Title

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Journal

Molecular Nutrition & Food Research, 63(15)

ISSN

1613-4125

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Publication Date

2019-08-01

DOI

10.1002/mnfr.201801055

Peer reviewed

HHS Public Access

Author manuscript

Mol Nutr Food Res. Author manuscript; available in PMC 2020 August 01.

Published in final edited form as:

Mol Nutr Food Res. 2019 August ; 63(15): e1801055. doi:10.1002/mnfr.201801055.

Blueberry supplementation mitigates altered brain plasticity and behaviour after traumatic brain injury in rats

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Abstract

Scope: Traumatic brain injury (TBI) compromises neuronal function required for hippocampal synaptic plasticity and cognitive function. Despite the high consumption of blueberries, information about its effects on brain plasticity and function under conditions of brain trauma is limited. We assessed the efficacy of dietary blueberry (BB) supplementation to mitigate the effects of TBI on plasticity markers and associated behavioural function in a rodent model of concussive injury.

Methods and results: Rats were maintained on a diet supplemented with blueberry (BB, 5% w/w) for 2 weeks after TBI. We found that that BB supplementation mitigated a loss of spatial learning and memory performance after TBI, and reduced the effects of TBI on anxiety-like behaviour. BB supplementation prevented a reduction of molecules associated with the BDNF system action on learning and memory such as cyclic-AMP response element binding factor (CREB), calcium/calmodulin-dependent protein kinase II (CaMKII). In addition, BB supplementation reversed an increase of the lipid peroxidation by product 4-hydroxy-nonenal (4-HNE) after TBI. Importantly, synaptic and neuronal signaling regulators changed in proportion with the memory performance suggesting an association between plasticity markers and behaviour.

Conclusion: Our data indicate that BB supplementation has a beneficial value for mitigating the acute aspects of the TBI pathology.

GRAPHICAL ABSTRACT

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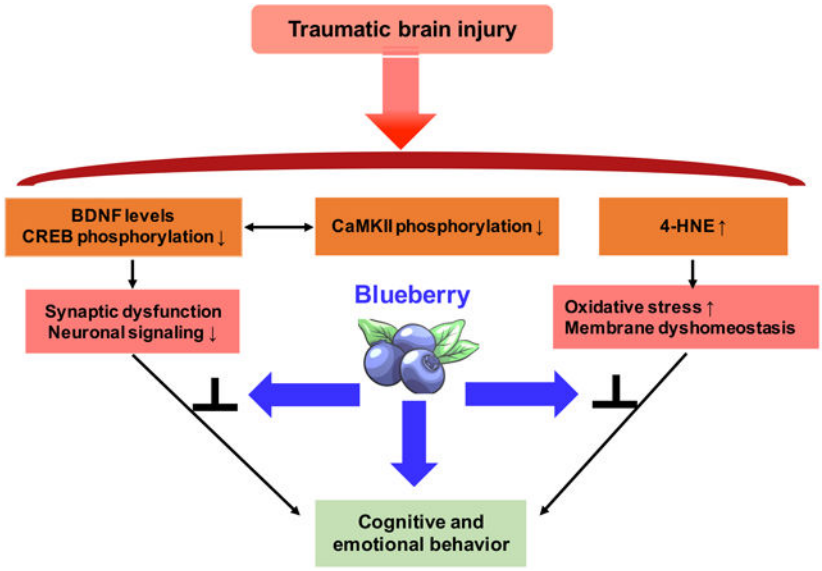
Conflict of Interest

None.



The beneficial effects of dietary supplementation of blueberry (BB) in mitigating the acute aspects of the traumatic brain injury (TBI) pathology involves action on BDNF and its downstream effector cAMP-responsive element-binding protein (CREB) that play an important role in synaptic function. The BDNF-mediated calcium/calmodulin-dependent kinase II (CaMKII) signaling system, required for learning and memory, is affected by TBI and BB. At the same time, formation of 4-hydroxynonenal (4-HNE) renders neurons vulnerable to increased plasma membrane damage following TBI that may, in turn, affect neuronal signaling. These molecular changes eventually lead to cognitive deficits. The present data indicate that BB supplementation plays a crucial role in regulating synaptic function, neuronal signalling and plasma membrane function. It is likely that BB supplementation can work to counteract TBI-induced manifestations of pathology with impact on cognitive processes.

Keywords

Blueberry; Traumatic Brain Injury; Plasticity; Brain-derived neurotrophic factor; Oxidative stress; Cognition

1. Introduction

Traumatic brain injury (TBI) accounts for approximately 90% of brain injuries, and is associated with cognitive dysfunction and long-term disability [1]. As a result of domestic incidents, military combat, traffic accidents and sports, TBI can compromise broad aspects of neuronal function. Patients often experience problems in the domains of learning, memory and affective functions that can profoundly influence quality of life [2,3]. Existing therapeutic strategies for TBI have not been successful in counteracting the heterogeneous TBI pathology nor improving the quality of life of patients [4]. Hence, identifying interventions with broad applicability seems necessary for effective management of TBI.

Dietary polyphenols have significant positive effects on brain health via protecting neurons against injury and enhancing neuronal function [5,6]. Evidence supports the neuromodulatory effects of flavonoid-rich blueberry, particularly in promotion of brain plasticity [7], and counteracting behavioural deficits [8]. In the United States, demand for blueberries has increased, with 2017 fresh per capita consumption of 1.79 pounds/person [9]. Several reports indicate that blueberry dietary supplementation improves memory, learning and general cognitive function [10-14], and protects against neuronal injury associated with stroke [15]. Moreover, it has been shown that blueberries possess potent antioxidant capacity through their ability to reduce free radical formation [16] or upregulating endogenous antioxidant defenses [17]. These studies suggest that blueberry supplementation can have the potential to be used to overcome the broad pathology of TBI. Given the lack of information about the effects of blueberry intake immediately after TBI, we have performed studies to assess the effects of blueberry extracts during the acute phase of TBI.

Evidence suggests that TBI is characterized by dysfunction in synaptic plasticity, elevated levels of free radicals, plasma membrane dysfunction [18], which can contribute to the behavioural dysfunction. Oxidative stress is part of the pathology of TBI and compromises neuronal function [19,20]. In particular, excessive free radical formation leads to accumulation of lipid oxidation by-products such as 4-hydroxynonenal (4-HNE) [21] with subsequent impairments in plasma membrane fluidity, receptor signaling across the membrane to deteriorate synaptic plasticity and reduce neuronal excitability [22].

Deficiencies in brain derived neurotrophic factor (BDNF) reduce the brain plasticity necessary to cope with the effects of TBI [23]. BDNF activates cAMP-responsive element-binding protein (CREB), a multifaceted transcriptional regulator involved in synaptic plasticity essential for learning and memory [24]. BDNF is known to bind to TrkB receptors, leading to activation of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), required for synaptic processes involved in behaviour [25]. Several observations indicate that the flavonoids exert action through modulation of signaling pathways to promote synaptic and neuronal function [26,27]. Accordingly, in the current study, we investigated whether blueberry (BB) supplementation would counteract TBI pathology by involving BDNF-related pathways involved in synaptic plasticity and oxidative stress to influence cognitive behaviours.

2. Materials and Methods

2.1. Diet

Freeze-dried highbush whole blueberry fruit powder (*Vaccinium corymbosum* L.; 50:50 blend of Tifblue and Rubel; U.S. Highbush Blueberry Council, Folsom, CA). This blend (per 100 g) contained bioactive phytocompounds (33 mg/g total phenolics; 10.2 mg/g anthocyanins; 85.9 IU β-carotene), and other macro- and micronutrients (2.38 mg proteins; 32.2 g fructose; 18.8 mg vitamin C). Diet supplemented with 5% w/w BB was mixed with pulverized standard rodent chow (#5001, *Lab Diet*, St. Louis, MO). 1.6% fructose, 1.45% glucose and 0.0009% vitamin C were mixed with the standard rodent chow to match the levels of sugars in the BB supplemented diet and used as the rodent control diet (RD).

2.2. Animals

Sprague–Dawley male rats were purchased from Charles River Laboratories (Wilmington, MA) at 10 weeks of age and were acclimatized for vivarium 1 week prior to commencement of experimental procedures. Rats were housed in environmentally controlled conditions (temperature 22–24 °C and humidity) with 12-h light/dark cycle in a controlled room with free access to food and water. All procedures were approved by the University of California at Los Angeles (UCLA) Chancellor's Animal Research Committee (ARC) and were conducted with adherence to the guidelines set out by the United States National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.3. Dosage information/ Dosage regimen

After acclimatization, rats underwent fluid percussion injury or sham surgery and were pair housed to a specific diet group with either regular diet (RD - regular rodent diet) or blueberry (BB) supplemented diet (5% w/w BB) for 2 weeks immediately. The BB dose was chosen based on previous *in vivo* studies which demonstrated that administration of blueberry offsets oxidative stress and reverses cognitive impairment [28,29]. The groups were: (1) Sham-RD as control group, (2) TBI-RD, and (3) TBI-BB. The rats (n = 8/group) were subjected to fluid percussion injury (FPI) or sham surgery. Rats were subjected to learning on Barnes maze at post-injury day (PID) 14 for 5 days, and after a 2-day interval, memory was assessed at PID 21. Rats were tested for anxiety-like behaviour on elevated plus maze on PID 22 (Supplementary Figure 1). All behavioural assessments were conducted between 9:00 and 13:00 hours. Rats were provided with diets prepared daily and fed *ad libitum* in powder form. To determine the voluntary food intake (g), food was weighed daily to measure consumption in each cage. Since the rats were pair-housed, food intake was divided by two to yield an approximate intake/rat.

2.3. Fluid percussion injury

We employed our standard lateral fluid percussion injury (FPI) protocol as described earlier [30]. Briefly, 3% isoflurane (1.0 mL/min in 100% oxygen) was provided in a chamber (VetEquip Inc., CA, USA), and then maintained with 2–2.5% isoflurane via nose cone while rats were in a stereotaxic frame. Body temperature was controlled (37–38 °C) by a heating pad. Under aseptic surgical conditions, a midline skin incision was made to expose the skull. Using a high-speed drill (Dremel, WI, USA), craniotomy (3.0 mm diameter) was made 3.0 mm posterior to bregma and 6.0 mm lateral (left) of midline to expose the intact dura. A hollow plastic injury cap was placed over the craniotomy, secured with dental acrylic cement and was later filled with 0.9% saline. When the dental cement hardened, the anesthesia was discontinued and the rat was attached to the FPI device via the head cap. At the first response of hind-limb withdrawal to a paw pinch, rats received a moderate fluid percussion pulse (2.7 atm). Upon resumption of spontaneous breathing the head cap was removed and the skin was sutured. Neomycin was applied on the suture and the rats were placed in a heated recovery chamber to be fully ambulatory before being returned to their cages. The sham animals were prepared using the identical surgically procedure but without the fluid pulse.

2.4. Behavioural analyses

2.4.1. Barnes maze test—Barnes maze testing was performed 2 weeks after experimental TBI with two trials per day with a 5-min test period (detailed in Supplemental Information). For learning assessment, rats were given two trials per day for five consecutive days at approximately the same time every day. Subsequently, memory retention was assessed at post-injury day 21. Latency to finding the escape hole and search strategies were analyzed for each trial. Three search strategies were identified using following categorization: spatial, peripheral, and random using data recorded with AnyMaze software.

2.4.2. Elevated-plus Maze (EPM)—EPM test was performed to assess anxiety-like behavior 2 weeks after experimental TBI with two trials as described in the Supplemental Information. Specifically, rats were individually placed in the closed arm of the EPM apparatus and permitted free exploration for 5 min during which their movements were camera recorded. The behaviors scored were time spent and number of entries into the open arm using automated video tracking system (AnyMaze, San Diego Instruments, CA, USA) [30]. EPM testing was conducted after Barnes maze memory test.

2.5. Tissue harvesting and immunoblotting

Upon completion of the experiment, hippocampal tissues were harvested frozen in dry ice, and stored at –80 °C until use for immunoblotting. The left side hippocampus (ipsilateral to injury) were homogenized in a lysis buffer containing 20 mM Tris–HCl (pH 8.0), 137 mM NaCl, 1% NP40, 10% glycerol, 1 mM phenylmethylsulfonylfluoride (PMSF), 10 μg/ml aprotinin, 0.1 mM benzethonium chloride, 0.5 mM sodium vanadate. The homogenates were then centrifuged (12000 *g* at 4 °C) and the supernatants were collected. Total protein was then determined using a BCA Protein Assay kit (Pierce, IL, USA), using bovine serum albumin (BSA) as standard. Equal amounts of protein were separated by sodiumdocecylsulphate-polyacrylamide gels and then transferred onto polyvinylidene difluoride membranes (Millipore, MA, USA). Membranes were probed with anti-actin or anti-BDNF, anti-pCREB, anti-CREB, (1:1000, Millipore, MA, USA), anti-CaMKII, anti-4-hydroxynonenal (4-HNE) (1:500; Santa Cruz Biotechnology, CA, USA) followed by secondary antibody (anti-rabbit or anti-goat or anti-mouse IgG horseradish peroxidase-conjugate, 1:10,000; Santa Cruz Biotechnology, CA, USA). Immunoreactive proteins were visualized using enhanced chemiluminescence reagents (Millipore, MA, USA). Band intensities were quantified using Image J32 Software. β-actin (anti β-actin; 1:5000) was used as an internal control for normalization western blot such that data were standardized according to β-actin values. Blots for each experimental group were normalized to Sham-RD values within the same gel.

2.6. Statistical Analyses

Protein data are expressed as mean ± standard error of the mean (SEM). Body weight data expressed as mean ± standard deviation. Statistical analysis was performed by software GraphPad Prism 7.04. A level of 5% probability was considered as statistically significant. The Barnes maze learning data analysis (n=8) were analyzed by repeated measures analysis of variance (ANOVA). Protein results are expressed as percentage (%) of Sham-RD group.

One-way ANOVA followed by Tukey *post-hoc* test for multiple comparison used for protein data analysis (n = 5–7 rats/group). The association among the endpoints were assessed using Pearson correlation (two-tailed).

3. Results

3.1. Food intake and body weight

There were no differences in the daily average food intake among the various rat groups: Sham/RD: 31.6 ± 1.9 g, TBI/RD: 29.3 ± 1.3 g and TBI/BB: 30.2 ± 2.3 g. There were no significant differences in the body weight gain among the groups by the end of the experiments (data not shown).

3.2. Cognitive assessment

3.2.1. Effect on learning—To assess whether BB supplementation can reverse the TBI-induced learning deficits, rats were trained for 5 days to find the escape hole in the Barnes maze guided by spatial cues. On day 5, rats subjected to TBI displayed a significant delay to find the escape hole compared with the Sham-RD rats as an evidence of impaired learning (Figure 1A). Interestingly, as observed by ANOVA, BB supplementation reversed learning deficits after TBI ($F_{2,95} = 3.89$, $p = 0.023$).

3.2.2. Use of search strategies—We performed the search strategy analysis to evaluate the efficiency of rats to locate the escape hole. Sham-RD animals used spatial strategies starting the first day and continued progressing over time (Figure 1B). TBI rats appeared to have lost their capacity to navigate using spatial learning cues throughout the test time and exhibited reliance mostly on random strategies (Figure 1C). In turn, rats exposed to BB supplementation appeared to regain the capacity to use spatial leaning cues (Figure 1D).

3.2.3. Effect on memory performance—Rats were subjected to the memory probe test two days after completion of learning acquisition. The ANOVA revealed a significant main effect of group ($p = 0.0001$; Figure 1E) in memory performance. The group comparisons indicated that rats subjected to TBI displayed larger latency to locate the escape hole than the Sham rats, and BB supplementation counteracted the memory deficits induced by TBI ($F_{2,20} = 19.11$, $p = 0.0001$).

3.3. Effect on elevated plus maze performance

Exploration of the open arms in the elevated plus maze is considered a reliable index of anxiety-like behaviour. There were no significant effects on time spent exploring the open arm among TBI rats in regular diet or BB supplementation in the open field ($F_{2,20} = 0.34$, $p = 0.712$; Figure 1F).

3.4. BDNF-related plasticity markers: BDNF, CREB and CaMKII phosphorylation

ANOVA revealed a significant group effect on BDNF levels ($F_{2,12} = 6.38$, $p = 0.012$). As shown in Figure 2A and confirmed by the Tukey's comparisons test, TBI rats showed significant reduction in BDNF levels compared to sham rats fed regular diet. BB

supplementation was able to maintain BDNF levels near Sham in rats exposed to TBI. We found that the BDNF levels increased in proportion to a reduction in latency time in the Barnes maze memory test (r = −0.638, $p = 0.011$; Figure 2B), suggesting that BDNF was a factor for the memory performance. To assess the effects of BB supplementation on molecular systems involved with the action of BDNF, we evaluated the protein levels of cyclic-AMP response element binding protein (CREB). The CREB family of transcription factors plays a major role in regulating synaptic plasticity and cognition [32]. The ANOVA revealed a significant group effect for CREB phosphorylation ($F_{2,16} = 9.69$, $p = 0.001$). Tukey's comparison test showed that TBI reduced levels of CREB phosphorylation (Figure 3A) in rats fed a regular diet whereas BB supplementation counteracted these effects. In addition, we found that the levels of CREB phosphorylation increased in proportion to a reduction in memory latency (r = −0.492, $p = 0.038$, Figure 3B), suggesting that CREB may contribute to the effects of BB supplementation on memory performance. Moreover, CREB phosphorylation changed in proportion to the levels of BDNF (r = 0.614, $p = 0.016$, Figure 3C) and suggests that the regulations of BDNF and CREB may be coordinated.

We also studied CaMKII phosphorylation in our paradigm based on the involvement of CaMKII on hippocampal memory consolidation [33], and its close interaction with the BDNF system [34]. The ANOVA showed a significant group effect for CaMKII phosphorylation levels ($F_{2,16} = 9.05$, $p = 0.002$). Subsequent analyses revealed that TBI reduced phosphorylation of CaMKII which was ameliorated by the BB supplementation (Figure 4A). We found that the CaMKII phosphorylation negatively correlated with latency time in Barnes maze test (r = −0.613, $p = 0.005$; Figure 4B), suggesting that CaMKII contributed to the observed memory performance.

3.5. Oxidative stress markers

Oxidative stress is known to play a role in TBI pathology [35]. We assessed the carbonyl-containing molecule 4-HNE which is the end product of lipid peroxidation affecting plasma membrane integrity and neuronal survival and function. The ANOVA for 4-HNE revealed a significant group effect ($F_{2,14} = 3.96$, $p = 0.043$). As depicted in Figure 5A-B, TBI enhanced the levels of 4-HNE in rats fed a regular diet compared with Sham animals fed regular diet. In turn, BB supplementation diminished the levels of 4-HNE in TBI rats. Linear regression analysis showed a positive correlation between the latency in the memory test of the Barnes maze and levels of 4-HNE, suggesting that higher oxidative stress may reduce spatial memory performance (r = 0.467, $p = 0.050$; Figure 5C).

4. Discussion

In the present study, we found that BB supplementation can attenuate important aspects of the acute TBI pathology. We report that BB supplementation immediately following TBI mitigates behavioural deficits in spatial learning and memory. BB supplementation counteracted the effects of TBI on proteins associated with the action of BDNF (CREB and CaMKII) on plasticity and behaviour. In addition, BB supplementation counteracted the increase of the end product of lipid peroxidation, 4-HNE. The results showing that markers of neuronal plasticity and lipid peroxidation change in proportion to memory performance

suggest a possible association between these molecular parameters and behaviour. Taken together, the present findings emphasize the beneficial effects of BB supplementation in fostering brain plasticity in the TBI pathology.

4.1. Impact of BB supplementation on behaviour

In agreement with previous reports [18,30], we found that TBI impairs spatial learning as evidenced by an increase in latency in the Barnes maze, while BB supplementation decreased latency time to find the escape hole at each training day. We assessed the use of spatial learning strategies in our paradigm to provide a complementary measure of cognitive function less dependent on motor behaviour. Interestingly, we found that BB supplementation appeared to counteract a lost capacity of TBI rats to employ spatial leaning cues. This information together with results of the shorter latencies strongly suggest that BB supplementation protects TBI animals from a loss in spatial learning performance. In this regard, recent functional neuroimaging study in humans has established a connection between BB intake and cognitive function [36]. Further, in the elevated plus maze test, rats exposed to TBI showed a tendency to reduce time spent in the open arms, which encompasses with clinical reports that psychiatric disorders are often observed in TBI patients [37]. TBI-induced behavioural deficits probably stems from the impairments in BDNF-TrkB signaling that has been implicated in various cognitive and affective disorders [38]. We cannot ascertain the cellular identity of the reported protein alterations. Although neuronal cells are the primary locus for learning and memory processing, non-neuronal cell types such as astrocytes and microglia can also contribute to these alterations [39,40]. Moreover, it known that astrocytes and microglia provide support to synaptic transmission that is fundamental for neuronal function involved in cognitive processing [41-43].

4.2. Effects of BB supplementation on plasticity markers

In the present investigation, we also found that TBI significantly reduced levels of hippocampal BDNF, and that BB dietary supplementation normalized these the levels. Previous report indicated that deficiencies in BDNF signaling is associated with impairments in cognition [44]. Alternatively, cognition is strongly reliant on long-term potentiation (LTP) and hippocampal BDNF, and the interaction between BDNF and its tyrosine kinase receptor (TrkB) is required for induction of LTP [45]. Previously we have shown the protective effects of BDNF on the TBI pathology [46]. Presently, our findings show that BB supplementation counteracted the BDNF reduction induced by TBI, paralleling improvements in cognitive function. It is well established that BDNF regulates synaptic plasticity and learning through interaction with the transcription factor CREB [47]. Interestingly, our results also showed that BB supplementation normalized levels of CREB in TBI animals, and that these changes were proportional to changes in BDNF levels. These findings are consistent with reports showing that BB dietary supplementation enhances BDNF-mediated plasticity with improved spatial and object recognition memory [48,49]. Moreover, the significant positive correlation between levels of BDNF and CREB indicates that BDNF and CREB are co-regulated in our paradigm. In addition, evidence indicates an association between BDNF and CREB, and this interaction is important for regulation of learning and memory [50]. The latter possibility can also be inferred from our results showing a negative correlation between CREB signaling and latency in the Barnes maze.

We also found that BB supplementation preserves levels of hippocampal CaMKII phosphorylation after TBI, and changes in CaMKII correlated negatively with latency to locate the escape hole in the Barnes maze memory test. CaMKII, the main protein of the postsynaptic density and key BDNF signaling element, upon autophosphorylation increases synaptic efficacy [51] and long-term synaptic memory [52]. In fact, CaMKII dysregulation has been associated with several neuropsychiatric diseases [53]. It is possible that the effects of BB on the BDNF levels results in autophosphorylation of tyrosine residues that rise intracellular Ca^{2+} levels leading to CaMKII activation [54].

4.3. Impact of BB supplementation on oxidative stress

Elevated levels of free radical formation are a common sequel of TBI pathology that can result in lipid peroxidation [55]. In particular, lipid peroxidation has negative consequences for the function of the plasma membrane. It has been reported that optimal maintenance of membrane function is essential to support neuronal signaling that underlie synaptic plasticity, and that membrane function loss following TBI may be associated with cognitive deficits [18]. Phospholipids are components of the plasma membrane that are particularly important for regulating cellular signaling and neuronal excitability [56]. The fatty acids residues in phospholipids are sensitive targets to oxidative free radical attack to induce lipid peroxidation. Lipid peroxidation products can impair the barrier function, ion-channel activity and neurotransmitter release associated synaptic activity [57]. Higher 4-HNE formation causes ionic disruption and membrane disturbance which contributes to additional reactive oxygen species (ROS) production. We presently found that TBI caused a marked increase in 4-HNE levels indicative of decreased neuronal excitability. Elevated levels of 4-HNE can form adducts with proteins, promotes oxidative stress [58] and contributes to membrane damage following TBI [59]. BB is considered to have strong antioxidant capacity, important in its ability to attenuate oxidative stress [60]. The fact that BB reduced the levels of 4-HNE supports the notion that BB attenuated TBI-related oxidative damage with positive consequences for neuronal excitability and plasma membrane function. Further, our reported positive correlation of spatial memory performance with 4-HNE levels suggest that a reduction of 4-HNE is important for behavioural outcome, in agreement with behavioural impact of 4-HNE on spatial memory performance [61].

Interestingly, BB powder supplementation showed a positive effect on various aspects of brain function and plasticity in spite of the fact that the powder contains several components with recognized unhealthy effects. For example, the BB powder has high contents of sugars, particularly fructose, which upon consumption reduced levels of the same plasticity markers being decreased by BB supplementation in our study [62]. It is important to note that in the present study, we matched the two diets for sugars (fructose and glucose) and vitamin C. Therefore, it is likely that the flavonoid components are largely responsible for the observed positive effects of the BB powder possessing anti-oxidant property [63,64]. These results seem to indicate that the combination of fructose with flavonoids in natural foods has an overall healthy action, which further suggests the importance of consuming natural foods. Also, the protective effects of BB against TBI pathology may be attributed to the presence of other bioactive compounds present in the powder such as β-carotenes and anthocyanins. Anthocyanins, the important class of flavonoids has reported to be effective in promoting

cognitive performance in animals through changes in synaptic plasticity via protein kinase signaling components such as c-Jun *N*-terminal kinase (JNK)/Akt and phosphatidylinositol-3 kinase (PI3K)/Akt [65,66]. Similarly, human intervention studies with anthocyanins have shown to promote a range of cognitive domains that include attention, visuospatial memory and executive function [67,68]. Additionally, previous reports with anthocyanins have also reported hippocampal localization of glycosylated derivatives [69]. In turn, β-carotenes and vitamins have strong potential to promote neuronal plasticity as reviewed by [70] and to delay cognitive decline [71,72].

5. Conclusions

Our data show that BB supplementation immediately following TBI mitigates behavioural impairment and neuronal dysfunction. These effects may be achieved by modulating proteins important for neuronal signaling and synaptic pathology coupled with reduction in oxidative processes ((Supplementary Figure 2). These findings support the contention that BB supplementation has therapeutic potential and clinical beneficial value.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This work was supported by the U.S. Highbush Blueberry Council and the National Institutes of Health Grant NS50465.

Abbreviations:

TBI	traumatic brain injury
BDNF	brain-derived neurotrophic factor
CREB	cAMP response-element binding protein
CAMKII	calcium/calmodulin-dependent protein kinase II
4-HNE	4-hydroxy-nonenal
ROS	reactive oxygen species
TrkB	tyrosine kinase B
p-CREB	phosphorylated CREB
pCAMKII	phosphorylated calcium/calmodulin-dependent protein kinase II

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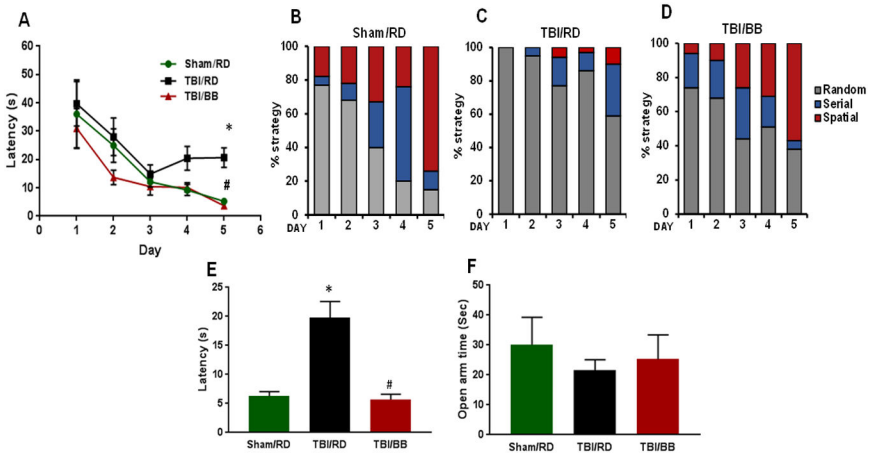

Figure 1.

Blueberry (BB) supplementation protects against spatial learning deficits after TBI, as indicated by the latency to locate the escape hole on Barnes maze during acquisition training (Days 1–5) (**A**), percent time using spatial, serial or random search strategies among Sham/RD (**B**), TBI/RD (**C**) and TBI/BB (**D**) groups, and latency to locate escape hole during memory test on Barnes maze (**E**). BB supplementation appeared to counteract a reducing trend in time spent in the open arms of the elevated plus maze to assess anxiety-like behaviour (**F**). Data presented as means (± SEM). $*p < 0.05$, compared to Sham-RD, $^{\#}p < 0.05$, compared to TBI-RD; Student's *t*-test or ANOVA followed by post hoc Tukey test, as appropriate.

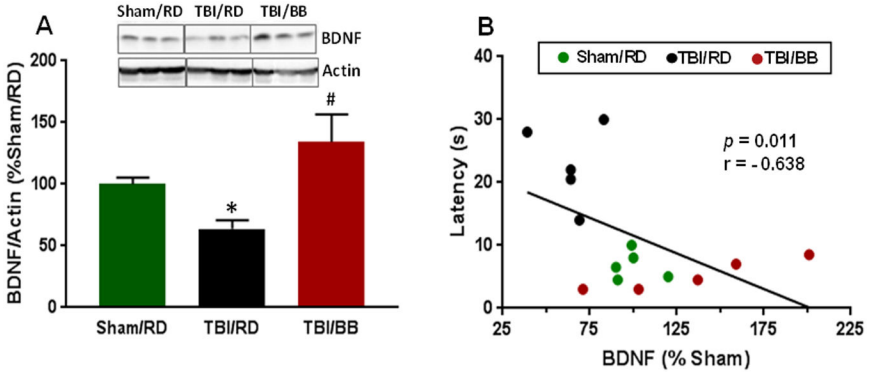

Figure 2.

Effects of blueberry (BB) supplementation on (**A**) brain-derived neurotrophic factor (BDNF) levels and (**B**) scatter plot showing a negative correlation between the BDNF levels and the memory latency time on the Barnes maze among all rats. TBI resulted in decrease in levels of BDNF while BB supplementation enhanced the levels in TBI rats. Data presented as means (± SEM). $^{*}p < 0.05$, compared to Sham-RD, $^{\#}p < 0.05$, compared to TBI -RD; ANOVA followed by post hoc Tukey test. Marker points reflect individual score.

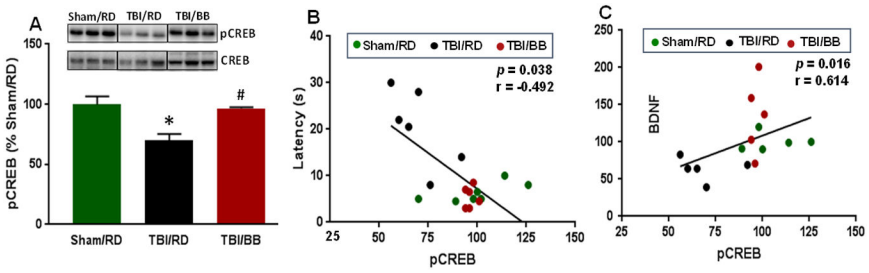

Figure 3.

Effects of blueberry (BB) supplementation on (**A**) CREB phosphorylation, (**B**) correlation analysis of CREB phosphorylation with the memory latency time on the Barnes maze and (**C**) the association between the pCREB/BDNF levels. Data presented as means (± SEM). *$p < 0.05$, compared to Sham-RD, $^{\#}p < 0.05$, compared to TBI-RD; ANOVA followed by post hoc Tukey test. Marker points reflect individual score.

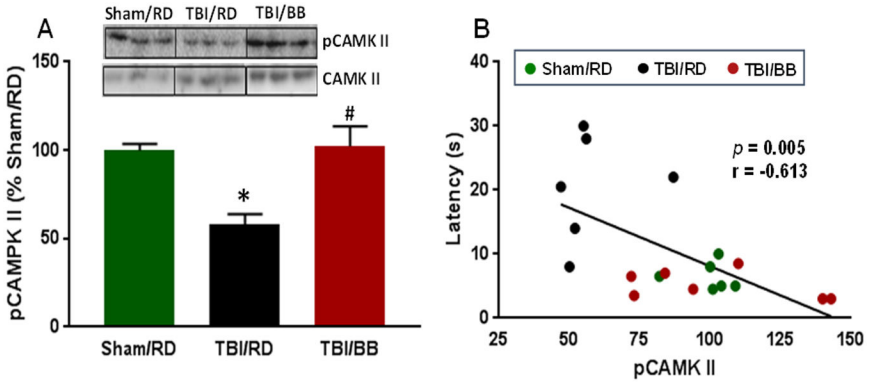

Figure 4.

Effects of blueberry (BB) supplementation on (**A**) CaMKII phosphorylation and (**B**) scatter plot shows a negative correlation between the pCaMKII/CaMKII levels and the memory latency time on the Barnes maze among all rats. Data presented as means (± SEM). *$p <$ 0.05, compared to Sham-RD, $^{\#}p < 0.05$, compared to TBI-RD; ANOVA followed by post hoc Tukey test. Marker points reflect individual score.

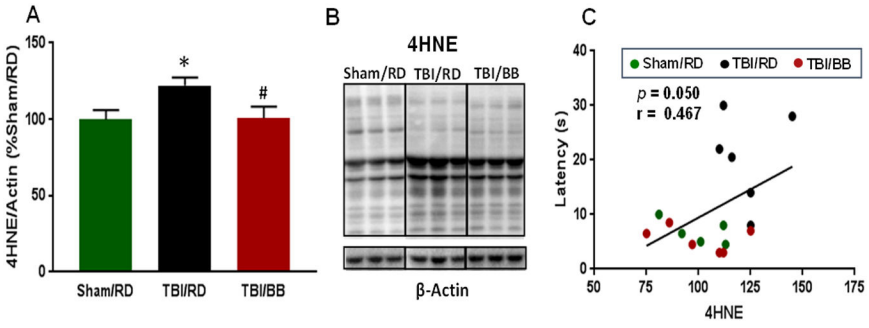

Figure 5.

Effects of blueberry (BB) supplementation on (**A**) 4-HNE levels, (**B**) representative western blots and (**C**) scatter plot showing a positive correlation between the 4-HNE levels and the latency time on the Barnes maze among all rats. Data presented as means (± SEM). $^{*}p < 0.05$, compared to Sham-RD, $^{\#}p < 0.05$, compared to TBI-RD; ANOVA followed by post hoc Tukey test. Marker points reflect individual score.