Beta-caryophyllene, An Anti-Inflammatory Natural Compound, Improves Cognition

Stacey J. Bell[1*], Fernando Gomez-Pinilla[2], Pei-Ra Ling[3]

[*1]Scientific consultant, BrainCare, LLC, 110 Woodland Street, Reno, NV 89523, USA.

[2]Professor of Neurosurgery and Integrative Biology and Physiology, UCLA, PO Box 956901, Los Angeles, CA 90095-6901, USA.

[3]Consultant, Cambridge, MA 02139, USA.

RESEARCH

Please cite this paper as: Bell SJ, Gomez-Pinilla F, Ling PR. Beta-caryophyllene, an anti-inflammatory natural compound, improves cognition. Journal of Food & Nutritional Sciences [2021] 3(2): 31-43.

***Corresponding Author:**

Stacey J. Bell, D.Sc.

Scientific consultant, BrainCare, LLC, 110 Woodland Street, Reno, NV 89523, USA, Tel: (617) 999 6150; E-mail: stacey.bell@nutrientfoods.com

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 the 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). At baseline, week 4, and week 8, cognitive function using four brain games taken online and quality of life were measured. At baseline, the average age was 67 ± 5 years, and the subjects were obese according to mean body mass indexes.

Baseline mean brain scores for the four tests were near or below average; the percentage changes for all scores were small (2-5% increases, except for two tests for those taking two BCP capsules, which had increases of 7% for Spatial Planning and 10% for Double Trouble). The percentage of participants who improved in all tests over 8 weeks was between 40% and 73%; similar improvements were observed between those taking one BCP capsule or two capsules. Interesting, over 8 weeks, the biggest improvements were observed for the combined summed means of all four tests for those with baseline mean scores at or below 100 (those taking one BCP capsule improved 5.0 ± 9.8%, and those taking two capsules improved 9.0 ± 12.9%).

Of the ten quality of life questions, four did not change significantly over 8 weeks. Questions related to memory, forgetfulness, remembering things, and focus improved significantly over 8 weeks ($P < 0.05$). A feeling of wellbeing and general health worsened in both groups, but the study was conducted in the early period of the Coronavirus disease-19 pandemic, so this may explain these findings.

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 BCP from cloves is an easy way to improve cognitive function in an elderly population, and it could be especially beneficial to those with the poorest cognition.

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: beta-amyloid plaque and neurofibrillary tangles [1-3]. More recently, neuroinflammation has been added to the mix, and it is known to play a neuropathological role in cognitive decline. Moreover, systemic inflammation is also associated with cognitive decline [4].

Inflammation of the brain has prompted testing anti-inflammatory drugs to treat or slow the progression of Alzheimer's disease.

Because 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 to remove pathogens before they do damage.

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, leading to reduced 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 works 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 [7], pain and inflammation in a neuropathic pain model [9], and oxidative stress reduction in the glial cells [6]. BCP has also shown promise for treating anxiety and depression [10], being a plausible therapeutic in diabetes and associated complications [11], preventing and ameliorating non-alcoholic fatty liver disease [12], lessening acute and chronic pain [13], and having an anti-convulsive effect against seizures [14]. In addition, BCP may offer benefit to promote weight loss and reduce cardiovascular disease [15]. 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. 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 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.

Systemic inflammation is associated with impaired cognition. Down-regulation of neuroinflammation is an attractive way to mitigate this. It is possible that activation of CB2R from BCP could lead to beneficial cognitive effects by reducing the neuroinflammatory response. 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, who were 60-80 years of age. 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. Cognitive testing and quality of life were assessed at baseline, week 4, and week 8. Each week, participants recorded usage of the BCP, body weight, and 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) [18]. 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 submisson of all data at week 8.

Diet

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.

Cognitive testing using Cambridge Brain Sciences, Toronto, Canada

(https://www.cambridgebrainsciences.com/)

The Cambridge Brain Sciences (CBS) included a battery of four tests to measure various aspects of cognition and that affected different regions of the brain. The tests were performed at baseline and weeks 4 and 8. Normal scores for each test ranged from 87 to 113. Improvement meant that the mean score increased from baseline to week 8, no change meant that the score at week 8 was the same as baseline, and worse indicated that the score at week 8 was lower than at baseline.

The first test is the Spatial Planning test, which addresses reasoning and planning. It tests for non-Alzheimer's dementia but has a high correlation with aging. It has a high reproducibility and is useful to distinguish impaired and unimpaired populations. The parts of the brain implicated include: frontal lobe, mid-dorsolateral frontal cortex, caudate nucleus, thalamus, lateral premotor, and anterior cingulate.

The second test is Double Trouble and addresses concentration and response inhibition. It can detect early Alzheimer's disease and age-related cognitive decline. It has a high reproducibility and is useful in distinguishing impaired and unimpaired aging. The parts of the brain involved include the right prefrontal cortex and dorsolateral region.

The third test is the Monkey Ladder, which relates visuospatial and working memory. The test can detect early Alzheimer's disease, non-Alzheimer's dementia, and age-related cognitive decline. The parts of the brain involved included: the mid-dorsolateral prefrontal cortex, premotor cortex, and posterior parietal cortex.

The fourth test is the Feature Match (aka Feature Selection), which addresses concentration and attention, and correlates well with aging. It can detect early Alzheimer's disease, non-Alzheimer's dementia. The parts of the brain involved include the mid-ventrolateral frontal cortex and the right inferior frontal gyrus.

Quality of life questions

Quality of life was determined weekly based on ten questions. Each was rated on a scale of 1 to 5, with 5 being the best and 1 being the worst. The questions asked were: general feeling, pain, mood, energy level, over-all memory, general health, forget the point you were trying to make while speaking, forget where you put your keys, sustain focus when reading, and alertness. Data were compared at baseline, week 4, and week 8, and the percentage change between the mean scores at baseline and week 8 was calculated.

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.). For the quality of life questions, results are compared by Student's t-test between baseline and week 4, and baseline and week 8 within each group. The 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.

For Cambridge Brain Sciences tests, some responses were flagged as being a highly unusual response (found in less than 1% of Cambridge Brain Sciences normative database). It may be that the participant did not understand the instructions for the test or was distracted. These scores were considered to be invalid and excluded from the final analysis. Data were presented as scores at

baseline, week 4, and week 8; the percentage change was also computed.

Subsequently, for each participant, the combined mean scores of all four tests were summed for at baseline and at week 8. The participants were then grouped according to these mean baseline score of all four tests as: below average (less than or equal to 100) and average and above (greater than 100).

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 certain diet to protect the brain.

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.

Cambridge Brain Sciences

Cambridge Brain Sciences actual scores and percentage change

One BCP capsule. Mean test scores at baseline indicated that the participants were either on the low side of normal which was 87-99 (88 ± 12 Double Trouble and 96 ± 6 Feature Match) or closer to the mid-point of 100 (102 ± 11 Spatial Planning, and 101 ± 6 Monkey Ladder) (Table 2). Considering all the subjects who had a sub-optimal score at baseline (i.e., less that 87), 31% improved during the study to a normal score on any test (i.e., 87 or greater).

Those taking one BCP capsule experienced average improvement for each test between 2% and 5%. For the Double Trouble test, 56% had sub-optimal scores at baseline and by week 8, only 44% had sub-optimal score. No meaningful changes in normalization were observed for the other three tests.

Two BCP capsules. Mean test scores at baseline were similar to those taking one capsule (Table 2). The means of three tests (Spatial Planning, Double Trouble, and Feature Match) were below 100 (96 ± 10, 88 ± 12, and 99 ± 7, respectively). Only the Monkey Ladder baseline mean exceeded 100 (102 ± 6). The biggest improvements in mean test scores that were significant for both ($P < 0.05$) were for Double Trouble (10%) and followed by 7% for Spatial Planning. A smaller increase in mean score between baseline and week 8 was observed for Monkey Ladder (2%), and there was no change in Future Match.

Improvement, worsening, and no change in Cambridge Brain Sciences tests between baseline and week 8

The overall percentage of those who improved, worsened, and remained the same for the four tests showed that more than half of all participants improved on each test, except for Feature Match for those taking two BCP capsules (Table 3). The percentage of those taking one BCP capsule that improved for Spatial Planning was 73%, for Double Trouble was 60%, Monkey Ladder was 70%, and Feature Match was 54%. The percent that had worse scores were 19% for Spatial Planning, 36% for Double Trouble, 26% for Monkey Ladder, and 33% for Feature Match. The remaining percentage of participants had no change.

The percentage of those taking two BCP capsules that improved for Spatial Planning was 72%, Double Trouble was 64%, Monkey Ladder was 68%, and Feature Match was 40% (Table 3). The percentage that had worse scores were 20% for Spatial Planning, 23% for Double Trouble, 4% for Monkey Ladder, and 44% for Feature Match. The remaining percentage of participants had no change.

Summed combined means of the four Cambridge Brain Sciences test scores at baseline and week 8

The mean combined sum of the four Cambridge Brain Sciences test scores at baseline was below the average of 100 (Table 4). Those taking one BCP capsule had a mean baseline of four test scores of 96.8, which increased to 99.8 at week 8, indicating a 3.6 ± 10.0% increase. For those taking two BCP capsules, mean baseline summed score was 97.2 and increased to 101.0, which was a 4.6 ± 12.1% increase.

Looking at only those with mean combined summed baseline scores for four tests of less than or equal to 100 revealed that those taking one BCP capsule experienced a 5.0 ± 9.8% increase (baseline was 92.3; week 8 was 96.8) (Table 4). Those taking two BCP capsules with baseline mean summed scores of 100 or less, experienced a 9.0% ± 12.9% increase (baseline was 91.0; week 8 was 98.8). In contrast, those with baseline mean combined summed scores over 100 for the four Cambridge Brain Sciences tests did not change appreciably at week 8 (-0.7 ± 9.4% for those taking one BCP and -1.5% ± 7.5% for those taking two BCP capsules).

Quality of life

Four quality of life indicators, pain level, mood, energy, and alertness, had no significant change over the 8-week study regardless of the number of BCP capsules taken. Significant changes in quality of life indicators are presented in Tables 5a-f. Memory improved 15% for those taking two BCP capsules at weeks 4 and 8 as compared to baseline ($P < 0.005$) (Table 5a). For those taking one BCP, memory improved to a lesser extent compared to baseline and did not reach statistical significance (7% at weeks 4 and 8). The mean scores for remembering the point you were trying to make increased in both groups (Table 5b). Those taking one BCP capsule experienced a 13% improvement at week 8 compared to baseline ($P = 0.05$); no change was observed at week 4. Those taking two capsules reported significant benefit at weeks 4 and 8 of 18% at each interval compared to baseline ($P = 0.001$).

Both dietary interventions improved in their ability to remembering things but those taking two BCP capsules improved more (Table 5c). For those taking two capsules, significant improvement occurred at week 4 and remained the same at week 8 (19%; $P < 0.0005$). Those taking one BCP capsule improved 6% at week 4 and 12% at week 8; neither change was significantly different from baseline. The ability to focus was improved over time in both dietary interventions at week 4 and week 8 (Table 5d). Compared to baseline mean scores, those taking two BCP capsules improved more than those taking one capsule (at week 4, 20% vs. 6%; and at week 8, 27% compared to 17%, respectively). And, the improvements were only significant for those taking two BCP capsules ($P < 0.05$ at week 4 and $P < 0.005$ at week 8).

Participants experienced a worsening general feeling of wellbeing during the study (Table 5e). At week 4, those taking one BCP capsule experienced a 25% reduction in wellbeing ($P < 0.05$) and those taking two capsules had a

17% decline at week 4 (P = 0.06). At week 8, both groups continued with a poor feeling of wellbeing, but the data were not significantly different from baseline. A decline in general health was observed in those taking one BCP capsule (Table 5f). Health worsened significantly by 26% (P = 0.005) at week 4 and 20% (P = 0.01) at week 8. No significant changes were observed in those taking two BCP capsules (worsening of 3% at week 4, and improvement of 3% at week 8).

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. This was the first report to our knowledge where BCP favorably affected cognition.

Small benefits were observed when the mean of each for the four Cambridge Brain Sciences tests were looked at individually. Of the eight tests (four tests for each intervention), only two improved more than the anticipated 5% achieved by from learning [19]. More than half (but mostly 60-70%) of the participants improved on the cognitive tests. Only one test, Feature Match, had only 40% of the group improve after consuming two BCP capsules. This was the only test in either dietary intervention where a higher percentage of participants experienced a worse score at week 8 compared to baseline. This test specifically addresses concentration and attention, and correlates well with aging, but other tests measured these same attributes and more improvement was observed.

The participants with lower mean combined summed baseline and week 8 test scores were more likely to improve over 8 weeks. Those taking one BCP capsule improved 5%, which is what would have been expected from taking multiple cognitive tests over a short period of time [19]. The group taking two BCP capsules had nearly double what would be expected (9%) improvement. This finding lends support for 180 mg of BCP to improve cognition in an elderly population, who at the onset of the study, had poor cognition. There were no other obvious changes to the participants during the study; the group was weight stable and reported they were compliant with the dietary interventions.

Neuroinflammation seems to increase the risk for dementia, including Alzheimer's disease [1, 2]. At autopsy, the brains of individuals with Alzheimer's disease show evidence of inflammation [2]. The mechanism of action is unknown for how BCP works in the brain to improve cognition but it likely affects the microglial cells, which are referred to as the brain's resident macrophages. Based on culture and animal models, BCP appears to protect the nervous system from inflammation and oxidative stress [6, 7, 15, 20, 21].

Reducing inflammation and oxidative stress provide beneficial effects against neuroinflammatory and neurodegenerative pathologies [22]. Neuroinflammation is a process leading to nervous system degeneration characterized by the activation of macrophages or microglial cells, in the case of the brain. This results in production of inflammatory mediators like various cytokines, nitric oxide, and nuclear factor kappa B. Administration of BCP dampens these mediators [7, 15, 22].

For its anti-oxidant effect, BCP modulates the redox state mainly through stimulation of nuclear factor-like 2 (Nrf2), which is a transcription factor stimulated by oxidative stress [6, 22]. The Nrf2 increases genes involved in cell survival and in the reduction of oxidative stress. BCP also appears to serve as an anti-oxidant by augmenting quinone oxidoreductase activity [23].

Another way that BCP may improve cognition relates to its ability to reduce amyloid plaques, which is considered a factor in worsening cognition in patients with Alzheimer's disease [8, 22].

Self-reported quality of life indicators improved significantly for those questions related to cognition memory, forgetting the point you were trying to make, focus, and remembering things. These findings support the positive outcomes of the four online cognitive testing results. In contrast, a feeling of wellbeing and general health and worsened. This could be explained because the study was conducted in the Summer of 2020, when the Coronavirus disease-19 was a new pandemic, treatments were lacking, and vaccines were not available.

The results of the study seem believable as the participants were representative of an aging population, concerned about failing cognition. Most baseline mean scores for each test in both treatment groups (5/8) were at or below the average score of 100. The group was obese according to a BMI of 32 kg/m^2. A study in more than 6,500 people aged 50 years and older had a 34% increased risk of dementia independent of sex, baseline age, apolipoprotein E-e4 (APOE-e4), education, physical activity, smoking and marital status [24].

The main limitation of the study was that there was no control group; however, it is hoped that these findings encourage future studies. The improvements observed in association with an increased amount of BCP suggests that a control group would have performed less favorably than either dose. It is possible that higher amounts of BCP may work even better and should be explored. Another limitation is that the participants had subjective cognitive decline. More robust measurements of cognition at baseline should be applied in future studies to determine changes based on a more homogeneous cohort.

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 and were more than could have been expected from retaking the online cognitive tests for those with the lowest mean combined test scores at baseline and taking 180 mg of BCP. The exact mechanism of action was not identified but likely the cognitive benefits were related to the anti-oxidant and anti-inflammatory properties of BCP.

Table 1. Baseline comparisons between dietary intervention groups*

	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
$\leq$ 25	2 (7 %)	0 (0 %)
$\leq$ 30	9 (31%)	11 (38 %)
$\leq$ 35 Class 1 Obesity+	10 (34 %)	14 (48 %)
$\leq$ 40 Class 2 Obesity	7 (24 %)	2 (7 %)
> 40 Class 3 Obesity	1 (3 %)	2 (7 %)

*Data presented as mean $\pm$ standard deviation

+Based on reference 18

Table 2. Cambridge Brain Sciences actual scores and percentage change over 8 weeks

Test	**Baseline**	**Week 4**	**Week 8**
Taking one beta-caryophyllene (BCP) capsule			
Spatial Planning	102 ± 11	104 ± 9	+ 2.3 %
Double Trouble	88 ± 12	93 ± 15	+ 5.3 %

Monkey Ladder	101 ± 6	103.8 ± 6.7	+ 2.5 %
Feature Match	96 ± 6	98.3 ± 6.9	+ 2.0 %
Taking two beta-caryophyllene (BCP) capsules			
Spatial Planning	96 ± 10	102. 5 ± 7.8 *	+ 7 %
Double Trouble	88 ± 12	97.3 ± 15.2 *	+ 10 %
Monkey Ladder	102 ± 6	103.7 ± 8.1	+ 2 %
Feature Match	99 ± 7	98.4 ± 7.6	No change

*P ≤ 0.05 compared to baseline by Student's t-test

Table 3. Improvement, worsening, and no change in Cambridge Brain Sciences tests between baseline and week 8

	Spatial Planning	**Double Trouble**	**Monkey Ladder**	**Feature Match**
Taking one beta-caryophyllene (BCP) capsule				
N	26	25	23	24
Improved	19 (73%)	15 (60%)	16 (70%)	13 (54%)
No change	2 (8%)	0	1 (4%)	3 (13%)
Worsened	5 (19%)	9 (36%)	6 (26%)	8 (33%)
Taking two beta-caryophyllene (BCP) capsules				
N	25	22	25	25
Improved	18 (72%)	14 (64%)	17 (68%)	10 (40%)
No change	2 (8%)	3 (14%)	7 (28%)	4 (16%)
Worsened	5 (20%)	5 (23%)	1 (4%)	11 (44%)

Table 4. Summed means of the four Cambridge Brain Sciences test scores at baseline and week 8 (lower scores indicate worse cognitive function)

Grouping of participants by dietary intervention and mean baseline score of the four tests	**Mean of four tests scores at baseline**	**Mean of four tests scores at week 8**	**Percent-age change (mean ± S.D.)**
Taking one BCP capsule*			
Mean of four tests	96.8	99.8	3.6 ± 10.0
Mean of four tests that were less than or equal to 100	92.3	96.8	5.0 ± 9.8
Mean of four tests that were greater than 100	110.0	108.7	-0.7 ± 9.4%
Taking two BCP capsules			
Mean of four tests	97.2	101.0	4.6 ± 2.1

Mean of four tests that were less than or equal to 100	91.0	98.8	9.0 ± 2.9
Mean of four tests that were greater than 100	105.7	104.2	-1.5 ± 7.5

*BCP = beta-caryophyllene

Table 5 (a-f). Changes in quality of life attributes (each attribute was rated on a scale of one to five, with one being the worst and five being the best)

Table 5a. Memory (higher mean 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)**
Baseline	3.1 ± 0.8	3.3 ± 0.6
Week 4 vs. Baseline	3.3 ± 0.7 + 7%	3.8 ± 0.6* + 15%
Week 8 vs. Baseline	3.3 ± 0.6 +7%	3.8 ± 0.6* + 15%

*P < 0.005 compared to baseline

Table 5b. Forgetting the point you want to make (higher mean 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)**
Baseline	3.2 ± 0.7	3.4 ± 0.6
Week 4 vs. Baseline	3.5 ± 0.7 + 9%	4.0 ± 0.6** + 18%
Week 8 vs. Baseline	3.6 ± 0.6* + 13%	4.0 ± 0.7** + 18%

*P = 0.05 compared to baseline

** P = 0.001 compared to baseline

Table 5c. Difficulty remembering things (higher mean 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)**
Baseline	3.3 ± 0.8	3.7 ± 0.8
Week 4 vs. Baseline	3.5 ± 0.9 + 6.0%	4.4 ± 0.6* + 19%
Week 8 vs. Baseline	3.7 ± 0.8 + 12%	4.4 ± 0.7* + 19%

*P ≤ 0.005 compared to baseline

Table 5d. The ability to focus (higher mean 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)
Baseline	3.0 ± 1.0	3.0 ± 1.0
Week 4 vs. Baseline	3.2 ± 1.0 + 6%	3.6 ± 0.9* + 20%
Week 8 vs. Baseline	3.5 ± 1.1 + 17%	3.8 ± 0.8** + 27%

*P < 0.05, compared to baseline

**P < 0.005, compared to baseline

Table 5e. General feeling of wellbeing (higher mean 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)
Baseline	3.2 ± 1.1	3.5 ± 0.8
Week 4 vs. Baseline	2.4 ± 1.1* - 25%	2.9 ± 1.1 - 17%
Week 8 vs. Baseline	2.5 ± 1.2 -22%	3.2 ± 1.0 - 9%

*P = 0.02 compared to baseline

Table 5f. General health (higher mean 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)
Baseline	3.5 ± 0.9	3.2 ± 1.0
Week 4 vs. Baseline	2.6 ± 1.0* - 26%	3.1 ± 1.1 - 3%
Week 8 vs. Baseline	2.8 ± 0.8** - 20%	3.3 ± 0.9 + 3%

*P < 0.005, compared to baseline

**P = 0.01, compared to baseline

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 filed a patent for improving cognitive function with BCP (number 17/459,889).

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.

REFERENCES

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

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

3. Cheng Y, Dong Z, Liu S. β-Caryophyllene ameliorates the Alzheimer-like phenotype in APP/PS1 Mice through CB2 receptor activation and the PPARγ pathway. (20140 Pharmacology. 94(1-2):1-12.

4. Walker KA, Gottesman RF, Wu A, Knopman DS, Gross AL, Mosley TH Jr, Selvin E, Windham BG. (2019) Systemic inflammation during midlife and cognitive change over 20 years: The ARIC Study. Neurology. 12; 92(11): e1256-e1267.

5. Basha RH and 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, de Bem AF. (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, 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: 1153-1166.

8. Tolon RM, Nunez E, Pazo MR, Benito C, et al. (2009) The activation of cannabinoid CB2 receptors stimulates in situ and in vitro beta-amyloid removal by human macrophages. 1283:148-154.

9. Klauke L, Racz I, Pradier B, Markert A, et al. (2014) The cannabinoid CB2 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, et al. (2014) β-Caryophyllene, a CB2 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, Ojha SK. Therapeutic Potential of β-Caryophyllene: A Dietary Cannabinoid in Diabetes and Associated Complications. Nutrients. 2020 Sep 28; 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; pp. 356-362.

14. de Oliveira CC, de Oliveira CV, Grigoletto J, Ribeiro LR, Funck VR, Grauncke AC, de Souza TL, Souto NS, Furian AF, Menezes IR, Oliveira MS. (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, Gallo MP, Maffei ME, Bovolin P. (2020) Protective Effects of (E)-β-Caryophyllene (BCP) in Chronic Inflammation. Nutrients. 12(11): 3273.

16. Biosfered. PipeNig®, a black pepper (Piper nigrum) extract with standardized content of (E)-β-caryophyllene, Nutrafoods (2018) 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. National Heart, Lung, and Blood Institute. Classification of Overweight and Obesity by BMI, Waist Circumference, and Associated Disease Risks https://www.nhlbi.nih.gov/health/educational/lose_wt/BMI/bmi_dis.htm

19. Hampshire A, Highfield R, Parkin BL, Owen AM. (2012) Fractionating Human Intelligence. Neuron 76(6): 1225-1237.

20. Machado KDC, Islam MT, Ali ES, Rouf R, Uddin SJ, Dev S, Shilpi JA, Shill MC, Reza HM, Das AK, Shaw S, Mubarak MS, Mishra SK, Melo-Cavalcante AAC. A systematic review on the neuroprotective perspectives of beta-caryophyllene. Phytother Res. 2018 Dec; 32(12): 2376-2388.

21. Ames-Sibin AP, Barizão CL, Castro-Ghizoni CV, Silva FMS, Sá-Nakanishi AB, Bracht L, Bersani-Amado CA, Marçal-Natali MR, Bracht A, Comar JF. β-Caryophyllene, the major constituent of copaiba oil, reduces systemic inflammation and oxidative stress in arthritic rats. J Cell Biochem. 2018 Dec; 119(12): 10262-10277.

22. Francomano F, Caruso A, Barbarossa A, Fazio A, La Torre C, Ceramella J, Mallamaci R, Saturnino C, Iacopetta D, Sinicropi MS. β-Caryophyllene: A Sesquiterpene with Countless Biological Properties. Applied Sciences. 2019; 9(24):5420.

23. Flores-Soto ME, Corona-Angeles JA, Tejeda-Martinez AR, Flores-Guzman PA, Luna-Mujica I, Chaparro-Huerta V, Viveros-Paredes JM. β-Caryophyllene exerts protective antioxidant effects through the activation of NQO1 in the MPTP model of Parkinson's disease. Neurosci Lett. 2021 Jan 18; 742: 135534.

24. Ma Y, Ajnakina O, Steptoe A, Cadar D. Higher risk of dementia in English older individuals who are overweight or obese. Int J Epidemiol. 2020 Aug 1; 49(4): 1353-1365.

PEER REVIEW

Not commissioned. Externally peer reviewed.