

Peanut protein-polyphenol aggregate complexation suppresses allergic sensitization to peanut by reducing peanut-specific IgE in C3H/HeJ mice

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ARTICLE INFO

Keywords:

Peanut allergy
IgE
CD63
Polyphenol
Blueberry
Cranberry

ABSTRACT

Peanut allergy is usually lifelong and accidental exposure impose formidable risk. The aim of this study was to assess the capacity of peanut proteins complexed to polyphenol extracts to reduce allergic response in C3H/HeJ mice. Mice were sensitized to peanut flour followed by exposure to amino acid diets fortified with peanut protein-polyphenol aggregates of either with low (15%; w/w) or high (40%; w/w) complexation ratios of blueberry (BB-Low and BB-High) and cranberry (CB-Low and CB-High) extracts. Treatment groups on diets with high complexation ratios of blueberry and cranberry aggregates showed significant reduction in peanut specific plasma Immunoglobulin E (IgE). Western blot analysis of spleen lysates showed CD63 protein expression was reduced in a dose-dependent manner in blueberry and cranberry complexed peanut protein supplemented diet groups. Our results demonstrate for the first time that complexation of polyphenols to peanut flour can potentially lower plasma IgE of peanut-sensitized C3H/HeJ mice.

1. Introduction

Nut and seafood allergies can persist through the life of affected individuals. In fact, the quality of life of children with allergies is deemed to be worse than children with diabetes (Huang et al., 2011). Among food allergies, the prevalence of peanut allergy is highest among the Western countries and has been observed to be increasing in Asian countries (Joo Chan, Richardo, & Lim, 2018). In recent years, scientific studies were undertaken to understand the mechanisms by which peanut allergens react with the human immune system. Overall, sixteen peanut allergens have been identified of which Ara h 1, Ara h 2 and Ara h 3 are considered to be more potent and are associated with severe allergic reactions in the USA (Palladino & Breiteneder, 2018).

These allergens can trigger an anaphylactic response in peanut-sensitive individuals by inducing secretion of allergic mediators such as histamine, platelet-activating factor (PAF), and leukotrienes from mast cells and basophils (Finkelman, 2010).

Current strategies for treatment of peanut allergies include strict avoidance and rescue medication upon accidental exposure to peanuts. An alternative strategy includes oral immunotherapy (OIT) which

increases the threshold of peanut sensitivity in individuals who are allergic to peanuts. Other technological strategies include modifying or removing allergens from foods (Bavaro et al., 2018). However, these strategies are either intensive, requiring clinical intervention as in the case of OIT, pose logistic problems when implementing at the population level and/or require significant lifestyle modifications (Heine, 2018).

Cross-linking of dietary proteins by polyphenols can change their structural and immunological properties and can affect the protein's physical, thermal and morphological properties (Radosavljevic et al., 2013; Liu, Ma, McClements, & Gao, 2017). Phenolic compounds can interact with proteins both covalently and non-covalently (Vesic et al., 2015). Our own findings have established that fortification of peanut flour with polyphenolic extracts rich in anthocyanins and proanthocyanidins attenuates the allergic response by reducing the Immunoglobulin E (IgE) binding and degranulation capacities (Plundrich et al., 2015; Plundrich, Bansode, Foegeding, Williams, & Lila, 2017; Bansode, Plundrich, Randolph, Lila, & Williams, 2018). We have demonstrated that peanut matrices fortified with polyphenols extracted from cranberry and lowbush blueberry exhibited significantly reduced

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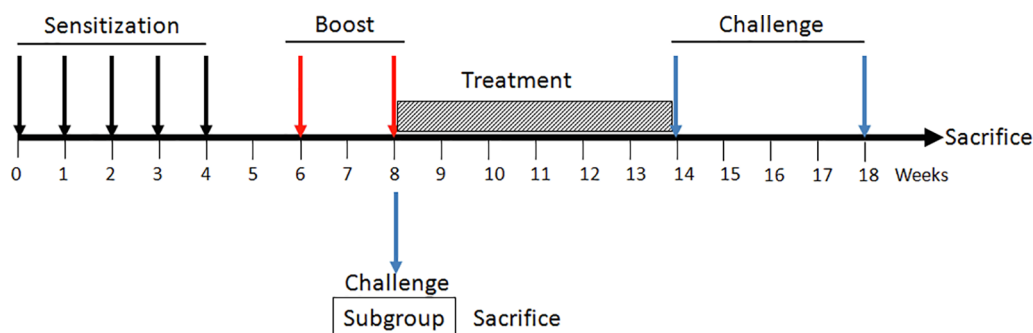


Fig. 1. Timeline of experimental protocol.

IgE binding capacity and inhibited the release of β -hexosaminidase and histamine by mast cells (Plundrich et al., 2017). It was also shown that these polyphenols complexation to peanut proteins are covalent in nature (Plundrich et al., 2017). In another study, we showed that peanut skin polyphenol-enriched peanut protein aggregates not only showed hypoallergenic properties *in vitro* but also reduced phosphorylation of p44/42 Mitogen-activated protein kinases (MAPK) and increased phosphorylation of p38 MAPK and stress-activated protein kinase/c-Jun NH2-terminal kinase (SAPK/JNK) in the rat basophilic leukemia (RBL-2H3) cells (Bansode et al., 2018). Our findings strongly suggest that aggregation of polyphenols to peanut proteins reduces peanut allergenicity by mitigating calcium-mediated MAPK-dependent cell degranulation.

Despite evidence highlighting the importance of polyphenol fortification of peanut flour in reducing peanut allergenicity *in vitro*, there has been limited research to validate these beneficial effects in either animal or clinical studies. In this study, peanut protein- polyphenol colloidal aggregate particles (formulated with blueberry and cranberry pomace polyphenolic extracts bound to edible peanut (PN) proteins) were used to investigate the potential hypoallergenic properties in an *in vivo* model of allergenicity using C3H/HeJ mice. Specifically, this study explored the earliest detectable biomolecular cues of allergenicity in animal models by exhibiting minimal anaphylactic reaction. The results of this work should further validate the hypoallergenic properties of the peanut protein-polyphenol complexation and lead to potential commercial implementation in the food industry.

2. Materials and methods

2.1. Preparation of colloidal aggregate particles

Lowbush blueberry (*Vaccinium angustifolium* Ait.) pomace (Jasper Wyman & Son, Cherryfield, ME, USA) and cranberry (*V. macrocarpon* L.) pomace (Ocean Spray, Lakeville-Middleboro, MA, USA) were freeze dried and polyphenols extracted using ethanol as solvent as described earlier (Plundrich et al., 2017). Food grade alcohol was deliberately used in these experiments to keep the process in line with production of a food ingredient. Methods using food grade aqueous alcohol for polyphenol extractions were developed and optimized by our group and have been reported earlier (Roopchand et al., 2013). The polyphenols were complexed with freshly ground 12% fat light roasted peanut flour containing $50 \pm 2\%$ protein (Golden Peanut and Tree Nuts, Alpharetta, GA, USA) to obtain dry, stable protein-polyphenol colloidal aggregate particles containing low cranberry (CB-Low), blueberry (BB-Low) (15%, w/w) and high cranberry (CB-High), blueberry (BB-High) (40%, w/w) polyphenolic content in the final aggregate particles as described by (Plundrich et al., 2015). No heat was applied to denature proteins. Complete methods on producing the colloidal aggregate particles have been reported previously (Plundrich et al., 2014). The colloidal mixtures contained less than 1% moisture, were frozen to prevent polyphenolic degradation and later were used to incorporate into the

animal diet formulations. The polyphenol profiles of the extracts and colloidal aggregates have been shown to contain anthocyanins, proanthocyanidins, and flavanols (Grace et al., 2013; Grace, Xiong, Esposito, Ehlenfeldt, & Lila, 2019; Hoskin, Xiong, Esposito, & Lila, 2019).

2.2. Preparation of animal diet

Amino acid-based TD.99366 diet was formulated with aggregate particles by mixing non-thermally and extruded to form pellets. The pellets which were warm to touch were dried at 50°C in a vacuum oven for 2 h to remove water and latter cooled to room temperature. The concentration of aggregate particles in the pellet diet were formulated to reflect daily consumption of 50 mg of peanut protein per day per mouse.

2.3. Animal study design

2.3.1. *In vivo* peanut challenge and efficacy evaluation, of peanut protein-polyphenol colloidal aggregate particles in the mouse model

Five-week-old female C3H/HeJ mice were purchased from Jackson Laboratory (Bar Harbor, ME, USA) and were maintained on Teklad Rodent Diet 8604 (Envigo, Madison, WI, USA) under specific pathogen-free conditions according to IACUC standard guidelines for the care and use of laboratory animals. At the beginning of the study all animals were switched to amino acid-based diet (TD.99366; Envigo, Madison, WI, USA). Freshly ground light roasted peanut flour was used as the antigen source. Mice were sensitized by intragastric gavage (ig) with peanut flour (10 mg/mouse suspended in water) weekly from week 0 to week 4, and booster doses (50 mg/mouse suspended in water) were administered at week 6 and 8 as shown in Fig. 1. Hypersensitivity to peanut was assessed at week 8 by challenging mice with 200 mg of peanut flour ig, and subsequent sacrifice of three mice and comparing against unsensitized mice fed on amino acid only diet (negative control). Peanut sensitivity in these groups was assessed by measuring markers of anaphylaxis according to methods described in the Online Supplemental Table S1. Peanut sensitivity was also established by measuring the increase in peanut specific plasma IgE and Immunoglobulin G (IgG) levels.

After 8 weeks, sensitized animals were randomly grouped into five treatment groups containing six animals per group and one group of unsensitized animals ($n = 6$). The treatment groups were: Unsensitized animals receiving amino acid diet only (negative control group, NEG) and five treatment groups with peanut-sensitized animals namely, a positive control group (CTL) receiving amino acid diet fortified with peanut flour (2.5% protein basis, w/w), two groups receiving amino acid diet fortified with cranberry polyphenols complexed with 2.5% (w/w) peanut protein (CB-Low and CB-High) and two groups receiving amino acid diet fortified with blueberry polyphenols complexed with 2.5% (w/w) peanut protein (BB-Low and BB-High). The control group and treatment group's TD.99366 diet complexed with polyphenolic extracts were given to respective groups for 6 weeks (between week 8

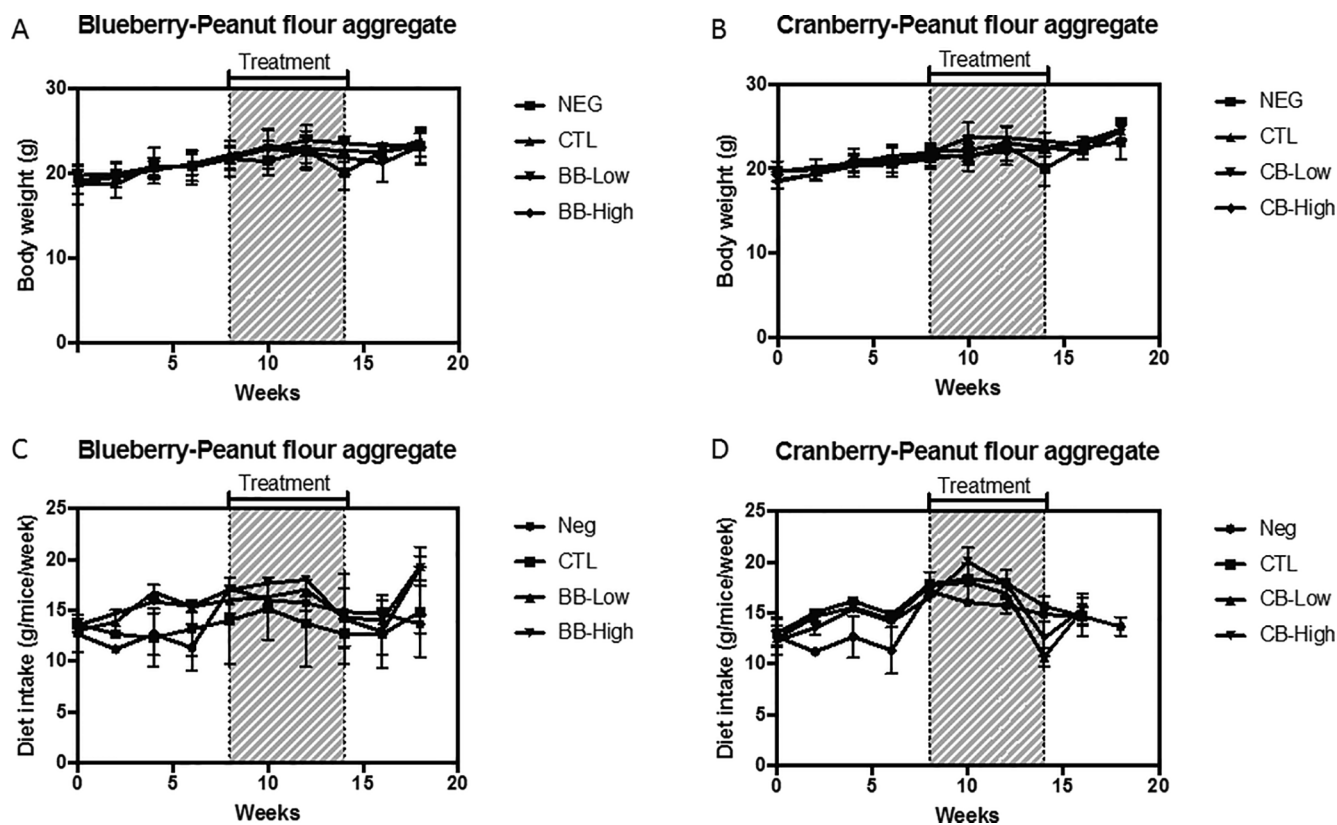


Fig. 2. Body weight and feed intake of mice. A. Body weight gain in blueberry polyphenol-peanut protein aggregate complex treatment groups. B. Body weight gain in cranberry polyphenol-peanut protein aggregate complex treatment groups. C. Food intake in blueberry polyphenol-peanut protein aggregate complex treatment groups. D. Food intake in cranberry polyphenol-peanut protein aggregate complex treatment groups.

Footnote: NEG: negative control (amino acid diet); CTL: Positive control (2.5% Peanut protein fortified amino acid diet); BB-Low: 2.5% (w/w, peanut protein basis) blueberry aggregated-peanut flour (aggregation of polyphenol, 15% w/w) in amino acid diet; BB-High: 2.5% (w/w, peanut protein basis) blueberry aggregated-peanut flour (aggregation of polyphenol, 40% w/w) in amino acid diet; CB-Low: 2.5% (w/w, peanut protein basis) cranberry aggregated-peanut flour (aggregation of polyphenol, 15% w/w) in amino acid diet; CB-High: 2.5% (w/w, peanut protein basis) blueberry aggregated-peanut flour (aggregation of polyphenol, 40% w/w) in amino acid diet. Treatment time is between 8 and 14 weeks. Amino acid diet: TD.99366 diet.

and 14). All treatment groups and the control group were further challenged with peanut flour (200 mg/mouse) on week 14 and again 4 weeks later (week 18). During the challenge period, cholera toxin serving as a mucosal adjuvant at 10 µg/mouse dissolved in water was administered to the mice except during the final challenge (i.e., week 18). Anaphylactic reaction scores and body temperatures were determined 30 min following each challenge.

2.3.2. Endpoint measurement of *in vivo* study

Immediately following the final peanut challenge (week 18), animals were scored for anaphylactic reactions, sacrificed, and blood samples were collected by cardiac puncture. Rectal temperatures were measured 30 min following challenge using a thermal probe (Harvard Apparatus, Newark, NJ, USA). Tissues samples aliquoted from the liver, and spleen were collected and frozen at -80°C until further analysis.

2.3.3. Hematoxylin and eosin staining of tissue samples

A section of spleen tissue samples was excised, placed into vials containing 10% buffered formalin and stored in refrigerated condition for further processing. The formalin fixed tissue samples were embedded in paraffin blocks, sectioned with a microtome, and mounted onto glass slides. The sections were stained with hematoxylin and eosin stains (H&E) and photographed using a BZ-X700 microscope (Keyence Corporation of America, Itasca, IL, USA).

2.3.4. Blood profile of IgE and IgG levels

Blood was collected in heparinized tubes immediately following

euthanasia by intracardiac puncture. Blood plasma was separated from the blood sample and analyzed for IgE and IgG levels as per manufacturers protocol (eBioscience, Vienna, Austria).

2.3.5. Spleen tissue expression of CD63 protein

Spleen tissue lysates were prepared by homogenizing spleen tissue in lysis buffer (Cell Signaling Technology, Inc., Boston, MA, USA) containing PhosSTOP™ phosphatase inhibitor and cOmplete™ protease inhibitor cocktail (Roche Diagnostics Corp., Indianapolis, IN, USA) in a Bead Ruptor Elite homogenizer (Omni Inc., NW Kennesaw, GA, USA). Lysates were standardized to equal protein content upon analysis using the Pierce™ BCA protein assay (Life Technologies Corp., Grand Island, NY, USA). The lysate samples were suspended in 2X Laemmli buffer, boiled for 5 min in a water bath, followed by centrifugation at 13,000g for 2 min. Following centrifugation, the supernatants were loaded onto a 10% Tris gel and SDS-PAGE gel electrophoresis was run at constant voltage of 60 V for 2 hrs. After SDS-PAGE, the proteins were transferred on to a nitrocellulose membrane. The membrane was probed for CD63 primary antibody, a strong indicator of the severity of peanut-induced IgE-mediated allergy and housekeeping protein β -actin was used to normalize the data (Santa Cruz Biotechnologies, Inc., Dallas, TX, USA). The nitrocellulose membranes were incubated in a secondary antibody (Bio-rad, Hercules, CA, USA) followed by horseradish peroxidase (HRP) enzyme substrate (Life Technologies Corporation, Grand Island, NY, USA) and visualized using Fotodyne charge-coupled device (CCD) imager (Fotodyne Incorporated, Hartland, WI, USA) and quantitated using ImageJ software (NIH, Bethesda, MD).

2.4. Statistical analysis

All data analysis was performed using analysis of variance (ANOVA) using JMP 11.0 (SAS, Cary, NC, USA). Experiments were conducted in triplicates unless stated otherwise. When significant ($p < 0.05$), means were separated using Student's *t* test.

3. Results

3.1. Diet intake, body weight and morphological changes

Following sensitization of the mice to peanut flour in combination with cholera toxin, the peanut sensitive mice were exposed to various diets ranging from amino acid diets supplemented with peanut flour, and low and high levels of blueberry and cranberry polyphenol colloidal aggregate particles, and key physiological characteristics associated with anaphylactic reactions were observed. Our results showed no adverse anaphylactic symptoms such as changes in skin, eye or mucous membrane during the study time period. No morphological or adverse anaphylactic changes were noticed during the final challenge stage at the dosages used for this study. No significant change in the body weights of the animals were observed between groups during the sensitization (0–8 weeks), treatment (8–14 weeks) and recovery (14–18 weeks) stage (Fig. 2). Feed intake of BB-low and BB-high diets were slightly higher than CTL but was not significantly different. The mice in CB-low and CB-high group showed slightly higher diet consumption than NEG group during sensitization period. The feed intake of CB-low and CB-high dropped during the fourteenth week and regained their intake during the recovery stage.

3.2. Plasma IgE and IgG levels

Blueberry and cranberry-based amino acid diet treatment groups at 40% polyphenol: protein ratio showed significant reduction in plasma IgE collected at the terminal timepoint (18-weeks) and is presented in Fig. 3. This reduction in IgE was dose-dependent and the highest polyphenol-peanut protein complexation showed IgE levels comparable to the basal levels of the unsensitized mice group (i.e., negative control). Plasma IgG levels increased in peanut sensitized animals. The trends were lower with increasing polyphenolic proportion, but the levels did not show significant reduction upon increase in polyphenol-peanut protein aggregation across all groups.

3.3. Histology of spleen tissue section

Hematoxylin and eosin staining of spleen tissue showed no significant difference in the morphology of the tissue (Figs. 4 and 5). Eosinophil infiltration in the peanut sensitive and polyphenol-peanut aggregated diet was not observed. Overall the effect was more specific to the role of blueberry and cranberry polyphenol aggregation in reducing the IgE levels in the peanut sensitized animals.

3.4. Western blot analysis of spleen lysates

Western blot analysis of spleen lysates showed an increase in CD63 protein expression in the peanut sensitized control group. CD63 protein expression was reduced in a dose-dependent manner in blueberry and cranberry complexed peanut protein supplemented diet groups (Fig. 6). The reduction in CD63 protein expression was 33.3 ± 3.9 and $53.5 \pm 0.6\%$ in BB-low and BB-high diet, respectively when compared to control group ($p < 0.05$). CD63 protein expression reduced by 35.6 ± 0.1 and $44 \pm 1.9\%$ in CB-low and CB-high diet groups, respectively, compared to the control group ($p < 0.05$).

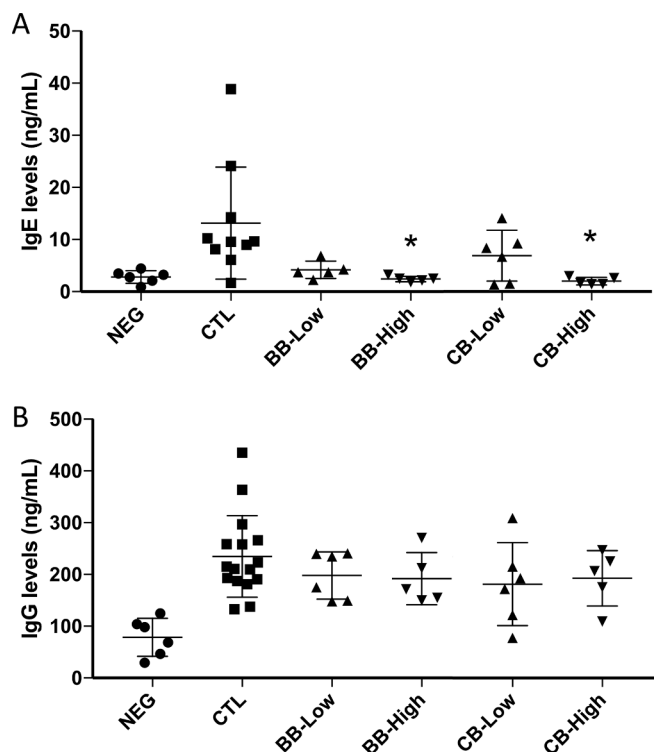


Fig. 3. Endpoint plasma IgE and IgG levels in mice. A. Plasma IgE levels in polyphenol-peanut protein aggregate complex treatment groups. B. Plasma IgG levels in polyphenol-peanut protein aggregate complex treatment groups.

Footnote: NEG: negative control (amino acid diet); CTL: Positive control (2.5% Peanut protein fortified amino acid diet); BB-Low: 2.5% (w/w, peanut protein basis) blueberry aggregated-peanut flour (aggregation of polyphenol, 15% w/w) in amino acid diet; BB-High: 2.5% (w/w, peanut protein basis) blueberry aggregated-peanut flour (aggregation of polyphenol, 40% w/w) in amino acid diet; CB-Low: 2.5% (w/w, peanut protein basis) cranberry aggregated-peanut flour (aggregation of polyphenol, 15% w/w) in amino acid diet; CB-High: 2.5% (w/w, peanut protein basis) blueberry aggregated-peanut flour (aggregation of polyphenol, 40% w/w) in amino acid diet. Endpoint measured after 18 weeks following terminal challenge. Amino acid diet: TD.99366 diet. *Significantly different at $p < 0.05$.

4. Discussion

Peanut allergy is considered an IgE-mediated disease (Burks, 2008). It is believed that the sensitization of food allergens occurs at the mucosal surface of the gastrointestinal tract. The food proteins are taken up by epithelial cells called M cells and are later transferred to dendritic cells. They are further processed to their peptide fragments and presented to the naïve T helper (Th) cells. These activated Th cells secrete interleukins and cytokines that stimulate B cells to synthesize IgE antibodies specific to the peanut allergens. The IgE antibodies bind to the FcεRI receptors of mast cell and basophil cells. In a study investigating wheat allergy using several polyphenol sources such as artichoke leaves, cranberries, apples and green teas, only cranberry and artichoke extracts limited cellular degranulation by reducing the mouse anti-gliadin IgE recognition (Perot et al., 2017).

When the peanut sensitive individual is exposed to peanut protein, the protein crosslinks with the IgE antibodies on the mast cells and basophils and triggers inflammatory response by releasing histamine, prostaglandins, leukotrienes and platelet-activating factor.

In our previous study we have demonstrated that polyphenols binding of peanut allergens inhibited IgE recognition of Ara h2 (Plundrich, Cook, Maleki, Fourches, & Lila, 2019). Our results showed that procyanidins C1 and chlorogenic acid (1) interacted with Ara h2; (2) changed Ara h2 secondary structure, and (3) decreased IgE binding

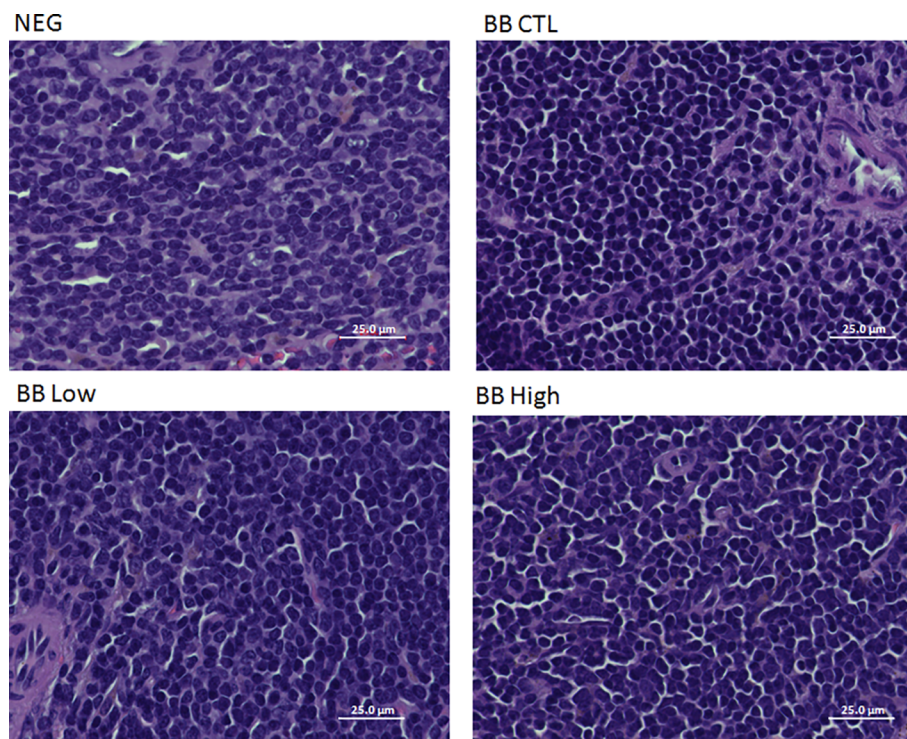


Fig. 4. Spleen histological sections of blueberry study group animals stained with hematoxylin and eosin stain.

Footnote: NEG: negative control (amino acid diet); CTL: Positive control (2.5% Peanut protein fortified amino acid diet); BB-Low: 2.5% (w/w, peanut protein basis) blueberry aggregated-peanut flour (aggregation of polyphenol, 15% w/w) in amino acid diet; BB-High: 2.5% (w/w, peanut protein basis) blueberry aggregated-peanut flour (aggregation of polyphenol, 40% w/w) in amino acid diet. Endpoint measured after 18 weeks following terminal challenge. Amino acid diet: TD.99366 diet. Magnification 40X (Scale bar = 25 μm).

capacity (Plundrich et al., 2019).

In this study, our goal was to validate the anti-allergic potential of the polyphenolic colloidal aggregates in an animal model system. Therefore, we conducted an *in vivo* study using C3H/HeJ mice by orally sensitizing these mice with peanut flour in combination with cholera toxin used as an oral adjuvant. The impact of peanut flour-amended amino acid diet with and without polyphenol complexation on the IgE-based immune response and anaphylactic reaction was assessed in these animals. Animals in various treatment groups did not show any

significant difference in body weight or feed intake. Interestingly, animals in treatment groups containing polyphenol aggregated with peanut flour showed reduced plasma IgE levels. Our results demonstrate for the first time that complexation of polyphenols to peanut flour can potentially lower plasma IgE of peanut sensitized C3H/HeJ mice. Previous studies have demonstrated oral administration of procyanidin C1 extracted from unripe apples inhibits high affinity receptors for IgE (FcεRI) in Balb/c mice (Nakano et al., 2008). Although, the anti-allergic properties of polyphenols have been reported mostly with

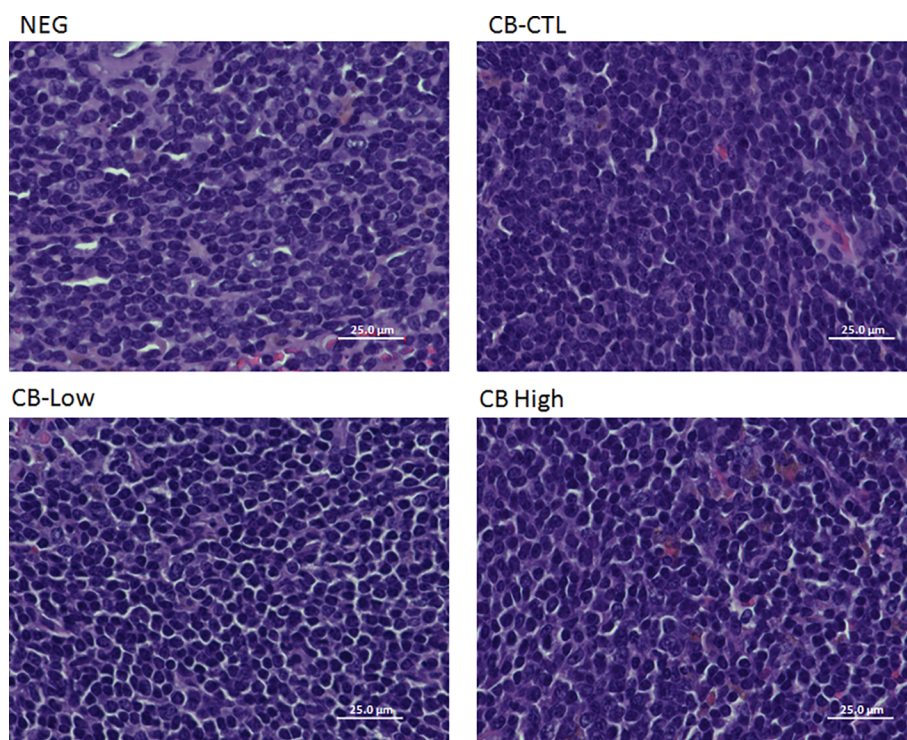


Fig. 5. Spleen histological sections of cranberry study group animals stained with hematoxylin and eosin stain.

Footnote: NEG: negative control (amino acid diet); CTL: Positive control (2.5% Peanut protein fortified amino acid diet); CB-Low: 2.5% (w/w, peanut protein basis) cranberry aggregated-peanut flour (aggregation of polyphenol, 15% w/w) in amino acid diet; CB-High: 2.5% (w/w, peanut protein basis) blueberry aggregated-peanut flour (aggregation of polyphenol, 40% w/w) in amino acid diet. Endpoint measured after 18 weeks following terminal challenge. Amino acid diet: TD.99366 diet. Magnification 40 × (Scale bar = 25 μm).

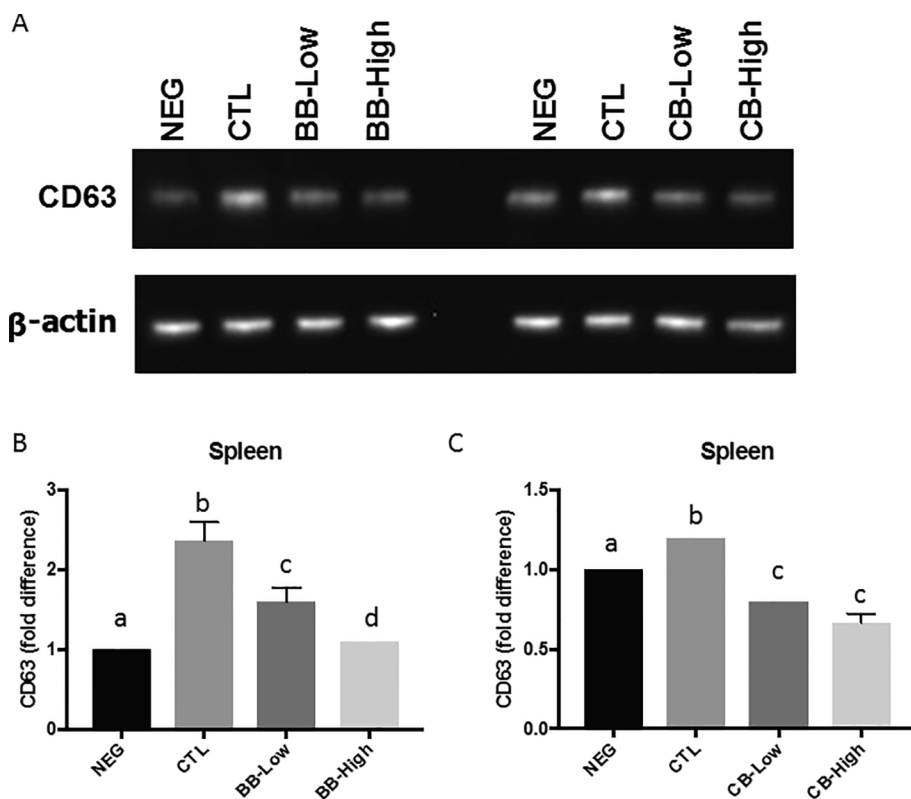


Fig. 6. A. Western blot analysis of spleen tissue lysate for CD63 and β -actin (house-keeping gene) collected after endpoint challenge. B. Histograms measured by densitometry for CD63 protein expression represented as fold change in blueberry polyphenol-peanut protein aggregate complex treatment groups. C. Histograms measured by densitometry for CD63 protein expression represented as fold change in cranberry polyphenol-peanut protein aggregate complex treatment groups.

Footnote: NEG: negative control (amino acid diet); CTL: Positive control (2.5% Peanut protein fortified amino acid diet); BB-Low: 2.5% (w/w, peanut protein basis) blueberry aggregated-peanut flour (aggregation of polyphenol, 15% w/w) in amino acid diet; BB-High: 2.5% (w/w, peanut protein basis) blueberry aggregated-peanut flour (aggregation of polyphenol, 40% w/w) in amino acid diet; CB-Low: 2.5% (w/w, peanut protein basis) cranberry aggregated-peanut flour (aggregation of polyphenol, 15% w/w) in amino acid diet; CB-High: 2.5% (w/w, peanut protein basis) blueberry aggregated-peanut flour (aggregation of polyphenol, 40% w/w) in amino acid diet. Endpoint measured after 18 weeks following terminal challenge. Samples from tissue lysates were pooled from three animals per group. Amino acid diet: TD.99366 diet; values with different letters are significantly different at $p < 0.05$.

ovalbumin sensitized murine models, studies involving peanut sensitized animal models and the effects of polyphenol-peanut complexation is severely lacking in the literature (Takano et al., 2007; Comstock, Gershwin, & Teuber, 2010; Kim et al., 2010; Oh et al., 2011; Wang, Lin, Liao, & Jan, 2013; Aswar, Kandhare, Mohan, & Thakurdesai, 2015).

Therefore, we conclude that peanut flour-polyphenol aggregate complex could inhibit specific IgE antibody production induced by oral sensitization and thus reduce the IgE-dependent anaphylaxis in peanut sensitized C3H/HeJ mice.

We further investigated the expression of basophil surface marker protein, CD63. The literature highlights various advantages and disadvantages of using CD63 expression as a marker of peanut allergy (Abuaf et al., 2008). In resting basophils, the expression of CD63 on the cell membrane is weakly upregulated and significantly increases upon cross-linking of surface IgE (Moneret-Vautrin, Sainte-Laudy, Kanny, & Frémont, 1999; Valent et al., 2001; Lambert, Guilloux, Dzvinga, Gourgaud-Massias, & Genin, 2003).

In a recent study, the ratio of CD63/anti-IgE was shown to be a better predictor of the severity and threshold of reactivity to peanut in patients with peanut allergy (Santos et al., 2015). In a study involving the CD63 knockout mouse model, bone marrow-derived mast cells showed a significant decrease in FC ϵ R1-mediated degranulation but no effect on ionomycin mediated degranulation was observed, thus implying the importance of CD63 in allergic inflammation (Kraft et al., 2013).

Condensed tannins have been shown to have anti-allergy and inhibitory properties against some physiological enzymes and receptors (Wang, Foo, & Polya, 1996; Zhu, Phillipson, Greengrass, Bowery, & Cai, 1997). In another study involving glycyrrhizic acid (GA), the major bioactive triterpene glycoside of glycyrrhiza showed the anti-allergic activity by restoring the immune balance of T_H1/T_H2 cells (Han, Sun, He, & Che, 2017). We have also evaluated the stability of peanut protein-polyphenol complexes *in vitro* using gastric digestion with pepsin (Plundrich et al., 2015). We found that the basic subunit of the peanut allergen Ara h 2 and Ara h 3 hydrolyzed more rapidly in complexed peanut flour compared to uncomplexed peanut flour signifying that

polyphenolic complexation renders peanut proteins less immunoreactive when passing through the digestive tract (Plundrich et al., 2015).

Considering these findings, it can be fairly assumed that polyphenolic complexation with peanut protein could prevent the allergenic response of the peanut allergen through inhibition of IgE and reduction of degranulation mediated by CD63 activation in mast cells in mice.

5. Conclusions

Our results demonstrate for the first time that complexation of polyphenols to peanut protein could inhibit specific IgE antibody elevation in peanut sensitized C3H/HeJ mice. From our previous studies which investigated the effects of complexation on the allergic response *in vitro*, it can be clearly deduced that complexation of peanut protein with polyphenolic extracts rich in anthocyanins and proanthocyanidins attenuates the allergic response by reducing the Immunoglobulin E (IgE) binding (Plundrich et al., 2017). In addition, we have also shown that basophil surface marker protein, CD63 expression is significantly down regulated implying the reduction of allergic response in peanut sensitized mice supplemented with peanut proteins complexed with polyphenolic extracts.

Acknowledgements

This research project was made possible by Grant No. 2015-38821-24370 from the USDA-National Institute of Food and Agriculture. The statements made herein are solely the responsibility of the authors.

Declaration of Competing of Interest

No conflicts of interest are declared for any of the authors.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://>

doi.org/10.1016/j.foodchem.2019.125025.

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Supplemental table 1. Scoring system for determining humane endpoint

Categories		Score
Body weight changes		
0	Normal	
1	< 10% weight loss	
2	10-15% weight loss	
3	>20% weight loss	
Body condition score (see diagram for details)		
0	Body condition score >3	
1	BCS>2 and <3	
2	BCS>1 and <2	
3	BCS of 1 or less	
Physical appearance		
0	Normal	
1	Lack of grooming	
2	Rough coat, nasal/ocular discharge	
3	Very rough coat, abnormal posture, enlarged pupils	
Measurable clinical signs		
0	Normal	
1	Small changes of potential significance	
2	Body temp. change of 1-2°C, cardiac and respiratory rate increased up to 30%	
3	Body temp. change of >2°C, cardiac and respiratory rate increased up to 50%, or markedly reduced	
Unprovoked behavior		
	Normal	
	Minor changes	
	Abnormal, reduced mobility, decreased alertness, inactive	
	Unsolicited vocalizations, self-mutilation, either very restless or immobile	
Behavioral responses to external stimuli		
0	Normal	
1	Minor depression/exaggeration of response	
2	Moderately abnormal responses	
3	Violent reactions, or comatose	
Total		

A total score between 3-5 in any one category, regardless of the total score should warrant mandatory evaluation/decision by a veterinarian or humane euthanasia.