RESEARCH ARTICLE

The Effects of Germinated Black Glutinous Rice on Antioxidant Defense and Lipid Peroxidation in Male Aged Rats

Tantip Boonsong[1], Onrawee Khongsombat[2,3]

[1] *Department of Biochemistry, Faculty of Medical Sciences, Naresuan University, Phitsanulok, Thailand*
[2] *Department of Physiology, Faculty of Medical Sciences, Naresuan University, Phitsanulok, Thailand*
[3] *Center of Excellence in Medical Biotechnology, Naresuan University, Phitsanulok, Thailand*

Abstract

Reactive Oxygen Species (ROS) are thought to be involved in oxidative damage and contribute to the aging process. It has been reported that unpolished rice or brown rice contained valuable nutrients such as vitamins, gamma-oryzanol, ferulic acid, phytic acid, and most significantly, gamma-aminobutyric acid (GABA) which was intensely increased in germinated brown rice. In this study, we determined the effects of pre-germinated black glutinous rice (PGR) (*Oryza sativa* var. *glutinosa*) on antioxidant enzyme activities such as superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) and malondialdehyde (MDA) in plasma and the hippocampus of male aged rats. Male Sprague-Dawley rats aged 12 months were randomly divided into three diet groups comprising of normal diet, normal diet with non-germinated black glutinous rice (NGR), and normal diet with PGR, respectively for 36 days. At the end of the experiment period, blood and the hippocampus tissue samples were assayed for lipid contents (cholesterol and triglyceride), malondialdehyde (MDA) levels, and the enzyme activity of SOD, GPx, and CAT. The SOD activity in plasma of aged rats fed PGR was significantly higher than that of the control and the NGR groups ($p<0.05$), but no significant changes were seen in the hippocampus from aged rats in different diet groups. Moreover, GPx and CAT activities in plasma and brain remained unchanged. The data also demonstrated that the plasma level of MDA in rats fed PGR was significantly decreased compared with the control ($p<0.05$) although no statistically significant differences were found in the hippocampus. The plasma levels of total cholesterol, triglyceride, and HDL were unchanged in aged rats fed with NGR and PGR diets. However, the level of plasma LDL was significantly decreased ($p<0.05$). The present study demonstrated that supplemented with PGR diet could improve lipid profile and antioxidative status in aged rats by decreasing plasma LDL cholesterol and MDA contents as well as increasing SOD activity, respectively. In conclusion, the data obtained suggests that PGR diet could be beneficial as a functional food to modulate the lipid contents and also as dietary antioxidants, nevertheless it is necessary to verify their roles through further study on the lipid profile and antioxidative mechanisms.

Keywords: Aging, germinated black glutinous rice, antioxidant enzyme, lipid peroxidation

Address correspondence and reprint request to: Tantip Boonsong, Department of Biochemistry, Faculty of Medical Sciences, Naresuan University, Phitsanulok 65000, Thailand.
E-mail address: tantipb@nu.ac.th.

ผลของข้าวเหนียวดำเพาะงอกต่อการต้านอนุมูลอิสระและปฏิกิริยาออกซิเดชั่นของลิพิดในหนูขาวแก่เพศผู้

ธารทิพย์ บุญส่ง[1], อรระวี คงสมบัติ[2,3]

[1] *ภาควิชาชีวเคมี คณะวิทยาศาสตร์การแพทย์ มหาวิทยาลัยนเรศวร จังหวัดพิษณุโลก ประเทศไทย*

[2] *ภาควิชาสรีรวิทยา คณะวิทยาศาสตร์การแพทย์ มหาวิทยาลัยนเรศวร จังหวัดพิษณุโลก ประเทศไทย*

[3] *สถานวิจัยเพื่อความเป็นเลิศทางวิชาการด้านเทคโนโลยีชีวภาพทางการแพทย์ มหาวิทยาลัยนเรศวร จังหวัดพิษณุโลก ประเทศไทย*

บทคัดย่อ

สารอนุมูลอิสระทำให้เกิดความเสียหายในกระบวนการออกซิเดชันและส่งผลเร่งให้เกิดกระบวนการแก่ชรา มีรายงานว่าข้าวที่ไม่ได้ผ่านการขัดสีหรือข้าวกล้องอุดมไปด้วยสารอาหารที่มีคุณประโยชน์ ได้แก่ วิตามิน แกมมาออริซานอล กรดเฟอรูลิก กรดไฟติก และที่สำคัญคือกาบา ซึ่งจะมีปริมาณเพิ่มขึ้นอย่างมากในข้าวกล้องงอก การศึกษานี้ผู้วิจัยสนใจศึกษาผลของข้าวเหนียวดำเพาะงอก (PGR) ที่มีฤทธิ์ต่อการทำงานของเอนไซม์ต้านอนุมูลอิสระได้แก่ ซูเปอร์ออกไซด์ดิสมิวเทส (SOD), กลูตาไธโอนเปอร์ออกซิเดส (GPx), คาตาเลส (CAT) และระดับของมาลอน-ไดอัลดีไฮด์ (MDA) ในพลาสมาและสมองส่วนฮิปโปแคมปัสในหนูแก่เพศผู้ ในการศึกษานี้ใช้หนูขาวเพศผู้อายุ 12 เดือน โดยหนูถูกแบ่งแบบสุ่มออกเป็นสามกลุ่ม คือ กลุ่มที่ได้รับอาหารปกติ กลุ่มที่ได้รับอาหารที่ผสมข้าวเหนียวดำไม่งอก (NGR) และกลุ่มที่ได้รับอาหารที่ผสม PGR หนูทั้งหมดถูกเลี้ยงด้วยอาหารดังกล่าวเป็นเวลา 36 วัน จากนั้นทำการเจาะเลือดและเก็บตัวอย่างสมองส่วนฮิปโปแคมปัสเพื่อนำไปตรวจวิเคราะห์ปริมาณไขมัน (โคเลสเตอรอล, ไตรกลีเซอร์ไรด์) ระดับ MDA และฤทธิ์การทำงานของเอนไซม์ SOD, GPx และ CAT ผลการศึกษาพบว่าการทำงานของเอนไซม์ SOD ในพลาสมาของหนูแก่ที่ได้รับ PGR สูงกว่าของหนูกลุ่มควบคุม และหนูกลุ่มที่ได้รับ NGR แต่ไม่พบการเปลี่ยนแปลงในเนื้อเยื่อสมองของหนูแก่ทั้งสามกลุ่ม และยังพบว่า MDA ในพลาสมาของหนูแก่ที่ได้รับ PGR มีระดับต่ำกว่าของหนูกลุ่มควบคุม แต่ไม่พบความแตกต่างของระดับ MDA ในเนื้อเยื่อสมองส่วนฮิปโปแคมปัสของหนูทั้งสามกลุ่ม นอกจากนี้พบว่าหนูแก่ที่ได้รับ NGR และ PGR ไม่มีการเปลี่ยนแปลงของระดับของโคเลสเตอรอล ไตรกลีเซอไรด์ และ HDL ในพลาสมา แต่มีระดับ LDL ลดลง การศึกษานี้แสดงให้เห็นว่าการให้ PGR เสริมในอาหารปกติส่งผลดีต่อหนูแก่โดยมีผลทำให้ระดับไขมัน LDL และ MDA ลดลง และเพิ่มฤทธิ์การทำงานของเอนไซม์ SOD จากผลการศึกษาสรุปได้ว่า PGR อาจมีประโยชน์ในการใช้เป็นอาหารเสริมสุขภาพที่ช่วยควบคุมระดับไขมันและต้านอนุมูลอิสระได้ อย่างไรก็ตามควรมีการศึกษาเพิ่มเติมเพื่อให้เข้าใจบทบาทในระดับกลไกต่อกระบวนการดังกล่าวต่อไป

คำสำคัญ: ความแก่, ข้าวเหนียวดำเพาะงอก, เอนไซม์ต้านอนุมูลอิสระ, ปฏิกิริยาออกซิเดชันของลิพิด

Introduction

Elderly population from more than 60 countries will increase to 2 million or more, by 2030.[1] In Thailand, the number of older Thai people (aged over 60) will increase up to 17% in 2025 and 27% in 2050.[2] Meanwhile, pathological disorders, including osteoarthritis, hypertension, heart disease, cancer, diabetes, stroke, and neurological disorders, are likely to increase as the mean population age increases. One of the major causes that leads to age related changes has been identified as the production of free radicals and/or reactive intermediates resulting from the metabolism of molecular oxygen, including alterations in antioxidant enzyme activities[3], disturbance of cellular redox balance, oxidative alterations in cellular components (i.e. lipids, proteins, and/or DNA)[4] and increasing levels of lipid peroxidation.[5] Singh et al.[6] found that the level of lipid peroxidation was significantly increased while the levels of antioxidant defense enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPx) in plasma were decreased in healthy males and females with increasing ages, suggesting that old age was associated with an increase in systemic oxidative stress.[7] Therefore, the protection from free radical-induced oxidative stresses may delay or prevent free radicals damages to the cells.

Dietary antioxidants, vitamin E, vitamin C, and carotenoids, were shown to contribute to cellular protection against free radicals.[8,9] At present, consumption of unpolished rice or brown rice (*Oryza sativa* L.) has become more popular as it is rich in valuable nutrients such as vitamins, gamma-oryzanol, ferulic acid, phytic acid[10], and especially gamma-aminobutyric acid (GABA) which was intensely increased in germinated brown rice[10] and has been claimed to play a role in disease prevention. Previous studies demonstrated that intake of germinated brown rice could prevent heart disease[11], Alzheimer's disease[12] and regulate blood sugar level.[10] In addition, increase in phenolic acid content in pre-germinated brown rice may reflect their antioxidant properties.[13] In addition to brown rice, other colored rices including red, purple or black rice has been studied for their bioactive compounds such as antioxidants. Germinated Thai black glutinous rice (*Oryza sativa* var. *glutinosa* or black sticky rice or Niew Dam) was reported to contain high content of anthocyanin (17.89-99.53 mg/100 g of rice) and antioxidant activity.[14]

This study aimed to evaluate antioxidant enzyme activities of SOD, GPx, and catalase (CAT) in plasma and hippocampus of aged rats fed with non-germinated and pre-germinated Thai black glutinous rice compared with control aged rats. We also measured the level of plasma malondialdehyde (MDA), end product of lipid peroxidation, to assess the alteration in antioxidant system in aged rats.

Materials and Methods

Animal

Male Sprague-Dawley rats aged 12 months were obtained from National Laboratory Animal Center, Mahidol University, Thailand. They were housed in standard stainless steel cages at 25±2 °C with a 12-hour light/dark cycle and placed at the Naresuan University Center for Animal Research, Naresuan University, Thailand, throughout the experimental period. The experiment protocol was approved by the Ethical committee for the Use of Animal, Naresuan University (NU-AE 560303). The animals were randomly divided into three groups comprising of normal diet (CON) (N=7), normal diet with unpolished non-germinated rice (NGR) (8 g/day) (N=7), and normal diet with pre-germinated rice (PGR) (8 g/day) (N=9), respectively. All samples were kindly given by Sudarat Jiamyangyuen from the Department of Agro-Industry, Naresuan University. The GABA contents in PGR and NGR diets are 16-18 mg/100 g and 6-8 mg/100 g, respectively (unpublished data). Treatments were given for a total of 36 days. At the end of the experimental period, the animals were anesthetized with sodium pentobarbital (50 mg/kg). Five milliliters of blood and the hippocampus specimens were taken for analysis of plasma lipid profile, lipid peroxidation and antioxidant enzyme activities.

Lipid content analysis

Total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL), and low-density lipoprotein cholesterol (LDL) were analyzed by Beckman Coulter AU480 (Beckman Coulter Inc., Brea, CA, USA), at Allied Health Sciences Promotion Center, Faculty of Allied Health Sciences, Naresuan University, Thailand.

Antioxidant enzyme activity measurement

The activities of antioxidant enzymes (SOD, GPx and CAT) were determined according to the method of commercial kit (Cayman Chemical, Ann Arbor, MI, USA) using an iEMS Reader MF microplate reader (Labsystems, Helsinki, Finland). All brain tissue samples were assayed for protein levels by Bradford assay (Bio-Rad, Hercules, CA, USA). The SOD, GPx and CAT activities in tissue were expressed as U/mg protein. The same analytical procedures used for the brain tissue were applied to plasma samples.

MDA level analysis

The MDA level in brain tissue and plasma samples were measured by high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection at a wavelength of 310 nm using a method modified from Tüközkan et al.[15] The samples were analyzed on a Shimadzu HPLC system (Shimadzu, Tokyo, Japan). MDA separation was carried out using a Prodigy ODS3 C18 analytical column (250×4.6 mm, 5 µm) (Phenomenex, Torrance, CA, USA). The mobile phase was acetonitrile-water (38:62, v/v) containing 0.2% (v/v) acetic acid with a flow rate of 1 mL/min. MDA peak was determined according to its retention time and confirmed by spiking with added exogenous standard. Level of MDA was calculated from standard curve prepared from 1,1,3,3-tetramethoxypropane (TMP) and were expressed as µM for the plasma samples and as µM/mg protein for the brain specimens.

Results

The effects of PGR and NGR on lipid contents

As shown in Figure 1, plasma levels of total cholesterol, triglyceride, and HDL were unchanged in aged rats fed with NGR and PGR diets. However, the level of plasma LDL was significantly decreased ($p<0.05$).

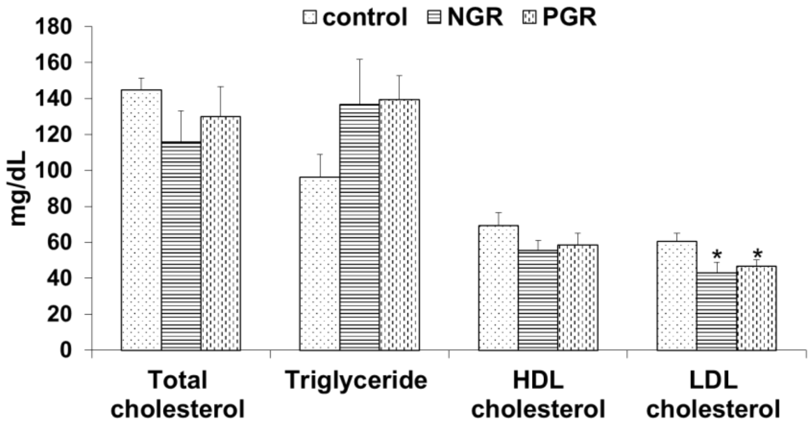

Figure 1. The plasma total cholesterol, triglyceride, HDL cholesterol, and LDL cholesterol (mg/dL) levels in rats fed with NGR (N=4) and PGR (N=6) compared with the control (N=4). Each value represents the mean ± SD. *Significantly different from control (p<0.05).

The effects of PGR and NGR on antioxidant enzyme activities and MDA levels

The results showed that the SOD activity in plasma of rats fed with PGR was significantly increased compared to that of NGR-treated and control groups (p<0.05) (Table 1). The GPx and CAT activities were slightly increased but not statistically significant in PGR groups. However, no significant changes in antioxidant enzyme activities were seen in the hippocampus of aged rats in both diet-treated groups. Moreover, the levels of plasma MDA in aged rats fed with PGR diet was significantly decreased compared to the control diet group (p<0.05) (Table 2).

Table 1. The activities of antioxidant enzymes (SOD, GPx and CAT) in plasma and hippocampus of aged rats fed with NGR and PGR diets.

Group	SOD		GPx		CAT	
	Plasma (U/mL)	Hippocampus (U/mg protein)	Plasma (U/mL)	Hippocampus (U/mg protein)	Plasma (U/mL)	Hippocampus (U/mg protein)
Control	2.14±016 (N=5)	2.74±0.64 (N=7)	62.40±25.24 (N=6)	25.75±7.69 (N=7)	112.75±25.65 (N=7)	58.48±7.41 (N=3)
NGR	1.89±0.20 (N=6)	2.22±0.57 (N=7)	57.31±11.29 (N=7)	21.56±4.90 (N=7)	113.05±0.02 (N=7)	54.02±3.78 (N=3)
PGR	3.20±0.9*# (N=7)	2.63±0.80 (N=9)	68.77±20.94 (N=6)	22.54±6.13 (N=9)	123.37±0.02 (N=7)	57.09±3.36 (N=5)

Values were expressed as the mean ± SD. Control=aged rats fed with normal diet; NGR=aged rats fed with non-germinated rice; PGR=aged rats fed with pre-germinated brown rice. * Significantly different from control (p<0.05); # Significantly different from NGR (p<0.05).

Table 2. The MDA levels in plasma and hippocampus of aged rats fed with NGR and PGR diets.

Groups	MDA	
	Plasma (μM)	**Hippocampus (μM/mg protein)**
Control	0.10±0.03 (N=4)	0.03±0.02 (N=6)
NGR	0.10±0.05 (N=3)	0.02±0.01 (N=6)
PGR	0.06±0.02* (N=6)	0.03±0.02 (N=8)

Values were expressed as the mean ± SD. Control=aged rats fed with normal diet; NGR=aged rats fed with non-germinated rice; PGR=aged rats fed with pre-germinated brown rice. * Significantly different from control (p<0.05); # Significantly different from NGR (p<0.05).

Discussion

In this study, NGR and PGR diets were given orally for 36 days to aged rats with the purpose to monitor changes in lipid profile and antioxidative status. Previous studies have reported that brown rice and pre-germinated brown rice had a significant effect in improving plasma lipid profile.[16-18] Consistently, the results obtained from this study showed that the plasma LDL cholesterol levels were significantly decreased in aged rats fed with either NGR or PGR diets (p<0.05). This observation could be explained by the fact that NGR and PGR contained valuable bioactive compounds such as dietary fibers, vitamins, gamma-oryzanol, and GABA.[10] Some of these compounds might be responsible for this LDL lowering effect.

A previous study by Kahlon and Chow[19] reported that rice bran components in brown rice, probably gamma-oryzanol and vitamin E, specifically bound to bile acids more than other cereal brans and also had strong antioxidative activities, which protected the cells from oxidative damage and membrane degeneration.[20] The aging process has been demonstrated to be accelerated via the accumulation of free radicals in the cells[21], as well as the consequent oxidative damage to cellular components (i.e. lipids). In our body, there are several antioxidant defense mechanisms which contribute to cellular protection against oxidative damage. These include antioxidant enzymes (i.e. SOD, GPx and CAT) and non-enzymatic antioxidants (i.e. glutathione). However, whether the process of aging changes the activities of these antioxidant enzymes is still controversial. In our study, the SOD activity was found to be significantly increased in plasma of aged rats fed with PGR (Table 1), but no significant changes were seen in the activities of GPx and CAT in all treated rats. Furthermore, we observed, an increase in the GPx activity of rats fed with PGR, although this change is not statistically significant. Thus, we proposed that natural antioxidants in PGR diet may exert antioxidative effects[10] and may improve SOD and GPx activities in plasma of aged rats fed with PGR diet. A study by Juliano et al.[22] showed that gamma-oryzanol can scavenge the 2,2-diphenyl-1-picrylhydrazyl radical and reduce lipid peroxidation. The antioxidative effects of PGR diet can also be assessed by determining the reduction of MDA level. Our results showed that feeding aged rats with PGR diet significantly reduced the MDA level compared to the control groups ($p<0.05$). Thus, PGR diet may have beneficial effects on free radical scavenging and decreasing the lipid peroxidation during aging. However, in this study, the activities of SOD, GPx and CAT as well as the MDA levels in both diet treated groups were in concord with previous study performed in brains of aged rats.[23-25] Nevertheless, it was inconsistent with the other report which showed a significant increase in the Mn-SOD activity in the hippocampus of 27-30 months old male rats compared to young male rats and observable changes in Mn-SOD activity in the brain were region-specific.[26] These results suggest that the treatments may not certainly affect oxidative status in brain of aged rats, as shown by the lack of changes in the level of MDA and the activities of antioxidant enzymes.

Conclusion

The present study demonstrated that supplementation with PGR diet in aged rats could improve lipid profile as shown by reducing plasma LDL cholesterol level and antioxidative status by decreasing MDA level and increasing SOD activity. This suggests that PGR diet could be beneficial as a functional food to modulate the lipid contents and also as dietary antioxidants. Nevertheless, further study is necessary to verify the effect of PGR diet on the lipid profile and antioxidative mechanisms.

Conflict of interest statement

The authors have no conflicts of interest to declare.

Acknowledgements

This work was supported by the Agricultural Research Development Agency (ARDA) [No. CRP5507020130]. The authors would like to gratefully thank Laddawan Lalert and Gayvalin Pramoolsilpa for taking care of animal treatment, Sasimol Udomruk and Chatchadawalai Chokchaitaweesuk for experimental analysis, and Professor Gavin Reynolds for manuscript proof reading and editing.

References

1. Tabloski PA. Global aging: implications for women and women's health. J Obstet Gynecol Neonatal Nurs. 2004 Sep;33(5):627-38.
2. Ghazy Mujahid. Population ageing in East and South-East Asia: Current situation and emerging challenges [Internet]. Bangkok, Thailand: UNFPA Country Technical Services Team for East and South-East Asia; 2006 [cited 2018 Nov 8]. Available from: https://asiapacific.unfpa.org/sites/default/files/pub-pdf/papers%20in%20pop%20agening%20no.%201%20%282006%29.pdf

3. Stohs S, El-Rashidy F, Lawson T, Kobayashi R, Wulf B, Potter J. Changes in glutathione and glutathione metabolizing enzymes in human erythrocytes and lymphocytes as a function of age of donor. Age. 1984 Jan;7(1):3-7.
4. Powers SK, Jackson MJ. Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production. Physiol Rev. 2008 Oct;88(4):1243-76.
5. Pfeffer SR, Swislocki NI. Age-related decline in the activities of erythrocyte membrane adenylate cyclase and protein kinase. Arch Biochem Biophys. 1976 Nov;177(1):117-22.
6. Singh K, Kaur S, Kumari K, Singh G, Kaur A. Alterations in lipid peroxidation and certain antioxidant enzymes in different age groups under physiological conditions. J Hum Ecol. 2009 Aug;27(2):143-7.
7. Antier D, Carswell HV, Julia BM, Hamilton CA, Mhairi MI, Groves S, et al. Increased levels of superoxide in brains from old female rats. Free Radic Res. 2004 Feb;38(2):177-83.
8. Carr A, Frei B. Does vitamin C act as a pro-oxidant under physiological conditions? FASEB J. 1999 Jun;13(9):1007-24.
9. Rhee CO SS, Lee YS. Volatile flavor components in cooked black rice. Korean J Food Sci Technol. 2000 Jan;32:1015-23.
10. Patil SB, Khan MK. Germinated brown rice as a value added rice product: A review. J Food Sci Technol. 2011 Dec;48(6):661-7.
11. Zhao R, Ghazzawi N, Wu J, Le K, Li C, Moghadasian MH, et al. Germinated brown rice attenuates atherosclerosis and vascular inflammation in low-density lipoprotein receptor-knockout mice. J Agric Food Chem. 2018 Apr;66:4512-20.
12. Mamiya T, Asanuma T, Kise M, Ito Y, Mizukuchi A, Aoto H, et al. Effects of pre-germinated brown rice on β-amyloid protein-induced learning and memory deficits in mice. Biol Pharm Bull. 2004 Jul;27(7):1041-5.
13. Tian S, Nakamura K, Kayahara H. Analysis of phenolic compounds in white rice, brown rice, and germinated brown rice. J Agric Food Chem. 2004 Jul; 52(15):4808-13.
14. Sutharut J, Sudarat J. Total anthocyanin content and antioxidant activity of germinated colored rice. Int Food Res J. 2012 Jan;19(1): 215-221.
15. Tüközkan N, Erdamar H, Seven I. Measurement of total malondialdehyde in plasma and tissues by high-performance liquid chromatography and thiobarbituric acid assay. Firat Tip Dergisi. 2006 Apr;11(2):88-92.
16. Mohd Esa N, Abdul Kadir KK, Amom Z, Azlan A. Improving the lipid profile in hypercholesterolemia-induced rabbit by supplementation of germinated brown rice. J Agric Food Chem. 2011 Jun;59(14):7985-91.
17. Miura D, Ito Y, Mizukuchi A, Kise M, Aoto H, Yagasaki K. Hypocholesterolemic action of pre-germinated brown rice in hepatoma-bearing rats. Life Sci. 2006 Jun;79(3):259-64.
18. Roohinejad S, Omidizadeh A, Mirhosseini H, Saari N, Mustafa S, Mohd Yusof R, et al. Effect of pre-germination time of brown rice on serum cholesterol levels of hypercholesterolaemic rats. J Sci Food Agric. 2010 Jan;90(2):245-51.

19. Kahlon T, Woodruff C. In vitro binding of bile acids by rice bran, oat bran, barley and β-glucan enriched barley. Cereal Chem. 2003 May;80(3):260-3.
20. Xu Z, Hua N, Godber JS. Antioxidant activity of tocopherols, tocotrienols, and γ-Oryzanol components from rice bran against cholesterol oxidation accelerated by 2,2'-Azobis (2-methylpropionamidine) dihydrochloride. J Agric Food Chem. 2001 Apr;49(4):2077-81.
21. Sohal R, Allen R. Relationship between oxygen metabolism, aging and development. Adv Free Radic Biol Med. 1986;2(1):117-60.
22. Juliano C, Cossu M, Alamanni MC, Piu L. Antioxidant activity of gamma-oryzanol: mechanism of action and its effect on oxidative stability of pharmaceutical oils. Int J Pharm. 2005 Aug;299(1-2):146-54.
23. Doğru-Abbasoğlu S, Tamer-Toptani S, Uğurnal B, Kocak-Toker N, Aykac-Toker G, Uysal M. Lipid peroxidation and antioxidant enzymes in livers and brains of aged rats. Mech Ageing Dev. 1997 Nov;98(2):177-80.
24. Cand F, Verdetti J. Superoxide dismutase, glutathione peroxidase, catalase, and lipid peroxidation in the major organs of the aging rats. Free Radic Biol Med. 1989;7(1):59-63.
25. Barja de Quiroga G, Pérez-Campo R, López Torres M. Anti-oxidant defences and peroxidation in liver and brain of aged rats. Biochem J. 1990 Nov;272(1):247-50.
26. Carrillo M-C, Kanai S, Sato Y, Kitani K. Age-related changes in antioxidant enzyme activities are region and organ, as well as sex, selective in the rat. Mech Ageing Dev. 1992 Sep;65(2-3):187-98.