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Anthocyanin in black rice, soybean and purple corn increase fecal butyric acid and prevent liver inflammation in high fat diet-induced obese mice

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Abstract

Epidemiological evidence indicates that anthocyanin consumption reduces the incidence of chronic and degenerative diseases. Therefore, the present study aimed to determine whether black rice anthocyanin (BRA), black soybean anthocyanin (BSA), and purple corn anthocyanin (PCA) could mitigate oxidative stress and inflammation associated obesity in C57BL/6 mice fed with high-fat diet. BRA, BSA, or PCA were administered at doses of 200 mg/kg throughout the 12-week experiment and reduced the body weight by 9.6%, 13.3%, or 16.6%, respectively. Furthermore, BRA, BSA or PCA administration could effectively increase fecal butyric acid levels, elevate hepatic SOD and GP_X activities, decrease lipid peroxidation, and downregulate the gene expression levels of TNFα, IL-6, iNOS, and NF-κB. Hence, BRA, BSA, or PCA might ameliorate diet-induced obesity by alleviating both oxidative stress and inflammation.

Keywords: Anthocyanin; Obesity; Inflammation; fecal short chain fatty acids

Abbreviations: BRA: black rice anthocyanin; BSA: black soybean anthocyanin; GPx: glutathione peroxidase activity; HDLC: high density lipoprotein cholesterol; HFD: high-fat diet:IL-6: interleukin -6; iNOS: inducible nitric oxide synthase; LDLC: low density lipoprotein cholesterol; LFD: low-fat diet; MDA: malondialdehyde; PCA: purple corn anthocyanin; ROS: reactive oxygen species; SCFA: short chain fatty acids; SOD: superoxide dismutase; TC: total cholesterol; TG: triglyceride; TNF-α: tumor necrosis factor-α

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1. Introduction

Obesity has become a global disease that carries considerable morbidity and mortality[1, 2] and is a complex metabolic disorder that results from extreme disequilibrium between energy uptake and expenditure[3, 4]. Orlistat, lorcaserin and fixed-dose drugs, namely phentermine and topiramate, have been approved for weight loss, but these drugs have adverse effects and high rates of secondary failure[5]. Therefore, food functional components that have anti-obesity effects need investigation[6, 7].

Anthocyanins belong to the flavonoid group of polyphenols, which are common in our daily diets, particularly in red, blue, black, or purple cereals, fruits and vegetables[8]. In recent years, anthocyanins have attracted scientific interest because of their health-promoting properties in humans[9, 10]. Anthocyanin-rich extracts from purple corn[11, 12], black soybean [13, 14], purple sweet potato [15], black rice [16], blueberry[17, 18], mulberry[19, 20], cherry [21] and blackcurrant [22] prevent bodyweight gain and metabolic aberrations in diet-induced obese animal models. In spite of the numerous publications, the information regarding the anti-obesity mechanisms of is still not fully understood.

Black rice (*Oryza sativa* L.), black soybean (*Glycine max* L.), and purple corn (*Zea mays* L.) are popular cereals in Asia because of their health benefits that are associated with their nutritious phytochemicals, especially anthocyanins[23]. Recent studies have suggested that consuming anthocyanins from black rice, black soybean and purple corn may suppress bodyweight gain[11-14, 16]. However, studies on how black rice anthocyanin (BRA), black soybean anthocyanin (BSA), and purple corn anthocyanin (PCA) alter bodyweight have not been conducted. Therefore, this study aims to determine whether BRA, BSA, and PCA can alter bodyweight by alleviating both

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oxidative stress and inflammation in diet-induced obesity.

2. Materials and Methods

2.1 Materials

Black rice, black soybean and purple corn were obtained from the Agricultural Logistics Center in Tianjin. Anthocyanins were isolated according to the procedures by Prior *et al*[24] with slight modifications. In brief, black rice, black soybean or purple corn were weighed out and extracted thrice with methanol/formic acid (9:1, v/v). The combined extract was subjected to vacuum evaporation to remove the solvent and subsequently loaded onto an equilibrated Amberlite XAD-7 column. The column was saturated with 1% formic acid, and the binding anthocyanins were eluted with 1% formic acid in methanol. The methanol eluent was collected and subjected to vacuum evaporation. Once evaporated, the concentrate was extracted with ethyl acetate until the organic layer no longer had any change in color. The aqueous layer was lyophilized and stored at −80 °C until further use. BRA is composed of cyanidin-3, 5-diglucoside (3.43%), cyanidin-3-glucoside (84.48%), peonidin-3-glucoside (5.53%), BSA consists of delphinidin-3-glucoside (27.17%), cyanidin-3-glucoside (0.33%), petunidin-3-glucoside (69.89%), pelargonidin-3-glucoside (1.36%), peonidin-3-glucoside (1.24%) and PCA contains cyanidin-3-glucoside (52.02%), peonidin-3-glucoside (13.58%), cyanidin-3-(6-malonyl-glucoside) (27.33%) and peonidin-3-(6-malonyl-glucoside) (7.07%). All the other chemicals were of reagent grade.

2.2 Animals and experimental design

A total of 72 maleC57BL/6 mice (aged 4 weeks) were purchased from the Beijing Laboratory Animal Center of the Academy of Military Medical Sciences, and maintained in a room with alternating 12 h/12 h light/dark cycles at 23± 3 °C, and

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provided with diet and water *ad libitum*. All procedures in the experiment were approved by the Animal Ethics Committee of Tianjin University of Science and Technology (TUST20160218) and conformed to the National Institutes of Health Guide for Care and Use of Laboratory Animals.

All experimental mice were acclimatized for one week and divided into six groups based on a randomized block design: a normal control group fed with low-fat diet (NC), a control group fed only with high-fat diet (HFD), a positive control group fed with HFD plus with orlistat at 100 mg/kg, and an anthocyanin group fed with HFD plus BRA, BSA or PCA at a doses of 200 mg/kg. The human-equivalent of anthocyanin doses based on body surface area was approximately about 2 mg/kg of body weight. The detailed nutritional information on the HFD and LFD diets are shown in supplemental Table 1. No untoward effects were observed during the 12 week experimental period. At the end of the experiment, all mice were anesthetized with ketamine–HCl following a 12 h fasting and sacrificed by decapitation. Serum samples, liver, and adipose tissues were immediately collected, weighed on ice, and stored at −80 °C until the further use.

2.3 Serum parameter analyses

Mouse serum triglyceride (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDLC), and high density lipoprotein cholesterol (HDLC) were measured on a Sysmex Analyzer KX-21 (Beckman Kurt Trading Co., Ltd.) according to the manufacturer's directions. Serum superoxide dismutase (SOD) activities and malondialdehyde (MDA) levels were analyzed using the hydroxylamine method and the thiobarbituric acid method, respectively.

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2.4 Hepatic lipid profiles and antioxidants

Mouse hepatic lipid profiles were determined according to the method by Folch *et al*.[25] Concentrations of TG and TC were estimated using commercially available kits (Bomeibio, China). Glutathione peroxidase activities (GPx) in the liver were measured with cellular GPx assay kit (Beyotime, China). MDA levels and SOD activities were characterized using the same commercial kits for serum analysis.

2.5 Analysis of fecal short chain fatty acids (SCFA)

SCFA composition and mouse feces concentration were analyzed by gas chromatography equipped with a flame ionization detector and chromatographic column HP-INNO Wax (30 m×320 μm×0.25 μm) (Agilent Technologies Inc., CA, USA) according to Periago *et al*[26]. SCFA (Supelco 46975-U, Sigma) was diluted to different concentrations. Grinded feces samples were treated with 2-methyl butyrate, and derivatized through esterification with isopropanol-pyridine (3:2) and propyl-chloroformate. Chromatographic conditions included hydrogen as a carrier gas, at a flow rate of 40 mL min^{-1}, air flow rate of 450 mL min^{-1}, nitrogen as a make-up gas, flow rate of 34 mL min^{-1}, injection port temperature of 280 ℃, detector temperature of 250 ℃, injection volume of 1 μL, sample flow rate of 1 mL/min, split ratio of 10:1，and temperature program of keeping at 60 ℃ constant for 5 min, followed by heating up to 230 ℃ with a constant heating rate of 10 ℃ /min.

2.6 Quantitative real-time PCR

Total RNA from the mouse liver samples was extracted using Trizol (Invitrogen Technologies, USA) according to the manufacturer's instructions. RNA extracts were reverse transcribed into cDNA, and the expression of tumor necrosis factor α (TNFα),

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interleukin-6 (IL-6), interferon gamma (IFN-γ), nuclear factor κB (NF-κB), and inducible nitric oxide synthase (iNOS) genes were examined by polymerase chain reaction (PCR, Bio-Rad) using the One Step SYBR Prime Script PLUS RT-PCR kit (TaKaRa, Japan). The primer sequences used in the experiments are shown in Supplemental Table 2.

2.7 Statistical analysis

The data were expressed as "mean ± standard deviation". The significance of treatment effects was performed with Duncan's multiple range tests after Statistical Package for the Social Sciences one-way ANOVA (SPSS PASW Statistic 19.0, SPSS Inc. Chicago, IL, USA). The significant level was $p<0.05$.

Results

Effects of BRA, BSA and PCA on the body weight of C57BL/6 mice

To determine whether BRA, BSA, and PCA affect body weight of bn C57BL/6 mice, BRA, BSA, or PCA was administered daily for 12 weeks. When HFD mice were administered with BRA, BSA or PCA at 200 mg/kg or orlistat at 100 mg/kg for 12 weeks, the body weights were effectively decreased by 9.6%, 13.3%, 16.6%, or 9.8%, respectively compared with the HFD group (Figure 1). The difference in daily food intake (~2.8 g/day) was no significant throughout the experiment. Moreover, the weights of visceral organs remained constant after the 12 week experiment. These results may suggest that decreasing food utility rate rather than appetite suppression causes the weight-reducing effects of BRA, BSA, or PCA[10, 27].

Effects of BRA, BSA and PCA on serum parameters

BRA, BSA and PCA significantly decreased serum TG, TC, LDL-C and MDA levels

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relative to the HFD control (Figure 2). Furthermore, BRA and PCA effectively elevated the HDL-C concentration and significantly increased the SOD activities comparison with the HFD group, whereas the BSA showed no significant differences in HDL-C and SOD activities. Such differences might be attributed to the structural differences of anthocyanin in terms of aglycone and sugar moieties[8, 28].

Effects of BRA, BSA, and PCA on hepatic lipids and antioxidants

The lipid and antioxidant levels in mouse liver were examined (Figure 3). The results suggested that mice fed with HFD had higher levels of hepatic TG and TC compared with those given with LFD. However, BRA and PCA could markedly reduce HFD-induced hepatic TC levels. Moreover, the marked elevation of total SOD and GP_X activities in the mouse liver in the BRA, BSA, and PCA groups were compared with those in the HFD group. By contrast, BRA, BSA, and PCA groups had significantly lower hepatic lipid peroxidation than the HFD group. These results indicated that BRA, BSA and PCA partially prevented HFD-induced oxidative stress [29, 30].

Effects of BRA, BSA and PCA on fecal short chain fatty acids

Individual and total SCFA were characterized in feces samples (Figure 4, Supplemental table 3). Figure 4 shows that the mouse feces contained six types of SCFA including acetic acid, propionic acid, butyric acid, isobutyric acid, isovaleric acid and valeric acid. The lowest amounts of acetic acid, propionic acid, butyric acid and valeric acid were present in HFD. Fecal butyric acid quantities in BRA, BSA, and PCA groups were significantly higher than those in the MC group, which indicated

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that BRA, BSA, and PCA could accelerate the fatty acid decomposition.[31-33]

Effects of BRA, BSA and PCA on inflammation

Expressions levels of inflammatory cytokine in the liver tissue were determined by quantitative real-time PCR (Fig. 5). HFD-fed mice have higher quantities of TNFα, IL-6, iNOS, and NF-κB genes than the LFD-fed mice. BRA, BSA, and PCA supplementation remarkably downregulated expression levels of TNFα, IL-6, NF-κB, and iNOS genes compared with the HFD group. These phenomena suggested that BRA, BSA and PCA may alleviate the HFD-induced liver inflammation[34-36].

Discussion

As pigments that contribute to the intense colors of many fruits and cereals, anthocyanins exhibit numerous health-promoting effects, such as cardiovascular protection, anti-diabetic properties, vision improvement, anti-inflammatory effects, and cancer protection[9, 10, 37]. In this study, we explored the weight loss effect of purified BRA, BSA, and PCA on HFD-induced obesity and determined whether these cereal anthocyanins prevented bodyweight gain by alleviating both oxidative stress and inflammation.

Convincing epidemiological evidence indicated that HFD can induce significant bodyweight gain and elevated lipid profiles in human and animals[38, 39]. Similar results were observed in the current investigation. Moreover, HFD markedly increased the expression of inflammatory cytokine levels, and significant decreased the total SOD and GPx activities. Administration of purified BRA, BSA, or PCA into HFD-fed mice

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199 at a dosage of 200 mg/kg effectively decreased the bodyweight, significantly
200 increased the fecal butyric acid contents, and the total SOD and GP_X activities ,
201 reduced lipid peroxidation, and markedly downregulated the expression levels of
202 TNFα, IL-6, iNOS, and NF-κB. Studies have shown that consumption of purified
203 strawberry anthocyanin (mainly pelargonidin-3-glucoside)[40], sweet cherry
204 anthocyanin (mainly cyanidin-3-rutinoside)[21], mulberry anthocyanin (mainly
205 cyanidin-3-glucoside)[41], blueberry anthocyanin (mainly malvidin-3-glucoside and
206 malvidin-3-galactoside) [24], and honeysuckle anthocyanin (mainly
207 cyanidin-3-glucoside)[42] decreases bodyweight and lipids profiles. In the present study,
208 PCA reduced the bodyweight more effectively than BSA and BRA did; moreover, and
209 BRA and PCA markedly reduced hepatic TC levels, and effectively elevated serum
210 HDL-C and SOD activities, whereas BSA showed no significant differences in
211 hepatic TC, serum HDL-C and SOD activities. The reasons behind such differences
212 remain unclear but may be attributed to their specific chemical structures[8]. Future
213 investigations should focus on careful and accurate characterization of different
214 anthocyanins to better elucidate the structure–anti-obesity relationships[10].
215 Extensive investigations demonstrated that HFD-induced obesity could increase
216 excessive oxidative stress by elevating lipid peroxidation and reducing antioxidant
217 enzyme activities[43, 44]. Epidemiological studies suggested that consuming polyphenols
218 may protect against lipid oxidation, and increase serum antioxidant status[29, 45]. In
219 study, BRA, BSA, or PCA reduced the lipid peroxidation in the liver and serum
220 whereas the hepatic total activities of SOD and GP_X were significant increased.
221 Therefore, BRA, BSA or PCA can prevent obesity via oxidative stress reduction[46].

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Many epidemiological and experimental studies have documented the link of obesity to low-grade inflammatory states[44, 47, 48], which elevate the expression of inflammatory cytokine levels, such as TNF-a, IL-6, and iNOS, and decrease the expression of anti-inflammatory cytokine. In the present study, HFD-fed mice presented the pathophysiological condition of inflammation accompanied by obesity, which was indicated by the high expression levels of TNFα, IL-6, iNOS, and NF-κB genes. BRA, BSA, or PCA decreased the production of inflammatory cytokines. BRA, BSA, or PCA demonstrated anti-obesity; however, controlled clinical trials that directly demonstrate the beneficial effects of anthocyanins in bodyweight management remained absent Therefore, further studies are necessary before finalizing the application of anthocyanins as a treatment for human obesity.

In summary, our results indicated that administration of BRA, BSA or PCA at 200 mg/kg or orlistat at 100 mg/kg reduced the bodyweight by 9.6%, 13.3%, 16.6% or 9.8%, respectively. Furthermore, BRA, BSA or PCA consumption could increase fecal butyric acid contents, elevate hepatic SOD and GP_X activities, decrease lipid peroxidation, and downregulate the gene expression levels of TNFα, IL-6, iNOS, and NF-κB. Therefore, BRA, BSA, or PCA ameliorate diet-induced obesity by alleviating both oxidative stress and inflammation.

Conflicts of interest

The authors declare no conflict of interest.

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References

1. T. Deng, C. J. Lyon, S. Bergin, M. A. Caligiuri and W. A. Hsueh, Obesity, Inflammation, and Cancer, *Annual Review of Pathology: Mechanisms of Disease*, 2016, **11**, 421-449.
2. C. M. Apovian and N. Gokce, Obesity and cardiovascular disease, *Circulation*, 2012, **125**, 1178-1182.
3. S. G. Kumar, M. A. Rahman, S. H. Lee, H. S. Hwang, H. A. Kim and J. W. Yun, Plasma proteome analysis for anti-obesity and anti-diabetic potentials of chitosan oligosaccharides in ob/ob mice, *Proteomics*, 2009, **9**, 2149-2162.
4. D. W. Haslam and W. P. James, Obesity, *Lancet*, 2005, **366**, 1197-1209.
5. E. E. Ladenheim, Liraglutide and obesity: a review of the data so far, *Drug Des Devel Ther*, 2015, **9**, 1867-1875.
6. R. Sunkara and M. Verghese, Functional Foods for Obesity Management, *Food and Nutrition Sciences*, 2014, **05**, 1359-1369.
7. N. Vasudeva, N. Yadav and S. K. Sharma, Natural products: a safest approach for obesity, *Chin J Integr Med*, 2012, **18**, 473-480.
8. A. Smeriglio, D. Barreca, E. Bellocco and D. Trombetta, Chemistry, Pharmacology and Health Benefits of Anthocyanins, *Phytotherapy research : PTR*, 2016, **30**, 1265-1286.
9. M. A. Lila, B. Burton-Freeman, M. Grace and W. Kalt, Unraveling Anthocyanin Bioavailability for Human Health, *Annual review of food science and technology*, 2016, **7**, 375-393.
10. D. Li, P. Wang, Y. Luo, M. Zhao and F. Chen, Health benefits of anthocyanins and molecular mechanisms: Update from recent decade, *Crit Rev Food Sci Nutr*, 2017, **57**, 1729-1741.
11. T. Tsuda, F. Horio, K. Uchida, H. Aoki and T. Osawa, Dietary cyanidin 3-O-beta-D-glucoside-rich purple corn color prevents obesity and ameliorates hyperglycemia in mice, *Journal of Nutrition*, 2003, **133**, 2125-2130.
12. M. Bhaswant, S. R. Shafie, M. L. Mathai, P. Mouatt and L. Brown, Anthocyanins in chokeberry and purple maize attenuate diet-induced metabolic syndrome in rats, *Nutrition*, 2016, DOI: 10.1016/j.nut.2016.12.009.
13. S. H. Kwon, I. S. Ahn, S. O. Kim, C. S. Kong, H. Y. Chung, M. S. Do and K. Y. Park, Anti-obesity and hypolipidemic effects of black soybean anthocyanins, *J Med Food*, 2007, **10**, 552-556.
14. M. Lee, S. R. Sorn, Y. Park and H. K. Park, Anthocyanin Rich-Black Soybean Testa Improved Visceral Fat and Plasma Lipid Profiles in Overweight/Obese Korean Adults: A Randomized Controlled Trial, *J Med Food*, 2016, **19**, 995-1003.
15. S. J. Shi, U. J. Bae, S. O. Ka, S. J. Woo, S. O. Noh, Y. S. Kwon, K. H. Jung, J. H. Wee and B. H. Park, Aqueous extracts of purple sweet potato attenuate weight gain in high fat-fed mice, *Int J Pharmaceut*, 2013, **9**, 42-49.
16. S. L. Yao, Y. Xu, Y. Y. Zhang and Y. H. Lu, Black rice and anthocyanins induce inhibition of cholesterol absorption in vitro, *Food Funct*, 2013, **4**, 1602-1608.
17. M. Shi, H. Loftus, A. J. McAinch and X. Q. Su, Blueberry as a source of bioactive compounds

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DOI: 10.1039/C7FO00449D

for the treatment of obesity, type 2 diabetes and chronic inflammation, *J Funct Foods*, 2017, **30**, 16-29.

18. T. Wu, Z. Jiang, J. Yin, H. Long and X. Zheng, Anti-obesity effects of artificial planting blueberry (Vaccinium ashei) anthocyanin in high-fat diet-treated mice, *International journal of food sciences and nutrition*, 2016, **67**, 257-264.

19. T. Wu, J. Yin, G. Zhang, H. Long and X. Zheng, Mulberry and cherry anthocyanin consumption prevents oxidative stress and inflammation in diet-induced obese mice, *Mol Nutr Food Res*, 2016, **60**, 687-694.

20. H. H. Lim, S. J. Yang, Y. Kim, M. Lee and Y. Lim, Combined treatment of mulberry leaf and fruit extract ameliorates obesity-related inflammation and oxidative stress in high fat diet-induced obese mice, *J Med Food*, 2013, **16**, 673-680.

21. T. Wu, Q. Tang, Z. Yu, Z. Gao, H. Hu, W. Chen, X. Zheng and T. Yu, Inhibitory effects of sweet cherry anthocyanins on the obesity development in C57BL/6 mice, *International journal of food sciences and nutrition*, 2014, **65**, 351-359.

22. D. Esposito, T. Damsud, M. Wilson, M. H. Grace, R. Strauch, X. Li, M. A. Lila and S. Komarnytsky, Black Currant Anthocyanins Attenuate Weight Gain and Improve Glucose Metabolism in Diet-Induced Obese Mice with Intact, but Not Disrupted, Gut Microbiome, *Journal of agricultural and food chemistry*, 2015, **63**, 6172-6180.

23. M. T. Escribano-Bailon, C. Santos-Buelga and J. C. Rivas-Gonzalo, Anthocyanins in cereals, *Journal of chromatography. A*, 2004, **1054**, 129-141.

24. R. L. Prior, E. W. S, R. R. T, R. C. Khanal, X. Wu and L. R. Howard, Purified blueberry anthocyanins and blueberry juice alter development of obesity in mice fed an obesogenic high-fat diet, *Journal of agricultural and food chemistry*, 2010, **58**, 3970-3976.

25. L. Folch, M. Lees and G. H. Sloane Stanley, A simple method for the isolation and purification of the total lipids from animal tissues, *The Journal of biological chemistry*, 1957, **226**, 497-509.

26. M. J. Periago, G. Martin-Pozuelo, R. Gonzalez-Barrio, M. Santaella, V. Gomez, N. Vazquez, I. Navarro-Gonzalez and J. Garcia-Alonso, Effect of tomato juice consumption on the plasmatic lipid profile, hepatic HMGCR activity, and fecal short chain fatty acid content of rats, *Food Funct*, 2016, **7**, 4460-4467.

27. T. Wu, X. Qi, Y. Liu, J. Guo, R. Zhu, W. Chen, X. Zheng and T. Yu, Dietary supplementation with purified mulberry (Morus australis Poir) anthocyanins suppresses body weight gain in high-fat diet fed C57BL/6 mice, *Food Chem*, 2013, **141**, 482-487.

28. E. Pojer, F. Mattivi, D. Johnson and C. S. Stockley, The Case for Anthocyanin Consumption to Promote Human Health: A Review, *Compr Rev Food Sci F*, 2013, **12**, 483-508.

29. C. Del Bo, D. Martini, M. Porrini, D. Klimis-Zacas and P. Riso, Berries and oxidative stress markers: an overview of human intervention studies, *Food Funct*, 2015, **6**, 2890-2917.

30. R. J. Thoppil, D. Bhatia, K. F. Barnes, E. Haznagy-Radnai, J. Hohmann, A. S. Darvesh and A. Bishayee, Black Currant Anthocyanins Abrogate Oxidative Stress through Nrf2-Mediated Antioxidant Mechanisms in a Rat Model of Hepatocellular Carcinoma, *Current cancer drug targets*, 2012, **12**, 1244-1257.

31. J. Tan, C. McKenzie, P. J. Vuillermin, G. Goverse, C. G. Vinuesa, R. E. Mebius, L. Macia and C. R. Mackay, Dietary Fiber and Bacterial SCFA Enhance Oral Tolerance and Protect against Food Allergy through Diverse Cellular Pathways, *Cell Rep*, 2016, **15**, 2809-2824.

View Article Online
DOI: 10.1039/C7FO00449D

32. D. R. Donohoe, N. Garge, X. Zhang, W. Sun, T. M. O'Connell, M. K. Bunger and S. J. Bultman, The microbiome and butyrate regulate energy metabolism and autophagy in the mammalian colon, *Cell Metab*, 2011, **13**, 517-526.
33. J. Tan, C. McKenzie, M. Potamitis, A. N. Thorburn, C. R. Mackay and L. Macia, The role of short-chain fatty acids in health and disease, *Adv Immunol*, 2014, **121**, 91-119.
34. C. A. Morais, V. V. de Rosso, D. Estadella and L. P. Pisani, Anthocyanins as inflammatory modulators and the role of the gut microbiota, *The Journal of nutritional biochemistry*, 2016, **33**, 1-7.
35. A. Cassidy, G. Rogers, J. J. Peterson, J. T. Dwyer, H. Lin and P. F. Jacques, Higher dietary anthocyanin and flavonol intakes are associated with anti-inflammatory effects in a population of US adults, *The American journal of clinical nutrition*, 2015, **102**, 172-181.
36. S. G. Lee, B. Kim, Y. Yang, T. X. Pham, Y. K. Park, J. Manatou, S. I. Koo, O. K. Chun and J. Y. Lee, Berry anthocyanins suppress the expression and secretion of proinflammatory mediators in macrophages by inhibiting nuclear translocation of NF-kappaB independent of NRF2-mediated mechanism, *The Journal of nutritional biochemistry*, 2014, **25**, 404-411.
37. B. Yousuf, K. Gul, A. A. Wani and P. Singh, Health Benefits of Anthocyanins and Their Encapsulation for Potential Use in Food Systems: A Review, *Crit Rev Food Sci Nutr*, 2016, **56**, 2223-2230.
38. M. White, Food access and obesity, *Obes Rev*, 2007, **8 Suppl 1**, 99-107.
39. D. W. L. Ma, Food, Diet and Obesity, *Trends in Food Science & Technology*, 2009, **20**, 45.
40. J. M. Alvarez-Suarez, F. Giampieri, S. Tulipani, T. Casoli, G. Di Stefano, A. M. Gonzalez-Paramas, C. Santos-Buelga, F. Busco, J. L. Quiles, M. D. Cordero, S. Bompadre, B. Mezzetti and M. Battino, One-month strawberry-rich anthocyanin supplementation ameliorates cardiovascular risk, oxidative stress markers and platelet activation in humans, *The Journal of nutritional biochemistry*, 2014, **25**, 289-294.
41. J. J. Chang, M. J. Hsu, H. P. Huang, D. J. Chung, Y. C. Chang and C. J. Wang, Mulberry anthocyanins inhibit oleic acid induced lipid accumulation by reduction of lipogenesis and promotion of hepatic lipid clearance, *Journal of agricultural and food chemistry*, 2013, **61**, 6069-6076.
42. T. Wu, Z. Yu, Q. Tang, H. Song, Z. Gao, W. Chen and X. Zheng, Honeysuckle anthocyanin supplementation prevents diet-induced obesity in C57BL/6 mice, *Food Funct*, 2013, **4**, 1654-1661.
43. I. Savini, V. Gasperi and M. V. Catani, in *Obesity*, Springer, 2016, pp. 65-86.
44. I. Bondia-Pons, L. Ryan and J. A. Martinez, Oxidative stress and inflammation interactions in human obesity, *J Physiol Biochem*, 2012, **68**, 701-711.
45. S. Khurana, M. Piche, A. Hollingsworth, K. Venkataraman and T. C. Tai, Oxidative stress and cardiovascular health: therapeutic potential of polyphenols, *Canadian journal of physiology and pharmacology*, 2013, **91**, 198-212.
46. F. B. Carvalho, J. M. Gutierres, C. Bohnert, A. M. Zago, F. H. Abdalla, J. M. Vieira, H. E. Palma, S. M. Oliveira, R. M. Spanevello, M. M. Duarte, S. T. Lopes, G. Aiello, M. G. Amaral, N. L. Pippi and C. M. Andrade, Anthocyanins suppress the secretion of proinflammatory mediators and oxidative stress, and restore ion pump activities in demyelination, *The Journal of nutritional biochemistry*, 2015, **26**, 378-390.
47. S. V. Joseph, I. Edirisinghe and B. M. Burton-Freeman, Fruit Polyphenols: A Review of

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Anti-inflammatory Effects in Humans, *Crit Rev Food Sci Nutr*, 2016, **56**, 419-444.

48. E. J. Park, J. H. Lee, G. Y. Yu, G. He, S. R. Ali, R. G. Holzer, C. H. Osterreicher, H. Takahashi and M. Karin, Dietary and genetic obesity promote liver inflammation and tumorigenesis by enhancing IL-6 and TNF expression, *Cell*, 2010, **140**, 197-208.

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Figure Captions

Fig. 1 Effects of BRA, BSA and PCA on body weight gains for the male C57BL/J6 mice. NC, low-fat diet control; HFD, high-fat diet control; OL, high-fat diet plus Orlistat at 100 mg/kg; BRA, high-fat diet plus black rice anthocyanin at 200 mg/kg; BSA, high-fat diet plus black soybean anthocyanin at 200 mg/kg; PCA, high-fat diet plus purple corn anthocyanin at 200 mg/kg.

Fig. 2 Effects of BRA, BSA and PCA on Serum parameters in the male C57BL/6 mice. A, TG; B, TC; C, HDL-C; D, LDL-C; E, MDA; F, SOD. NC, low-fat diet control; HFD, high-fat diet control; OL, high-fat diet plus Orlistat at 100 mg/kg; BRA, high-fat diet plus black rice anthocyanin at 200 mg/kg; BSA, high-fat diet plus black soybean anthocyanin at 200 mg/kg; PCA, high-fat diet plus purple corn anthocyanin at 200 mg/kg. The means marked with superscript letters are significantly different relative to others.

Fig. 3 Effects of BRA, BSA and PCA on hepatic lipids and antioxidants. A, TG; B, TC; C, MDA; D, SOD; E, GP_X. NC, low-fat diet control; HFD, high-fat diet control; OL, high-fat diet plus Orlistat at 100 mg/kg; BRA, high-fat diet plus black rice anthocyanin at 200 mg/kg; BSA, high-fat diet plus black soybean anthocyanin at 200 mg/kg; PCA, high-fat diet plus purple corn anthocyanin at 200 mg/kg. The means marked with superscript letters are significantly different relative to others.

Fig. 4 Effects of BRA, BSA and PCA on Fecal fatty acid composition and content. NC, low-fat diet control; HFD, high-fat diet control; OL, high-fat diet plus Orlistat at 100 mg/kg; BRA, high-fat diet plus black rice anthocyanin at 200 mg/kg; BSA, high-fat diet plus black soybean anthocyanin at 200 mg/kg; PCA, high-fat diet plus purple corn anthocyanin at 200 mg/kg. The means marked with superscript letters are significantly different relative to others.

Fig. 5 Effects of BRA, BSA and PCA on the expression of inflammatory cytokine. A, TNFα; B, IL-6; C, NF-KB; D, iNOS genes. NC, low-fat diet control; HFD, high-fat diet control; OL, high-fat diet plus Orlistat at 100 mg/kg; BRA, high-fat diet plus black rice anthocyanin at 200 mg/kg; BSA, high-fat diet plus black soybean anthocyanin at 200 mg/kg; PCA, high-fat diet plus purple corn anthocyanin at 200 mg/kg. The means marked with superscript letters are significantly different relative to others.

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411 **Figure 1**

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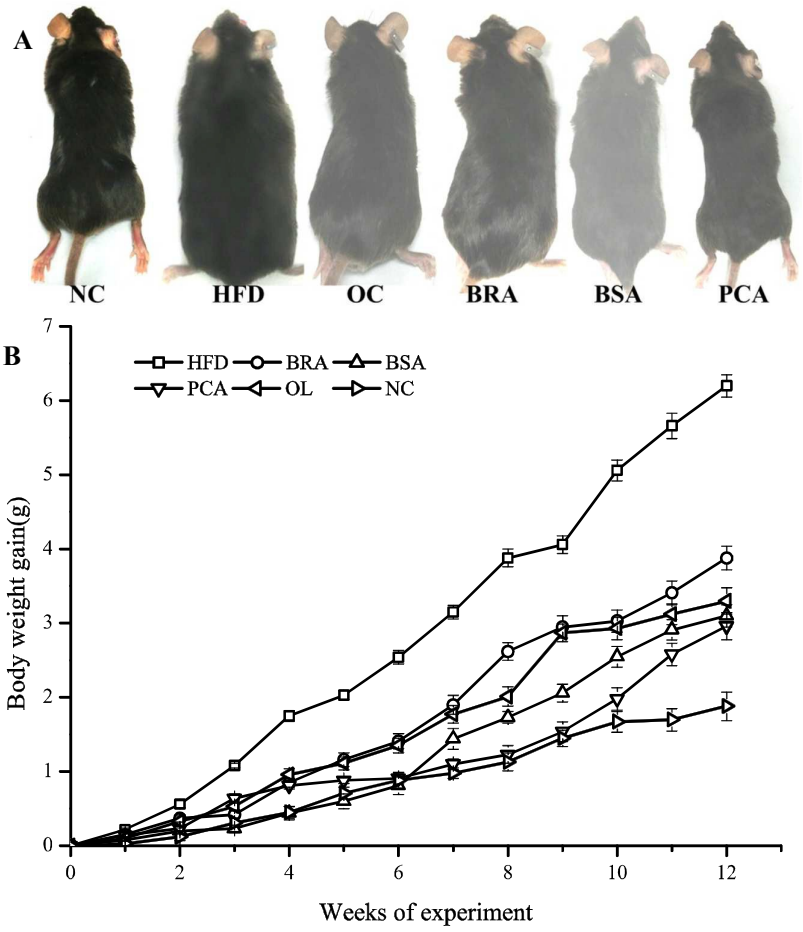

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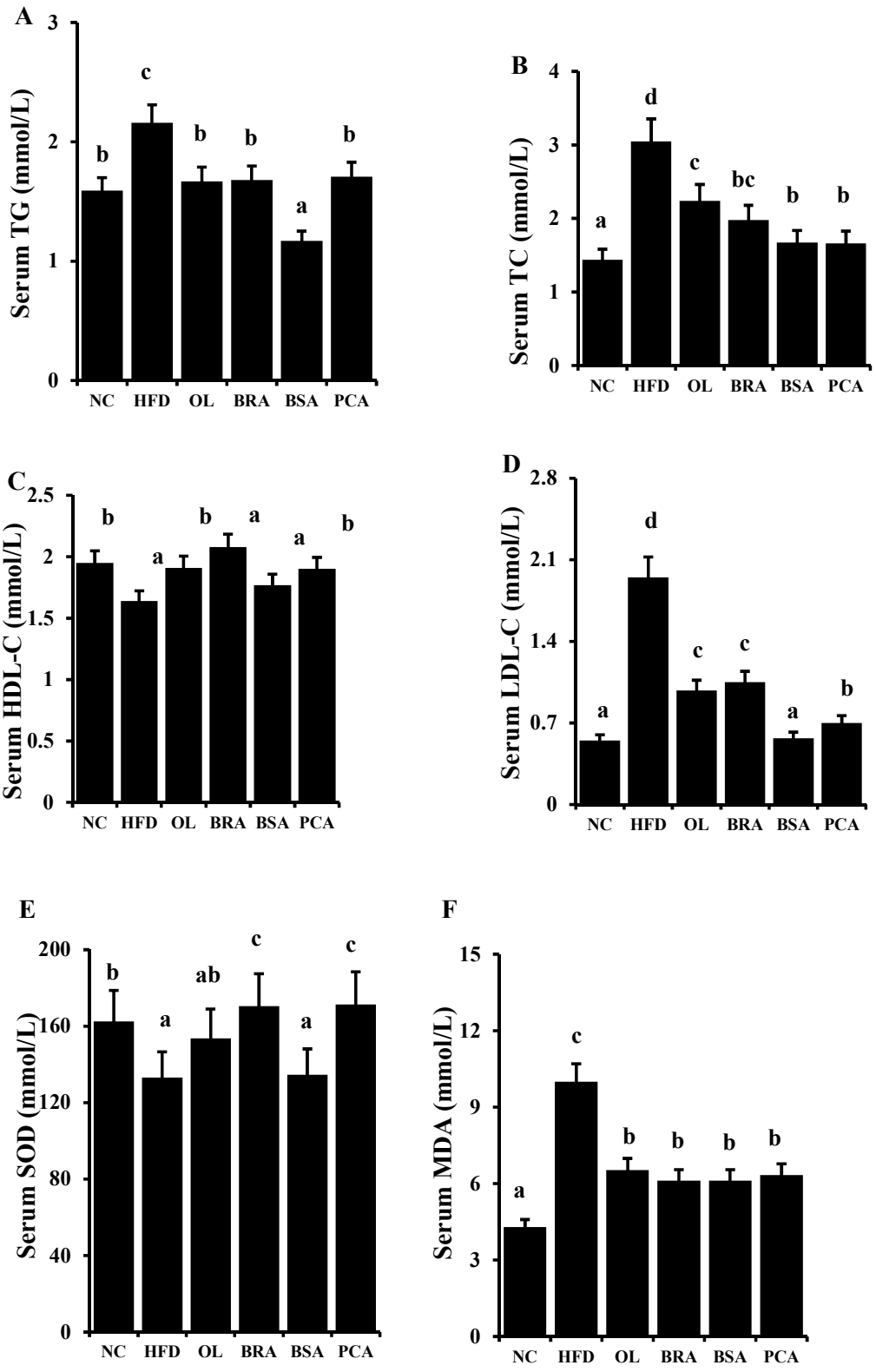
Figure 2



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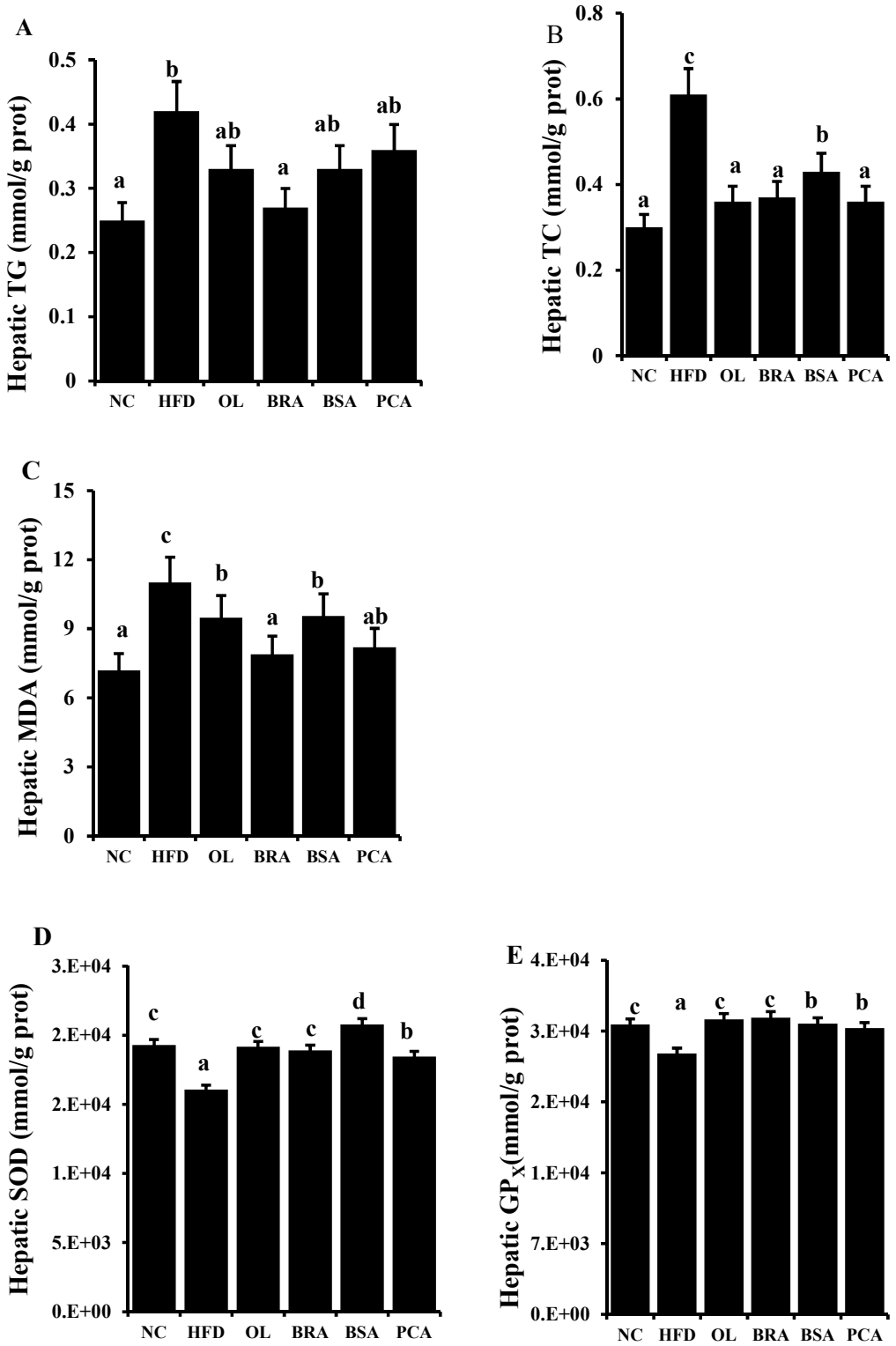
423 **Figure 3**



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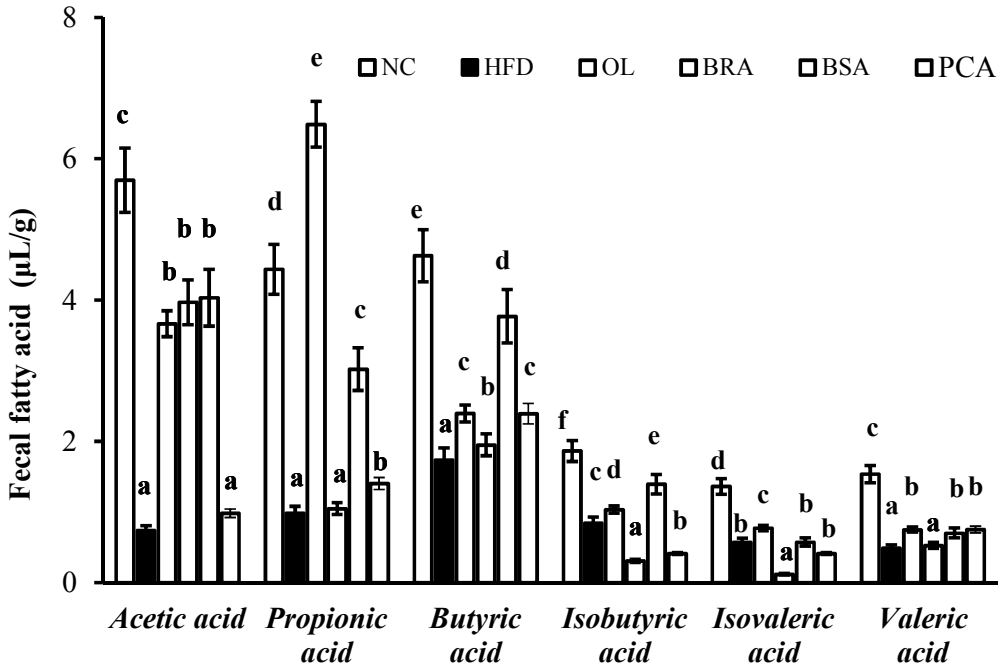

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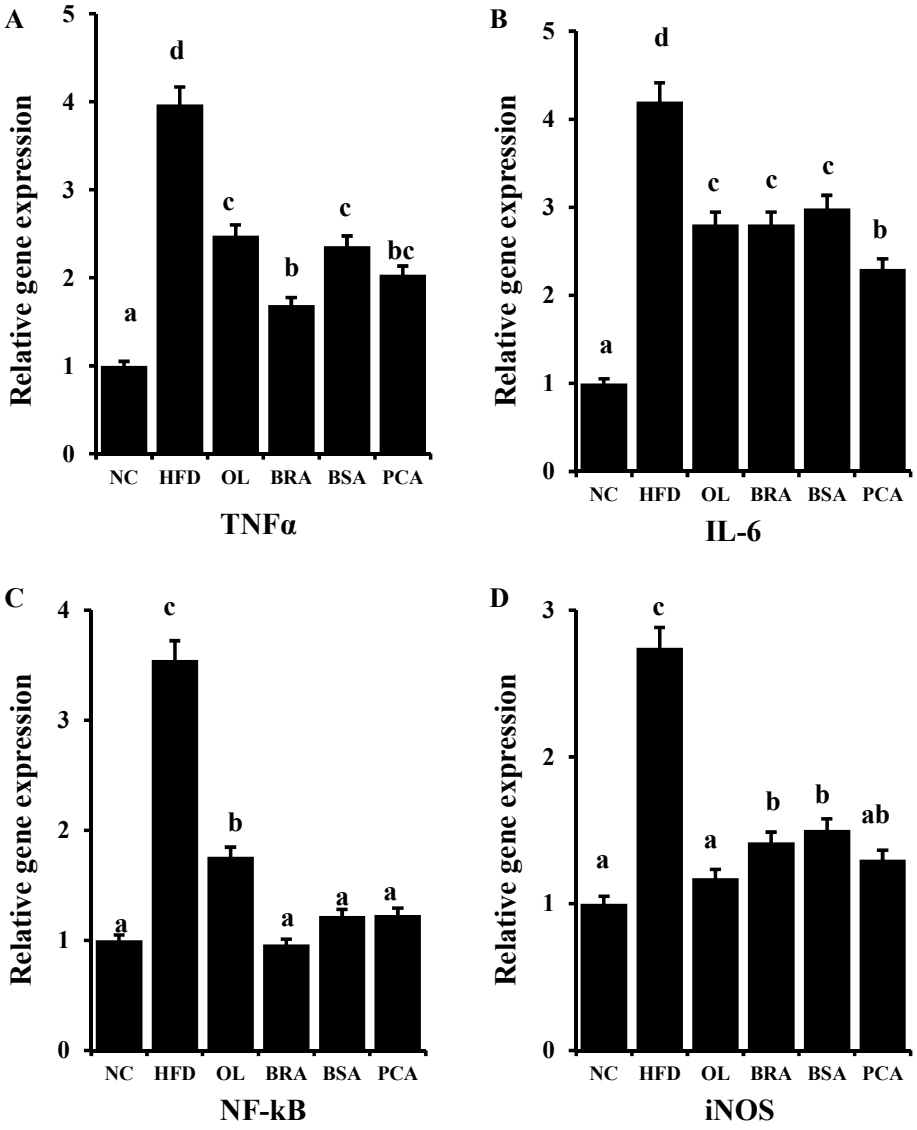
430 **Figure 5**



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