, few seeds and nuts). However, the group tended to avoid alcohol and dining at fast food restaurants, which reduce dementia risk.

Compliance with the BCP capsules was excellent and no other dietary or lifestyle changes were imposed. As no treatments are available to treat dementia, the current study suggests that the use of 180 mg of BCP from cloves is an easy way to improve cognitive function in an elderly population.

Keywords: Brain Inflammation; Cognition; Beta-Caryophyllene; Aging Brain; Nutrition and Cognition

Introduction

Impaired cognition, and in particular, Alzheimer's disease used to be characterized by two core pathologies: formation of beta-amyloid plaque and neurofibrillary tangles [1-3]. More recently, neuroinflammation has been added to the mix, and it is known to play a role in the development of the overall Alzheimer's pathology including cognitive

decline. Since, systemic inflammation is also associated with cognitive decline, inflammation of the brain and periphery are potential targets for anti-inflammatory drugs to treat or slow the progression of Alzheimer's disease [4].

Because brain inflammation is an important dementia risk factor, much of the focus today is on the microglial cells, which were only characterized in the mid-1980s [2]. These cells are considered to be the brain's macrophages and are able to secrete both pro- and anti-inflammatory mediators such that it is crucial to maintain homeostatic mechanisms that tip microglia function to the healthy side.

Growing scientific interest is on beta-caryophyllene (BCP), a natural, bicyclic sesquiterpene found in many plants (e.g., oregano, cinnamon, clove, rosemary, wild sage, thyme, and black pepper) [5,6]. Beta-caryophyllene can cross the blood-brain barrier and thus act on the central nervous system. It is an endocannabinoid that activates and selectively binds to the cannabinoid receptor 2 (CB2R), which is mainly localized in the microglial cells.

Activation of CB2R is implicated in the reduction of pro-inflammatory cytokines, and subsequent neuro-inflammation (e.g., lower amounts of IL-1 beta, IL-6, IL-8, and TNF-alpha) [6]. Endocannabinoids like BCP exert their anti-inflammatory properties, at least in part, by the activation of the peroxisome proliferator-activated receptor-gamma (PPAR-gamma) pathway [7]. PPAR-gamma is a member of the superfamily of nuclear receptors and has important anti-inflammatory activity, because it inhibits the activation of nuclear factor-kappa beta and the expression of the proinflammatory cytokines, IL-1-beta and TNF-alpha. Activation of CB2R can trigger phagocytosis of beta-amyloid in human macrophages like the microglial cells but not the astrocytoma cells [8].

Specific actions of beta-caryophyllene (BCP)

It is likely that BCP protects the brain and body based on its anti-inflammatory/anti-oxidant properties. Transgenic mice mimicking Alzheimer's disease experienced reduced inflammation that prevented cognitive decline after oral ingestion of BCP [3]. BCP has been shown to improve conditions related to its anti-inflammation property: inflammatory bowel disease, pain and inflammation in a neuropathic pain model, and oxidative stress reduction in the glial cells [6,7,9]. BCP has also shown promise for treating anxiety and depression, being a plausible therapeutic in diabetes and associated complications, preventing and ameliorating non-alcoholic fatty liver disease, lessening acute and chronic pain, and having an anti-convulsive effect against seizures [10-14]. In addition, BCP may offer benefit to promote weight loss and reduce cardiovascular disease. In an animal model, BCP was able to decrease the visceral fat index, total and LDL cholesterol, very low-density lipoprotein (VLDL), and pro-inflammatory cytokines [15]. These effects were reversed by treatment with CB2 cannabinoid receptors and PPAR-antagonists, suggesting that BCP activity is mediated by direct binding to CB2 receptors and by the activation of PPAR-agonists.

One clinical study showed that BCP from black pepper mitigates pain [16]. Of the 31 participants, half complained of either acute or chronic pain at the onset of the study. Each received 60 mg of BCP daily for eight weeks. By day 4, 60% reported mitigation of pain and the benefits lasted for one week. About one-third of the participants stated that the level of reduced pain was comparable to non-steroidal anti-inflammatory drugs (NSAIDs) that were used previously. Another group explored the effect of BCP on testosterone levels in women who complained of low libido [17]. The women had an olfactory exposure treatment of a control (glycerol) followed by 3% BCP, each over 20 minutes. Salivary testosterone increased without changing estrogen for the BCP treatment compared to the control period. These findings suggest that BCP may be a remedy with few side effects for women with decreased libido.

It is possible that activation of CB2R from orally consumed BCP may lead to beneficial cognitive effects by reducing the neuroinflammatory response. Admittedly, more involved dietary plans like the Mediterranean and MIND diets have been shown to reduce the risk of cognitive impairment [18,19]. Both diet plans include mostly fresh foods like fruits, vegetables, whole grains, healthy oils like olive oil, legumes, and seeds and nuts, which are not foods typically consumed by most Americans. The purpose of this 8-week, randomized, prospective study is to evaluate the effect of two different doses of BPC on cognition in an elderly population, who have not been diagnosed with mild cognitive impairment or dementia, yet have noticed poorer cognition themselves.

Methods

Eighty subjects were recruited by a company (L&E Research, Austin, Texas; https://www.leresearch.com/) with the anticipation of having 60 complete the study. Fifty-nine enrolled in the study with seven later withdrawing. Participants, who meet the entry criteria, were randomized to one or two servings of BCP daily and asked to complete baseline and weekly data collection forms, which were sent electronically (e.g., anthropometry, cognitive testing, quality of life). All signed a consent form which abided by the *Helsinki Declaration*, seventh revision. The study lasted eight weeks.

Entry criteria and recruitment

The entry criteria were to be aged 60-80 years, worried about their own brain function (e.g., losing memory, concentration, focus), and being overweight or obese according to body mass index (BMI; 25-40 kg/m^2) [20]. Each candidate needed to own a computer and be versatile on its use in order to complete weekly data collection forms and perform online brain games. Participants could not have been diagnosed with Alzheimer's disease, Parkinson's disease, mild cognitive impairment, or any other neurodegenerative condition, but could have other chronic conditions, as long as they were well controlled. No one with COVID-19 was admitted to the study, and throughout the 8 weeks, if anyone contracted the coronavirus, they were excluded from the study. Candidates had to be naïve to using supplements of beta-caryophyllene (BCP).

The participants were screened and recruited by L&E Research, who then remained in regular contact with them to assure weekly compliance with requested data. If more than one questionnaire was submitted, the last one was used. Participants were compensated $100 upon submission of all data at week 8.

Cognitive testing using the Cognitive Failures Questionnaire (CFQ)

(https://www.ocf.berkeley.edu/~jfkihlstrom/ConsciousnessWeb/Meditation/CFQ.htm)

The CFQ probes minor mistakes that most people make occasionally but sometimes they happen more frequently. The 25-question survey was developed to assess the frequency that individuals experience these mistakes in cognitive function, such as absent-mindedness, in everyday life – slips and errors of perception, memory, and motor functioning. The CFQ scoring system used for each question was: 0=Never; 1=Very rarely; 2=Occasionally; 3=Quite often; 4=Very often. Four summed sub-scale scores with similar attributes were also determined for a Total CFQ score; Forgetfulness (a tendency to forget something that is known or planned, for example, names, intentions, appointments, and words); Distractibility (mainly in social situations such as being absentminded or easily disturbed with one person or in a group); and False triggering (interrupted ability to pay attention leading to making errors in thinking and acting logically).

The CFQ cognitive test was sent to the participants electronically at baseline, week 4, and week 8. A lower score indicated better cognitive function.

Nutrition Intervention and Baseline Dietary Survey

Clove oil, (*Syzygium aromaticum*, was provided by Biosfered, Torino, Italy and served as the source of BCP. Capsules for the study were prepared by Tishcon Corp. (http://www.tishcon.com/), Westbury, New York. Each capsule contained 100 mg of clove oil containing 90% BCP and 350 mg of rice bran oil. The group taking one capsule daily received 90 mg of BCP and the group taking two capsules received 180 mg. Each capsule had a screw-off top. Participants were told to empty the contents of the capsule into the mouth and leave it under the tongue for a couple of minutes before swallowing. Everyone was told to take the one or two capsules in the morning. Capsules were provided at no charge to the participants.

At baseline, subjects provided information about their current dietary habits. Questions probed the frequency of consumption of sugar, fish, red meats and cold cuts, fruits and vegetables, seeds and nuts, and alcohol. Also, the frequency of eating at a fast food restaurant was asked.

Statistics

An independent consultant analyzed the data. Anthropometric data (height and weight) obtained from the recruiting company were not used in the analyses. Instead baseline data obtained when the study was about to start were used because these more closely aligned with subsequent, weekly body weight data provided by the participants.

Subjects were initially randomized to take one or two BCP-containing capsules daily and were evenly matched by: gender, body weight (body mass index $\geq$ 30 kg/m^2 or less than 30 kg/m^2), and age (60 to less than 70 years old, and 70 years and older). At week 4, four subjects were removed from the study due to the lack of BCP capsules. Two subjects were randomly removed from each group (2/28 in the two capsule/day group; 2/29 in the one capsule/day group). Random numbers were generated between 1 and 28 for the two/day group, which were then sorted by highest to lowest. The first two subjects were removed. The same randomization was performed to remove two subjects in the one capsule/day group. The randomization procedures for removal of subjects were performed by someone not involved in the study.

The data are presented as mean ± standard deviation (S.D.). Results from the Cognitive Failures Questionnaire are compared using the Student t-test in each intervention group between baseline and week 4 and between baseline and week 8. Significance was defined as $p \leq 0.05$.

At each time point, the percentage change was compared with the values at baseline. This calculation was only obtained from the means between each time point, and the statistical analysis was not made on these percentage changes.

Results

Fifty-nine subjects entered the study with an average age of 67 ± 5 years. Females comprised 58% of the group. The mean body weight was 88 ± 15 kg and body mass indexes (BMIs) showed that 2% were normal weight, 36% were overweight, and 62% obese.

Seven subjects withdrew. Four were randomly selected before week 4 to be withdrawn due to lack of capsules; two were removed from each dietary intervention group. Three others withdrew; one at baseline who didn't like the study (taking one capsule per day), another withdrew after week 2 because they wanted more compensation (taking two capsules daily), and the third person withdrew after completing week 5 because they didn't like taking the capsules (taking one capsule daily). The final number of subjects to complete the study was 52 subjects (88% retention), leaving each dietary intervention group with 26 subjects.

At baseline, subjects were fairly well matched between the groups for age, gender, and BMI (Table 1). There were more women in the one capsule per day group compared to those taking two (62% female vs. 55% female). The mean BMI was similar between the groups: 32 ± 6 kg/m^2 in the one capsule per day group and 32 ± 5 kg/m^2 in the two capsule per day group. More participants in the two BCP capsules daily had Class 1 Obesity (48% vs. 34% in the one capsule daily group). However, those in the one BCP capsule daily included more participants in the Class 2 Obesity group (24% vs. 7% in the two capsule per day group). None of the participants were told by their physician to follow a special diet to maintain or improve brain function.

	Group 1=29 (one capsule daily)	Group 2=29 (two capsules daily)
Age (years)	67 ± 5	68 ± 4
Gender (% female)	62	55
Body weight (kg)	90 ± 18	88 ± 11
Height (cm)	66.0 ± 4.7	65.0 ± 4.1
BMI (kg/m^2)	32 ± 6	32 ± 5
≤ 25	2 (7 %)	0 (0 %)
≤ 30	9 (31%)	11 (38 %)
≤ 35 Class 1 Obesity+	10 (34 %)	14 (48 %)
≤ 40 Class 2 Obesity	7 (24 %)	2 (7 %)
> 40 Class 3 Obesity	1 (3 %)	2 (7 %)

*Data presented as mean ± standard deviation
+Based on reference 20
Table 1: Baseline comparisons between dietary intervention groups.

Participants in both groups had 100% compliance with the dietary interventions over the 8-week study. Participants remained weight stable, which was the objective of the study so that weight change was not a confounding variable on cognitive changes (data not shown). For those taking one BPC capsule daily baseline was BMI 32 ± 6 kg/m^2 and at week 8 it was 31 ± 5 kg/m^2. For those taking two capsules daily, baseline BMI was 32 ± 5 kg/m^2 and at week 8 it was 32 ± 6 kg/m^2.

Cognitive Failures Questionnaire (CFQ)

Four summed sub-scale CFQ scores: For each of the four sub-scale scores, all measurements at week 4 and week 8 were lower than at baseline for both dietary intervention groups (i.e., one or two BCP capsules), indicating improvement in cognition (Tables 2a-2d). Taking two BCP capsules resulted in significant improvement for all four sub-scale scores at week 4 and week 8 compared to baseline (P ≤ 0.05). Unlike taking two BCP capsules, those taking one BCP capsule only had a significant improvement for Forgetfulness at week 8 compared to baseline (P ≤ 0.05) (Table 2b) and for False Triggering at weeks 4 and 8 compared to baseline (P ≤ 0.05) (Table 2d). The percentage improvement in the sub-scale scores for those taking two BCP capsules were about twice greater than experienced by those taking one BCP capsule (Tables 2a-2d).

Time	Group taking one capsule of beta-caryophyllene (BCP) daily (n=26)	Group taking two capsules of beta-caryophyllene daily (n=26)
Total CFQ		
Baseline	39 ± 12	39 ± 16
Week 4 vs.	34 ± 10	29 ± 14*
Baseline	14 % better	25 % better
Week 8 vs.	33 ± 11	27 ± 11**
Baseline	16 % better	30% better

*P ≤ 0.01 and **P ≤ 0.005 compared to baseline of two BCP capsules, using t-test, respectively.
Table 2a: Changes in Total Summed Sub-score of the Cognitive Failures Questionnaire (CFQ) (lower scores are better).

Time	Group taking one capsule of beta-caryophyllene (BCP) daily (n=26)	Group taking two capsules of beta-caryophyllene daily (n=26)
Forgetfulness		
Baseline	16 ± 5	16 ± 6
Week 4 vs.	14 ± 5	12 ± 5^
Baseline	14% better	27 % better
Week 8 vs.	13 ± 5*	12 ± 5^
Baseline	17 % better	27% better

*P ≤ 0.05 compared to baseline of one BCP capsule, by t-test
^P ≤ 0.01 compared to baseline of two BCP capsules, by t-test, respectively
Table 2b: Changes in Forgetfulness Summed Sub-score of the Cognitive Failures Questionnaire (CFQ) (lower scores are better).

Time	Group taking one capsule of beta-caryophyllene (BCP) daily (n=26)	Group taking two capsules of beta-caryophyllene daily (n=26)
Distractibility		
Baseline	13 ± 4	13 ± 5
Week 4 vs.	11 ± 4	10 ± 5*
Baseline	8 % better	20 % better
Week 8 vs. baseline	11 ± 3	9 ± 4*
Baseline	9 % better	29% better

*P ≤ 0.001 compared to baseline for two BCP capsules, using t-test, respectively
Table 2c: Changes in Distractibility Summed Sub-score of the Cognitive Failures Questionnaire (CFQ) (lower scores are better).

Time	Group taking one capsule of beta-caryophyllene (BCP) daily (n=26)	Group taking two capsules of beta-caryophyllene daily (n=26)
False triggering		
Baseline	12 ± 4	11 ± 6
Week 4 vs.	9 ± 4*	7 ± 4^
Baseline	18 % better	33 % better
Week 8 vs.	9 ± 4*	7 ± 4^
Baseline	19 % better	36 % better

*P ≤ 0.05 compared to baseline for one BCP capsule, using t-test, respectively
^P ≤ 0.01 compared to baseline for two BCP capsules, using t-test, respectively
Table 2d: Changes in False Triggering Summed Sub-score of the Cognitive Failures Questionnaire (CFQ) (lower scores are better).

The participants taking two BCP capsules experienced significant improvement in the Total CFQ sub-scale scores of 25% and 30% at weeks 4 ($P \leq 0.01$) and 8 ($P \leq 0.005$), respectively (Table 2a). Those taking one BCP capsule improved 14% at week 4 and 16% at week 8. For the Forgetfulness sub-score, those taking two capsules improved more than those taking one BCP capsules at week 4 (27% vs. 14%) and at week 8 (27% vs. 17%) (Table 2b). Those taking one BCP experienced a significant improvement at week 8 ($P \leq 0.05$), and those taking two BCP capsules achieved a significant benefit at both weeks 4 and 8 ($P \leq 0.01$) (Table 2b).

For the Distractibility sub-score, the participants taking two BCP capsules improved 20% at week 4 and 29% at week 8 ($P \leq 0.001$, respectively), in contrast to non-significant improvements for those taking one BCP capsule of 8% at week 4 and 9% at week 8 (Table 2c). The False Triggering sub-scores improved the most in the group taking two BCP capsules compared to any other sub-score for either one and two BCP capsule groups (Table 2d). For those taking one BCP capsule, significant improvement was observed at week 4 (18%, $P \leq 0.05$) and week 8 (19%, $P \leq 0.05$). With the group taking two BCP capsules, a greater improvement was observed at week 4 (33%, $P \leq 0.01$) and week 8 (36%, $P \leq 0.01$).

Changes in individual CFQ questions: Of the 25 questions on the CFQ questionnaire, improvement was observed for nearly all questions over 8 weeks, but only eight achieved significance. In most cases, greater improvement was found in those taking two BCP capsules compared to one; often it was two or three times more.

Changes in CFQ that achieved statistical significance (Table 3a-3h): Eight of the twenty-five CFQ questions improved significantly for both dietary options (one or two BCP capsules) (Tables 3a-3h). Baseline questions were 2.0 or greater for 10 of the 16 options (8 for one BCP capsule and 8 for two BCP capsules), indicating difficulty at baseline in these aspects of cognitive function. Mostly two capsules showed greater improvement than taking one BCP capsule, and no worsening of cognition was observed for either dietary intervention.

Time	Group taking one capsule of beta-caryophyllene (BCP) daily (n=26)	Group taking two capsules of beta-caryophyllene daily (n=26)
Baseline	1.9 ± 1.2	2.3 ± 0.8
Week 4 vs.	1.7 ± 0.9	1.5 ± 0.9*
Baseline	11% better	39 % better
Week 8 vs.	1.6 ± 0.9	1.4 ± 0.8*
Baseline	16% better	39% better

*$P \leq 0.005$ compared to baseline for two capsules, using t-test, respectively

Table 3a: Do you read something and find you haven't been thinking about it and must read it again?

Time	Group taking one capsule of beta-caryophyllene (BCP) daily (n=26)	Group taking two capsules of beta-caryophyllene daily (n=26)
Baseline	2.4 ± 0.9	2.3 ± 1.0
Week 4 vs.	1.7 ± 0.6*	1.4 ± 0.7^
Baseline	29% better	39 % better
Week 8 vs.	1.7± 0.6*	1.4 ± 0.8^
Baseline	29 % better	39 % better

*$P \leq 0.005$ compared to baseline for one capsule, using t-test, respectively

^ $P \leq 0.0005$ compared to baseline for two capsules, using t-test, respectively

Table 3b: Do you find you forget why you went from one place of the house to the other?

Time	Group taking one capsule of beta-caryophyllene (BCP) daily (n=26)	Group taking two capsules of beta-caryophyllene daily (n=26)
Baseline	1.8 ± 1.0	2.0 ± 1.0
Week 4 vs.	1.6 ± 0.8	1.1 ± 0.9*
Baseline	11% better	45% better
Week 8 vs.	1.5 ± 0.7	1.2 ± 0.7*
Baseline	17 % better	40% better

*P ≤ 0.005 compared to baseline for two BCP capsules, using t-test, respectively

Table 3c: Do you find you forget whether you've turned off a light or locked the door?

Time	Group taking one capsule of beta-caryophyllene (BCP) daily (n=26)	Group taking two capsules of beta-caryophyllene daily (n=26)
Baseline	2.3 ± 0.9	2.1 ± 1.2
Week 4 vs.	1.8 ± 0.9*	1.7 ± 0.9^
Baseline	22% better	19% better
Week 8 vs.	1.8 ± 0.7*	1.5 ± 0.9^
Baseline	22% better	29% better

*P ≤ 0.05 compared to baseline for one BCP capsule, using t-test, respectively

^P ≤ 0.01 compared to baseline for two BCP capsules, using t-test, respectively

Table 3d: Do you forget where you put something like a newspaper or a book?

Time	Group taking one capsule of beta-caryophyllene (BCP) daily (n=26)	Group taking two capsules of beta-caryophyllene daily (n=26)*
Baseline	1.2 ± 0.9	0.8 ± 1.0
Week 4 vs.	1.0 ± 0.8	0.7 ± 0.9
Baseline	17% better	13% better
Week 8 vs.	0.9 ± 0.7	0.3 ± 0.5*
Baseline	25% better	63% better

*P ≤ 0.05 compared to baseline for two BCP capsules, using t-test, respectively

Table 3e: Do you find you accidentally misplace things like placing the fruit in the cupboard and the bowl in the refrigerator?

Time	Group taking one capsule of beta-caryophyllene (BCP) daily (n=26)	Group taking two capsules of beta-caryophyllene daily (n=26)
Baseline	1.9 ± 0.8	1.8 ± 1.1
Week 4 vs.	1.6 ± 0.8	1.3 ± 0.9*
Baseline	16% better	28% better
Week 8 vs.	1.6 ± 0.9	1.2 ± 0.9*
Baseline	11% better	33% better

*P ≤ 0.05 compared to baseline for two BCP capsules, using t-test, respectively

Table 3f: Do you daydream when you ought to be listening to something?

Time	Group taking one capsule of beta-caryophyllene (BCP) daily (n=26)	Group taking two capsules of beta-caryophyllene daily (n=26)
Baseline	2.3 ± 0.8	2.7 ± 1.0
Week 4 vs.	2.3 ± 0.8	1.9 ± 0.8*
Baseline	no change	30% better
Week 8 vs.	2.2 ± 0.6	1.9 ± 0.7*
Baseline	4% better	30% better

*P ≤ 0.005 compared to baseline for two BCP capsules, using t-test, respectively

Table 3g: Do you start doing one thing at home and get distracted and do something else (unintentionally)?

Time	Group taking one capsule of beta-caryophyllene (BCP) daily (n=26)	Group taking two capsules of beta-caryophyllene daily (n=26)
Baseline	2.6 ± 0.8	2.5 ± 0.9
Week 4 vs.	2.3 ± 0.8	2.0 ± 1.0^
Baseline	12% better	20% better
Week 8 vs.	2.1 ± 0.7*	2.2 ± 0.8
Baseline	19% better	12% better

*P ≤ 0.01 compared to baseline for one BCP capsule, using t-test

^ P ≤ 0.01 compared to baseline for two BCP capsules, using t-test

Table 3h: Do you find you can't quite remember something although it's "on the tip of your tongue"?

Time	Group taking one capsule of beta-caryophyllene (BCP) daily (n=26)	Group taking two capsules of beta-caryophyllene daily (n=26)
Do you find you forget people's names?		
Baseline	2.5 ± 0.9	2.5 ± 0.9
Week 4 vs.	2.2 ± 1.0	2.0 ± 0.9
Baseline	12% better	20% better
Week 8 vs.	2.2 ± 0.8	2.0 ± 1.0
Baseline	12% better	20% better
Do you find you forget what you came to the store to buy?		
Baseline	1.7 ± 1.1	1.5 ± 0.9
Week 4	1.5 ± 0.8	1.0 ± 0.9
vs. Baseline	12% better	33% better
Week 8	1.4 ± 0.8	1.1 ± 0.9
vs. Baseline	18% better	27% better
Do you find you can't think of anything to say?		
Baseline	1.3 ± 0.7	1.1 ± 0.9
Week 4 vs.	1.1 ± 0.8	0.8 ± 0.8
Baseline	15% better	27% better
Week 8 vs.	1.3 ± 0.8	0.8 ± 0.7
Baseline	no change	27% better
Do you find you forget which way to turn on a road you know well but rarely use?		
Baseline	1.1 ± 0.8	0.9 ± 0.8
Week 4	0.8 ± 0.7	0.6 ± 0.8
vs. Baseline	27% better	33% better
Week 8	0.8 ± 0.7	0.5 ± 0.6

vs. Baseline	27% better	44% better
Do you lose track of paying bills?		
Baseline	1.3 ± 0.9	1.0 ± 0.9
Week 4 vs.	1.0 ± 0.8	0.9 ± 1.0
Baseline	23% better	10% better
Week 8 vs.	1.1 ± 0.8	0.7 ± 0.9
Baseline	15% better	30% better
Do you fail to notice signposts on the road?		
Baseline	1.2 ± 0.8	1.0 ± 0.8
Week 4 vs.	1.0 ± 0.7	0.7 ± 0.7
Baseline	17% better	30% better
Week 8 vs.	1.0 ± 0.8	0.8 ± 0.7
Baseline	17% better	20% better
Do you confuse left and right when giving people directions?		
Baseline	0.6 ± 1.0	0.9 ± 1.2
Week 4 vs.	0.7 ± 1.0	0.7 ± 0.8
Baseline	17% worse	22% better
Week 8 vs.	0.7 ± 0.9	0.7 ± 0.8
Baseline	17% worse	22% better
Do you bump into people?		
Baseline	0.7 ± 0.6	0.7 ± 1.0
Week 4 vs.	0.5 ± 0.6	0.6 ± 0.8
Baseline	29% better	14% better
Week 8 vs.	0.7 ± 0.6	0.5 ± 0.6
Baseline	No change	29% better
Do you fail to listen to people's names when you are meeting them for the first time?		
Baseline	2.2 ± 1.0	2.3 ± 1.1
Week 4 vs.	2.2 ± 1.1	1.8 ± 1.2
Baseline	No change	22% better
Week 8 vs.	1.9 ± 1.2	1.9 ± 1.1
Baseline	14% better	17% better
Do you say something and realize afterwards that it might be taken as an insult?		
Baseline	1.5 ± 0.9	1.4 ± 0.8
Week 4 vs.	1.1 ± 1.0	1.4 ± 0.9
Baseline	28% better	No change
Week 8 vs.	1.2 ± 0.7	1.2 ± 0.7
Baseline	20% better	14% better
Do you fail to hear people speaking to you when you are doing something?		
Baseline	2.0 ± 0.8	1.7 ± 0.8
Week 4 vs.	1.7 ± 0.9	1.5 ± 1.1
Baseline	15% better	12% better
Week 8 vs.	1.6 ± 0.9	1.3 ± 1.1
Baseline	20% better	23% better
Do you lose your temper and regret it?		

Baseline	1.1 ± 0.7	1.5 ± 0.9
Week 4 vs.	1.1 ± 0.7	1.1 ± 0.8
Baseline	No change	26% better
Week 8 vs.	1.0 ± 0.6	1.0 ± 0.7
Baseline	9% better	33% better
Do you fail to see what you want in a supermarket (although it's there)?		
Baseline	1.4 ± 0.9	1.3 ± 1.1
Week 4 vs.	1.2 ± 0.7	1.1 ± 1.0
Baseline	14% better	15% better
Week 8 vs.	1.3 ± 0.9	1.0 ± 0.8
Baseline	7% better	23% better
Do you find yourself suddenly wondering if you used a word correctly?		
Baseline	1.4 ± 1.0	1.4 ± 1.2
Week 4 vs.	1.2 ± 0.8	1.0 ± 1.1
Baseline	14% better	29% better
Week 8 vs.	1.2 ± 0.7	0.8 ± 0.9
Baseline	14% better	43% better
Do you have trouble making up your mind?		
Baseline	1.5 ± 0.9	1.9 ± 1.2
Week 4 vs.	1.3 ± 0.7	1.5 ± 1.1
Baseline	13% better	21% better
Week 8 vs.	1.4 ± 0.9	1.4 ± 0.9
Baseline	7% better	26% better
Do you find that you forget appointments?		
Baseline	0.8 ± 0.7	1.0 ± 1.0
Week 4 vs.	1.0 ± 0.7	0.7 ± 0.8
Baseline	25% worse	30% better
Week 8 vs.	0.9 ± 0.8	0.5 ± 0.5
Baseline	13% worse	50% better
Do you find that you drop things?		
Baseline	1.4 ± 0.9	1.5 ± 0.9
Week 4 vs.	1.2 ± 0.9	1.1 ± 0.7
Baseline	14% better	27% better
Week 8 vs.	1.2 ± 0.7	1.0 ± 0.7
Baseline	14% better	33% better

Table 3i: Non-significant changes in responses to CFQ.

The question related to "having to re-read something because you were thinking of something else" significantly improved with two BCP capsules at week 4 (39%) and week 8 (39%) ($P \leq 0.005$ for both time points; Table 3a). For both BCP capsule groups, significant benefit was observed in "remembering why you went from one room to another" (Table 3b). For those taking one BCP capsule, significant improvement was observed at week 4 (29%; $P \leq 0.005$) and week 8 (29%; $P \leq 0.005$) compared to baseline. For those taking two BCP capsules, compared to baseline, improvement was observed at week 4 (39%; $P \leq 0.0005$) and week 8 (39%; $P \leq 0.0005$).

The score for "remembering whether you turned a light off or locked a door" was significantly improved only for those taking two BCP capsules (Table 3c). Compared to baseline, at week 4 (45% better; $P \leq 0.005$) and week 8 (40% better $P \leq 0.005$). The "ability to remember where you put a newspaper or book" improved in both BCP groups (Table 3d). For those taking one BCP capsule, the percentage improvement at both

weeks 4 and 8 was 22% ($P \leq 0.05$) compared to baseline. Those taking two BCP capsules, compared to baseline, improved 19% at week 4 ($P \leq 0.01$) and at week 8 improved 29% ($P \leq 0.01$).

Accidently "misplacing something like putting fruit in the cupboard and the bowl in the refrigerator" was only significant for those taking two BCP capsules (Table 3e). Compared to baseline, at week 8 there was 63% improvement ($P \leq 0.05$). The participants taking two BCP capsules daydreamed significantly less when they were supposed to be listening to something (Table 3f). At both week 4 (28% better; $P \leq 0.05$) and week 8 (33%; $P \leq 0.05$) improvement occurred compared to baseline.

Becoming less distracted and doing something else unintentionally after starting a task improved significantly for those taking two BCP capsules at weeks 4 and 8 (30%; $P \leq 0.005$ for both time points) (Table 3g). The ability to remember things that are on the "tip of the tongue" improved significantly for those taking one BCP capsule at week 8 compared to baseline (19%; $P \leq 0.01$) and for those taking two BCP capsules at week 4 compared to baseline (20%; $P \leq 0.01$ (Table 3h).

Changes in CFQ that did not achieve statistical significance (Table 3i): Seventeen of the twenty-five CFQ questions did not achieve significance over the 8-week study for either dietary intervention (Table 3i). Most mean baseline responses (15/17) in both BCP groups have values of 2.0 or less, which indicated that they were near-normal, and, thus, significant improvements would be unlikely. Despite seemingly high percentage improvements (many of 20% to 30%), the large standard deviations made achieving significance less likely.

Individual questions that did not result in significance improvement included: forgetting someone's name, forgetting why entered a store, failing to remember anything to say, forgetting which way to turn on a road, not paying bills, forgetting to look at signposts, confusing left and right when giving directions, bumping into people, not listening when told someone's name, worrying that something said was insulting, failing to hear people when doing something else, loosing your temper, forgetting why went into the supermarket, using a word incorrectly, having trouble making up your mind, forgetting an appointment, and dropping things (Table 3i).

The only times that a response was worse at week 4 and 8 than baseline was in those taking one BCP capsule at week 4 and week 8 for confusing left and right when giving others directions; and at week 4 and week 8 for forgetting an appointment (Table 3i). However, none of these were significant.

Dietary History

Pre-study dietary habits that related to cognition showed little differences between the two groups (Table 4). Most participants (> 70%) consumed sugar-rich foods one to three times daily; more than half consumed fish once weekly; and more than half claimed to avoid fast food restaurants. The most common frequent response for consuming red meat and cold cuts was two to three times weekly. About half of the subjects consumed seven servings of fruits and vegetables daily. One-third consumed seeds and nuts about once weekly; a bit more (~40%) consumed them two to three times weekly. About half of the participants stated that they did not consume alcoholic beverages.

Frequency	Group 1=29 (one capsule daily) (%)	Group 2=29 (two capsules daily)
Daily consumption of sugary foods		
Never/rarely	24%	21%
1-3 times daily	72%	76%
4-6 times daily	3%	3%
Weekly servings of fish consumed		
Never/rarely eat fish	10%	21%
About 1 serving per week	66%	52%
2-3 servings per week	17%	28%
4-6 servings per week	7%	0%
Weekly eating at fast food restaurant		
Never/rarely	62%	52%
About 1 time per week	17%	28%
2-3 times per week	17%	17%
4-6 times per week	3%	3%

Weekly servings of red meats or cold cuts consumed		
Never/rarely	7%	3%
About 1 time per week	10%	14%
2-3 times per week	66%	45%
4-6 times per week	17%	35%
7 times per week (at least one serving daily)	0 (0%)	3%
Daily consumption of fruits and vegetables		
Never/rarely	0%	0%
1 per day	0%	0%
2-3 servings per day	21%	17%
4-6 servings per day	28%	35%
≥ 7 servings per day	52%	48%
Weekly consumption of seeds and nuts		
Rarely/Never	3%	7%
About 1 time per week	31%	28%
2-3 times per week	45%	38%
4-6 times per week	21%	17%
≥ 7 times per week (at least daily)	0%	10%
Weekly consumption of alcohol (includes, spirits, wine, beer)		
Rarely/Never	48%	59%
1-2 times per week	41%	21%
3-4 times per week	3%	17%
≥ 7 times per week	7%	3%

Table 4: Baseline dietary habits of the participants that are related to cognition.

Discussion

Neuroinflammation is an emerging cause of age-related dementia, and the microglial cells are of interest, as they are the resident macrophages in the brain [2,4]. Cloves contain a rich source of beta-caryophyllene (BCP), which has been shown in animal models to reduce inflammation [5-6,8,13]. We showed that BCP obtained from an extract of cloves improved cognition in an elderly population, who reported being worried about their memory. Both dietary interventions of BCP (90 mg or 180 mg) led to improvement in various aspects of cognition over 8 weeks, but taking two capsules resulted in more improvement. This was the first report to our knowledge where BCP favorably affected cognition.

Based on the results of the Cognitive Failures Questionnaire (CFQ), each of the four sub-scale scores measurements at week 4 and week 8 improved for both dietary intervention groups compared to baseline (i.e., one or two BCP capsules). Taking two BCP capsules resulted in significant improvement for all four sub-scale scores at week 4 and week 8 compared to baseline. In contrast, those taking one BCP capsule had a significant improvement only three of eight times. The percentage improvement in the sub-scale scores for those taking two BCP capsules were about twice greater than experienced by those taking one BCP capsule. Given the dose effects of BCP, it is possible that a larger dose than the ones tested could lead to additional cognitive improvement.

Pre-study dietary habits that related to cognition showed little differences between the two groups. Most participants ate a typical American diet is associated with an increased risk of dementia (e.g., consumed sugar-rich foods, had limited seafood, seeds and nuts). On the positive side, the subjects tended to avoid alcohol and dining at fast food restaurants. The anti-oxidant rich fruits and vegetables were consumed by the participants in amounts commensurate with the Mediterranean and MIND diets, which have been shown reduce the risk of dementia [18,19]. However, according to the *Dietary Guidelines for Americans 2020-2025*, most American don't adhere to these healthy diets [21]. For those aged 60 years and older, the Healthy Eating Index score was 63 out of a possible 100. The current study showed that it is much simpler to include a small amount of BCP than to change an entire diet.

The limitation of the study was the lack of a placebo group. However, this was a pilot study to determine if the doses selected had any impact on cognition, which they both

did. Given the improvement observed between 90 mg and 180 mg of BCP, it seems likely that a placebo group would not promote significant benefit. The participants self-reported that they noticed they had memory difficulties. It would have been preferable to have everyone screened and accrue those with similar levels of mild cognitive impairment.

In summary, there are no treatments for dementia, and use of natural compound from cloves is an easy way to improve cognition. The participants found the intervention satisfactory in that compliance was excellent. Results of cognitive testing improved for both dietary interventions, but better results were observed in those using 180 mg of BCP.

Conflicts of Interest and Source of Funding

BrainCare, LLC funded the study. Dr. Bell is a scientific consultant to BrainCare, LLC, the company that sells the clove extract; manufacturing of the capsules used in this study was done at an outside facility. Dr. Gomez-Pinilla is a member of the Scientific Advisory Board of BrainCare, LLC. Dr. Ling was paid as a statistical consultant and she worked independently of the other co-authors.

Author Contributions

All co-authors were involved with the manuscript. Dr. Bell made a substantial contribution to the study design and prepared the draft of the manuscript. Dr. Ling made important contributions to the data analysis. All co-authors were involved with interpretation of the data, and provided critical and intellectual reviews of the draft manuscript to get it to the point where it was suitable to be submitted for publication.

Acknowledgments

The authors are grateful to the participants, who dutifully completed their weekly data collections forms, including lengthy questionnaires about their cognitive function. Based on the feedback from the participants, they were pleased with their results. We are indebted to Kate Bauer for her careful work in assembling the data that was submitted by the participants. This study would not have been possible without Charles Marsland, co-founder of BrainCare, LLC, who identified the beta-caryophyllene (BCP) used in the study and determined how it should be manufactured. Mr. Marsland and Dr. Bell have a patent-pending for improving cognitive function with BCP (17/459,889).

References

1. Kinney JW, Bemiller SM, Murtishaw AS, Salazar AM, et al. (2018) Inflammation as a central mechanism in Alzheimer's disease. Alzheimer's Dement 4: 575-590.

2. Wyss-Coray T, Rogers J (2012) Inflammation in Alzheimer disease-a brief review of the basic science and clinical literature. Cold Spring Harb Perspect Med 2(1): a006346.

3. Cheng Y, Dong Z, Liu S (2014) β-Caryophyllene ameliorates the Alzheimer-like phenotype in APP/PS1 Mice through CB2 receptor activation and the PPARγ pathway. Pharmacology 94(1-2): 1-12.

4. Walker KA, Gottesman RF, Wu A, Knopman DS, Gross AL, et al. (2019) Systemic inflammation during midlife and cognitive change over 20 years: The ARIC Study. Neurology 92(11): e1256-e1267.

5. Basha RH, Sankaranarayanan C (2015) Protective role of β-caryophyllene, a sesquiterpene lactone on plasma and tissue glycoprotein components in streptozotocin-induced hyperglycemic rats. J Acute Medicine 5(1): 9-14.

6. Assis LC, Straliotto MR, Engel D, Hort MA, Dutra RC, et al. (2014) β-Caryophyllene protects the C6 glioma cells against glutamate-induced excitotoxicity through the Nrf2 pathway. Neuroscience. 279: 220-31.

7. Bento AF, Marcon R, Dutra RC, Cola M, Leite DF, et al. (2011) β-Caryophyllene Inhibits Dextran Sulfate Sodium-Induced Colitis in Mice through CB2 Receptor Activation and PPARγ Pathway. The Am J Pathology 178(3): 1153-1166.

8. Tolon RM, Nunez E, Pazo MR, Benito C, Castillo AI, et al. (2009) The activation of cannabinoid CB_2 receptors stimulates in situ and in vitro *beta*-amyloid removal by human macrophages. Brain Res 1283: 148-154.

9. Klauke L, Racz I, Pradier B, Markert A, Zimmer AM, et al. (2014) The cannabinoid CB_2 receptor-selective phytocannabinoid beta-caryophyllene exerts analgesic effects in mouse models of inflammatory and neuropathic pain. European Neuropsychopharmacology 24(4): 608-620.

10. Bahi A, Al Mansouri S, Al Memari E, M Al Ameri, Nurulain SM, et al. (2014) β-Caryophyllene, a CB_2 receptor agonist produces multiple behavioral changes relevant to anxiety and depression in mice. Physiology & Behavior 135: 119-124.

11. Hashiesh HM, Meeran MFN, Sharma C, Sadek B, Kaabi JA, et al. (2020) Therapeutic Potential of β-Caryophyllene: A Dietary Cannabinoid in Diabetes and Associated Complications. Nutrients 12(10): 2963.

12. Kamikubo R, Kai K, Tsuji-Naito K, Akagawa M (2016)

β-Caryophyllene attenuates palmitate-induced lipid accumulation through AMPK signaling by activating CB2 receptor in human HepG2 hepatocytes. Mol Nutr Food Res 60(10): 2228-2242.

13. Paula-Freire LIG, Andersen ML, Garna VS, Molska GR, Carlini ELA (2014) The oral administration of trans-caryophyllene attenuates acute and chronic pain in mice. Phytomedicine 21(3): 356-362.

14. de Oliveira CC, de Oliveira CV, Grigoletto J, Ribeiro LR, Funck VR, et al. (2016) Anticonvulsant activity of β-caryophyllene against pentylenetetrazol-induced seizures. Epilepsy Behav 56: 26-31.

15. Scandiffio R, Geddo F, Cottone E, Querio G, Antoniotti S, et al. (2020) Protective Effects of (*E*)-β-Caryophyllene (BCP) in Chronic Inflammation. Nutrients 12(11): 3273.

16. Biosfered (2018) PipeNig®, a black pepper (Piper nigrum) extract with standardized content of (E)-β-caryophyllene, Nutrafoods 17: N12-N23

17. Tarumi W, Shinohara K (2020) Olfactory Exposure to β-Caryophyllene Increases Testosterone Levels in Women's Saliva. Sex Med 8(3): 525-531.

18. Valls-Pedret C, Sala-Vila A, Serra-Mir M, Corella D, de la Torre R, et al. (2015) Mediterranean Diet and Age-Related Cognitive Decline: A Randomized Clinical Trial. JAMA Intern Med 175(7): 1094-1103.

19. Morris MC, Tangney CC, Wang Y, Sacks FM, Bennett DA, et al. (2015) MIND diet associated with reduced incidence of Alzheimer's disease. Alzheimers Dement 11(9): 1007-14.

20. National Heart, Lung, and Blood Institute. Classification of Overweight and Obesity by BMI, Waist Circumference, and Associated Disease Risks.

21. (2020) U.S. Department of Agriculture and U.S. Department of Health and Human Services. Dietary Guidelines for Americans, 2020-2025. 9th (Edn.),