Published in final edited form as:

J Nutr Biochem. 2016 November ; 37: 60–66. doi:10.1016/j.jnutbio.2016.07.009.

Cranberry extract attenuates hepatic inflammation in high fat-fed obese mice

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Abstract

Cranberry (*Vaccinium macrocarpon*) consumption has been associated with health beneficial effects. Non-alcoholic fatty liver disease (NAFLD) is a co-morbidity of obesity. In the present study, we investigated the effect of a polyphenol-rich cranberry extract (CBE) on hepatic inflammation in high fat-fed obese C57BL/6J mice. Following dietary treatment with 0.8% CBE for 10 weeks, we observed no change in body weight or visceral fat mass in CBE supplemented mice compared to high fat-fed control mice. We did observe a significant decrease in plasma alanine aminotransferase (31%) and histological severity of NAFLD (33% decrease in area of involvement, 29% decrease in lipid droplet size) compared to high fat-fed controls. Hepatic protein levels of tumor necrosis factor alpha and C-C chemokine ligand 2 were reduced by 28% and 19%, respectively, following CBE supplementation. CBE significantly decreased hepatic mRNA levels of toll-like receptor 4 (TLR4, 63%) and nuclear factor kappa B (NFκB, 24%), as well as a number of genes related to the nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing-3 inflammasome. In conclusion, CBE reduced NAFLD and hepatic inflammation in high fat-fed obese C57BL/6J mice. These effects appear to be related to mitigation of TLR4-NFκB related signaling, however further studies into the underlying mechanisms of these hepatoprotective effects are needed.

Keywords

Vaccinium macrocarpon; cranberry; non-alcoholic fatty liver disease; inflammation; polyphenols

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1. INTRODUCTION

Obesity is a growing public health problem worldwide. Approximately 34.9% of American adults were obese (body mass index [BMI] > 30) in 2012 [1]. Nonalcoholic fatty liver disease (NAFLD) is a common comorbidity of obesity and ranges in severity from simple steatosis (hepatic fat accumulation > 5%) to steatohepatitis (NASH). NASH in turn can lead to fibrosis, cirrhosis, and hepatocellular carcinoma [2]. An estimated 6 million US adults have NASH, and 10% of those have NASH-related cirrhosis [2].

Two major hypotheses have been proposed regarding the progression to NASH: a “two-hit” model and a “multiple parallel hits” model. In the two-hit model, the first hit is the development of steatosis, while the second hit, which can include inflammation, oxidative stress, and/or increased endotoxin circulation, induces the progression of steatosis to NASH [3, 4]. By contrast, the multiple parallel hits model posits that the second hit may develop simultaneously with or even before NAFLD development [5].

Immune cells, including macrophages and Kupffer cells, are important mediators of stress and injury response and coordinate local inflammatory response through the release of cytokines and chemokines. Macrophages ubiquitously express EGF-like module-containing mucin-like hormone receptor-like 1 (EMR1), a protein that can be used to quantify macrophage infiltration [6]. Macrophages can be activated to generate a context-dependent immune response. The classical M1 phenotype mainly produces pro-inflammatory cytokines while the alternative M2 phenotype primarily produces anti-inflammatory cytokines [7, 8].

Consumption of a high-fat diet is associated with increased toll-like receptor (TLR) 4 expression and nuclear factor- κ B (NF κ B) activation in monocytes [9, 10]. At least two mechanisms have been proposed to account for this activation. First, consumption of high fat diet and obesity have been shown to lead to compromised gut barrier function and increased systemic availability of gut bacteria-derived lipopolysaccharide (LPS) [11]. The binding of LPS to TLR4 triggers an inflammatory cascade by inducing NF- κ B-mediated transcription of pro-inflammatory cytokines and chemokines including tumor necrosis factor α (TNF α), interleukin 1 β (IL-1 β), chemokine (C-C motif) ligand 3 (CCL3 aka macrophage inflammatory protein [MIP]-1 α), and CCL2 (aka monocyte chemoattractant protein [MCP-1]). In addition, saturated free fatty acids (*e.g.* lauric, myristic, palmitic, and stearic acid) have also been shown to induce TLR4-mediated inflammation [12].

Activation of the nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing-3 (NLRP3) inflammasome plays a key role in the post-translational maturation of IL-1 β and is associated with the induction of hepatic fibrosis and injury [13, 14]. The formation of the NLRP3 inflammasome can be activated by a variety of stimuli including pathogen-associated molecular patterns and danger-associated molecular patterns [15]. Both LPS and TNF- α have been shown to increase the transcription of NLRP3, while NF κ B activation plays a key role in priming the inflammasome to form by inducing NLRP3 expression [16, 17].

Cranberries (*Vaccinium macrocarpon*, Ericaceae) are rich in polyphenols [18]. In addition to anthocyanins, flavonols, catechins, organic acids, resveratrol, and B-type proanthocyanidins

(PACs), cranberries contain a high amount of rare A-type PACs [19]. Previous studies have found cranberry bioactives improve lipid and cholesterol profiles in animals and humans, reduce blood markers of oxidative stress in humans, and reduce inflammation *in vitro* and in humans [20]. Daily oral bolus dosing with a commercially-available cranberry extract has recently been reported to decrease high fat diet-induced weight gain, hepatic triglyceride accumulation, and oxidative stress in high fat/high sucrose-fed mice [21]. The objective of this study was to investigate the effects of dietary supplementation with a polyphenol-rich cranberry extract on the progression of NAFLD to NASH in high fat-fed obese mice.

2. MATERIALS AND METHODS

2.1 Cranberry extract preparation and phytochemical characterization

A polyphenol-enriched cranberry extract (CBE) was prepared by macerating sulfite-free dried cranberries (Traverse Bay Dried Fruit Company, Travers City, MI) in a Waring blender, adding 10 volumes of acetone:water:acetic acid (80:19.9:0.1, v:v:v), and stirring overnight at room temperature [22]. The extract was vacuum filtered to remove solids and the acetone was removed via rotary evaporator. The resulting aqueous extract was loaded onto an Amberlite XAD-7HP column. Sugars were removed by elution with water. The polyphenol-containing fraction was eluted with acetone containing 0.1% acetic acid. The acetone in the resultant eluent was removed via rotary evaporator and the water was removed by freeze-drying.

Total phenolics in the extract were determined using the Folin-Ciocalteu method. In brief, 20 mg/mL extract was dissolved in DMSO and diluted to a final concentration range of 0.2–4 mg/mL with 10% DMSO. The extract was then combined with 79 volumes of 10% DMSO, 5 volumes of Folin-Ciocalteu reagent, and 15 volumes of 20% (w/v) sodium carbonate. The mixture was incubated at 37°C for 30 minutes and the absorbance was measured at $\lambda = 765$ nm. The resulting measurements were compared to a standard curve prepared with gallic acid.

Total monomeric anthocyanins were measured using a modified Somers assay. CBE was dissolved in tartrate buffer (5 g/L tartaric acid, 12% ethanol, pH 3.4) at 1 g/L and sodium metabisulfite buffer (3.75 mg/L NaMBS in tartrate buffer) and hydrochloric acid (1 M) were prepared. A 1:10 dilution of CBE was made in the metabisulfite buffer, incubated at room temperature for an hour, and absorbance was read at $\lambda = 520$ nm. A 1:50 dilution of CBE was made in the hydrochloric acid buffer, incubated in the dark at room temperature for three hours, and absorbance was read at $\lambda = 520$ nm. Total monomeric anthocyanin content (mg/g) was calculated using the following equation:

$$20 \times [(50 \times A_{520Hcl}) - 1.6667 \times (10 \times A_{520Sulfite})]$$

Total precipitable tannins were measured in duplicate using the methyl cellulose precipitation assay according to Mercurio et al. [23]. This method is typically used for grapes and wine but has been used successfully in other food products [24]. Briefly, the CBE was dissolved in water (1 g/L) and absorbance was read at $\lambda = 280$ nm for a control and

treatment sample after precipitation with 0.04% w/v methyl cellulose. Tannin concentration was determined using the following equation:

$$\frac{[Tannin_{extract}] \times V}{W}$$

where $[Tannin_{extract}]$ is the tannin concentration in the CBE extract (mg/L catechin equivalents [CE] based on an external standard curve), V is the final volume of the extract (L), and W is the initial weight of the extract (g).

The polyphenolic profile of the CBE was determined by normal-phase HPLC profiling with fluorescence detection and UPLC-MS to quantify the compounds using a modification of our previously reported method [25, 26]. In brief, normal-phase HPLC was performed using an Agilent Technologies 1260 Infinity HPLC equipped with a fluorescence detector ($\lambda_{ex} = 230$ nm; $\lambda_{em} = 321$ nm). Separation was achieved using a Develosil Diol column (100 Å, 250 x 4.6 mm, 5µm particle, Phenomenex, Torrance, CA) and a gradient of acetonitrile containing 2% acetic acid and methanol containing 2% acetic acid and 3% water as previously described. UPLC-MS analysis was performed as described previously with modification. In brief, analysis was performed on Waters (Milford, MA) Acquity H-class separations module. Analytes were separated using a Waters Acquity UPLC HSS T3 column (2.1 mm x 100 mm, 1.8 µm particle size) maintained at 40°C and a binary gradient of 0.1% formic acid in water and 0.1% formic acid in acetonitrile. UPLC eluent was analyzed by (-) electrospray ionization (ESI) coupled to tandem mass spectrometry (MS/MS) on a Waters Acquity triple quadrupole (TQD) MS. Ionization was performed in the negative mode, with a capillary voltage of 4.25 kV, a cone voltage of 30.0 V, an extractor voltage of 3.0 V, and source and desolvation temperatures of 150 and 400 °C respectively. Cone and desolvation gasses were N2 with flows rates of 75 and 900 L/h respectively. MS/MS was performed using Ar as the collision gas at a flow rate of 0.25 mL/min in the collision cell. Data acquisition was carried out with MassLynx software (version 4.1, Waters) in the selected ion recording (SIR) mode. All compound peaks were processed and quantified using the QuanLynx function of MassLynx software.

2.2 Animals and treatment

All animal experiments were approved by the Institutional Animal Care and Use Committee (protocol no. 37115) at the Pennsylvania State University (University Park, PA). Experimental diets, high fat (HF, 60% kcal from fat, D12492) and HF supplemented with 0.8% CBE (CBE, D13051702), were prepared by Research Diets (New Brunswick, NJ). The diets were matched in terms of nutrient and energy content (Suppl. Table 1). A number of cranberry extracts are available commercially as dietary supplements [27]. The recommended doses of these supplements range from 0.5–7 g per day. Based on allometric scaling (assuming energy requirement of 12 kcal and 2000 kcal for mice and humans, respectively), the dose used in the current study corresponds to a human dose of 4 g per day [28]. Male C57BL/6J mice (4 wk old) were purchased from Jackson Laboratories (Bar Harbor, ME) and maintained on a 12 h light/dark cycle with access to food and water *ad libitum*. After a two-week acclimatization period, mice were given a HF diet and allowed to

develop an obese phenotype over 11 weeks. Mice were then randomized based on body weight to either be maintained on the HF diet (HF group, $n = 24$) or to be switched to the CBE diet (CBE group, $n = 24$) for 10 weeks. Body weight and food intake were recorded weekly. At the end of week 21, mice were food-deprived for 7 h (0700–1400 h), anesthetized, and euthanized by exsanguination via cardiac puncture. Hearts, livers, spleens, kidneys, and visceral fat depots (epididymal, retroperitoneal, and mesenteric) were harvested, rinsed, and weighed. Plasma samples were isolated by centrifugation at 3200g for 15 min. All samples were snap-frozen and stored at -80°C until further analysis.

2.3 Fasting blood glucose and insulin resistance

Fasting blood glucose measurements were made at weeks 0, 4, 10, 15, 17, 19, and 21 using a handheld Contour glucose monitor (Bayer Healthcare, Tarrytown, NY). In brief, cage bedding was changed to prevent coprophagy, mice were food-deprived for 7 h (0700 – 1400 h), and blood was sampled from the tail vein. Plasma insulin was measured at euthanasia using an Ultra-Sensitive Mouse Insulin ELISA kit (Crystal Chem, Downers Grove, IL). Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated from the last fasting blood glucose measurement and the fasting plasma insulin measurement using the equation:

$$\text{HOMA-IR} = \frac{\text{glucose } \left(\frac{\text{mg}}{\text{dL}}\right) \times \text{insulin } \left(\frac{\text{mU}}{\text{L}}\right)}{405}$$

2.4 Biochemical analysis of plasma and liver samples

Plasma alanine aminotransferase (ALT) levels were determined using a spectrophotometric method ($\lambda_{\text{max}} = 340 \text{ nm}$) according to the manufacturer's protocol (Catachem, Inc, Bridgeport, CT). Plasma and liver levels of cytokines were determined using commercially available ELISAs (R&D Systems, Minneapolis, MN) according to the manufacturer's protocols. Caspase-1 activity was measured using a colorimetric method ($\lambda_{\text{max}} = 405 \text{ nm}$) according to the manufacturer's protocol (Abcam, Co., Cambridge, MA).

2.5 Histopathological assessment of hepatic steatosis

Hepatic steatosis was evaluated histopathologically by determining lipid droplet number, size, and total area. Analysis was performed in a manner analogous to that of Gu et al., with some modifications [29]. Liver samples were fixed in formalin, embedded in paraffin, cut into $5 \mu\text{m}$ sections, and stained with hematoxylin and eosin. The sections were viewed at $20\times$ magnification and images were obtained with a FV1000 digital camera (Olympus Imaging America Inc., Center Valley, PA, USA). The images were edited by making them black and white, inverting the color, adjusting "Fill Light," "Highlights," and "Shadows," and "Sharpen Image" using Picasa 3 (Google, Mountain View, CA, USA). The images were then opened in Adobe PhotoShop CS 8.0 (Adobe systems, San Jose, CA, USA), a scale was made based on $20\times$ magnification, shadows (i.e. lipid droplets) were selected, and the total number and area of the lipid droplets were calculated with the command "Record Measurements." Results were directly loaded into Excel (Microsoft Inc., Redmond, WA, USA) for analysis.

2.6 Quantitative reverse transcriptase polymerase chain reaction (qPCR)

Total RNA was extracted using the RNeasy Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's protocol. Total RNA was quantified with a Nanodrop 2000 spectrophotometer and reverse-transcribed to cDNA using a RT² HT First Strand Kit (SA Biosciences, Valencia, CA). Quantitative PCR was performed using an Applied Biosystems 7900HT Fast Real-Time PCR System (San Francisco, CA) and primers (Suppl. Table 2, The Pennsylvania State University Genomics Core Facility, University Park, PA) or TaqMan® hydrolysis probes (Suppl. Table 3, Life Technologies, Inc, Grand Island, NY) were used. The reactions included either 5 µL SYBR® Green PCR Master Mix, 0.3 µL of both the forward and reverse primers and 4.4 µL diluted cDNA or 5 µL PerfeCTa® qPCR SuperMix, ROX Quanta BioSciences (Gaithersburg, MD), 0.5 µL TaqMan® hydrolysis probe Life Technologies (Grand Island, NY, Table 1), and 4.5 µL diluted cDNA. Reactions were incubated in a 384-well plate at 95°C for 10 min, followed by 50 cycles of denaturation at 95°C for 15 s and annealing/extension at 60 °C for 10 min, 95°C for 15 s, 60°C for 15 s, and 95°C for 15 s. Data were recorded and analyzed with Sequence Detector Software (Applied Biosystems). Relative gene expression was determined using the 2^{-C_T} method, where $C_T = (C_{T, \text{target}} - C_{T, \text{reference}})$, with *Gapdh* as the reference gene. C_T values were used for statistical analysis and 2^{-C_T} values were used for graphical representation.

2.7 Statistical analysis

Data are presented as the mean $\pm$ standard error of the mean (SEM). Two-way ANOVA (time x diet) with Bonferroni post-test was used for body weight, food intake, and blood glucose comparisons over the course of the study. Student's *t*-test was used for all other data comparisons. A $p < 0.05$ was considered statistically significant. All analyses were performed using GraphPad Prism 6.0 (San Diego, CA).

3. RESULTS

3.1 Polyphenolic composition of CBE

Based on the Folin-Ciocalteu assay, CBE containing 80.9% total phenolics (expressed as GAE/g extract, Table 1). A small percentage of these phenolics consisted of monomeric anthocyanins including petunidin glycoside, peonidin galactoside, and others. Precipitable tannins made up approximately 20% of the CBE. The normal phase chromatogram exhibits a large peak with a retention time around 65 min that is comparable to that seen with polymeric (DP 7+) PACs (Fig. 1) [25, 26].

3.2 Effect of CBE on food intake, body weight, visceral fat mass, and organ weights

Addition of 0.8% CBE to the diet had no significant effect on food intake over the course of the experiment (data not shown). CBE treatment for 10 weeks had no significant effect on final body weight or visceral adipose tissue, although CBE-treated mice did have a 5.2% lower rate of body weight gain than HF-fed controls (Table 2). CBE-treatment had no significant effect on relative organ weights in HF-fed mice (Table 2).

3.3 Effect of CBE on plasma markers of insulin resistance, inflammation, and dyslipidemia

CBE supplementation had no significant effect on fasting blood glucose levels, fasting plasma insulin levels, or HOMA-IR, however plasma free fatty acids were reduced by 17.3% in CBE supplemented mice compared to HF-fed controls ($p < 0.05$, Table 3). Plasma levels of IL-1 β were significantly lower in CBE-supplemented mice (24%, $p < 0.05$) compared to the HF group (Table 3). No significant effect of CBE on plasma CCL2 and TNF- α were observed (Table 3).

3.4 Liver injury and NAFLD

Dietary supplementation with CBE reduced plasma ALT levels in HF-fed obese mice by 31.2% compared to HF-fed control mice ($p < 0.05$, Fig. 2). Histological examination showed that although there was no significant difference in the total number of hepatic lipid droplets in CBE-supplemented mice compared to HF-fed controls (Fig. 2), there was a significant decrease in the total lipid droplet area in the liver and total hepatic lipid area was also significantly reduced (Fig. 2).

3.5 Hepatic markers of inflammation

CBE supplementation reduced hepatic levels of CCL2 (19%, $p < 0.05$) and TNF- α (28%, $p < 0.01$) protein compared to HF-fed controls (Fig. 3). No significant effect of CBE on hepatic IL-1 β protein levels was observed (Fig. 3). CBE-supplemented mice also had lower hepatic expression of the NF- κ B-dependent pro-inflammatory genes *Tnfa* (47% decrease, $p < 0.01$) and *Cox2* (46% decrease, $p < 0.05$) compared to HF-fed mice (Fig. 3). The hepatic mRNA expression of *Iilb* and *Ucp2* were also dramatically decreased in CBE-supplemented mice by 55% ($p < 0.01$) and 57% ($p < 0.01$), respectively, compared to the HF group (Fig. 3). Although hepatic levels of *Nos2* ($p = 0.15$) and *Ccl2* ($p = 0.07$) appeared to be decreased in CBE-treated mice, the effects were not statistically significant (Fig. 3). The TLR4/NF- κ B signaling axis plays a critical role in chronic inflammation. CBE supplemented mice had 69% lower hepatic *Tlr4* expression ($p < 0.01$) and 30% lower hepatic *Nfkb* expression ($p < 0.05$) compared to the HF-fed control mice (Fig. 3). CBE supplementation reduced hepatic mRNA levels of C-C chemokine receptor 2 (*Ccr2*), the CCL2 receptor expressed on recruited monocytes, by 56% and *Ccl3* by 55% compared to HF-fed control mice ($p < 0.01$, Fig. 3). We observed no significant effect of CBE supplementation on the hepatic expression of the macrophage-associated genes *Emr1* (Fig. 3).

3.6 NLRP3 inflammasome

The NLRP3 inflammasome is a critical regulator of IL-1 β activation [30]. CBE-supplemented mice had significantly lower hepatic expression of *Nlrp3* (43%, $p < 0.01$) and thioredoxin-interacting protein (*Txnip*, 30%, $p < 0.001$) compared to HF-fed control mice. The gene expression of hepatic peroxisome proliferator- α (*Ppara*), the transcription factor responsible for regulating *Txnip* expression, was also significantly decreased due to CBE supplementation (24%, $p < 0.05$, Fig. 4). Hepatic *Casp1* expression was also 35% lower in the CBE group compared to the HF group ($p < 0.05$) and caspase 1 activity tended to decrease, however, the change did not reach statistical significance ($p = 0.08$, Fig. 4).

4. DISCUSSION

Chronic systemic and hepatic inflammation associated with obesity can lead to the progression of NAFLD to NASH. In this study, we examined the effect of dietary supplementation with CBE on liver inflammation and hepatocellular injury in HF-fed obese C57BL/6J mice. We found that dietary supplementation with CBE reduced hepatic mRNA *Tnfa*, *Ccl3*, and *Cox2* expression, as well as hepatic protein levels of CCL2 and TNF- α compared to HF-fed controls without significantly altering final body weight or visceral fat mass. The effects observed correlated with a decrease in plasma ALT levels and a histological improvement in hepatic steatosis (*i.e.* decreased total lipid droplet area), indicating reduced hepatocellular damage. These results imply that CBE reduced progression from steatosis to steatohepatitis. These results differ somewhat from a previous study examining the effect of a commercially-available cranberry extract (Nutra Canada, Quebec, Canada) on markers of metabolic syndrome in HF/high sucrose-fed mice [21]. Those authors found that treatment with cranberry extract (200 mg/kg, once daily) by oral gavage for 8 weeks not only affected liver weight and hepatic triglyceride levels, but also significantly decreased final body weight and visceral adipose tissue weight. The inconsistencies between our study and this previous one could be related to differences in the mouse model (HF/high sucrose vs HF) used, differences in experimental design (prevention vs intervention), differences in phytochemical composition of the cranberry extract tested, and/or the route of CBE administration (gavage vs dietary administration). Oral gavage dosing with CBE is likely to produce higher acute concentrations of cranberry phytochemicals in the intestine and liver compared to dietary administration. Such differences in pharmacokinetics may result in differential pharmacodynamics impacts.

The TLR4/NF- κ B pathway plays a central role in the transcription regulation of pro-inflammatory genes including IL-1 β , COX-2, and TNF- α . In the present study, we found that CBE supplementation significantly reduced hepatic mRNA levels of both *Tlr4* and *Nfkb*. These findings, in conjunction with decreases in hepatic TNF- α protein levels and reduced hepatic *Cox2* and *Tnfa* mRNA levels, suggest that CBE reduces hepatic inflammation in part by modulating the TLR4/NF- κ B-signaling axis. Anhê et al. (2015) previously reported that CBE can reduce the ratio of NF- κ B and its chaperone, inhibitor κ κ B, and blunt expression of TNF- α in the liver of HF/high sucrose-fed mice, results consistent with our current findings [21]. One limitation of the present study is that we were not able to measure protein levels or localization of NF- κ B because of degraded samples. Previously it has been shown that changes in mRNA levels of NF- κ B do not necessarily correspond to changes in localization and activity. That fact that the expression of several NF- κ B responsive genes (*i.e.* *Iilb* and *Cox2*) is changed by CBE treatment provides us with some confidence that this transcription factor plays an important role in the observed effects, but future studies looking at protein levels and using reporter assays are necessary to confirm the importance of NF- κ B.

CCL2 is a key chemokine responsible for the recruitment of CCR2-presenting bone marrow-derived monocytes into the liver during inflammation [4, 31, 32]. *Ccl3* is another chemokine that is highly expressed in recruited monocytes during liver inflammation [33]. The expression of both is regulated by NF- κ B. In the present study, we found that CBE

supplementation decreased the expression of *Ccr2* and *Ccl3* by 56% and 55%, respectively. Moreover, hepatic CCL2 protein expression was reduced by CBE supplementation. This could imply that macrophage recruitment is decreased by CBE supplementation and macrophage polarization or inflammatory response by hepatocytes may be altered.

Uncoupling protein-2 (UCP2) is embedded in the inner membrane of mitochondria in all tissues and is thought to have a role in regulating reactive oxygen species (ROS) [34]. UCP2 has also been suggested to have a role in promoting the pathogenesis of NASH and its expression is increased in response to increased plasma free fatty acids and hepatic steatosis. Up-regulation of *Ucp2* gene expression in the liver has also been linked to increased oxidative stress [35]. In the present study, CBE supplementation decreased the hepatic gene expression of *Ucp2*, an indication that CBE may reduce hepatic oxidative stress.

The NLRP3 inflammasome functions as a post-translational regulator of IL-1 β and its activation is associated with the induction of hepatic fibrosis and injury [13, 14]. ROS are sensed by a thioredoxin- TXNIP complex, which subsequently dissociates allowing TXNIP to interact with NLRP3 and facilitate the assembly of the inflammasome. The active inflammasome converts pro-caspase-1 to active caspase-1, which can subsequently cleave pro-IL-1 β into active IL-1 β . We observed that CBE supplementation significantly decreased the hepatic mRNA expression of *Nlrp3*, *Txnip*, and *Casp1*, indicating decreased production of inflammasome components. We also observed a significant decrease in plasma IL-1 β levels. The expression of *Illb* and *Nlrp3* are regulated by NF- κ B activation while *Txnip* is regulated by *Ppara*, and the present results may indicate that CBE reduces IL-1 β and its maturation through reduced transcription rather than by modulating the activity of the NLRP3 inflammasome. CBE supplementation tended to decrease hepatic caspase 1 activity, however the effect did not reach statistical significance ($p = 0.083$) and is similar to the insignificant decrease in hepatic protein expression of IL-1 β .

In conclusion, the present study demonstrates that supplementation of CBE in a HF diet can attenuate the inflammation associated with the progression of steatosis to steatohepatitis, and provides additional support to the efficacy of this foodstuff for the mitigation of non-alcoholic fatty liver disease. Future studies are needed to identify the active components in cranberry extract and to further investigate the mechanisms underlying the observed results.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding Source: This study was supported in part by grant from the National Institutes of Health (No. AT004678) and a United States Department of Agriculture Hatch Project (No. 4565) to JDL. SLG was supported by a United States Department of Agriculture National Needs Fellowship.

The authors wish to thank Roberta Horner and the Penn State University Animal Diagnostics Laboratory for preparation of liver samples for histopathological analysis. The authors thank Dr. Connie J. Rogers, Department of Nutritional Sciences, Penn State University, for helpful discussions on this research project.

Abbreviations

ALT	alanine aminotransferase
CASP1	caspase 1
CBE	polyphenol-rich cranberry extract
CCL2	chemokine (C-C motif) ligand 2
CCR2	C-C chemokine receptor type 2
COX-2	cyclooxygenase 2
DP	degree of polymerization
EMR1	EGF-like module-containing mucin-like hormone receptor-like 1
HF	high fat diet
HOMA-IR	homeostatic model assessment-insulin resistance
IL-1β	interleukin 1 β
MCP-1	monocyte chemotactic protein 1
MIP-1α	macrophage inflammatory protein 1 α
NAFLD	non-alcoholic fatty liver disease
NASH	non-alcoholic steatohepatitis
NFκB	nuclear factor κ B
NLRP3	NACHT, LRR and PYD domains-containing protein 3
NOS2	inducible nitric oxide
PAC	proanthocyanidin
ROS	reactive oxygen species
TLR4	toll like receptor 4
TNFα	tumor necrosis factor α
TXNIP	thioredoxin interacting protein
UCP2	uncoupling protein 2

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Figure 1.
Representative HPLC chromatogram of CBE used in the present study.

Figure 2.

Impact of CBE supplementation on markers of liver injury and NAFLD in HF-fed obese mice. HF-fed Obese C57BL/6/J mice were treated for 10 wks with 0.8% CBE. Plasma ALT levels were determined spectrophotometrically as a marker of liver injury. The degree of lipidosis, total number of lipid droplets, and mean lipid droplet size were determined histologically after staining liver sections with hematoxylin and eosin. Two sections from 4–5 mice from each group were used and 3 fields per section were evaluated. Values are expressed as mean $\pm$ SEM. Values are statistically significantly different by Student's *t*-test compared to HF-fed controls (* $p < 0.05$, ** $p < 0.01$).

Figure 3.

Effect of CBE supplementation on markers of hepatic inflammation in HF-fed obese mice. HF-fed obese C57BL6/J mice were treated for 10 wks with 0.8% CBE. Protein levels of hepatic CCL2, TNF-α, and IL-1β were determined by ELISA. Hepatic mRNA expression of *Tlr4*, *Nfkb*, and downstream target genes, as well as markers of macrophage infiltration (*Emr1*, *Ccr2*, *Ccl3*) were evaluated by qPCR. Values are expressed as mean ± SEM. Values are statistically significantly different by Student's *t*-test (* $p < 0.05$, ** $p < 0.01$).

Figure 4.

Effect of CBE on hepatic expression of NLRP3 inflammasome-related genes and caspase 1 activity in HF-fed obese mice. Hepatic expression of NLRP3 inflammasome-related genes and caspase-1 activity were determined by qPCR and a commercially-available enzyme assay, respectively. Values are expressed as mean $\pm$ SEM. Values are statistically significantly different by Student's *t*-test (* $p < 0.05$, ** $p < 0.01$).

Table 1

Polyphenolic compositional analysis of CBE.

Description	Units	Concentration
Total phenolic content	mg GAE/g extract	809 ± 24
Total monomeric anthocyanins	mg/g extract	18.2 ± 0.1
Protein precipitable tannins	mg CE/g extract	202 ± 28
Specific Polyphenols	mg/g extract	
A-type PaC dimer		0.862
B-type PaC dimer		0.100
B-type PaC trimer		0.044
Cyanidin-6-acetyl-3-glucoside		0.001
Cyanidin galactoside or glucoside		0.659
Delphinidin arabinoside		0.076
Delphinidin-6-acetyl-3-glucoside		0.291
Malvidin-6-acetyl-3 glucoside or galactoside 1		0.188
Malvidin-6-acetyl-3 glucoside or galactoside 2		0.347
Peonidin arabinoside 1		0.118
Peonidin arabinoside 2		0.955
Peonidin galactoside		3.97
Petunidin glycoside		0.638

Table 2

Effect of dietary CBE on body, organ, and fat weight in HF-fed obese mice.

	Units	HF	CBE
BW wk 11	g	46.69 ± 0.73	45.51 ± 0.53
Final BW	g	53.42 ± 0.58	51.96 ± 0.50
Rate BW gain	g/wk	1.55 ± 0.03	1.47 ± 0.02*
Organs			
Heart	%BW	0.35 ± 0.007	0.34 ± 0.015
Kidneys	%BW	0.76 ± 0.015	0.77 ± 0.017
Spleen	%BW	0.23 ± 0.007	0.21 ± 0.005
Liver	%BW	5.78 ± 0.14	5.47 ± 0.15
Fat pads			
Epididymal	g	1.66 ± 0.15	1.61 ± 0.13
Mesenteric	g	1.64 ± 0.07	1.53 ± 0.09
Retroperitoneal	g	1.50 ± 0.07	1.52 ± 0.07

Values are expressed as mean ± SEM. Means in a row are statistically significantly different by Student's *t*-test (**p* < 0.05).

Table 3

Effect of dietary CBE on plasma glycemic and inflammatory markers in HF-fed obese mice.

	HF	CBE
Final fasting blood glucose (mmol/L)	8.6 ± 1.8	8.4 ± 1.3
Fasting plasma insulin (pmol/L)	1020.9 ± 73.9	1232.9 ± 118.0
HOMA-IR	54.4 ± 4.1	64.4 ± 5.8
Free fatty acids (μM)	602.5 ± 22.6	498.0 ± 22.8**
CCL2 (pg/mg protein)	2.45 ± 0.28	2.55 ± 0.30
TNF-α (pg/mg protein)	0.282 ± 0.016	0.268 ± 0.011
IL-1β (pg/mg protein)	1.125 ± 0.119	0.859 ± 0.041*

Values are expressed as mean ± SEM. Means in a row are statistically significantly different by Student's *t*-test (* $p < 0.05$; ** $p < 0.01$).