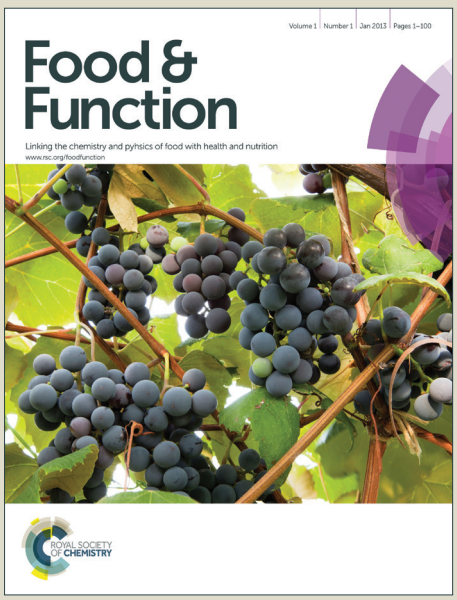
Food & Function

Accepted Manuscript



This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/foodfunction

Ameliorative effect of black rice anthocyanin on senescent mice induced by D-galactose

Xiaoling Lu*, Yanhua Zhou，Tao Wu，Lei Hao

Key Laboratory of Nutrition and Safety, Ministry of Education, Department of Food Sciences & Bioengineering, Tianjin University of Science & Technology, Tianjin 300457, PR China

*Corresponding to: Xiaoling Lu, Professor, Tianjin University of Science & Technology. Tel. /fax: +86 22 60601445. *E-mail address*: lu_xiaoling@sina.com.

Abstract

This study investigated the ameliorative effect of black rice anthocyanin (BACN) in senescent mice induced by D-galactose. The male mice were randomly divided into five groups, namely the normal group, the model group and dosage groups (15, 30 and 60 mg/kg). The model group and three dosage groups were injected subcutaneously with D-galactose continuously. The results suggested that superoxide dismutase (SOD) and catalase (CAT) were significantly increased upon black rice anthocyanin treatment, while MDA and the activity of monoamine oxidase (MAO) significantly decreased. The expressions of superoxide dismutase genes (*SOD1* and *SOD2*) in liver were up-regulated in black rice anthocyanin group, while the expression of *MAO-B* gene was down-regulated. These findings demonstrated that the ameliorative effect of BACN might be achieved partly by altering endogenous antioxidant enzymatic and aging-related enzymatic activities and regulating *SOD1, SOD2* and *MAO-B* gene expressions.

Keywords: Black rice; Anti-aging; Anthocyanin; D-galactose

1 Introduction

Aging is a complex biological process. Among the aging theories, the free radical theory has received widespread attention recently. It plays an essential role in aging-related research[1,2]. Accumulation of oxidative damages caused by reactive oxygen species (ROS) is one of the major factors responsible for aging [3]. Oxidative stress leads to an imbalance between ROS and the antioxidant [4, 5]. Free radicals are highly reactive molecules with unpaired electrons which can cause damage to cell membranes, lipids, proteins, and DNA[6, 7]. To scavenge the excess ROS, antioxidant defense system (endogenous antioxidant system and exogenous antioxidants intake) is especially necessary. Endogenous antioxidant system, including superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px), removes ROS in cell [6, 8]. Exogenous antioxidants intake including ascorbic acid, tocotherol, and plant flavonoids, are also responsible for scavenging ROS [6, 9, 10].

Monoamine oxidase (MAO), an aging-related enzyme, is widely distributed in many animal organs. It is the main metabolism enzyme of monoamine neurotransmitter. Abnormal MAO could cause tissue organ dysfunction and various diseases, and then speed up the aging process. According to the differences of substrate, distribution location and selective inhibitors, MAO has been separated into MAO-A and MAO-B. The activity of MAO-B increases with aging in the human brain and the rats [11, 12], while MAO-A has no correlation with aging. Consequently, it is necessary to study the change of MAO-B in the aging process.

Black rice (*Oryza sativa* L.) is a special cultivar of rice that contains abundant

colour pigments and it's rich natural anthocyanin compounds in different layers of the pericarp, seed capsule and aleurone of rice [13, 14]. It has been widely consumed as a health-promoting food[15, 16]. Some reports showed anthocyanin has functional effects, such as antioxidant, antiallergic, antiscratching effects[16, 17]. Others have shown that it could reduce serum lipid profiles [18, 19] and atherosclerotic plaque formation[15, 20, 21], and inhibit cancer [14, 22]. Furthermore, Shan et al. [23] revealed purple sweet tomato anthocyanins attenuated D-gal-induced cognitive impairment partly via enhancing the antioxidant and anti-inflammatory capacity. Balu et al.[24] demonstrated that grape seed extract enhanced the antioxidant status and decreased the incidence of free radical induced protein oxidation in aged rats thereby protecting the central nervous system from the reactive oxygen species . Peng et al. [25] claimed that blueberry extract prolong lifespan of drosophila melanogaster. However, no report to date has studied the anti-aging effect of black rice anthocyanin in mice. In the present study, we used D-galactose treated mice as experimental aging models and evaluated the ameliorative effect of BACN on senescent mice.

2 Materials and methods

2.1 Chemicals

Black rice anthocyanin (20 mg/g) was supplied by Chenguang Biotech Group Co., Ltd. SOD, MDA, CAT and MAO test kits were purchased from Nanjing Jiancheng Bioengineering Institute. Cyanidin-3-O-glucoside was purchased from

Yuanye Biotechnology. All other chemicals were of analytical purity.

2.2 Quantification of black rice anthocyanin

To obtain a more comprehensive knowledge of the composition of BACN, we determined the content of anthocyanin components by HPLC-MS [25-28]. BACN was diluted with 1% formic acid solution and filtered using a 0.22-μm filter before the HPLC analysis. The HPLC system (Thermo, USA) was equipped with a Hypersil Gold column (100×2.1mm, 3 μm i.d.) and was used for analyses, operating at a constant temperature of 30℃ and a flow rate of 0.2 mL/min. An eluting mixture of acetonitrile and 1% formic acid (10:90; v/v) was used. The HPLC system was connected in series with an ion-trap mass spectrometer, which was fitted with an electrospray ionization source operating in the positive mode. Nitrogen was used as the carrier gas at a temperature of 275℃, a flow rate of 30 arb, a nebulizer pressure of 45 psi, a quadrupole temperature of 30℃ and a capillary voltage of 4000V. Data were collected using the full scan mode over a mass range of m/z 300-700 at 1.0 s per cycle. Anthocyanin components of BACN were quantified as cyanidin-3-O-glucoside equivalents using external calibration curves of an authentic standard.

2.3 Animals experiments

Male mice (n=50; KUNMING background, at 8 weeks of age) were purchased from the Peking University Health Science Center Department of Laboratory Animal Science. Preceding the study, all animals consumed the same diet for 1 week. They were then randomized into five groups based on their body weight. Five animals were

housed in each cage and maintained at 25℃ in an atmosphere-controlled room with a 12 h light-dark cycle. During the experiment, mice had free access to food and tap water, and their body weights were recorded weekly. The study protocol was approved by the Institutional Animal Care and Use Committee of Tianjin University of Science & Technology (TUST20130905). All handling and management procedures were in accordance with the guidelines of experimental animal administration.

D-galactose and BACN solution were made freshly at the beginning of each experiment. D-galactose was dissolved in 0.9% normal saline for subcutaneous administration. BACN was diluted with distilled water and administered orally in a constant dose of 0.1 mL/10 g body weight. Normal group administered distilled water orally and received normal saline administered subcutaneously. Model group mice administered distilled water orally and received 120 mg/kg of D-galactose administered subcutaneously. BACN (15，30 and 60 mg/kg,) were administered to low, medium and high dose group mice. The doses of BACN were selected as 5 times, 10 times and 20 times of anthocyanin ADI. The dose of D-galactose was selected based on those reported in literature. The study was carried out for a period of 6 weeks.

After the last administration, the animals were fasted overnight and sacrificed after anesthetization. Their brains, livers and kidneys were quickly removed, rinsed in ice cold normal saline (0.9% w/v NaCl), blotted dry, weighed separately, and immediately frozen in liquid nitrogen and kept at -80℃until used. Left first half of brains used to make histopathology put into10% neutral formalin solution. 10% (w/v)

tissue homogenate was prepared in normal saline using glass homogenizer. The homogenate was centrifuged at 3000g for 10 min at 4 ℃, and the supernatant was used for biochemical assays.

2.4 Biochemical Assays

The activities of endogenous antioxidant enzymes (SOD, CAT), aging-associated enzymes MAO and the content of lipid peroxide (MDA) in brain, liver and kidney were determined using commercially available kits according to the manufacturer's instructions.

2.5 Histological analysis

Brain tissues were harvested from the sacrificed mice, and the fragments from tissues were fixed in 10% neutral formalin solution, embedded in paraffin, and then stained with haematoxylin and eosin (HE), sealed piece with neutral balsam.

2.6 Gene expression analysis

Total RNA was extracted from liver tissue with Trizol reagent by following manufacturer's instructions. The total RNA was digested with RNase-free DNase for 2 min at 42 ℃. 1μg of total RNA was used for cDNA synthesis using real-time RT-PCR SYBR GREEN Ⅱ.Concentrations of reagents used were determined using the manufacturer's instructions. β-actin gene was used as an internal control. Real-Time PCR primers for SOD1, SOD2, MAO-B and β-actin were as follows: for SOD1, sense, 5'-GGTTCCACGTCCATCAGTAT-3'and anti-sense,

5’-AGTCACATTGCCCAGGTCTC-3’, which afforded a 134-bp fragment; for SOD2, sense, 5’-TGGGAGTCCAAGGTTCAGG-3’ and anti-sense, 5’-GATTAGAGCAGGCAGCAATC-3’, which produced a 81-bp fragment; for MAO-B, sense, 5’-ACAACCTACTTCCCTCCCG-3’ and anti-sense, 5’-GCTGTTTCAGTGCCTGCAA-3’, which afforded a 92-bp fragment; for β-actin, sense, 5’-GAGGGAAATCGTGCGTGAC-3’ and anti-sense, 5’-CGCTCGTTGCCAATAGTGA-3’, which gave a 147-bp fragment. The reaction mixture was subjected to PCR to amplify sequences to the desired primers. Amplification was performed in a MyiQ2 cycler (Bio-Rad, USA) with cycles of denaturation at 95 ℃, annealing at 56 ℃, and extension at 68 ℃ for 20 s, respectively. Gene expression was calculated on the basis of the comparative threshold cycle (CT) value. The changes in gene expression ratio were calculated using iQ5 data analysis software.

2.7 Statistical analysis

The data were presented as means±sd. The values were evaluated by one-way analysis of variance (ANOVA) followed by Dunan’s multiple range tests. All analyses were performed using the SPASS system. $P<0.05$ was considered to be significant.

3 Results

3.1 Quantitative analyses of anthocyanin components of BACN

HPLC-MS analysis was performed to estimate the content of anthocyanin components in BACN. Three peaks were detected (Fig.1.). The properties of peaks

1-3 were shown in Table 1. On the basis of peak areas, BACN contained cyanidin-3, 5-O-diglucoside (3.43%), cyanidin-3-O-glucoside (84.48%), peonidin-3-O-glucoside (5.53%).

3.2 Daily behavior, body weight and the organs index

Significant difference in daily behavior was seen among the five groups. Model mice showed obvious aging appearance. They were dull, depressed, aching, and easy to grab. BACN -treated mice were noticeably better than model mice. The normal mice showed the best state.

The body weight gain was similar among the five groups. BACN did not affect the weight gain compared with the normal mice. After 1 week of intragastric administration, the body weight gain was less or even a slight decline. Mice gained weight after a period of time to adjust. At the beginning and end of the experiment, there was no significant difference in body weight among the five groups (Table 2).

Compared with normal mice, the thymus index and the spleen index of model mice significantly reduced ($P<0.05$, Table 2). Intragastric administration of 30 or 60 mg/kg BACN restored the spleen index and thymus index in a dose-dependent manner, suggesting that BACN favorably modulate the immune system and delay organs aging in mice.

3.3 Effects of BACN on activities of SOD in senescent mice induced by D-galactose

In model group, D-galactose significantly decreased SOD activity in the three

organs (brain, liver and kidney) (Table 3). Intragastric administration of 30 or 60mg/kg BACN increased the activity of SOD in the three tissues ($P<0.05$).

3.4 Effects of BACN on activities of CAT in senescent mice induced by D-galactose

D-galactose significantly suppressed CAT activity in the brain, liver and kidney (Table 4). Compared with model group, intragastric administration of 15, 30 or 60m g/kg BACN enhanced the activity of CAT in brain and liver tissues ($P<0.05$), while 30 or 60m g/kg BACN in kidney.

3.5 Effects of BACN on contents of MDA in senescent mice induced by D-galactose

MDA, one of the products of lipid peroxidation, could reflect the degree of lipid peroxidation *in vivo*. The MDA content of model group mice significantly increased in the three tissues (Table 5). Intragastric administration of 30 or 60 mg/kg BACN reduced the MDA content in different degree. Intragastric administration of 30 mg/kg BACN could significantly reduce MDA content in the brain, liver and kidney ($P<0.05$).

3.6 Effects of BACN on activities of MAO in senescent mice induced by D-galactose

D-galactose significantly increased MAO activity in the brain, liver and kidney ($P<0.01$) (Table 6). Intragastric administration of 30 or 60 mg/kg BACN suppressed

the activity of MAO in the three tissues ($P<0.05$), compared with the model group.

3.7 Effect of BACN on the brain pathology in senescent mice

Based on the result of HE staining under the light microscope (Fig.2.), compared with normal mice, neuronal cells in model mice were scattered, misaligned, swelling, and the amount was significantly reduced. These reflected the apparent characteristics of aging. In contrast, Intragastric administration of 30 or 60 mg/kg BACN showed an obvious increase in neuronal cell density, which relieved aging-related degenerative changes.

3.8 Effect of BACN on mRNA of SOD1, SOD2, MAO-B in senescent mice induced by D-galactose

As to the gene expression, both *SOD1* and *SOD2* were significantly down-regulated in model group compared with the normal group ($P<0.01$, Fig. 3). It was also observed that the gene expression of *SOD1* and *SOD2* was decreasing markedly after intragastric administration of 60 mg/kg BACN compared with the model group. In contrast, *MAO-B* gene was significantly down-regulated in 30 or 60m g/kg BACN groups compared with the model group ($P<0.05$ or $P<0.01$, Fig. 3).

4 Discussion

The present study demonstrates that BACN possess the anti-aging effect on senescent mice induced by D-galactose. Results showed that BACN could improve the physical signs of senescent mice and delay the senility of the immune organs. Besides that, results also suggested that BACN could increase the activities of

endogenous antioxidant enzymes, decreased aging-related enzyme activity, and relieve aging-related degenerative changes in the brain. Although there is no direct evidence to show that BACN intake increases human lifespans, it has been reported that daily consumption of BACN in sufficient amounts could prolong life by averting death caused by chronic disease, such as hyperlipidemia, cancer etc[29,30].

The underlying mechanisms by which BACN delays senility remain poorly understood. One possible mechanism is probably related to the free radical scavenging activity of black rice anthocyanin. It is generally believed that the free radical species can cause deterioration of an organism while the antioxidant can delay the process of aging[6, 31]. HPLC-MS analysis results showed that BACN used in the present study contained three anthocyanin (cyanidin-3, 5-O-diglucoside, cyanidin-3-O-glucoside, peonidin-3-O-glucoside) (Fig. 1 and Table 1). The content of cyanidin-3-O-glucoside is the largest of the three components. It is known that these anthocyanins are effective antioxidants[32-34]. In fact, the present study demonstrated that black rice anthocyanintreatment increased SOD, CAT activities and reduced MDA content (Table 3, 4, 5). SOD, as an important enzyme of defending against oxidative damage, could catalyze superoxide anion radical to molecular oxygen and H_2O_2, after that H_2O_2 is metabolized to harmless water and oxygen by CAT and GPH-Px. SOD, CAT, and GPH-Px, could protect cells from oxidative damage caused by ROS, suggesting that oxidative stress is at least one of the factors leading to age. In addition, MAO-B, as an aging-related enzyme, played an important role in delaying senescence. In the study, MAO-B activity decreased with BACN intake. It could

reduce monoamines neurotransmitter decomposition, ensure the content of monoamines, especially in the brain, and weaken the nervous system degenerative diseases, which slow down the process of neural aging [35,36].

It was noteworthy that gene expression of *SOD1* and *SOD2* was up-regulated upon BACN treatment. In contrast, gene expression of *MAO-B* was down-regulation compared with the model group, suggesting that anti-aging effect of BACN was associated at least in part by up-regulation of endogenous antioxidant enzymes of SOD1, SOD2 and down-regulation aging-related enzyme of MAO.

In conclusion, BACN could be involved in the anti-aging effect, and anti-aging mechanisms might be as follows: BACN could enhance antioxidant activity in brain, liver and kidney, improve aging-related enzyme activity and alter gene expression. Although the report supports the potential anti-aging effect of BACN, further investigations should be conducted to substantiate its anti-aging effect.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

This study was supported by grants from the National Science and technology support plan of China (2011BAD23B02). We wish to thank the crew at the laboratory of Nutrition and Food Additive.

References

1. Zhong WQ, Liu N, Xie YG, et al., *International Journal of Biological Macromolecules,*2013, **60**, 355-359.

2. Zhang XL, Shi GF, Liu XZ, et al., *Cell Biochemistry Function*, 2011, **29**(4), 342-7.

3. Harman D., *Journal of Gerontology,* 1956, **11**(3), 298-300.

4. Da Costa LA, Badawi A and El-Sohemy A., *Annals of Nutrition & Metabolism, 2012, 60 Suppl 3,* 27-36.

5. Halliwell B., *Journal of Neurochemistry,* 1992, **59**(5), 1609-23.

6. Ames BN, Shigenaga MK and Hagen TM., Proceedings of the National Academy of Sciences of the united states of America, 1993, **90**(17), 7915-22.

7.Khurana S, Piche M, Hollingsworth A, et al., *Canadian Journal of Physiology and Pharmacology 2013*, **91**(3), 198-212.

8. Paredes-Lopez O, Cervantes-Ceja ML, Vigna-Perez M, et al., *Plant Foods for Human Nutrition*, 2010, **65**(3), 299-308.

9. Fernandez-Sanchez A, Madrigal-Santillan E, Bautista M, et al., *International Journal of Molecular Sciences,* 2011, **12**(5), 3117-32.

10. Jaacks LM, Gordon-Larsen P, Mayer-Davis EJ, et al., *International Journal of Epidemiology,* 2013, **42**(3), 828-37.

11. Benedetti MS and Keane PE, *Journal of Neurochemistry*, 1980, **35**(5), 1026-32.

12. Willcox DC, Scapagnini G and Willcox BJ. *Mechanisms of Ageing and Development 2014. 10.1016/j.mad.2014.01.002*

13. Hu C, Zawistowski J, Ling W, et al., *Journal of Agricultural and Food Chemistry* 2003, **51**(18), 5271-7.

14. Yoon J, Ham H, Sung J, et al., *Nutrition Research and Practice,* 2014, **8**(2), 125-31.

15. Xia M, Ling WH, Ma J, et al., *The Journal of Nutrition*,2003, **133**(3), 744-51.

16. Chiang AN, Wu HL, Yeh HI, et al., *Lipids* 2006, **41**(8), 797-803.

17. Han SJ, Ryu SN, Trinh HT, et al., *Journal of Food Science*, 2009, **74**(8), H253-8.

18. Ling WH, Wang LL and Ma J., *The Journal of Nutrition*, 2002, **132**(1), 20-6.

19. Kim HW, Lee AY, Yeo SK, et al., *Food Research International*, 2013, **53**(1), 373-90.

20. Wu T, Qi X, Liu Y, et al. , *Food Chemistry*, 2013, **141**(1), 482-7.

21. Ling WH, Cheng QX, Ma J, et al., *The Journal of Nutrition,* 2001, **131**(5), 1421-6.

22. Chen PN, Kuo WH, Chiang CL, et al. *Chemico-Biological Interactions,* 2006, **163**(3), 218-29.

23. Shan Q, Lu J, ZhengYL, et al., *Journal of Biomedicine and Biotechnology,* 2009; 2009:564737.

24. Muthaiya B, Purushotham S, Ganesan M, et al., *International Journal of Developmental Neuroscience*, 2005,23(6),501-7.

25. Peng C, Zuo YY, Kwan KM, et al. *Experimental Gerontology* , 2012, 47(2), 170-8.

26. Lee MJ, Park JS, Choi DS, et al., *Journal of Agricultural and Food Chemistry,* 2013, **61**(12), 3148-58.

27. Navas MJ, Jimenez-Moreno AM, Bueno JM, et al., *Critical Reviews in Analytical Chemistry,* 2012, **42**(4), 284-312.

28. Huang Z, Wang B, Williams P, et al., *LWT - Food Science and Technology,* 2009, **42**(4), 819-24.

29. QIN Yu and LW-H, *Food Science,* 2008, **29**(10), 540-42.

30. Martin C, Zhang Y, Tonelli C, et al., *Annual Review of Plant Biology*, 2013, **64**, 19-46.

31. Venskutonis PR, Kraujalis P.,*Comprehensive Reviews in Food Science and Food Safety*, 2013, **12**(4), 381-412.

32. Wu T, Yu Z, Tang Q, et al. *Food & Function,* 2013, **4**(11), 1654-61.

33. Einbond LS, Reynertson KA, Duo XD, et al., *Food Chemistry,* 2004, **84**(1), 23-28.

34. Pojer E, Mattivi F, Johnson D, et al., *Comprehensive Reviews in Food Science and Food Safety,* 2013, **12**(5), 483-508.

35. Sohal RS and Weindruch R. *Science* 1996, **273**(5271), 59-63.

36. Kong F, Chen S, Cheng Y, et al. *PLoS One* 2013, **8**(4), e61385.

Figure Captions

Fig.1.The HPLC profile of black rice anthocyanin （BACN） at 520nm. The properties of peaks 1-3 and the compounds at each peak are described in Table 1. The chemical structure of black rice anthocyanin.

Fig.2. Brain pathology in senescent mice induced by D-galactose; HE staining 200×. A: Normal group shows normal neuronal cells with normal cellularity and normal distribution. B: Model group demonstrates marked cell swelling and disordered distribution. C, D and E: intragastric administration of 15，30 or 60 mg/kg black rice anthocyanin（BACN）showed an obvious increase in neuronal cell density.

Fig.3. Effect of black rice anthocyanin （BACN） on mRNA of SOD1, SOD2, MAO-B in senescent mice induced by D-galactose, a-d Mean values within the column with unlike superscript letters were significantly different ($P<0.05$).

302 **Table 1.** Identification and distribution of anthocyanin components in black rice
303 anthocyanin (BACN)

Peak	Anthocyanin components	RT (min)	m/z[M+H]+
1	Cyanidin-3,5-O-diglucoside	3.972	611.06
2	Cyanidin-3-O-glucoside	5.599	449.60
3	Peonidin-3-O-glucoside	8.705	464.05

304

305 **Table 2.** Effects black rice anthocyanin (BACN) on body weight and organs index of
306 senescent mice induced by D-galactose
307 (Mean values and standard deviations, n 10 per group)

Group	Initial weight(g)	Final weight(g)	Thymus index(mg/g)	Spleen index(mg/g)
Normal	25.79±3.31	34.56±3.95	1.34±0.19 [a]	3.70 ±0.46[a]
Model	25.88±4.05	30.66±3.91	1.08 ±0.22[b]	3.16±0.56 [b]
15 mg/kg BACN	26.70±3.37	33.82±5.39	1.27 ±0.16[b]	3.45±0.63 [b]
30 mg/kg BACN	26.26±3.45	33.35±5.51	1.29 ±0.20[a]	3.40±0.18 [ab]
60 mg/kg BACN	26.28±3.20	33.83±4.50	1.30 ±0.22[a]	3.65±0.46 [a]

308 [a,b]Mean values within the column with unlike superscript letters were significantly different
309 ($P<0.05$).
310

311 **Table 3.** Effects of black rice anthocyanin (BACN) on activities of SOD in senescent
312 mice induced by D-galactose
313 (Mean values and standard deviations, n 10 per group)

Groups	Brain(U/mg protein)	Liver(U/mg protein)	Kidney(U/mg protein)
Normal	179.12±32.34 [a]	139.92±12.64[a]	26.15±5.88 [a]
Model	138.91±24.79 [b]	93.48±15.43 [b]	19.99 ±4.38[b]
15 mg/kg	152.35±22.35 [b]	128.48±11.84 [a]	22.24±5.80 [b]
30 mg/kg	166.15±31.63 [a]	127.30±11.36 [a]	26.41±6.60 [a]
60 mg/kg	170.83±20.99 [a]	128.36±12.01 [a]	25.50±3.05 [a]

314 [a,b]Mean values within the column with unlike superscript letters were significantly different
315 ($P<0.05$).
316

317 **Table 4.** Effects of black rice anthocyanin (BACN) on activities of CAT in senescent
318 mice induced by D-galactose
319 (Mean values and standard deviations, n 10 per group)

Groups	Brain(U/mg protein)	Liver(U/mg protein)	Kidney(U/mg protein)
Normal	15.94±1.69 [a]	41.49±6.38 [a]	13.91±3.84 [a]
Model	11.47±1.94 [b]	22.28±4.78 [b]	9.34±2.12 [b]
15 mg/kg	13.82±2.00 [a]	33.71±7.59 [a]	11.20±4.11 [b]
30 mg/kg	14.53±2.12 [a]	34.00±8.26 [a]	12.25±2.64 [a]
60 mg/kg	15.05±2.09 [a]	36.91±9.29 [a]	13.75±2.23 [a]

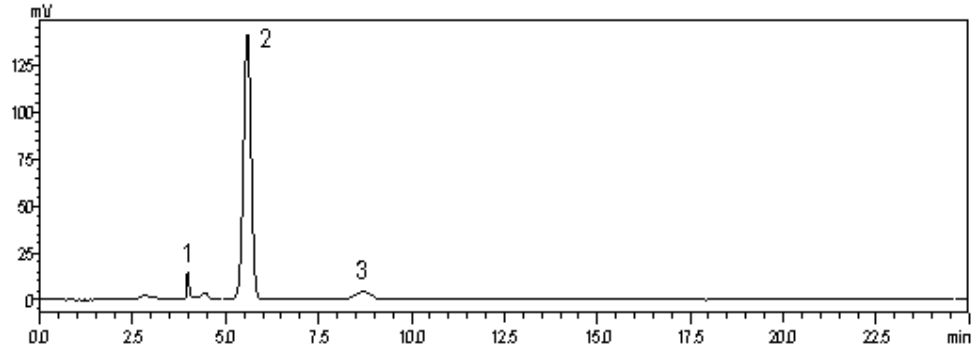
320 [a,b]Mean values within the column with unlike superscript letters were significantly different
321 ($P<0.05$).
322

323 **Table 5.** Effects of black rice athocyanin (BACN) on contents of MDA in senescent
324 mice induced by D-galactose
325 (Mean values and standard deviations, n 10 per group)

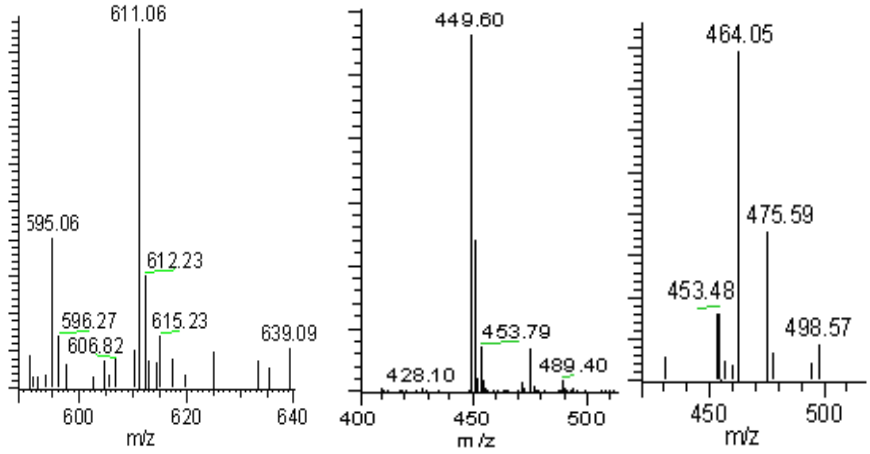
Groups	Brain (nmol/mg protein)	Liver (nmol/mg protein)	Kidney (nmol/mg protein)
Normal	81.90±10.66 [a]	218.59±31.68 [a]	105.99±30.40 [a]
Model	109.42±20.05 [b]	287.03±60.83 [b]	147.63±50.74 [b]
15 mg/kg	104.93±13.91 [b]	277.22±52.47 [b]	111.75±26.20 [a]
30 mg/kg	93.82±12.66 [ac]	234.35±41.40 [a]	114.48±36.93 [a]
60 mg/kg	97.57±12.93 [c]	266.48±84.48 [ab]	120.26±32.69 [ab]

326 [a,b,c]Mean values within the column with unlike superscript letters were significantly different
327 ($P<0.05$).
328

329 **Fig.1**

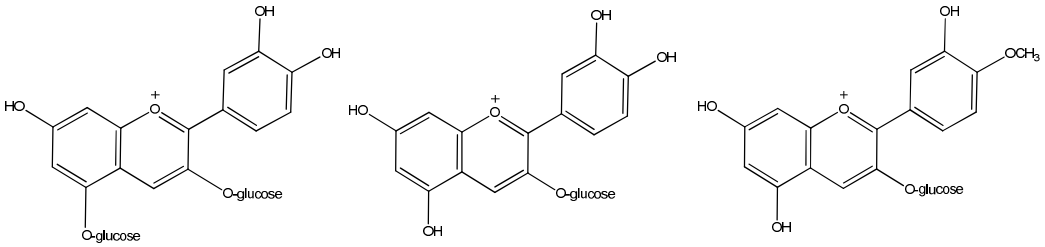


330



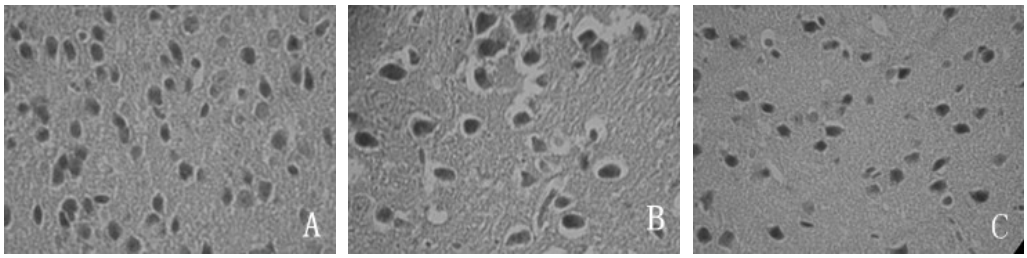
331

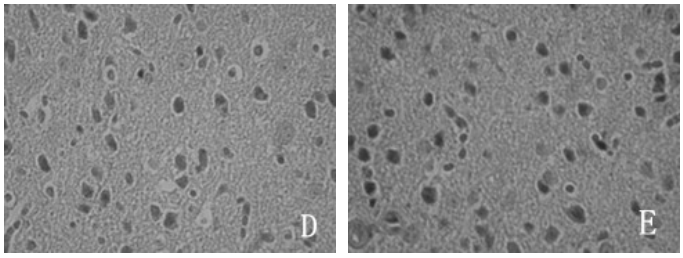
332



333 Cyanidin-3,5-diglucoside Cyanidin-3-O-glucoside Peonidin-3-O-glucoside

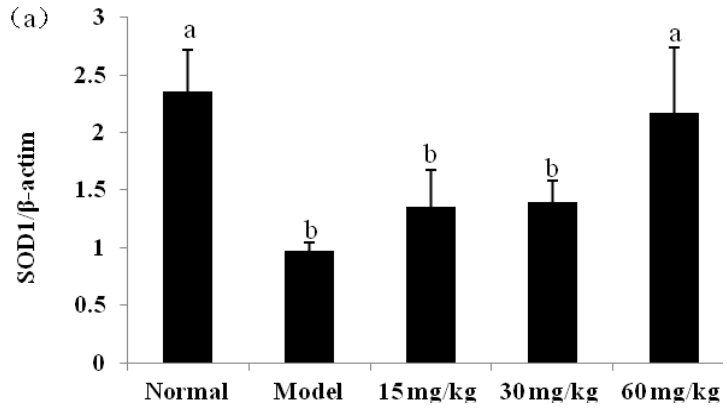
334 **Fig 2**

335 

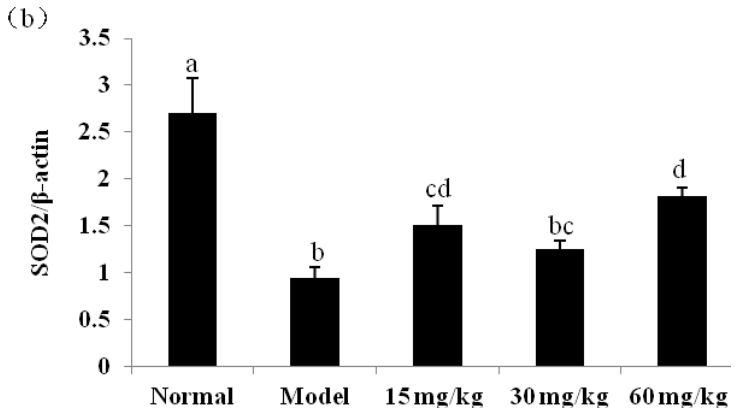
336 

337

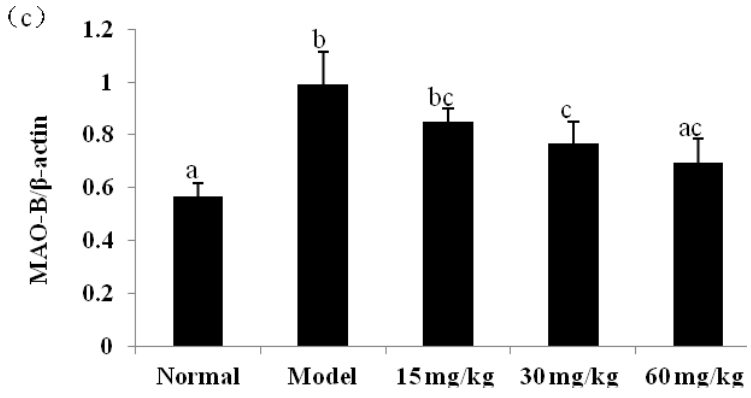
338 **Fig 3**



339



340



341